EICOSANOIDS IN CANCER

EDITED BY: Emanuela Ricciotti, Nune Markosyan, Emer Smyth and Paola Patrignani

PUBLISHED IN: Frontiers in Pharmacology

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-557-2
DOI 10.3389/978-2-88971-557-2

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

EICOSANOIDS IN CANCER

Topic Editors:
Emanuela Ricciotti, University of Pennsylvania, United States
Nune Markosyan, University of Pennsylvania, United States
Emer Smyth, Columbia University, United States
Paola Patrignani, University of Studies G. d'Annunzio Chieti and Pescara, Italy

Citation: Ricciotti, E., Markosyan, N., Smyth, E., Patrignani, P., eds. (2021). Eicosanoids in Cancer. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-557-2

Table of Contents

frontiers in Pharmacology

EDITORIAL
published: 09 September 2021
doi: 10.3389/fphar.2021.765214

Editorial: Eicosanoids in Cancer

*Nune Markosyan[1], Emer M. Smyth[2], Paola Patrignani[3] and Emanuela Ricciotti[4]**

[1]Department of Medicine, Abramson Family Cancer Research Institute, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, United States, [2]Department of Pathology and Cell Biology, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY, United States, [3]Department of Neuroscience, Imaging and Clinical Sciences, School of Medicine, CAST (Center for Advanced Studies and Technology), "G. d'Annunzio" University, Chieti, Italy, [4]Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, Institute for Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, PA, United States

Keywords: eicosanoids, cancer, arachidonic acid, oxylipins, inflammation

Editorial on the Research Topic

Eicosanoids in Cancer

Eicosanoids, a family of bioactive lipid mediators discovered less than 100 years ago, have broad functions in homeostasis and in various physiological and pathological conditions. They are metabolites of arachidonic acid formed primarily through the action of cytosolic phospholipase A2-α. These bioactive lipids consist of three major groups of metabolites: prostaglandins (PG)s and thromboxane, formed by the cyclooxygenases (COX)s; leukotrienes (LT)s, lipoxins, and hydroxyeicosatetraenoic acids formed by the 5-lipoxygenase (LOX); epoxygenated fatty acids, formed by the cytochrome P450 (CYP). The importance of the eicosanoids was emphasized by the Nobel Prize, awarded in 1982, to Sune Bergström, Bengt Samuelsson and John Vane "for their discoveries concerning prostaglandins and related biologically active substances". When produced on demand, these oxidized lipids are responsible for inflammation and its subsequent resolution with eventual return to pre-inflammation levels (Schmid and Brüne, 2021). However, unresolved, chronic inflammation, and pathogen/antigen persistence characterized by sustained PG and LT production and immune-suppression becomes a fertile soil for malignant transformation and tumor immune evasion. Based on these tumor-promoting features, inflammation was designated as an enabling characteristic among the hallmarks of cancer (Hanahan and Weinberg, 2011). As a consequence, eicosanoids became attractive targets in gastrointestinal (Wang and DuBois, 2018), colon (Guillem-Llobat et al., 2016; Patrignani et al., 2017), breast (Kundu et al., 2014; Markosyan et al., 2014), pancreatic (Markosyan et al., 2019), prostate, and lung (Hanaka et al., 2009) cancers, and in melanoma (Zelenay et al., 2015).

Eicosanoids in Cancer research topic combines 1) reviews presenting the current understanding of the role of COXs, (Johnson et al.; Smith et al.; Ching et al.), LOX (Tian et al.), and CYPs (Evangelista et al; Luo and Liu) pathway products in cancer, 2) articles presenting alternative regulators of eicosanoid pathways in cancer such as miR-574-5p (Emmerich et al.), nitro fatty acids (Piesche et al.), and extracellular ATP (Sharma et al.), 3) novel therapeutic approaches focusing on downstream targets in eicosanoid pathways and genes upregulated by COX-2 (Ching et al.; Hidalgo-Estevez et al.), as well as harnessing the potential of dietary manipulations to circumvent pro-tumorigenic effects of eicosanoids (Storniolo et al.; Luo and Liu).

A timely and thorough review by Johnson et al. focuses on the role of prostaglandins in the suppression of anti-tumor immunity through their paracrine effects on the immune cells in the tumor microenvironment. Given the revolution in cancer treatment afforded by immune checkpoint blockade and cellular immunotherapy, investigation into the mechanisms of resistance and sensitization to immunotherapy is becoming imperative. Data presented in the review suggests that blocking prostaglandin pathways can relieve immunosuppression or synergize with immunotherapies. Smith et al. discuss the role of all three groups of eicosanoids in

OPEN ACCESS

Edited and reviewed by:
Dieter Steinhilber,
Goethe University Frankfurt, Germany

****Correspondence:***
Emanuela Ricciotti
emanuela@pennmedicine.upenn.edu

Specialty section:
This article was submitted Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *26 August 2021*
Accepted: *31 August 2021*
Published: *09 September 2021*

Citation:
Markosyan N, Smyth EM, Patrignani P and Ricciotti E (2021) Editorial: Eicosanoids in Cancer. Front. Pharmacol. 12:765214. doi: 10.3389/fphar.2021.765214

gynecological malignancies and Ching et al. review the data on PGE_2 receptors EP2 and EP4 signaling in cancer. The terminal prostanoid synthase inhibition and receptor antagonism are increasingly attractive approaches in cancer treatment as they are free of the dire side effects that have complicated the use of non-steroidal anti-inflammatory drugs and selective COX-2 inhibitors. EP4 antagonism emerges as a promising approach in cancer treatment, especially in combination with chemotherapy or checkpoint blockade.

As putative inflammatory mediators, elevated LTs are associated with tumor initiation, progression, neo-angio- and lymphogenesis, epithelial-to-mesenchymal transition, and metastasis. However, as discussed in the review by Tian et al., there is a body of evidence indicating that these lipid mediators may 1) alleviate immune-suppression through recruitment of $CD8^+$ T cells, 2) maintain viable lymphatic vasculature, increasing the efficacy of immunotherapy. Similarly, several isoforms of CYPs are overexpressed in solid tumors, and due to their drug metabolizing capacity and pro-angiogenic/vasodilator functions, are implicated in chemoresistance, tumor growth and metastasis. Evangelista et al. review the contrasting roles of CYPs in physiological conditions and cancer and point out the difficulty of inhibition of these pathways in cancer due to their importance in non-cancer settings. Analyzing the pleotropic functions of CYPs in cancer, Luo et al. conclude that CYP- mediated eicosanoids derived from ω-3 fatty acids lack the pro-tumorigenic effects of their ω-6 derived counterparts and can be beneficial in cancer prevention and treatment. A study by Storniolo et al. furthers the notion of the possibility to alter the effect of eicosanoids in cancer by manipulating the composition of dietary fats, with high concentrations of ω-3 fatty acids inducing apoptosis of colorectal cancer cells and low concentrations being mitogenic for the same cell line.

A series of research articles within the Eicosanoids in Cancer research topic identify novel regulators of prostaglandin synthesis enzymes that can be targeted in inflammation as well as in cancer. Micro RNAs are more often post-transcriptional repressors of gene expression, but as shown by Emmerich et al., in human lung cancer cells, miR-574-5p induces PGE_2 synthetic enzyme microsomal prostaglandin E synthase-1 (mPGES1) expression and promotes tumor growth. High expression of *PTGES*, the gene encoding mPGES1 protein, is associated with low $CD8^+$ T cells infiltration and shorter patient survival (Kim et al., 2019), and a small molecular inhibitor of mPGES1 was shown to suppress tumor growth in a preclinical neuroblastoma mouse model (Kock et al., 2018). miR-574-5p emerges as a new target to control intra-tumoral mPGES1 expression, hence PGE_2 levels, and tumor growth. Sharma et al. explore a possible mechanism of COX-2 upregulation following chemotherapy. In cervical, neuronal, and breast cancer cell lines, doxorubicin induced cell death results in increased release of ATP that coincides with up-regulation of COX-2 and metalloproteinases-2 and increased tumor cell invasion and migration. Further studies are needed to explore the potential of ATP-binding purinergic receptors as potential targets in cancer therapeutics. A review by Piesche et al. summarizes the data on nitro fatty acids (NFA) that can undergo Michael addition reactions and modify the function of target proteins such as Peroxisome Proliferator-Activated Receptor-γ, NF-κB, 5-LOX, and soluble epoxide hydrolase. This ability of NFAs can potentially be used to control inflammation and enhance cancer treatments. Finally, Hidalgo-Estevez et al. suggest that targeting genes regulated by COX-2 such as Dual Specificity Phosphatase 4 and 10, Programmed cell death 4, Trophoblast cell-surface antigen 2 and many from the Tumor growth factor β and p53 pathways can be a viable alternative to the use of COX-2 inhibitors in colon cancer.

Although widely studied as inflammatory mediators, the role of many eicosanoids in cancer has not been completely elucidated. PGE_2 has justifiably received the most attention, but more research is needed to understand the contribution of other prostanoids, LTs, and CYP metabolites in cancer initiation, progression, spread, and treatment. Most importantly, anti-tumor therapies targeting these inflammatory pathways should be specifically tailored based on the tissue eicosanoid profile and receptor expression as well as their interplay with other cancer treatments.

AUTHOR CONTRIBUTIONS

ER: Conceptualization. Reviewing. Original draft preparation. NM: Writing, Reviewing. Original draft preparation. EMS: Reviewing and Editing. PP: Reviewing and Editing.

REFERENCES

Guillem-Llobat, P., Dovizio, M., Bruno, A., Ricciotti, E., Cufino, V., Sacco, A., et al. (2016). Aspirin Prevents Colorectal Cancer Metastasis in Mice by Splitting the Crosstalk between Platelets and Tumor Cells. *Oncotarget* 7 (22), 32462–32477. May 31. doi:10.18632/oncotarget.8655

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of Cancer: the Next Generation. *Cell* 144 (5), 646–674. Mar 4. doi:10.1016/j.cell.2011.02.013

Hanaka, H., Pawelzik, S. C., Johnsen, J. I., Rakonjac, M., Terawaki, K., Rasmuson, A., et al. (2009). Microsomal Prostaglandin E Synthase 1 Determines Tumor Growth *In Vivo* of Prostate and Lung Cancer Cells. *Proc. Natl. Acad. Sci. U S A.* 106 (44), 18757–18762. Nov 3. doi:10.1073/pnas.0910218106

Kim, S. H., Roszik, J., Cho, S. N., Ogata, D., Milton, D. R., Peng, W., et al. (2019). The COX2 Effector Microsomal PGE2 Synthase 1 Is a Regulator of Immunosuppression in Cutaneous Melanoma. *Clin. Cancer Res.* 25 (5), 1650–1663. Mar 1. doi:10.1158/1078-0432.CCR-18-1163

Kock, A., Larsson, K., Bergqvist, F., Eissler, N., Elfman, L. H. M., Raouf, J., et al. (2018). Inhibition of Microsomal Prostaglandin E Synthase-1 in Cancer-Associated Fibroblasts Suppresses Neuroblastoma Tumor Growth. *EBioMedicine* 32, 84–92. Jun. doi:10.1016/j.ebiom.2018.05.008

Kundu, N., Ma, X., Kochel, T., Goloubeva, O., Staats, P., Thompson, K., et al. (2014). Prostaglandin E Receptor EP4 Is a Therapeutic Target in Breast Cancer Cells with Stem-like Properties. *Breast Cancer Res. Treat.* 143 (1), 19–31. Jan. doi:10.1007/s10549-013-2779-4

Markosyan, N., Chen, E. P., and Smyth, E. M. (2014). Targeting COX-2 Abrogates Mammary Tumorigenesis: Breaking Cancer-Associated Suppression of

Immunosurveillance. *Oncoimmunology* 3, e29287, 2014 . Jun 30. doi:10.4161/onci.29287

Markosyan, N., Li, J., Sun, Y. H., Richman, L. P., Lin, J. H., Yan, F., et al. (2019). Tumor Cell-Intrinsic EPHA2 Suppresses Anti-tumor Immunity by Regulating PTGS2 (COX-2). *J. Clin. Invest.* 129 (9), 3594–3609. Jun 4. doi:10.1172/JCI127755

Patrignani, P., Sacco, A., Sostres, C., Bruno, A., Dovizio, M., Piazuelo, E., et al. (2017). Low-Dose Aspirin Acetylates Cyclooxygenase-1 in Human Colorectal Mucosa: Implications for the Chemoprevention of Colorectal Cancer. *Clin. Pharmacol. Ther.* 102 (1), 52–61. Jul. doi:10.1002/cpt.639

Schmid, T., and Brüne, B. (2021). Prostanoids and Resolution of Inflammation - beyond the Lipid-Mediator Class Switch. *Front. Immunol.* 12, 714042, 2021 . Jul 12. doi:10.3389/fimmu.2021.714042

Wang, D., and DuBois, R. N. (2018). Role of Prostanoids in Gastrointestinal Cancer. *J. Clin. Invest.* 128 (7), 2732–2742. Jul2. doi:10.1172/JCI97953

Zelenay, S., van der Veen, A. G., Böttcher, J. P., Snelgrove, K. J., Rogers, N., Acton, S. E., et al. (2015). Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. *Cell* 162 (6), 1257–1270. Sep 10. doi:10.1016/j.cell.2015.08.015

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared past collaborations with one of the authors PP.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 13 March 2020
doi: 10.3389/fphar.2020.00196

Proteomics-Based Characterization of miR-574-5p Decoy to CUGBP1 Suggests Specificity for mPGES-1 Regulation in Human Lung Cancer Cells

*Anne C. Emmerich[1,2], Julia Wellstein[1,2], Elena Ossipova[3], Isabell Baumann[1,2], Johan Lengqvist[3], Kim Kultima[4], Per-Johan Jakobsson[3], Dieter Steinhilber[2] and Meike J. Saul[1,2]**

[1] Department of Biology, Technische Universität Darmstadt, Darmstadt, Germany, [2] Institute of Pharmaceutical Chemistry, Goethe-Universität Frankfurt, Frankfurt, Germany, [3] Rheumatology Unit, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden, [4] Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden

OPEN ACCESS

Edited by:
Emanuela Ricciotti,
University of Pennsylvania,
United States

Reviewed by:
Lihong Chen,
Dalian Medical University, China
Andreas Koeberle,
The University of Tokyo, Japan

***Correspondence:**
Meike J. Saul
saul@bio.tu-darmstadt.de

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: 19 November 2019
Accepted: 13 February 2020
Published: 13 March 2020

Citation:
Emmerich AC, Wellstein J, Ossipova E, Baumann I, Lengqvist J, Kultima K, Jakobsson P-J, Steinhilber D and Saul MJ (2020) Proteomics-Based Characterization of miR-574-5p Decoy to CUGBP1 Suggests Specificity for mPGES-1 Regulation in Human Lung Cancer Cells. Front. Pharmacol. 11:196. doi: 10.3389/fphar.2020.00196

MicroRNAs (miRs) are one of the most important post-transcriptional repressors of gene expression. However, miR-574-5p has recently been shown to positively regulate the expression of microsomal prostaglandin E-synthase-1 (mPGES-1), a key enzyme in the prostaglandin E_2 (PGE_2) biosynthesis, by acting as decoy to the RNA-binding protein CUG-RNA binding protein 1 (CUGBP1) in human lung cancer. miR-574-5p exhibits oncogenic properties and promotes lung tumor growth *in vivo* via induction of mPGES-1-derived PGE_2 synthesis. In a mass spectrometry-based proteomics study, we now attempted to characterize this decoy mechanism in A549 lung cancer cells at a cellular level. Besides the identification of novel CUGBP1 targets, we identified that the interaction between miR-574-5p and CUGBP1 specifically regulates mPGES-1 expression. This is supported by the fact that CUGBP1 and miR-574-5p are located in the nucleus, where CUGBP1 regulates alternative splicing. Further, in a bioinformatical approach we showed that the decoy-dependent mPGES-1 splicing pattern is unique. The specificity of miR-574-5p/CUGBP1 regulation on mPGES-1 expression supports the therapeutic strategy of pharmacological inhibition of PGE_2 formation, which may provide significant therapeutic value for NSCLC patients with high miR-574-5p levels.

Keywords: miR-574-5p, CUGBP1, non-canonical miR function, decoy, proteomics, mPGES-1, lung cancer

Abbreviations: Δ, knockdown; CUGBP1, CUG-RNA binding protein 1; FISH, fluorescence *in situ* hybridization; hnRNP E2, Heterogenous Nuclear Ribonucleoprotein E2; IL, Interleukin; IPA, Ingenuity pathway analysis; miR, microRNA; mPGES-1, microsomal prostaglandin E-synthase-1; NDUFS2, NADH- Ubiquinone Oxidoreductase Core Subunit S2; nt, nucleotides; oe, overexpression; PGE_2, prostaglandin E_2; RBP, RNA-binding protein; RIP, RNA immunoprecipitation; SEM, standard error of mean; SMAD, Mothers against decapentaplegic homolog; TMT, tandem mass tag; UTR, untranslated region.

INTRODUCTION

MicroRNAs (miRs) represent a large family of small non-coding RNAs of approximately 21 nucleotide length. MiRs have proven to be important post-transcriptional regulators of gene expression in eukaryotes (Jansson and Lund, 2012). It is predicted that miRs control the expression of about 60% of human protein-coding genes (Friedman et al., 2009; Schwanhäusser et al., 2011) and participate in the regulation of almost all cellular processes. Importantly, they have emerged as critical regulators of tumorigenesis and progression of different types of cancer, since certain miRs can lead to a dysregulation of processes like cell differentiation (Harfe, 2005), cell cycle (Carleton et al., 2007), and apoptosis (Jovanovic and Hengartner, 2006). Mechanistically, miRs are known to repress expression of their target genes by base-pairing to 3′ untranslated regions (UTRs) (Wienholds and Plasterk, 2005). Depending on the level of complementarity, miR binding leads to either translational repression or degradation of the mRNA. Both regulatory miR functions result in strongly impaired gene expression and a decreased protein level (Guo et al., 2010).

In contrast to this, over the last years different studies have discovered new non-canonical miR functions. MiRs are not only able to inhibit gene expression, but also to activate gene expression. They can bind to RNA-binding proteins (RBPs) and sequester them from their target mRNA. This function is independent of the miR's seed region and operates exclusively by interference with a RBP. It was shown for the first time for miR-328 which acts as RNA decoy for the heterogeneous nuclear ribonucleoprotein E2 (hnRNP E2), a global gene expression repressor (Eiring et al., 2010; Saul et al., 2016, 2019b).

Recently, a new miR/RBP interaction was discovered. MiR-574-5p reveals a binding site for CUG-RNA binding protein 1 (CUGBP1) in its mature form (Saul et al., 2019a). Therefore, it can directly interact with CUGBP1 and promotes lung tumor growth *in vivo* by inducing microsomal prostaglandin E-synthase-1 (mPGES-1) expression, the terminal synthase responsible for the production of the pro-tumorigenic lipid mediator prostaglandin E_2 (PGE_2) (Nakanishi and Rosenberg, 2013). In human lung tumor and under inflammatory circumstances, miR-574-5p is strongly upregulated and increases mPGES-1 expression by preventing CUGBP1 binding to the mPGES-1 3′UTR which leads to an enhanced alternative splicing and the generation of a novel 3′UTR isoform (Saul et al., 2019a). The newly discovered link between miR-574-5p overexpression and PGE_2-mediated tumor growth *in vivo* suggests that pharmacological inhibition of PGE_2 formation might be a potential therapeutic approach in combination with standard therapies for lung cancer patients with high miR-574-5p levels (Saul et al., 2019a). Thereby, miR-574-5p could serve as a stratification and biomarker to identify suitable candidates, an approach which is also supported by earlier studies (Foss et al., 2011; Del Vescovo et al., 2014).

To identify new CUGBP1 targets and characterize the global impact of the novel RNA decoy mechanism of miR-574-5p and CUGBP1, with the aim of demonstrating the specificity of the biomarker miR-574-5p, we applied a tandem mass tag (TMT)-based proteomics approach in A549 lung cancer cells. In this study, we were able to identify and validate novel CUGBP1 targets like Mothers against decapentaplegic homolog (SMAD) 2 which is an important signal transducer and transcription factor. Moreover, we demonstrate that the miR-574-5p/CUGBP1 decoy mechanism could be specific for mPGES-1 in human lung cancer due to a very unique splicing pattern in the 3′UTR of mPGES-1.

MATERIALS AND METHODS

Cell Line and Cell Culture Conditions

The human lung adenocarcinoma cell line A549 (ATCC) was cultured in Dulbecco's modified Eagle medium (DMEM, Life Technologies, Thermo Fisher Scientific) with 10% (v/v) fetal bovine serum (FBS, Life Technologies, Thermo Fisher Scientific), 100 U/ml penicillin, 100 μg/ml streptomycin and 1 mM sodium pyruvate (PAA the Cell Culture Company). Cell culture was carried out in a humidified atmosphere of 5% CO_2 at 37°C.

RNA Interference

A549 cells were seeded at a density of 5×10^5 cells/well in a 6-well plate and treated with 20 pmol siRNA oligonucleotides the next day using Lipofectamine 2000® (Invitrogen) according to manufacturer's instructions. A previously published anti-CUGBP1 siRNA (5′-GCUGUUUAUUGGUAUGAUU-3′) was used for transient knockdown (Δ) of CUGBP1 (Vlasova-St Louis and Bohjanen, 2011). As control, a scramble siRNA with an unspecific sequence (5′-UCUCUCACAACGGGCAUUU-3′) was used. 24 h after transfection, A549 cells were incubated with 5 ng/ml of Interleukin (IL)-1β (Sigma-Aldrich) for further 24 h. The efficiency of the knockdown was determined by Western blot analysis as described in Saul et al. (2019a).

Transfection of miR-574-5p Mimic or Inhibitor (miR Overexpression or miR Knockdown)

A549 cells were seeded at a density of 5×10^5 per well in a 6-well plate. For overexpression of miR-574-5p, 100 pmol miRIDIAN miR-574-5p mimics (HMI0794, Sigma-Aldrich) or negative control mimic (HMC0002, Sigma-Aldrich) were transfected using Lipofectamine 2000® according to the manufacturer's instructions. In the same way, the A549 cells were transfected with 200 pmol miR-574-5p-LNA™ inhibitor (4101451-001, Exiqon) or negative control LNA (199006-001, Exiqon) for the knockdown of miR-574-5p. 24 h after transfection, A549 cells were incubated with 5 ng/ml of IL-1β (Sigma-Aldrich) for further 24 h. The transfection efficiency was determined by qRT-PCR.

Fraction Preparation

In order to find compartment specific regulations, soluble and microsomal proteins of A549 cells were separately analyzed. The fractionation was performed as previously described (Saul et al., 2016). Protein content of Western blot samples was

determined by Bradford assay (Bio-Rad Laboratories), for proteomics samples the protein amount was determined by Pierce BCA Protein Assay (Thermo Fisher Scientific) following manufacturing instructions.

Trypsin Digestion, TMT Labeling and Peptide Fractionation

The protein pellets were solubilized in 50 µl buffer containing 0.05 M triethylammonium bicarbonate, 4 M urea, 0.01% SDS and 2% RapiGest SF Surfactant (Waters). Equal protein amount from each sample (50 µg) was taken as starting point for further sample preparation. Disulfide reduction was performed by adding 5 µl 1 M DTT (in H_2O) for 30 min at 56°C followed by sulfhydryl alkylation performed by adding 4 µl iodoacetamide solution (1 M in H_2O) and incubation at RT for 1 h in the dark. Trypsin (modified sequencing grade, Promega) was added in a ratio of 1:30 (trypsin: protein) and the samples were incubated at 37°C overnight. Peptide labeling was performed using tandem mass tags (TMT 6-plex, Thermo Fisher Scientific) according to the instructions of the manufacturer (**Table 1**). Labeled peptide samples were pooled to final soluble and microsomal fractions and excess reagents were removed by solid phase extraction (STRATA XC Phenomenex). Liquid chromatography tandem MS of a TMT-labeled sample was performed on QExactive mass spectrometer (Thermo Fisher Scientific).

Peptide pre-fractionation was done essentially as described (Cao et al., 2012). Briefly, TMT-labeled protein digests were separated over a 60 min gradient (3–55% B-buffer) on a 2.1 × 250 mm XBridge BEH300 C18 column (Waters) using a flow rate of 200 µL/min. A- and B-buffers consisted of 20 mM ammonia in MilliQ-grade water or 20 mM ammonia in 80% acetonitrile, respectively. Individual fractions were collected per minute and the fractions covering the peptide elution range were concatenated to yield 12 final pooled fractions. These fractions were evaporated to dryness by vaccume drying and stored frozen until nanoLC-MS data capture.

Mass Spectrometry

Online LC-MS was performed using a Q-Exactive mass spectrometer (Thermo Scientific). Peptide samples were trapped on an Acclaim PepMap trap column (C18, 3 µm, 100Å, 75 µm × 20 mm), and separated on a 15-cm long C18 picofrit column (100 µm internal diameter, 5 µm bead size, Nikkyo Technos, Tokyo, Japan) installed on to the nano-electrospray ionization source. Solvent A was 97% water, 3% acetonitrile, 0.1% formic acid; and solvent B was 5% water, 95% acetonitrile, 0.1% formic acid. At a constant flow of 0.25 µl/min, the curved gradient went from 3% B up to 48% B in 50 min.

FTMS master scans with 70,000 resolution (and mass range 400–1200 m/z) were followed by data-dependent MS/MS (17,500 resolution) on the top 10 precursor ions using higher energy collision dissociation (HCD) at 31% normalized collision energy. Precursors were isolated with a 2 m/z window. Automatic gain control (AGC) targets were 3e6 for MS1 and 2e5 for MS2. Maximum injection times were 250 ms for MS1 and 200 ms for MS2. Dynamic exclusion was used with 20 s duration. Precursors with unassigned charge state or charge state 1 were excluded. An underfill ratio of 1% was used. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD016803.

Data Analysis

Acquired MS raw files were searched using Sequest-Percolator under the software platform Proteome Discoverer 1.4.1.14 (Thermo Fisher Scientific) against human Uniprot database (release 01.12.2015) and filtered to a 1% FDR cut off. We used a precursor ion mass tolerance of 10 ppm, and product ion mass tolerances of 0.02 Da for HCD-FTMS. The algorithm considered tryptic peptides with maximum 2 missed cleavages; carbamidomethylation (C), TMT 6-plex (K, N-term) as fixed modifications and oxidation (M) as dynamic modifications. Quantification of reporter ions was done by Proteome Discoverer on HCD-FTMS tandem mass spectra using an integration window tolerance of 10 ppm. Only unique peptides in the data set were used for quantification. Fold values were calculated comparing proteins from ΔCUGBP1 to Scramble, ΔmiR-574-5p to negative control LNA and miR-574-5p oe to negative control mimic. Fold values of +1.5/−1.5 were considered up-/downregulated. All regulated proteins in soluble as well as microsomal fractions from all three conditions were analyzed using Ingenuity Pathway Analysis (IPA) (Ingenuity Systems[1]). We predicted the five most affected canonical pathways. The canonical pathways with p-values ≤ 0.05 were defined as significant.

RNA Extraction

Total RNA was extracted with TRIzol reagent (Invitrogen) and treated with Turbo DNase (Ambion, Thermo Fisher Scientific) according to manufacturer's instructions. RNA concentration was determined with NanoDrop (Peqlab). DNase-treated RNA was used for reverse transcription. For mRNAs, the High-Capacity cDNA reverse transcription Kit (Applied Biosystems, Thermo Fisher Scientific) was used according to manufacturer's instructions. For miR detection, RNA was transcribed with the miScript II RT Kit (Qiagen) according to manufacturer's instructions.

Quantitative Reverse Transcription PCR (qRT-PCR)

qRT-PCR was performed with Applied Biosystems StepOne Plus™ Real-Time PCR System (Applied Biosystem, Thermo Fisher Scientific). 1 µl cDNA (1:2 diluted) was used per reaction. For mRNA quantification, qRT-PCR was performed using Fast SYBR Green PCR Master Mix (Applied Biosystems, Thermo Fisher Scientific). The sequences for primer pairs are listed in **Table 2**. qRT-PCR based miR quantification was performed using miScript system (Qiagen). It was performed using the miR-574-5p specific primer (MS00043617, Qiagen). qRT-PCR was

[1] www.ingenuity.com

performed according to the manufacturer's instructions. Fold inductions were calculated using $2(-\Delta Ct)$ value.

RNA Immunoprecipitation (RIP)

RNA immunoprecipitation was performed as previously described (Saul et al., 2019a). In short, 6×10^6 A549 cells per RIP were resuspended in lysis buffer containing 10 mM Tris-HCl (Carl Roth) pH 7.5, 10 mM KCl (Sigma-Aldrich), 1.5 mM $MgCl_2$, 0.5 mM DTT (Sigma-Aldrich), 0.9% Nonidet P-40 (Sigma-Aldrich), 20 µl ribonuclease inhibitor and protease inhibitor EDTA-free (Roche). The suspension was sonicated. Afterward, samples were centrifuged, supernatant was transferred into a fresh tube and 10% were taken as input sample. We used blocked GammaBind Plus Sepharose beads (GE Healthcare). Beads and antibodies were linked by mixing 50 µl bead suspension with 10 µg of CUGBP1 antibody (05-621 clone3B1, Merck) or normal mouse IgG antibody (12-371, Merck). The IP was then conducted by dividing the lysate equally to the CUGBP1-/IgG-bead mixture and incubating for 2 h at 4°C. Afterward, samples were washed and 10% of each precipitate was taken for Western blot analysis to validate the IP. The remaining precipitates were resuspended in 500 µl TRIzol reagent (Invitrogen) and RNA was isolated as described above. Subsequent qRT-PCR based mRNA or miR quantification was performed as previously described. All buffers and solutions contained protease inhibitor EDTA-free (Roche). Successful precipitation was verified via Western blot analysis with a primary antibody directed against CUGBP1 (ab129115, Abcam).

Western Blotting

Western blot analysis was performed as previously described (Ochs et al., 2013). Briefly, the Odyssey Imaging System (Li-COR Biosciences) was used which allows a linear quantification using near-infrared fluorescence. The membranes were incubated with primary antibodies that recognize CUGBP1 (ab9549, Abcam), SMAD2 (sc-6200, Santa Cruz), SMAD3 (ab28379, Abcam), SMAD4 (ab3219, Abcam), p38 (sc-535, Santa Cruz), NDUFS2 (ab96160, Abcam), mPGES-1 [160140, Cayman-Chemical (Westman et al., 2004)], β-Actin (sc-1616, Santa Cruz).

Bioinformatical 3′UTR Analysis

For the analysis of splice patterns, we concentrated on the list of proteins that were upregulated at least 1.5-fold in response to ΔCUGBP1 concerning the mass spectrometry data. All described 3′UTR sequences of those 399 proteins were downloaded from ensemble biomart [GRCh38/.p12, version 91, Ensembl variation resources (Hunt et al., 2018)], resulting in a list of 1916 transcripts. Those were aligned with 42 different binding motifs of CUGBP1 that were known so far [downloaded from the online tool Splice Aid F (Marquis et al., 2006; Giulietti et al., 2013)].

Then, three criteria were applied (high stringency analysis): (I) binding sites should be of 39 or 46 nucleotides (nt) length, (II) there should be 2 binding sites and (III) those binding sites should span a potential intron of 1000 nt. For a second less stringent approach (referred to as low stringency analysis), we investigated transcripts (I) with binding sites of at least 8 nt length (II), at least 2 binding sites and (III) those binding sites should span a potential intron of 100 nt. Analysis was conducted using Microsoft Excel.

Immunofluorescence

Immunofluorescent staining was performed as previously described (Saul et al., 2019a). A549 cells were seeded on glass cover slips (12 mm, Neolab) at a density of 2.5×10^5 per well in a 6-well plate and incubated for 24 h. Cells were washed with PBS, prior to fixation with 4% formaldehyde (FA, Carl Roth) for 10 min. After washing with PBS 3 times for 3 min, cells were permeabilized with 0.5% Triton X-100 (Sigma-Aldrich) in PBS for 10 min. Then, cells were blocked with 2% BSA (Sigma-Aldrich) in PBS for 20 min. The primary antibody directed against CUGBP1 (ab9549, Abcam) was diluted in blocking solution (1:500) and incubated for 1 h at room temperature together with fixed cells. Afterward, cells were washed 3 times with 0.01% Tween20 (Carl Roth) in PBS for 5 min and incubated for 45 min at room temperature with the secondary antibody goat anti-mouse IgG (Alexa Fluor® 594, ab150116, Abcam) diluted in blocking solution (1:500). Finally, cells were washed with 0.01% Tween20 (Carl Roth) in PBS as described, counterstained for 5 min with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI, Sigma-Aldrich) and mounted in Mowiol 4-88 mounting medium (Sigma-Aldrich).

Fluorescent *In situ* Hybridization (FISH)

FISH was performed as previously described (Saul et al., 2019a). 2.5×10^5 A549 cells were seeded on glass cover slips (12 mm, Neolab) in 6-well plates and incubated for 24 h. Cells were washed with PBS and prefixed with 1% FA in PBS for 10 min. After 3 washing steps of 3 min with PBS, cells were permeabilized with 0.5% Triton X-100 in PBS for 20 min on ice. After additional three washing steps of 3 min with PBS, cells were refixed with 4% FA for 10 min followed by further washing with PBS 3 times for 3 min. Subsequently, samples were prehybridized for 30 min at 40°C in microRNA ISH buffer (Qiagen) and then hybridized for 1 h at 54°C with 100 nM double digoxigenin (DIG) labeled miR-574-5p probe (Qiagen) diluted in microRNA ISH buffer. After hybridization, samples were washed twice for 5 min with 2x saline-sodium citrate buffer (Gibco, Carlsbad, CA, United States) at hybridization temperature and once at room temperature followed by a 20 min blocking step with 2% BSA in PBS. Subsequently, cells were incubated for 1 h at room temperature with rabbit anti-DIG antibody (9H27L19, Invitrogen, Thermo Fisher Scientific) diluted 1:40 in blocking solution. Afterward, cells were washed with 0.01% Tween20 in PBS 3 times for 5 min and incubated at room temperature for 45 min with secondary antibody goat anti-rabbit IgG (Alexa Fluor® 594, 111-585-144, Jackson ImmunoResearch) diluted 1:300 in blocking solution. Then, cells were washed with 0.01% Tween20 in PBS, counterstained with DAPI and mounted as described.

Microscopy

The Leica TCS SPE confocal point scanner mounted on a Leica DMi8 stand equipped with an oil immersion 63 × Apochromat was used in order to take confocal images of immunofluorescence and miR FISH samples. For all samples, the 405 and 561 nm

laser lines were used to perform excitation. All images were analyzed using the ImageJ software and show one focal plane of the middle of the nucleus.

Statistics

Results are shown as mean + Standard error of mean (SEM) of at least three independent experiments. Statistical analysis was carried out by Student's paired or unpaired *t*-test (two-tailed) using GraphPad Prism 5.0. Differences were considered as significant for $p \leq 0.05$ (indicated as * for $p \leq 0.05$, ** for $p \leq 0.005$, and *** for $p \leq 0.001$).

RESULTS

Analysis of Protein Expression Changes in Response to ΔCUGBP1, ΔmiR-574-5p, and miR-574-5p oe in IL-1β-Stimulated A549 Cells

A stable isotope labeling based proteomics study was conducted to identify changes in the proteome of A549 lung cancer cells stimulated with IL-1β (**Figure 1**). A549 cells with ΔCUGBP1, ΔmiR-574-5p or miR-574-5p oe were compared to their corresponding controls. Knockdown of CUGBP1 was validated via Western blot analysis and showed a reduction of 67% in the soluble fraction and 83% in the microsomal fraction of A549 cells (**Supplementary Figure S1**). Knockdown and overexpression of miR-574-5p were quantified with qRT-PCR analysis and revealed a significant ∼80% decrease of miR-574-5p, while miR-574-5p was ∼300-fold upregulated in the miR-574-5p oe samples (**Supplementary Figure S1**). For the LC-MS/MS analysis, all samples were digested and were labeled with TMT 6-plex to allow quantitative protein comparison (**Table 1**). In all three conditions (ΔCUGBP1, ΔmiR-574-5p or miR-574-5p oe) we identified roughly the same numbers of total protein (in soluble fraction around 2450 and in microsomal fraction around 3970 proteins). But only small percentages of them revealed an up- or downregulation in comparison to their corresponding controls (**Figure 2**). Overall, we set the criteria for upregulation to a TMT ratio of ≥1.5 (fold change ≥ 1.5) and downregulation was considered with a TMT ratio of ≤0.5 (fold change ≤ −1.5). In ΔCUGBP1 cells, canonical targets of CUGBP1 as well as decoy targets were supposed to show an upregulation in response to the knockdown. In the soluble fraction, 2% were indeed upregulated (61 proteins) and 8% (187 proteins) of all detected proteins were downregulated. In the microsomal fraction, 9% (338 proteins) were elevated, while 4% (152 proteins) showed a downregulation. In miR-574-5p oe cells, we expected direct miR targets to be decreased and decoy targets to be increased in response to high miR-574-5p levels. In the soluble fraction, 2% (40 proteins) were up- and 3% (78 proteins) were downregulated. While a decrease was measured for 8% of microsomal proteins (303 proteins), only 3% were upregulated (124 proteins). In the soluble fraction, 1% (29 proteins) of the total protein amount was decreased upon ΔmiR-574-5p, while 14 proteins showed an increase. However, the microsomal proteins showed higher percentages, 9% were upregulated (345 proteins), while even 13% (504 proteins) showed a downregulation.

Overall, the results show that on average 11% of all analyzed proteins are regulated by CUGBP1, while 9.7% of all detected proteins show an expression change related to miR-574-5p. But the distribution of the miR-574-5p regulated proteins varies considerably depending on the protein fraction.

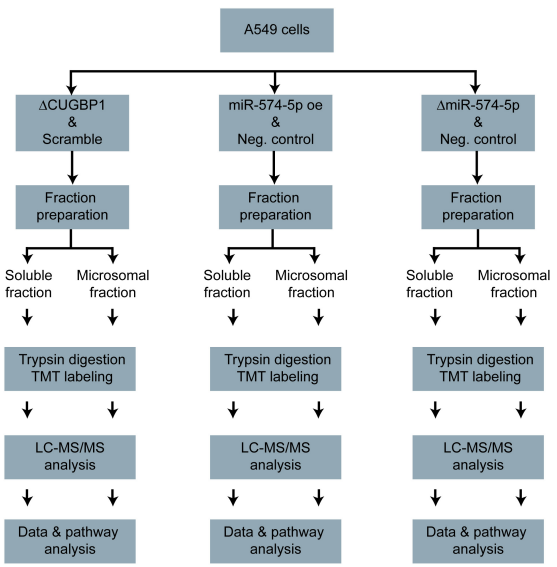


FIGURE 1 | Proteomics study workflow. A549 cells with ΔCUGBP1, ΔmiR-574-5p, or miR-574-5p oe and respective controls. Soluble and microsomal proteins were isolated, trypsin digested and TMT-labeled. Afterward, the LC-MS/MS measurement was conducted, the data were analyzed and the pathway analysis was performed. Δ, knockdown; oe, overexpression; TMT, tandem mass tag.

IPA Predicts Top Canonical Pathways and Upstream Regulators

Next, we analyzed all regulated proteins in soluble as well as microsomal fraction using IPA (Ingenuity Systems). The most

TABLE 1 | TMT labeling of protein samples in the proteomics study.

Sample name	Fraction	Label reagent
Control ΔCUGBP1 (Scramble)	Soluble	126
ΔCUGBP1	Soluble	127
Control miR-574-5p oe	Soluble	128
miR-574-5p oe	Soluble	129
Control ΔmiR-574-5p	Soluble	130
ΔmiR-574-5p	Soluble	131
Control ΔCUGBP1 (Scramble)	Microsomal	126
ΔCUGBP1	Microsomal	127
Control miR-574-5p oe	Microsomal	128
miR-574-5p oe	Microsomal	129
Control ΔmiR-574-5p	Microsomal	130
ΔmiR-574-5p	Microsomal	131

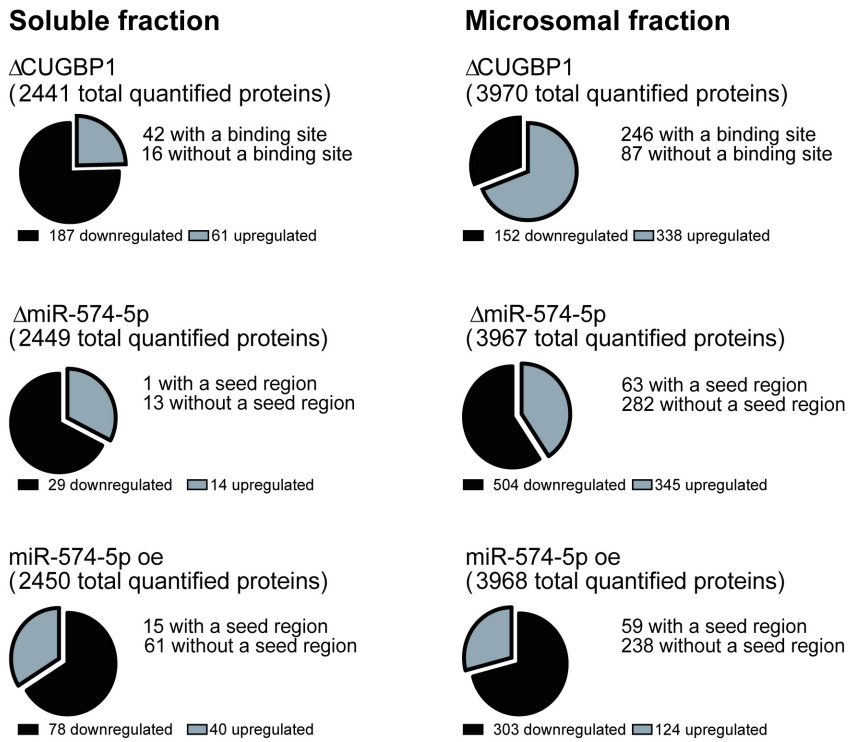


FIGURE 2 | Numbers of regulated proteins. Numbers of differentially expressed proteins upon ΔCUGBP1, ΔmiR-574-5p, or miR-574-5p oe in soluble and microsomal fraction of the proteomics study. Cut-offs were set to 1.5-fold for upregulation and -1.5-fold for downregulation. CUGBP1 binding sites of upregulated proteins were predicted using splice aid www.introni.it/spliceaid.html, while miR-574-5p seed regions were identified with the online tool microrna.org. of proteins revealing an upregulation upon ΔmiR-574-5p or a downregulation upon miR-574-5p oe. Δ, knockdown; oe, overexpression.

affected canonical pathways as well as upstream regulators were predicted. Canonical pathways with p-values ≤ 0.05 were termed as significant. The analysis revealed that the Eukaryotic Initiation Factor 2 (eIF2) Signaling, Regulation of Eukaryotic Initiation Factor 4 (eIF4) and 70 kDa ribosomal S6 kinase (p70S6K) Signaling, tRNA Charging, Protein Ubiquitination Pathway and mechanistic Target of Rapamycin (mTOR) Signaling are the most affected canonical pathways in the soluble fraction (**Supplementary Table S4**). In the microsomal fraction, top regulated pathways were eIF2 Signaling, Protein Ubiquitination Pathway, Mitochondrial Dysfunction, Regulation of eIF4 and p70S6K Signaling as well as Oxidative Phosphorylation. Top predicted upstream regulators were Hepatocyte Nuclear Factor 4 Alpha (HNF4A), Cystatin D (CST5), Rapamycin-Insensitive Companion of mTOR (RICTOR), MYC Proto-Oncogene, MYCN Proto-Oncogene, microtubule associated protein tau (MAPT) and Tumor Protein P53 (TP53) (**Supplementary Table S3**), which further underlines the cancer context of CUGBP1 and miR-574-5p.

Validation of TMT Based Quantification of Proteomics Results Using Western Blot Analysis

In order to validate the results of the proteomics study (**Supplementary Tables S1, S2**), distinct proteins were selected to be confirmed via Western blot analysis. Since this was a complex study with many different conditions (**Figure 1**), we concentrated not necessarily on physiologically connected proteins. We firstly selected NADH-Ubiquinone Oxidoreductase Core Subunit S2 (NDUFS2) which is a postulated CUGBP1 target in HeLa cells (Rattenbacher et al., 2010). Although there were no strong changes of microsomal NDUFS2 in the proteomics study, soluble NDUFS2 showed a trend toward slight upregulation in response to ΔCUGBP1 and miR-574-5p oe. This upregulation and also the values for the microsomal fraction could be confirmed using Western blot analysis (**Supplementary Figure S2A**, right panel). In response to ΔmiR-574-5p, soluble NDUFS2 revealed a slight decrease in the proteomics data which was also confirmed by Western blot analysis (**Supplementary Figure S2A**, left panel).

In addition to this, SMAD3 was another target which we aimed to validate. It was strongly downregulated in response to ΔCUGBP1 in the soluble fraction of the proteomics study and we were also able to confirm this via Western blot (**Supplementary Figure S2B**, left panel). While the proteomics study revealed no strong regulation of SMAD3 in microsomal fraction, IPA predicted it to be activated upon ΔCUGBP1 (**Supplementary Table S3**). Indeed, we observed an upregulation of SMAD3 proteins in the microsomal fraction performing Western blot analysis (**Supplementary Figure S2B**, right panel). In this case, we could validate the pathway analysis which is based on the proteomics data (**Supplementary Tables S1, S2**).

In response to ΔmiR-574-5p, SMAD3 was slightly upregulated in the microsomal fraction which was also a tendency in the proteomics study, although weaker. In general, the Western blot data of the two miR-574-5p conditions showed higher variations in SMAD3 levels.

SMAD2, another member of the same protein family, was also predicted in the IPA to be upregulated upon ΔCUGBP1 (**Supplementary Table S3**). Indeed, we were able to confirm this regulation by Western blot analysis. We observed a significant fourfold upregulation of SMAD2 in the microsomal fraction (**Supplementary Figure S2C**, right panel). Interestingly, in the soluble fraction SMAD2 expression was significantly reduced (**Supplementary Figure S2C**, left panel) which indicates that its regulation might be highly compartment specific. In soluble and microsomal fraction, SMAD2 and SMAD3 revealed a slight downregulation in response to miR-574-5p oe and an upregulation in response to ΔmiR-574-5p in the microsomal fraction. This indicated that both genes might be canonical miR-574-5p targets. Therefore, we analyzed the 3′UTRs of SMAD2 and SMAD3 using the online tool microrna.org (Betel et al., 2010) but found that none of them contains a seed region of miR-574-5p. This suggests that the effects on protein level are caused by secondary effects.

To complete the family of SMADs, it was coherent to analyze SMAD4, as the only other SMAD that was detected in the proteomics study. However, neither in the proteomics study nor in the Western Blot images, it did depict any regulation upon ΔCUGBP1 (**Supplementary Figure S3A**) which validated the mass spectrometry data, but excluded SMAD4 as interesting potential target.

Finally, we studied p38 (MAPK14) which was one of the strongest downregulated proteins upon ΔCUGBP1 in the soluble fraction of the proteomics study. In fact, we verified this severe decrease of p38 protein using Western blot analysis (**Supplementary Figure S3B**). Nevertheless, this effect seems to be secondary, as CUGBP1 targets are supposed to be upregulated in response to the knockdown.

Overall, these results validated the proteomics data (**Supplementary Tables S1, S2**). Since we measured an increase in NDUFS2, SMAD2 and SMAD3 as a response to ΔCUGBP1, we considered them as potential novel CUGBP1 targets.

Identification of New CUGBP1 Targets

Next, we aimed to identify new targets of the decoy mechanism, but also CUGBP1 targets using bioinformatical approaches. Therefore, we analyzed proteins which were increased upon ΔCUGBP1 and analyzed their 3′UTRs for CUGBP1 binding sites. We downloaded the 3′UTR sequences of the specific proteins from the database UCSC Genome Browser (December 2013 GRCh38/hg38)[2] (Kent et al., 2002) and analyzed them by using the online tool SpliceAid 2 (Piva et al., 2009) to identify potential CUGBP1 binding motifs. Thereby, the algorithm detects GU-rich elements (GREs) and other binding motives (e.g., CUGUCUG) in the provided 3′UTR sequences. In fact, a high number of the upregulated proteins does have a potential CUGBP1 binding site: in the soluble fraction 69% (42 proteins) and in the microsomal fraction 73% (246 proteins) of all detected proteins in the proteomics study (**Figure 2**).

Since CUGBP1 itself was mainly detectable in the microsomal fraction (**Supplementary Figure S1A**), we focused on this fraction for further analysis. As a positive control we used mPGES-1 which is already described as CUGBP1 target and decoy target (Saul et al., 2019a). The three candidates SMAD2, SMAD3 and NDUFS2 all depicted an upregulation in response to ΔCUGBP1 visualized by Western blot analysis (**Figures 3A,C,D**) and indeed also contained binding sites for CUGBP1 in their 3′UTRs. SMAD2 and SMAD3 both exhibit multiple binding motifs all over their 3′UTRs, while NDUFS2 with a rather short 3′UTR, harbors only one binding site.

We also observed an increase on protein level of mPGES-1 upon ΔCUGBP1 which further supports our results from the mass spectrometry (**Figure 3B**). This data provides first evidence that SMAD2, SMAD3 and NDUFS2 could be CUGBP1 targets in A549 cells.

RIP Confirmed Binding of CUGBP1 to mRNAs of Novel Targets

In order to further validate the new postulated targets SMAD2, SMAD3, NDUFS2 as well as mPGES-1, binding of CUGBP1 needed to be confirmed. Therefore, we performed immunoprecipitation of CUGBP1 with a specific antibody and then quantified the bound mRNAs via qRT-PCR. As mock control we used a normal mouse IgG antibody. Binding was assumed, if the specific mRNA showed an enrichment in CUGBP1-IP, compared to IgG-IP. Successful RIP was verified using Jun Proto-Oncogene AP-1 Transcription Factor Subunit (cJUN) as positive control (**Figure 3E**) which revealed a significant 2.4-fold enrichment. Cyclooxygenase (COX)-2 was used as negative control, since it has no binding site and was not influenced by CUGBP1 (Saul et al., 2019a). Indeed, we could show that CUGBP1 binds to three of the mRNAs (**Figure 3F**): SMAD2 mRNA was significantly 6.4-fold enriched. NDUFS2 showed a 2.2-fold enrichment and mPGES-1 was 4.3-fold enriched in comparison to IgG control. However, SMAD3 was not bound by CUGBP1 and showed no enrichment of the mRNA although it contains a binding site in the 3′UTR. Interestingly, it was also revealed that CUGBP1 binds its own mRNA, as it showed a slight enrichment compared to IgG, indicating an autoregulatory mechanism.

Overall, we confirmed CUGBP1 binding to three of the four postulated targets: SMAD2, NDUFS2 and mPGES-1. Since we assumed decoy targets to be a subpopulation of CUGBP1 targets, we aimed to investigate if these new candidates were also regulated by miR-574-5p.

Identification of Novel miR-574-5p/CUGBP1 Decoy Targets by Investigating a "Decoy Regulation Pattern" on Protein Level

In order to find new targets of the miR-574-5p/CUGBP1 decoy mechanism, we analyzed the proteomics data in regard to a

[2]https://genome.ucsc.edu/

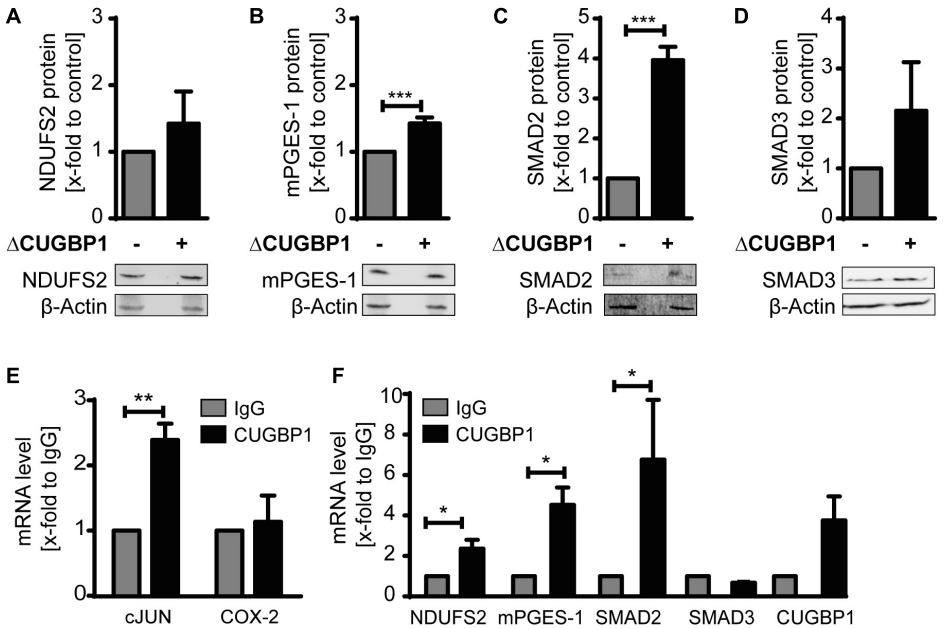


FIGURE 3 | Identification of novel CUGBP1 targets. Western blots of IL-1β-stimulated A549 cells with ΔCUGBP1 showed increased levels of **(A)** NDUFS2, **(B)** mPGES-1, **(C)** SMAD2 and **(D)** SMAD3 compared to control. β-Actin was used as loading control. Fold induction is given as mean (+SEM) of three independent experiments, *t*-test $^{***}p \leq 0.001$. **(E,F)** RIP with antibodies against CUGBP1 or IgG (mock) showed enrichment of bound mRNAs quantified via q RT-PCR. **(E)** cJUN was used as positive control, COX-2 as negative control. **(F)** mRNAs of NDUFS2, mPGES-1, SMAD2 and CUGBP1 were enriched in CUGBP1-IP, while SMAD3 mRNA showed no enrichment. Relative enrichment normalized to IgG is given as mean (+SEM) from three independent experiments. *t*-test $^{*}p \leq 0.05$; $^{**}p \leq 0.005$. RIP: RNA immunoprecipitation.

"decoy regulation pattern" in the three conditions (ΔCUGBP1, ΔmiR-574-5p or miR-574-5p oe) and aimed to verify it via Western blot analysis. As subpopulation of CUGBP1 targets, decoy targets should be increased in response to ΔCUGBP. Further, they are supposed to be downregulated upon ΔmiR-574-5p, because lower miR levels cause higher binding capacity of CUGBP1 which in turn has a negative effect on the protein level. *Vice versa*, miR-574-5p oe should lead to an upregulation of potential decoy targets, as more miRs are available to prevent binding of CUGBP1. Here, we also included those proteins showing a tendency for the "decoy regulation pattern," such as only two conditions that met the criteria or only slight up- or downregulations, for instance NDUFS2 showed only a −1.33-fold induction in response to ΔCUGBP1. Another example is High-mobility group AT-hook 2 (HMGA2) protein which was strongly upregulated upon ΔCUGBP1 and downregulated upon ΔmiR-574-5p but showed only a 1.14-fold TMT ratio in response to miR-574-5p oe. As mentioned above, we focused only on the microsomal fraction, since CUGBP1 was predominantly expressed there (**Supplementary Figure S1**). We analyzed the newly postulated CUGBP1 target proteins and several other candidates via Western blot. Although the control decoy target mPGES-1 could be successfully validated, none of the other potential candidates could be confirmed revealing the "decoy regulation pattern" in the Western blot images. Neither SMAD2, SMAD3 nor NDUFS2 showed the adequate "decoy regulation pattern" concerning the two miR conditions (**Supplementary Figure S2**). Other candidates such as glyoxalase domain-containing protein 4 (GLOD4) or HMGA2 which were detected by mass spectrometry, could not be validated herein. Probably, the sensitivity of the Western blot system was too low and the proteins were only expressed on a very basal level by the cells.

We next analyzed the proteomics data again using more stringent criteria. Taking a closer look how many proteins showed a strong upregulation of ≥1.5-fold in response to ΔCUGBP1 and a downregulation of ≤−1.5-fold after ΔmiR-574-5p and at least an upregulation of ≥1.5-fold in response to miR-574-5p oe. With these criteria set, zero proteins were found in the soluble fraction (**Figure 4A**), while in the microsomal fraction only seven proteins (0.2%) matched this regulation pattern in all three conditions (**Figure 4B**). This gave us the first hint that the CUGBP1/miR-574-5p decoy seems to be a very specific mechanism, considering that originally around 3970 proteins were detected in the microsomal fraction in the proteomics study. Those seven proteins were: Ubiquitin Conjugating Enzyme E2 R2 (UBE2R2), Centrosomal Protein of 41 kDa (CEP41), RNA polymerase-associated protein LEO1 (LEO1), General Transcription Factor IIE Subunit 2 (GTF2E2), Polyadenylate-Binding Protein-Interacting Protein 2 (PAIP2), Solute carrier family 39 member 6 (SLC39A6), GRIP1 Associated Protein 1 (GRIPAP1). Of note, mPGES-1 was not among the seven proteins, since it did not reveal the exact pattern in the proteomics data either, probably due to the limitations of the mass spectrometry procedure. While GRIPAP1 has no described 3′UTR, the other 3′UTRs were analyzed concerning CUGBP1

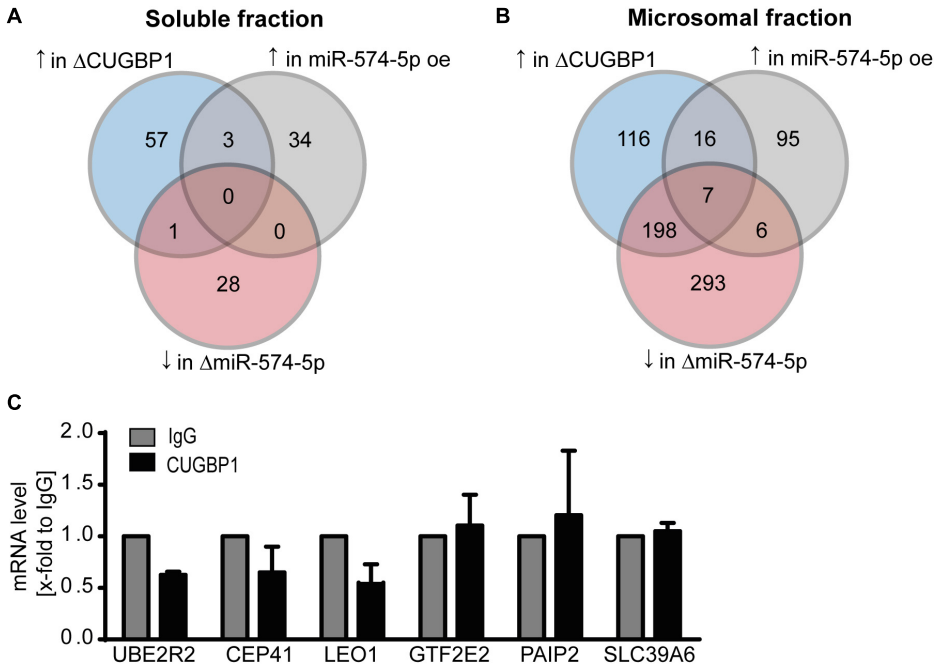


FIGURE 4 | RIP excludes seven potential decoy candidates. Stringent criteria applied to results of the proteomics study: number of proteins which reveal an upregulation (↑) upon ΔCUGBP1, an upregulation upon miR-574-5p oe and a downregulation (↓) upon ΔmiR-574-5p in **(A)** soluble fraction and **(B)** microsomal fraction. **(C)** In RIP assays of A549 cells, none of the mRNAs was enriched in CUGBP1-IP samples compared to IgG. Relative enrichment normalized to IgG is given as mean (+SEM) from three independent experiments. Δ, knockdown; oe, overexpression; RIP, RNA immunoprecipitation.

binding motifs and it was revealed that all of them did contain a binding site in their 3′UTRs. By conducting a RIP binding assay, we analyzed whether the potential decoy candidates were bound by CUGBP1. It turned out that none of these six remaining mRNAs showed an enrichment in the CUGBP1-IP compared to mock control (**Figure 4C**). They were excluded as decoy targets in A549 cells, since none of the candidates was bound by CUGBP1. Thus, based on our Western blot and RIP data, mPGES-1 is the only target that was identified to be regulated by the decoy mechanism.

Subcellular Localization of CUGBP1 and miR-574-5p in A549 Cells

In order to approach the decoy search on a different level, we wanted to find out exactly where in the cell the interaction of CUGBP1 and miR-574-5p takes place. Therefore, subcellular localization of the two binding partners was visualized using immunofluorescence staining and FISH assay, respectively. miR-574-5p was detected using a complementary DIG labeled LNA probe which was visualized with a DIG binding antibody. For localization of CUGBP1, immunofluorescence staining with a specific antibody directed against CUGBP1 was performed. DAPI served as nuclear marker. In fact, it was possible to show that both, miR-574-5p and CUGBP1 were mainly located within the nuclei of A549 cells (**Figure 5A**). While miR-574-5p showed very weak cytoplasmic signals, CUGBP1 showed a stronger presence in the cytoplasm, but was still predominantly located in the nucleus. The cellular localization of both binding partners did not change upon IL-1β stimulation. Therefore, we concluded that the decoy mechanism mainly takes place in the nucleus and miR-574-5p interferes with CUGBP1 functions there, just like it is the case for the mPGES-1 mRNA regulation.

Bioinformatical 3′UTR Analysis Revealed That Splicing Pattern of mPGES-1 3′UTR Is Very Unique

Since both CUGBP1 and miR-574-5p were located in the nucleus, it was consequential to concentrate on alternative splicing which is the main function of CUGBP1 within the nucleus. In the case of mPGES-1, as the decoy target model, CUGBP1 binds to two binding sites in the mPGES-1 3′UTR and influences alternative splicing which creates a shorter 3′UTR isoform. Therefore, we used this as a model and analyzed other potential decoy candidates concerning this splicing pattern. Sequences of the 3′UTRs from all 399 proteins which showed an upregulation in response to ΔCUGBP1 in soluble or microsomal fraction of the proteomics study were downloaded, using ensemble biomart GRCh38/.p12, version 91, Ensembl variation resources (Hunt et al., 2018). These 1914 3'UTR sequences were aligned with 42 described CUGBP1 binding motifs [using the online tool Splice Aid F (Giulietti et al., 2013)]. In a high stringency analysis, we searched for the exact splicing pattern of mPGES-1, and found that none of the transcripts represented this exact pattern, with two 39 and 46 nt binding sites and 1000 nt in between (**Figure 5B**, upper panel). Therefore, it was also considered that the splicing pattern might not have to be identical which is why

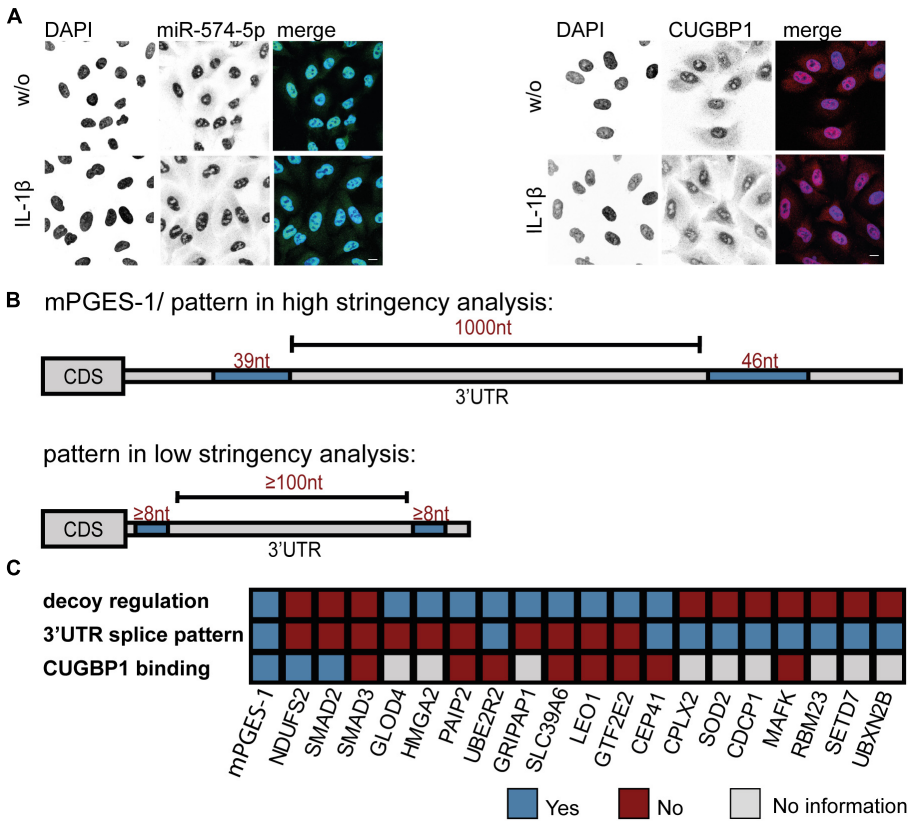


FIGURE 5 | Bioinformatical 3′UTR analysis confirms specificity of mPGES-1-derived splicing pattern. **(A)** FISH assay and immunofluorescence staining of A549 cells ± IL-1β. miR-574-5p FISH was performed using DIG-labeled miRCURY LNA probes (green). Immunofluorescence staining with specific antibody visualized subcellular localization of CUGBP1 (red). Nuclei were counterstained with DAPI (blue). Scale bar represents 10 μm in each image. Representative images of three independent staining experiments are shown. **(B)** Bioinformatical 3′UTR analysis of upregulated proteins from proteomics study after ΔCUGBP1. High stringency/mPGES-1 splice pattern (upper panel) or low stringency pattern (lower panel) with two or more CUGBP1 binding sites (≥8 nucleotides), with potential intron (≥100 nucleotides). **(C)** Summary of the decoy target search. 20 different proteins were analyzed concerning their "decoy regulation pattern" on protein level, the specific (low stringency) splice pattern in the 3′UTR and binding of CUGBP1 to their mRNA in RIP assays. Nt, nucleotides; FISH, fluorescence *in situ* hybridization; RIP, RNA immunoprecipitation. Δ, knockdown.

we loosened the criteria in a second low stringency analysis. We searched for transcripts containing two CUGBP1 binding sites of at least 8 nt length and with at least 100 nt in between (**Figure 5B**, lower panel). As depicted in **Table 3**, 575 (30%) of the originally 1914 3′UTRs, contained any CUGBP1 binding site. Restricting it to binding sites that are 8 nt or longer, only 118 transcripts (6%) were left. Setting the criterion that there have to be at least two distinct binding sites of at least 8 nt length, only 33 transcripts were left (1.7%). Finally, including the criterion that these binding sites have to be at least 100 nt apart to provide some kind of intron in between, only 11 transcripts (0.6%) fulfilled all the criteria. Those 11 transcripts belong to nine different genes (**Supplementary Table S5**): Superoxide dismutase 2 (SOD2), MAF BZIP Transcription Factor K (MAFK), CUB domain-containing protein 1 (CDCP1), RNA Binding Motif Protein 23 (RBM23), SET Domain Containing Lysine Methyltransferase 7 (SETD7), UBX Domain Protein 2B (UBXN2B), Complexin 2 (CPLX2), CEP41 and UBE2R2. Of these, only CEP41 and UBE2R2 depicted the "decoy regulation pattern." However, they were not bound by CUGBP1 in the RIP assay of A549 cells (**Figure 4C**) and therefore excluded as novel decoy targets.

DISCUSSION

Since their discovery in 1993 (Wightman et al., 1993), miRs were generally considered as gene expression repressors. On post-transcriptional level, miRs specifically bind to 3′UTRs of their target mRNAs which has a silencing effect by either mediating translational repression or degradation of the mRNA (Wienholds and Plasterk, 2005; Croce, 2009). However, over the last years different studies reported that miRs can also positively influence gene expression. They can bind to RNA binding proteins and antagonize their activity which leads to an elevated gene expression of their target genes (Eiring et al., 2010; Saul et al., 2016; Saul et al., 2019a,b). The newly discovered decoy of miR-574-5p and CUGBP1 has a crucial impact on physiological processes, since it regulates mPGES-1, thereby PGE_2 levels and subsequently lung tumor growth *in vivo* (Saul et al., 2019a).

TABLE 2 | Primers used for qRT-PCR analysis.

Target	Sequence
cJun fw	TCGACATGGAGTCCCAGGA
cJun rev	GGCGATTCTCTCCAGCTTCC
SMAD2 fw	GGGATGCTTCAGGTAGGACA
SMAD2 rev	TCTCTTTGCCAGGAATGCTT
SMAD3 fw	CGCAGAACGTCAACACCAAG
SMAD3 rev	GGCGGCAGTAGATGACATGA
COX-2 fw	CCGGGTACAATCGCACTTAT
COX-2 rev	GGCGCTCAGCCATACAG
NDUFS2 fw	GTTTTGCCCATCTGGCTGGT
NDUFS2 rev	CATGCCATGGCCTATGGTGAA
mPGES-1 fw	GAAGAAGGCCTTTGCCAAC
mPGES-1 rev	CCAGGAAAAGGAAGGGGTAG
UBE2R2 fw	ATGTGGCACCCCAACATT
UBE2R2 rev	TCCACCTTTCAGAAGGCAGT
CEP41 fw	ACAGAACCCAAGATACCAGCATAT
CEP41 rev	GGGAGCTGGTAAGATACACACA
SLC39A6 fw	GCACTTACTGCTGGCTTATTCA
SLC39A6 rev	CGGCTACATCCATGGTCACT
PAIP2 fw	CCATTTGCAGAGTACATGTGGA
PAIP2 rev	CCGTACTTCACCCCAGGAAC
GTF2E2 fw	CCATGCAGGAATCTGGACCA
GTF2E2 rev	AATCCTTCAGCACTCCAGCC
LEO1 fw	ACTGCCCAACTTTCTCAGTGT
LEO1 rev	AGATGATTGTGGTCGCCCTG

TABLE 3 | Numbers of transcripts in low stringency analysis.

Binding site length	Number of binding sites	Space between binding sites	Number of transcripts left
–	–	–	575
Binding site of ≥ 8 nt	–	–	118
Binding site of ≥ 8 nt	≥2 binding sites	–	33
Binding site of ≥ 8 nt	≥2 binding sites	≥100 nt apart	11

*For complete list of transcript IDs see **Supplementary Table S5**. Nt, nucleotides.*

Therefore, it was especially interesting to further characterize this rather unknown miR function which seems to have a more global significance than initially assumed.

In a mass spectrometry-based proteomics study, we aimed to further elucidate global distribution and novel targets of the decoy mechanism. To this end, we conducted a proteomics study with manipulated levels of CUGBP1 or miR-574-5p in A549 lung cancer cells. In this complex study we separately analyzed proteins from soluble and microsomal fraction and observed many compartment specific effects. Especially the R-Smads, SMAD2 and SMAD3 revealed opposite regulation upon ΔCUGBP1 in the two fractions. This supports the hypothesis that RBPs could allow different translational efficiencies concerning free and endoplasmic reticulum-bound ribosomes (Mansfield and Keene, 2009; Reid and Nicchitta, 2012). Moreover, in the two miR conditions (ΔmiR-574-5p and miR574-5p oe), higher percentages of regulated proteins were found in the microsomal fraction, compared to the soluble fraction. This suggests that miR-574-5p is likely to be mainly localized there and further supports the assumption that the decoy mechanism occurs in this fraction. In addition, we also observed that there are more CUGBP1 targets detectable in the microsomal fraction based on the fact that more proteins were upregulated than downregulated upon the knockdown. Whereas, secondary effects were apparently stronger in the soluble fraction, as 8% of all proteins were decreased upon ΔCUGBP1 while only 2% were increased. This also matches the observation that CUGBP1 itself is mainly found in the microsomal fraction.

Hence, we focused on the microsomal fraction in further analyses and identified several new CUGBP1 targets. The NADH-Ubiquinone Oxidoreductase NDUFS2 was already postulated to be directly targeted by CUGBP1 (Rattenbacher et al., 2010). We verified the interaction of CUGBP1 and NDUFS2 via RIP and could confirm the regulation using Western blot analysis. This suggests that CUGBP1 influences the respiratory chain and thereby a major biological process within the cell (Procaccio et al., 1998). Next, we analyzed the SMAD family. SMAD2, SMAD3 and SMAD4 were detected in the proteomics study and expression changes were successfully validated using Western blot analysis. Interestingly, SMAD7, another SMAD family member, was not detected in our proteomics study but nevertheless is described as CUGBP1 target in C2C12, a murine myoblast cell line (Lee et al., 2010). This gives an indication that the SMAD family and subsequent pathways could actually be regulated by CUGBP1 as a regulon. While SMAD2 was significantly enriched in RIPs, SMAD3 showed no enrichment in the CUGBP1-IP. This indicates that SMAD2 is indeed bound by CUGBP1, whereas SMAD3 is not at least under the conditions investigated here. We can probably speculate that the binding of SMAD3 mRNA only takes place under certain conditions, since CUGBP1 is known to bind its targets strictly context specific (Vlasova and Bohjanen, 2008).

Following, we were interested if these new CUGBP1 targets were also influenced by the decoy mechanism. Hence, we took a closer look on their regulation in the microsomal fraction upon miR-574-5p oe and ΔmiR-574-5p. It was expected that targets of the decoy mechanism would be upregulated in response to an overexpression and downregulated in response to a knockdown of the miR-574-5p, as it is the case for mPGES-1. However, microsomal SMAD2 and NDUFS2 did not exhibit this "decoy regulation pattern" which implies that they are not affected by the decoy mechanism. In fact, the only protein depicting the "decoy regulation pattern" in the Western blot analyses was mPGES-1. However, it should be mentioned that mPGES-1 did not reveal this pattern in the proteomics data. We would like to point out that a proteomics study, even if we have used an established method (Eriksson et al., 2008; Ochs et al., 2013; Bergqvist et al., 2019; Saul et al., 2019b), only provides initial indications of which proteins could be regulated. It does not map the exact expression changes, which is a common limitation of this approach. It is therefore possible that mPGES-1 as multi-pass membrane protein is not efficiently, quantitatively represented by the mass spectrometry due to

different sample preparation procedures. In contrast to mass spectrometry samples, protein samples in Western blot analyses are SDS-treated and boiled. Therefore, it is likely that Western blot analysis allows for a better quantification in these cases. This also means, it is possible that there are novel decoy targets which we did not detect with our proteomics approach. To ensure the general accuracy of the study, it is crucial to validate the mass spectrometry data. We addressed this for a selected amount of proteins and further supported this by additional validation of the pathway analysis which is in turn based on the proteomics study.

Interestingly, the decoy mechanism of miR-328 and hnRNP E2 affects a variety of targets and cellular processes (Saul et al., 2016, 2019b) while in this case the decoy of miR-574-5p and CUGBP1 seems to be quite specific for mPGES-1. Retrospectively, this is consistent with one of our previous studies in which we firstly demonstrated that miR-574-5p prevents CUGBP1 from binding to mPGES-1 mRNA. This has a crucial impact on lung tumor growth *in vivo* (Saul et al., 2019a) due to the influence on PGE_2 levels and the tumor microenvironment. However, those pro-tumorigenic effects are completely blocked with the administration of a selective mPGES-1 inhibitor and apparently are solely caused by the decoy-mediated mPGES-1 induction. Therefore, it seems likely that the decoy function mainly regulates mPGES-1 expression which might be a cell-type-specific effect.

The hypothesis is further supported by the fact that CUGBP1 and miR-574-5p are mainly located in the nucleus. It is implied that the role of CUGBP1 as regulator of alternative splicing is of greater importance here than its function as translational repressor (Dasgupta and Ladd, 2012). Therefore, it was assumed that miR-574-5p could solely interfere with this CUGBP1 function. Thus, we analyzed the splicing pattern that emerged from the only known decoy target mPGES-1. It has a specific pattern comprising of two, 39 and 46 nt long CUGBP1 binding sites, separated by a 1000 nt 3′UTR intron (Saul et al., 2019a). Alternative 3′UTR splicing leads to the generation of a mPGES-1 isoform with a much higher translational rate. This can be explained by the fact that inhibitory elements like Alu elements or canonical miR binding sites within the 3′UTR intron are removed (Daskalova et al., 2007; Mayr and Bartel, 2009; Fitzpatrick and Huang, 2012). In a high stringency analysis, we discovered that this specific splicing pattern is exclusively found for mPGES-1 and could not be observed for another potential CUGBP1 target. This can be explained by the fact that this splice pattern with a 3'UTR Alu element framed by two CUGBP1 sites is indeed very specific. Thus, we decided to loosen the criteria and looked for similar patterns in a second low stringency analysis which resulted in a list of 11 transcripts. Taking into account that we started with nearly 2000 transcripts, this gave us another strong hint that the decoy mechanism seems to be very specific. In the future it would be necessary to examine these transcripts more closely for example in relation to splice variants under inflammatory or pro-tumorigenic conditions. Further, it should be noted that we analyzed the 3′UTRs of the transcripts, as we used mPGES-1 as model for a decoy target. Despite the fact that CUGBP1 indeed mostly binds to 3′UTRs of target genes, it is also known to bind to exon-intron boundaries within the coding sequence (Xia et al., 2017) which we did not include within this study.

Taken together, in this study we discovered a variety of potential new canonical targets of CUGBP1 and successfully verified two of them. By influencing NDUFS2 expression, CUGBP1 is able to interfere with the respiratory chain and could have an impact on the mitochondrial complex I (Procaccio et al., 1998). The newly found interaction with SMAD2 mRNA opens further possibilities for CUGBP1, since SMAD2 mediates transforming growth factor β signaling and has a crucial impact on many signaling cascades (Riggins et al., 1996).

Unexpectedly, we found that the decoy mechanism of CUGBP1 and miR-574-5p seems to be quite target-specific in this type of lung cancer cells. Overall, we used several criteria to identify new targets of the CUGBP1/miR-574-5p decoy mechanism: First, potential candidates should show a "decoy regulation pattern" on protein level as we have seen for mPGES-1. Second, the splice site in the 3′UTR should contain two CUGBP1 binding sites with a potential intron in between. Third, binding of CUGBP1 to the respective binding sites occurs. Interestingly, only mPGES-1 was identified which fulfills all of these three criteria (**Figure 5C**). Our data suggest that decoy mechanisms can lead to the regulation of specific target genes which is potentially cell-type-specific. Interestingly, in A549 cells, mPGES-1 was identified as the only protein regulated by the decoy mechanism of CUGBP1 and miR-574-5p. This explains our previous observation that tumor growth induced by overexpression of miR-574-5p can be selectively blocked with an mPGES-1 inhibitor (Saul et al., 2019a). Concerning lung cancer, mPGES-1 could be a promising target for future lung cancer therapy in patients overexpressing miR-574-5p.

In light of the observation that not all patients benefit from a treatment with PGE_2-reducing medication (Edelman et al., 2015; Yokouchi and Kanazawa, 2015), determination of miR-574-5p levels might be helpful to identify PGE_2-dependent tumors. Hence, the levels of miR-574-5p could serve as stratification marker to identify subgroups of patients for the treatment with mPGES-1 inhibitors.

DATA AVAILABILITY STATEMENT

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD016803.

AUTHOR CONTRIBUTIONS

AE performed the experiments, analyzed the data, and wrote the manuscript. JW performed the immunostaining and FISH experiments. EO, IB, JL, and KK performed the MS analytic. P-JJ and DS conceived the study and designed the project. MS conceived the study, designed and supervised the project,

and wrote the manuscript. All authors conducted the quality assurance of the manuscript and reviewed the manuscript.

FUNDING

This project was supported by the Else Kröner-Fresenius-Stiftung [grant no: 2013_A265 and Else Kröner-Fresenius-Graduiertenkolleg (2014_A187)], the Deutsche Forschungsgemeinschaft (ECCPS), Athene Young Investigator program (Technische Universität Darmstadt; grant no: n/a), LOEWE-Zentrum Translationale Medizin und Pharmakologie (Innovationsfond, TP18), Swedish Research Council (grant no: 2017-02577); Innovative Medicines Initiative (EU/EFPIA) (ULTRA-DD grant no: 115766); Stockholm County Council (ALF grant no: 20160378), The Swedish Rheumatism Association (grant no: R-755861); Cancerfonden (grant no: CAN 2016/739); Radiumhemmets forskningsfonder (grant no: 151132); King Gustaf V's 80-year foundation (grant no: n/a); Funds from Karolinska Institutet (grant no: n/a).

ACKNOWLEDGMENTS

The authors like to report special thanks to Michaela Müller-McNicoll (Goethe University Frankfurt, Germany) for scientific discussion and technical support concerning the RIP assays. Furthermore, we would like to thank M. Cristina Cardoso (TU Darmstadt) for providing access to her microscope facilities. Finally, special thanks to Tobias Saul for his help with the bioinformatical analysis. Further, we acknowledge support by the German Research Foundation and the Open Access Publishing Fund of Technische Universität Darmstadt.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00196/full#supplementary-material

REFERENCES

Bergqvist, F., Ossipova, E., Idborg, H., Raouf, J., Checa, A., Englund, K., et al. (2019).). Inhibition of MPGES-1 or COX-2 results in different proteomic and lipidomic profiles in A549 lung Cancer cells. *Front. Pharmacol.* 10:636. doi: 10.3389/fphar.2019.00636

Betel, D., Koppal, A., Agius, P., Sander, C., and Leslie, C. (2010). Comprehensive modeling of MicroRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol.* 11:R90. doi: 10.1186/gb-2010-11-8-r90

Cao, Z., Tang, H.-Y., Wang, H., Liu, Q., and Speicher, D. W. (2012). Systematic comparison of fractionation methods for in-depth analysis of plasma proteomes. *J. Proteome Res.* 11, 3090–3100. doi: 10.1021/pr201068b

Carleton, M., Cleary, M. A., and Linsley, P. S. (2007). MicroRNAs and cell cycle regulation. *Cell Cycle* 6, 2127–2132. doi: 10.4161/cc.6.17.4641

Croce, C. M. (2009). Causes and consequences of MicroRNA dysregulation in Cancer. *Nat. Rev. Genet.* 10, 704–714. doi: 10.1038/nrg2634

Dasgupta, T., and Ladd, A. N. (2012). The Importance of CELF control: molecular and biological roles of the CUG-BP, elav-like family of RNA-binding proteins. *Wiley Interdiscip. Rev. RNA* 3, 104–121. doi: 10.1002/wrna.107

Daskalova, E., Baev, V., Rusinov, V., and Minkov, I. (2007). 3'UTR-Located ALU elements: donors of Potential MiRNA target sites and mediators of network MiRNA-Based regulatory interactions. *Evol. Bioinform. Online* 2, 103–120.

Del Vescovo, V., Grasso, M., Barbareschi, M., and Denti, M. A. (2014). MicroRNAs as lung Cancer biomarkers. *World J. Clin. Oncol.* 5, 604–620. doi: 10.5306/wjco.v5.i4.604

Edelman, M. J., Tan, M. T., Fidler, M. J., Sanborn, R. E., Otterson, G., Sequist, L. V., et al. (2015). Randomized, double-blind, placebo-controlled, multicenter Phase II study of the efficacy and safety of apricoxib in combination with either docetaxel or pemetrexed in patients with biomarker-selected non-small-cell lung Cancer. *J. Clin. Oncolo.* 33, 189–194. doi: 10.1200/JCO.2014.55.5789

Eiring, A. M., Harb, J. G., Neviani, P., Garton, C., Oaks, J. J., Spizzo, R., et al. (2010). MiR-328 functions as an RNA decoy to modulate HnRNP E2 regulation of MRNA translation in leukemic blasts. *Cell* 140, 652–665. doi: 10.1016/j.cell.2010.01.007

Eriksson, H., Lengqvist, J., Hedlund, J., Uhlén, K., Orre, L. M., Bjellqvist, B., et al. (2008). Quantitative membrane proteomics applying narrow range peptide isoelectric focusing for studies of small cell lung Cancer resistance mechanisms. *PROTEOMICS* 8, 3008–3018. doi: 10.1002/pmic.200800174

Fitzpatrick, T., and Huang, S. (2012). 3'-UTR-Located inverted alu repeats facilitate MRNA translational repression and stress granule accumulation. *Nucleus* 3, 359–369. doi: 10.4161/nucl.20827

Foss, K. M., Sima, C., Ugolini, D., Neri, M., Allen, K. E., and Weiss, G. J. (2011). MiR-1254 and MiR-574-5p: serum-Based MicroRNA biomarkers for early-stage non-small cell lung Cancer. *J. Thorac. Oncol.* 6, 482–488. doi: 10.1097/JTO.0b013e318208c785

Friedman, R. C., Farh, K. K.-H., Burge, C. B., and Bartel, D. P. (2009). Most mammalian MRNAs Are conserved targets of MicroRNAs. *Genome Res.* 19, 92–105. doi: 10.1101/gr.082701.108

Giulietti, M., Piva, F., D'Antonio, M., De Meo, P. D. O., Paoletti, D., Castrignanò, T., et al. (2013). SpliceAid-F: a database of human splicing factors and their RNA-Binding sites. *Nucleic Acids Res.* 41, D125–D131. doi: 10.1093/nar/gks997

Guo, H., Ingolia, N. T., Weissman, J. S., and Bartel, D. P. (2010). Mammalian MicroRNAs PREDOMINANTLY ACT TO DECREASE TARGET MRNA Levels. *Nature* 466, 835–840. doi: 10.1038/nature09267

Harfe, B. D. (2005). MicroRNAs in vertebrate development. *Curr. Opin. Genet. Dev.* 15, 410–415. doi: 10.1016/j.gde.2005.06.012

Hunt, S. E., McLaren, W., Gil, L., Thormann, A., Schuilenburg, H., Sheppard, D., et al. (2018). Ensembl variation resources. *Database* 2018, 1–12. doi: 10.1093/database/bay119

Jansson, M. D., and Lund, A. H. (2012). MicroRNA and Cancer. *Mol. Oncol.* 6, 590–610. doi: 10.1016/j.molonc.2012.09.006

Jovanovic, M., and Hengartner, M. O. (2006). MiRNAs and apoptosis: RNAs to Die For. *Oncogene* 25, 6176–6187. doi: 10.1038/sj.onc.1209912

Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., et al. (2002). The human genome browser at UCSC. *Genome Res.* 12, 996–1006. doi: 10.1101/gr.229102

Lee, J. E., Lee, J. Y., Wilusz, J., Tian, B., and Wilusz, C. J. (2010). Systematic analysis of Cis-elements in unstable MRNAS demonstrates That CUGBP1 Is a key regulator of MRNA decay in muscle cells. *PLoS One* 5:e11201. doi: 10.1371/journal.pone.0011201

Mansfield, K. D., and Keene, J. D. (2009). the ribonome: a dominant force in co-ordinating gene expression. *Biol. Cell* 101, 169–181. doi: 10.1042/BC20080055

Marquis, J., Paillard, L., Audic, Y., Cosson, B., Danos, O., Le Bec, C., et al. (2006). CUG-BP1/CELF1 Requires UGU-Rich sequences for high-affinity binding. *Biochem. J.* 400, 291–301. doi: 10.1042/BJ20060490

Mayr, C., and Bartel, D. P. (2009). Widespread Shortening of 3′UTRs by Alternative Cleavage and Polyadenylation Activates Oncogenes in Cancer Cells. *Cell* 138, 673–684. doi: 10.1016/j.cell.2009.06.016

Nakanishi, M., and Rosenberg, D. W. (2013). Multifaceted Roles of PGE2 in Inflammation and Cancer. *Semin. Immunopathol.* 35, 123–137. doi: 10.1007/s00281-012-0342-8

Ochs, M. J., Ossipova, E., Oliynyk, G., Steinhilber, D., Suess, B., and Jakobsson, P. J. (2013). Mass Spectrometry-Based proteomics identifies UPF1 as a critical gene expression regulator in monomac 6 cells. *J. Proteome Res.* 12, 2622–2629. doi: 10.1021/pr301193f

Perez-Riverol, Y., Csordas, A., Bai, J., Bernal-Llinares, M., Hewapathirana, S., Kundu, D. J., et al. (2019). The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Res.* 47, D442–D450. doi: 10.1093/nar/gky1106

Piva, F., Giulietti, M., Nocchi, L., and Principato, G. (2009). SpliceAid: a database of experimental rna target motifs bound by splicing proteins in humans. *Bioinformatics* 25, 1211–1213. doi: 10.1093/bioinformatics/btp124

Procaccio, V., de Sury, R., Martinez, P., Depetris, D., Rabilloud, T., Soularue, P., et al. (1998). (NDUFS2) Encoding the 49-KDa subunit of the mitochondrial respiratory complex i and immunodetection of the mature protein in mitochondria. *Mamm. Genome* 9, 482–484. doi: 10.1007/s003359900803

Rattenbacher, B., Beisang, D., Wiesner, D. L., Jeschke, J. C., von Hohenberg, M., St Louis-Vlasova, I. A., et al. (2010). Analysis of CUGBP1 targets identifies GU-repeat sequences that mediate rapid MRNA Decay. *Mol. Cell. Biol.* 30, 3970–3980. doi: 10.1128/MCB.00624-10

Reid, D. W., and Nicchitta, C. V. (2012). Primary role for endoplasmic reticulum-bound ribosomes in cellular translation identified by ribosome profiling. *J. Biol.Chemi.* 287, 5518–5527. doi: 10.1074/jbc.M111.312280

Riggins, G. J., Thiagalingam, S., Rozenblum, E., Weinstein, C. L., Kern, S. E., Hamilton, S. R., et al. (1996). Mad-related genes in the human. *Nat. Genet.* 13, 347–349. doi: 10.1038/ng0796-347

Saul, M. J., Baumann, I., Bruno, A., Emmerich, A. C., Wellstein, J., Ottinger, S. M., et al. (2019a). MiR-574-5p as RNA Decoy for CUGBP1 stimulates human lung tumor growth by MPGES-1 Induction. *FASEB J.* 33, 6933–6947. doi: 10.1096/fj.201802547R

Saul, M. J., Hegewald, A. B., Emmerich, A. C., Ossipova, E., Vogel, M., Baumann, I., et al. (2019b). Mass spectrometry-based proteomics approach characterizes the dual functionality of MiR-328 in monocytes. *Front. Pharmacol.* 10:640. doi: 10.3389/fphar.2019.00640

Saul, M. J., Stein, S., Grez, M., P-Jakobsson, J., Steinhilber, D., and Suess, B. (2016). UPF1 Regulates Myeloid Cell Functions and S100A9 Expression by the HnRNP E2/MiRNA-328 Balance. *Sci. Rep.* 6:31995. doi: 10.1038/srep31995

Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., et al. (2011). Global quantification of mammalian gene expression control. *Nature* 473, 337–342. doi: 10.1038/nature10098

Vlasova, I. A., and Bohjanen, P. R. (2008). Posttranscriptional regulation of gene networks by GU-rich elements and CELF proteins. *RNA Biol.* 5, 201–207. doi: 10.4161/rna.7056

Vlasova-St Louis, I., and Bohjanen, P. R. (2011). Coordinate regulation of MRNA decay networks by GU-Rich elements and CELF1. *Curr. Opin. Genet. Dev.* 21, 444–451. doi: 10.1016/j.gde.2011.03.002

Westman, M., Korotkova, M., af Klint, E., Stark, A., Audoly, L. P., Klareskog, L., et al. (2004). Expression of microsomal prostaglandin E synthase 1 in rheumatoid arthritis synovium. *Arthritis Rheum.* 50, 1774–1780. doi: 10.1002/art.20286

Wienholds, E., and Plasterk, R. H. A. (2005). MicroRNA function in animal development. *FEBS Lett.* 579, 5911–5922. doi: 10.1016/j.febslet.2005.07.070

Wightman, B., Ha, I., and Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic Gene Lin-14 by Lin-4 Mediates temporal pattern formation in C. *Elegans. Cell* 75, 855–862. doi: 10.1016/0092-8674(93)90530-4

Xia, H., Chen, D., Wu, Q., Wu, G., Zhou, Y., Zhang, Y., et al. (2017). CELF1 preferentially binds to exon-intron boundary and regulates alternative splicing in HeLa cells. *Biochim.Biophys. Acta Gene Regul. Mech.* 1860, 911–921. doi: 10.1016/j.bbagrm.2017.07.004

Yokouchi, H., and Kanazawa, K. (2015). Revisiting the role of COX-2 Inhibitor for Non-Small Cell Lung Cancer. *Transl. Lung Cancer Res.* 4, 660–664. doi: 10.3978/j.issn.2218-6751.2015.04.03

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 29 April 2020
doi: 10.3389/fphar.2020.00533

Cyclooxygenase 2-Regulated Genes an Alternative Avenue to the Development of New Therapeutic Drugs for Colorectal Cancer

Alicia M. Hidalgo-Estévez[1†], *Konstantinos Stamatakis*[2,3†], *Marta Jiménez-Martínez*[2], *Ricardo López-Pérez*[2] *and Manuel Fresno*[2,3*]

[1] Faculty of Biomedical and Health Sciences, Universidad Europea de Madrid, Madrid, Spain, [2] Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, Madrid, Spain, [3] Instituto Sanitario de Investigación Princesa, Madrid, Spain

OPEN ACCESS

Edited by:
Paola Patrignani, Università degli Studi G. d'Annunzio Chieti e Pescara, Italy

Reviewed by:
Melania Dovizio, Università degli Studi G. d'Annunzio Chieti e Pescara, Italy
Angel Lanas, University of Zaragoza, Spain

***Correspondence:**
Manuel Fresno
mfresno@cbm.csic.es

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *26 January 2020*
Accepted: *06 April 2020*
Published: *29 April 2020*

Citation:
Hidalgo-Estévez AM, Stamatakis K, Jiménez-Martínez M, López-Pérez R and Fresno M (2020) Cyclooxygenase 2-Regulated Genes an Alternative Avenue to the Development of New Therapeutic Drugs for Colorectal Cancer. Front. Pharmacol. 11:533. doi: 10.3389/fphar.2020.00533

Colorectal cancer (CRC) is one of the most common and recurrent types of cancer, with high mortality rates. Several clinical trials and meta-analyses have determined that the use of pharmacological inhibitors of cyclooxygenase 2 (COX-2), the enzyme that catalyses the rate-limiting step in the synthesis of prostaglandins (PG) from arachidonic acid, can reduce the incidence of CRC as well as the risk of recurrence of this disease, when used together with commonly used chemotherapeutic agents. These observations suggest that inhibition of COX-2 may be useful in the treatment of CRC, although the current drugs targeting COX-2 are not widely used since they increase the risk of health complications. To overcome this difficulty, a possibility is to identify genes regulated by COX-2 activity that could give an advantage to the cells to form tumors and/or metastasize. The modulation of those genes as effectors of COX-2 may cancel the beneficial effects of COX-2 in tumor transformation and metastasis. A review of the available databases and literature and our own data have identified some interesting molecules induced by prostaglandins or COX-2 that have been also described to play a role in colon cancer, being thus potential pharmacological targets in colon cancer. Among those mPGES-1, DUSP4, and 10, Programmed cell death 4, Trop2, and many from the TGFβ and p53 pathways have been identified as genes upregulated in response to COX-2 overexpression or PGs in colon carcinoma lines and overexpressed in colon tumor tissue. Here, we review the available evidence of the potential roles of those molecules in colon cancer in the context of PG/COX signaling pathways that could be critical mediators of some of the tumor growth and metastasis advantage induced by COX-2. At the end, this may allow defining new therapeutic targets/drugs against CRC that could act specifically against tumor cells and would be effective in the prevention and treatment of CRC, lacking the unwanted side effects of COX-2 pharmacological inhibitors, providing alternative approaches in colon cancer.

Keywords: cyclooxygenase, prostaglandin, Colon cancer, therapy, tumor development, metastasis, effector genes/proteins

COX-2 IN COLORECTAL CANCER

Colorectal cancer (CRC) is the third most common type of cancer and the second leading cause of cancer-related death in the world, with around 900,000 deaths per year (Globocan, 2018; Keum and Giovannucci, 2019). Even in patients who have undergone tumor resection, 40–50% relapse and die of metastases, being the overall 5-year survival less than 60%. Despite this health burden, only few therapeutic options exist for this disease, including combination of chemotherapy and anti-angiogenic agents (Kuipers et al., 2015; Karpisheh et al., 2019). Thus, currently available therapeutic options do not achieve the desired efficiency and development of new strategies is required.

There are two types of CRC: hereditary or familial colon cancer and sporadic colon cancer (Sancho et al., 2004; Ma et al., 2018). Nonetheless, sporadic colon cancer is the most common with an incidence of 75–80% in diagnosed cases. The alterations generated in two main molecular pathways subdivide this type of cancer. In 80–85% of sporadic CRCs there are mutations or deletions in suppressor genes such as KRAS, APC, DCC, and p53, promoting chromosomal instability (Sancho et al., 2004; Moran et al., 2010). In 1990, Fearon and Vogelstein proposed that the mutation in three or four of the aforementioned oncogenes and/or suppressor genes is necessary to initiate the tumorigenesis process, where the final properties of the tumor are determined from the accumulation and order in which changes appear (Fearon and Vogelstein, 1990). Later, it was described how the epithelial cells of the CRC acquire the genetic alterations in a strict order and involve the Wnt and TGFβ signaling pathways in cancer promotion (Arends, 2000). On the other hand, the non-canonical route, also called "mutator", comprises 15–20% of sporadic CRCs and depends mainly on the alteration due to the instability of microsatellite sequences (MSI, Microsatellite Sequences Instability) in the genome due to the accumulation of mutations in the genes for rupture repair (MMR, Mismatch Repair) produced during DNA replication (Imai and Yamamoto, 2008).

Many clinical trials and epidemiological studies have suggested that the use of non-steroidal anti-inflammatory drugs (NSAIDs), which are classic inhibitors of COX enzymatic activity reduce the risk of developing cancer in general, and more specifically in CRC (reviewed in (Thun et al., 2012; Rothwell, 2013; Piazuelo and Lanas, 2015; Patrignani and Patrono, 2016). Taking together all these data strongly suggest that Cox targeting is a mechanism for cancer prevention.

COX enzymes catalyse the rate limiting step in the conversion of arachidonic acid, derived from membrane phospholipids by phospholipase A2, into prostanoids (Williams and Dubois, 1996). Two main isoforms of this enzyme are known: COX-1 encoded by prostaglandin-endoperoxide synthase 1 (*PTGS1*) and COX-2 (encoded by *PTGS2*). Although both proteins show the same cyclooxygenase and peroxidase activity, their differences are found in substrates, cell expression, inhibition, and intracellular localization and induction (Garavito et al., 2002). COX-1 is constitutively expressed in most tissues and involved in physiological processes. However, COX-2 is not usually detectable in normal tissues but is induced by numerous cytokines, growth factors, hormones, and tumor promoters. Prostanoids modulate immune responses, renal function, blood clotting and play a role in many pathological conditions, such as inflammation, pain, fever, swelling, etc. (Iniguez et al., 2003; Iniguez et al., 2008; Sreeramkumar et al., 2012).

Considerable amounts of evidence in clinical settings further support a role of COX-2 in colorectal carcinogenesis and tumor progression [reviewed in (Subbaramaiah and Dannenberg, 2003; Wang and Dubois, 2010)]. Thus, COX-2 is expressed early during the adenoma-carcinoma sequence that occurs in CRC, suggesting an important role of this enzyme in colorectal carcinogenesis. COX-2 expression is upregulated in human colorectal adenocarcinomas when compared with normal adjacent colonic tissue. Moreover, polymorphisms of the *PTGS2* gene were associated with risk of CRC (Cox et al., 2004; Agundez et al., 2015). The use of Min/+ mice as a CRC model showed elevated levels of COX-1 and COX-2 in sporadically formed adenomas (Williams et al., 1996) and inhibition of COX-2 resulted in a substantial decrease in intestinal polyp number and size (Jacoby et al., 2000). In the same way, Apc716 mice developed a smaller number and size of tumor polyps when the COX-2 gene was eliminated (Oshima et al., 1996).

In addition, many reports of colorectal tumor cells either overexpressing COX-2 or having it silenced have correlated increased COX-2 expression with their invasive and metastatic properties in xenografted tumors in mice (Tsujii and Dubois, 1995; Tsujii et al., 1997; O'mahony et al., 1999; Chen et al., 2001; Sun et al., 2002; Yoshimoto et al., 2002; Charames and Bapat, 2006; Strillacci et al., 2006; Stamatakis et al., 2015). However, the molecular mechanisms by which COX-2 expression in intestinal epithelial cells leads to that phenotype have not been fully elucidated yet.

In view of the abundant biological and phenotypic evidence, several clinical trials have been performed, aimed to evaluate the efficacy of specific inhibitors of COX-2 (COXIBs) (Fitzgerald and Patrono, 2001) to prevent or delay the onset (or recurrence) of tumors in high-risk patients, including those with prior removal of colon tumors. These studies indicate that specific inhibition of COX-2 prevents the (re)appearance of tumors but also show cardiovascular side-effects [reviewed in (Sinicrope, 2006; Bertagnolli, 2007)].

Recent studies, remark the role of COX-2 in constitutive IDO1 expression by human tumors and substantiate the use of COX-2 inhibitors to improve the efficacy of cancer immunotherapy, either by reducing constitutive IDO1 expression, which contributed to the lack of T-cell infiltration in tumors that fail to respond to immunotherapy (Hennequart et al., 2017), or by synergizing with anti-checkpoint antibodies (Zelenay et al., 2015).

For all of the above, the study of the expression of COX-2 in the different phases of tumor progression and metastasis and the finding of new signaling pathways triggered by this enzyme are essential in order to develop new drugs that inhibit the effects of COX-2 both in cancer prevention and therapy (Rizzo, 2011).

PROSTANOIDS IN COLON CANCER

The activity of cyclooxygenases (COX) is coupled to several terminal synthases that produce the five primary different prostanoids: prostaglandin D2 (PGD_2), prostaglandin E2 (PGE_2), prostaglandin F2α ($PGF_{2\alpha}$), prostaglandin I2/prostacyclin (PGI_2), and thromboxane A2 (TXA_2) (Iniguez et al., 2003; Iniguez et al., 2008) being also some of them implicated in colon cancer.

PGE_2 in Colon Cancer

Among the prostanoids, PGE_2 has been proposed as the principal prostanoid promoting tumor growth and survival in CRC. PGE_2 is present in the healthy colon but its levels are elevated in CRC (Pugh and Thomas, 1994; Cathcart et al., 2012; Zuo and Shureiqi, 2013) and correlate with tumor size (Pugh and Thomas, 1994; Yang et al., 1998) and disease progression (Karpisheh et al., 2019). The elevation of PGE_2 levels may favor tumor growth and invasion *via* several mechanisms as stimulating cell proliferation, inducing local immunosuppression, inhibiting apoptosis, promoting angiogenesis, and increasing cell migration and invasion as well as drug resistance in colon cancer cells (Iniguez et al., 2003; Corral et al., 2007; Greenhough et al., 2009; Wang and Dubois, 2010; Karpisheh et al., 2019). Moreover, there is a positive feedback loop between COX-2 and PGE_2, in which COX-2 induces PGE_2 production, and that in turn increases further the expression of COX-2 in colon cancer cells (Stamatakis et al., 2015; Karpisheh et al., 2019).

Three PGE_2 synthases from PGH_2 have been described (Hara et al., 2010), two microsomal, mPGES1, mPGES-2, and the cytoplasmic cPGES, encoded by the PTGES, PTGES2, and PTGES3 genes, respectively. MPGES1 expression has been associated to CRC incidence and prognosis (Seo et al., 2009; Sasaki et al., 2012) and has been proposed to cooperate with COX-2 to enhance tumor growth (Kamei et al., 2003). cPGES is a non-inducible isoform, constitutively expressed in most tissues and associated with COX-1 activity while maintaining the production of PGE_2 (Tanioka et al., 2000).

Notably, we found co-localization of COX-2 and MPGES1 in human CRC biopsies and that PTGS2 and PTGES gene expression levels strongly correlate in human microarray databases. Interestingly, we have described the joint induction of mPGES-1 and COX-2 by PGE_2 due to the involvement of the transcription factor EGR1 (Early Growth Response Protein 1), representing a positive feedback loop between COX-2, mPGES-1, and PGE_2 (Stamatakis et al., 2015). Moreover, mPGES-1 overexpressing carcinoma cell lines have increased tumorigenic capacity *in vivo* indicating that high levels of any of the two enzymes is sufficient to enhance CRC growth (Stamatakis et al., 2015). Our results demonstrate that mPGES1 is induced by COX-2 overexpression, *via* autocrine PGs release, likely $PGF_{2\alpha}$ through an EGR1-dependent mechanism in colon carcinoma.

Thus, mPGES1 has gained attention recently as alternative target to COX-2 for CRC chemoprevention and chemotherapy (Sasaki et al., 2015). In this sense, mPGES1 inhibitors have demonstrated promising effects in inhibiting colon carcinoma growth (Dixon et al., 2013; Wang et al., 2018; Karpisheh et al., 2019), that may circumvent the *in vivo* cardiovascular toxicity associated with COX-2 inhibitors.

PGE_2 acts through its binding to prostanoid-E (EP) receptors. Four subtypes of EP receptors (EP1, EP2, EP3, EP4) have been identified. Their tissue-specific expression and activation trigger different signaling pathways that favor, for example, the cellular proliferation of the intestinal epithelium (Takafuji et al., 2000). EPs belong to the family of G-protein coupled receptors (GPCRs) and transduce PGE_2 signaling through different intracellular messengers. EP2 and EP4 preferably increase intracellular levels of cyclic adenosine monophosphate (cAMP) while signaling by EP3 mostly mobilizes cAMP and the activation of EP1 mobilizes calcium (Ca^{2+}) (Regan, 2003; Alfranca et al., 2006). In different mice models of colon cancer, genetic deletion of the EP1 (Kawamori et al., 2005), EP2 (Ma et al., 2015), and EP4 receptors inhibits colonic tumorigenesis (Mutoh et al., 2002).

Prostaglandin 2α in Colorectal Cancer

$PGF_{2\alpha}$ is mainly produced by the activity of PGF synthase (PGFS) from PGH_2 and specifically activates the GPCR type FP receptor (Breyer et al., 2001; Komoto et al., 2006). Signaling through the two FP isoforms leads to the activation of phospholipase C (PLC) and, consequently, an increase in intracellular Ca^{2+} and the activation of protein kinase C (PKC). However, $PGF_{2\alpha}$ can be synthesized from other PGs such as PGE_2 and PGD_2, and can signal through other receptors such as EP1 and EP3, thus varying the intracellular signaling pathways (Breyer et al., 2001; Komoto et al., 2006). For example, induction of COX-2 through FP can occur through Rho activation and transcription mediated by β-catenin/Tcf (Fujino and Regan, 2003). Along those lines, elevated levels of cAMP have been detected in cells isolated from colonic crypts after treatment with $PGF_{2\alpha}$ (Collins et al., 2009).

There is very little evidence of the possible role of $PGF_{2\alpha}$ in CRC other than its higher levels, together with PGE_2 (Nugent et al., 1996). Also, in the tumor tissue of patients with CRC, a differential regulation of the cancer-dependent FP receptor has been detected (Gustafsson et al., 2007). $PGF_{2\alpha}$ also promotes the invasion and mobility of epithelial cells of adenomas and colon carcinomas in collaboration with PGE_2 (Qualtrough et al., 2007).

Recently, we have shown that colorectal tumor cells produce $PGF_{2\alpha}$ though COX-2 and have provided some evidence that $PGF_{2\alpha}$ may also play a role in colorectal tumorigenesis (Stamatakis et al., 2015). Colorectal adenoma and carcinoma-derived cell lines secrete $PGF_{2\alpha}$ while they also express FP indicating potential autocrine effects. Interestingly, this secreted $PGF_{2\alpha}$ was able to increase mPGES1 but also COX-2 in colon carcinoma cells, *via* FP receptor and EGR1, further enhancing PGE_2 levels indicating a positive feedback loop between COX-2/mPGES1/PGE_2 and $PGF_{2\alpha}$ (Stamatakis et al., 2015). However, $PGF_{2\alpha}$ failed to directly induce cell proliferation in CRC cell lines (Cassano et al., 2000).

Other Prostanoids

The roles of the other COX-derived prostanoids in CRC tumors, as well as in carcinoma cell lines remains poorly understood (Cathcart et al., 2011). It has been reported that the levels of some prostaglandin receptors, such as IP (PGI_2 receptor) and DP (PGD_2 receptor), were reduced in CRC cells and the downregulation of

one of them, DP2, has been related to differentiation of healthy epithelium to tumor (Gustafsson et al., 2007). The expression of thromboxane synthase (TXS), the enzyme that catalyses the conversion of PGH2 to TXA_2, was significantly increased in CRC tumors compared to normal tissue. Furthermore, genetic or pharmacological reduction of TXS diminishes proliferation in CRC cell lines (Ekambaram et al., 2011). In addition it has also been described that the lack of the enzyme 15-PGDH, responsible for the inactivation of prostaglandins and lipoxins, is associated with CRC (Backlund et al., 2005) and together with the increase in the PGE_2 synthesis, induces tumor formation in Min (multiple intestinal neoplasia) CRC model in mice. (Myung et al., 2006). Besides the loss of 15-PGDH and induction of this enzyme has been associated with the suppression of inflammation-driven colon carcinogenesis in mice (Choi et al., 2014).

PROSTAGLANDIN PATHWAY AND COLORECTAL CANCER

The potential implication of the Cox-PG synthases-PG receptors in CRC can be also studied through expression levels of the pathway´s components using The Cancer Genome Atlas (TCGA) gene expression data. A summary of these findings is shown in **Figure 1**, based on the TCGA Colon Cancer dataset. These analyses, comparing tumor and normal colon epithelia, showed that gene expressions from several members of this pathway are upregulated in tumors, namely *PTGS2*, *PTGES*, and *TBXAS1*, indicating that the biosynthetic and signaling pathways of PGE_2 and TXA_2 are favored in established tumors. Conversely, expression downregulation in tumor tissue was observed for genes encoding components of the pathway leading to the signaling of other prostanoids, but most notably of *HPGD* that encodes the PGE_2-inactivating enzyme 15-PGDH. Analysis with other different datasets, i.e., the OncoMine microarray database (Compendia Bioscience), resulted in very similar data regarding *PTGS2*, *PTGES* (Stamatakis et al., 2015). Together, those analyses confirm the important role of COX2-2 and PGE_2 in colon cancer. Nonetheless, since COX is the rate-limiting enzyme in the synthesis of all prostanoids, an increased expresion of this enzyme could result in the elevation of other prostanoids besides PGE_2.

COX-2 DOWNSTREAM GENES IN CRC

As mentioned above, the treatment of patients with NSAIDs or COXIBs greatly decreases tumor progression in a number of clinical trials in CRC. However, since general inhibitors of COXs,

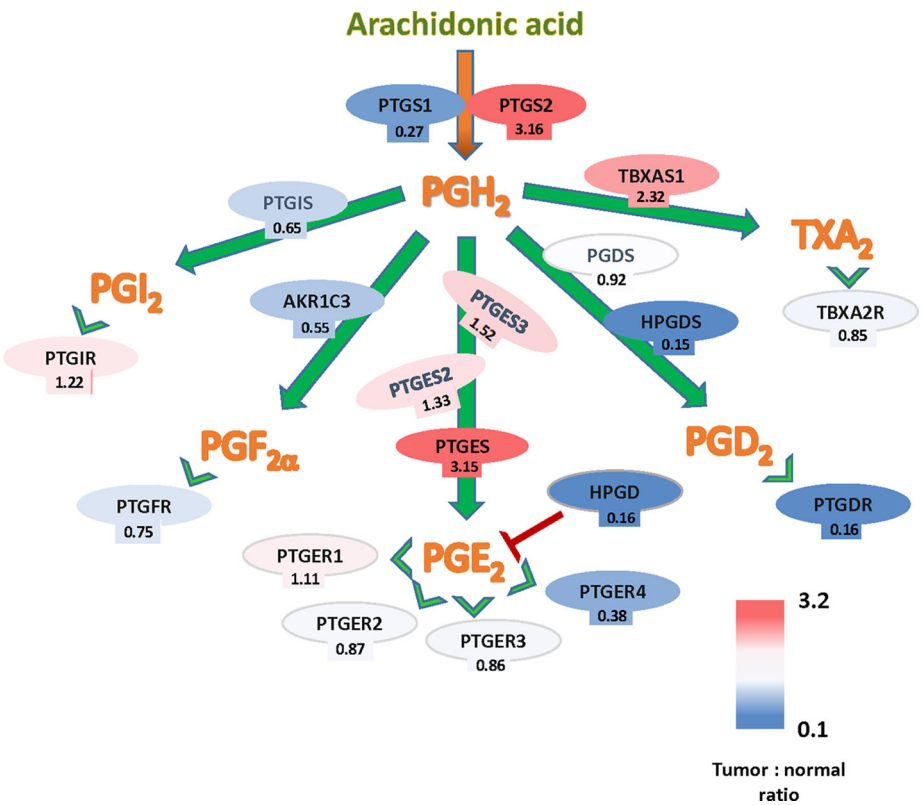


FIGURE 1 | Arachidonate-prostanoids pathway gene expression in colorectal cancer (CRC) tumors. Shown are mRNA expressions in tumors of enzymes and receptors from the arachidonate-prostanoids pathway. Numbers indicate the fold differences with normal adjacent tissue based on the The Cancer Genome Atlas (TCGA) Colon Cancer dataset.

such as aspirin, can cause gastrointestinal bleeding problems and specific inhibitors of COX-2 increase the risk of cardiovascular disease, alternative treatments are needed targeting the downstream effector of COX-2 protumoral activity, likely minimizing side effects, and if possible aiming exclusively to cancer cells (Cathcart et al., 2011).

Although PGE_2 blockade may represent a good therapeutic option, other PGs besides PGE_2 could be responsible for COX-2 effect in colon carcinoma cells. Besides, although prostanoids may modulate the growth and invasiveness of colon carcinoma cells, they also modulate the immune responses and inflammation and their inhibition may also have unwanted side effects.

To address this issue, we have concentrated in the effects of COX-2 in colon carcinoma cells. COX-2 activity regulates gene expression, which confers cells advantages for growth, migration, invasion, and metastasis (Stamatakis et al., 2015). We found that stable COX-2 overexpression in carcinoma cell lines (HT-29, HCT116, and Caco2) significantly affects gene transcription. Analysis of genes modified by COX-2 overexpression indicates that many genes involved in transcription, growth, apoptosis, angiogenesis, and migration were elevated (ArrayExpress, E-MEXP-2343). Moreover, a review of the available databases and literature in other carcinoma cell lines overexpressing COX-2 or repressed siRNA also identify some COX-2 downstream effectors responsible for the pro-tumorigenic properties of COX-2, although none of them performed a comprehensive gene expression analysis. Altogether, those analyses identified some interesting molecules. We selected some genes and validated them by quantitative RT-PCR in colon carcinoma cell lines, either induced by PGE_2 or by overexpression of COX-2 or mPGES1. The results of mRNA levels obtained for some of those genes are shown in **Table 1**. Besides, we performed analyses of mRNA expression and survival in colon adenocarcinomas using public databases. From all these analyses some expected genes emerged, but others are relatively new and amenable to drug targeting.

Dual-Specificity MAPK Phosphatase 10

Dual-specificity Mitogen Activated Protein Kinase (MAPK) phosphatase 10 (DUSP10), also named MPK5, was found altered by COX-2 overexpression in CRC cell lines in several data sets (Doherty et al., 2009) included our own. DUSP10 is able to dephosphorylate p38 and c-Jun NH_2-terminal kinase (JNK). Until recently, there were few reports regarding the putative role of DUSP10 in cancer. Most of those studies have found increased *DUSP10* mRNA in tumor tissue, thus suggesting a pro-tumorigenic role for this phosphatase (Jimenez-Martinez et al., 2019b). In CRC, the expression of certain DUSP10 polymorphisms are linked to an enhanced CRC risk (Duan et al., 2015).

We found that DUSP10 overexpression increased the growth of CRC cell lines and mouse xenografts, while the opposite phenotype was observed by DUSP10 silencing. Cancer progression is related to an uncontrolled cell division and DUSP10 overexpression produces the loss of cell-contact inhibition through the dephosphorylation of Yes-associated protein (YAP) at Ser397. This dephosphorylation retains YAP in the nucleus. In fact, we found that high amounts of DUSP10 and YAP1 are located in the nucleus of CRC cells (Jimenez-Martinez et al., 2019a). Additionally, the quantity of

TABLE 1 | Genes upregulated by COX-2, mPGES1, or PGE_2.

	Cox-2[1]	PGE_2[2]	mPGES[3]	COAD risk[4]	Metastasis risk[5]
PTGS2	Increased	Increased	Increased	No	Higher
PTGES	Highly increased	NT	NT	Higher	Higher
DUSP4	Increased	Increased	NT	NO	Higher
DUSP10	Increased	Increased	Increased	High	Higher
PMEPA	Increased	Increased	NT	NO	Higher
IL15Ra	Increased	NT	Increased	NO	NO
KLF4	Increased	NT	NO	Lower	Lower
CALD1	Increased	NT	NT	High	Higher
TACSTD2	Increased	NO	NT	Weak	Lower
NFAT5	Increased	NT	NO	Weak	Lower
DDIT3	Increased	NT	NT	Higher	Lower
Zc3h12c	Increased	NT	NT	Lower	NO
MMP7	Increased	NT	NT	NO	NO
CYR61	Increased	NT	NT	High	Higher
CXCL2	Increased	NO	NO	Lower	Lower
NEDD9	Increased	Increased	NT	NO	NO
PDCD4	Decreased	Decreased	NT	NO	NO
CXCR4	Decreased	NT	NT	NO	Higher
FGFR4	Decreased	NT	NO	Lower	Lower
NR0B2	Decreased	NT	NT	Lower	Lower
CA9	Decreased	NT	NT	NO	Lower
REG4	Highly decreased	NT		Lower	Higher

(1) Data are obtained from RT-PCR of COX-2 overexpresssing carcinoma cells (our data on HT-29, HCT116, or Caco2 or reported in the literature) compared to control cells. (2) Data are obtained from RT-PCR of PGE2 treated carcinoma cells (our data on HT-29, HCT116 and Caco2 or reported in the literature) compared to untreated control cells. (3) Data are obtained from RT-PCR of mPGES1 overexpresssing carcinoma cells (our data on HT-29, HCT116, and Caco2) compared to control cells. (4) Associated to significant worse prognosis in databases of patients with Colon adenocarcinoma http://www.oncolnc.org/; or (5) bioprofiling database (http://www.bioprofiling.de/). NT, Not tested or reported; NO, no effect; Higher means that high expression is associated with higher risk of death or metastasis; Lower means that high expression is associated with lower risk of death or metastasis.

nuclear DUSP10 in CRC tumor biopsies is directly correlated with high tumor stage CRC and poor prognosis and survival in a large cohort of CRC patients, being also associated to high expression of nuclear YAP1. All these data point at DUSP10 as a downstream protein in COX-2 signaling and provide evidence of the role of DUSP10 in CRC progression *via* YAP1 regulation (Jimenez-Martinez et al., 2019a). Interestingly, an inhibitor of DUSP10 have been described (Hommo et al., 2015), supporting its use in CRC.

Dual-Specificity MAPK Phosphatase 4

Another dual-specificity MAPK phosphatase, DUSP4/MKP-2, was also upregulated by COX-2. DUSP4 role in several cancers has been documented (Low and Zhang, 2016; Seternes et al., 2019). An altered expression of DUSP4 is related to colon tumorigenesis. Thus, elevated DUSP4 expression is associated with microsatellite instability in CRC patients (Gröschl et al., 2013) being also associated with liver and lung metastases of CRC (Saigusa et al., 2013). Moreover, DUSP4 overexpression in CRC cell lines produced upregulation of MAPK targets, such as EGR1, FOS, and MYC, and downregulation of the mismatch repair gene MSH2, all these effects leading to an increase in cell proliferation (Gröschl et al., 2013). In contrast, it has been more recently reported that the decrease in the expression of DUSP4 in CRC cell lines activated ERKs, causing cell proliferation and invasiveness (Ichimanda et al., 2018).

Interestingly, DUSP4 overexpression in CRC cell lines decreases their sensitivity to doxorubicin, a drug used to treat CRC (Kang et al., 2017). Moreover DUSP4 genetic inactivation increased the resistance to cetuximab, an epidermal growth factor receptor (EGFR)-blocking antibody, widely used and approved for treatment of metastatic CRC in cell lines and to another well-known EGFR inhibitor, erlotinib (Park et al., 2019). Interestingly, those effects are also related to COX-2 expression, since *DUSP4* was the highest induced gene in cetuximab-resistant CRC cells (Lu et al., 2016). All these data together point at DUSP4, enzyme regulated by COX-2, as a factor whose overexpression leads to CRC development and invasion, and which can be a promising therapeutic target. Moreover, DUSP4 specific inhibitors have been described (Park et al., 2014) supporting its clinical testing in CRC at least in some drug resistant tumors.

Trophoblast Cell-Surface Antigen 2

Trophoblast cell-surface antigen 2 (TROP2), also called Tumor-Associated Calcium Signal Transducer 2 (TACSTD2), is a cell-surface glycoprotein expressed during embryonic and fetal development which is involved in cell proliferation, cell binding, motility, and metastasis (Mcdougall et al., 2015). TROP2 overexpression can induce cancer growth and is associated with poor prognosis and drug resistance in cancer cells (Shvartsur and Bonavida, 2015).

Our COX-2, but not mPGES1, overexpressing colon carcinoma cells present high levels of *TACSTD2*. Besides, high expression of *PTGS2* and *TACSTD2* genes have been found also associated in lung cancer metastasis (Citterio et al., 2012). Previous studies in CRC patients, already showed the correlation between high expression of TROP2 and metastasis in CRC (Kapoor, 2013). The overexpression of TROP2 together with MMP7, another COX-2 induced gene (see below), is a predictor of worse prognosis and relapse in CRC (Fang et al., 2009). TROP2 expression enhances anchorage-independent growth in colon carcinoma cell lines (Wang et al., 2008) and its activation of TROP2 by Tumor Necrosis Factor (TNF)-alpha induces cell migration and invasion (Zhao and Zhang, 2018).

More importantly, an anti-TROP-2 antibody, sacituzumab govitecan, is a potential therapeutic drug for metastatic solid tumors (Starodub et al., 2015). Since TROP2 is consistently induced by COX-2 overexpression, this makes it an alternative target to COX-2 blockade therapy in CRC.

Matrix Metalloproteinase- 7

Matrix metalloproteinases (MMPs) are proteolytic enzymes which degrade and remodel the extracellular matrix (ECM) in physiological processes, such as in cell migration, and have also been involved in metastasis (Zucker and Vacirca, 2004). It has been reported that MMP7 is one of the most important MMPs in colorectal tumorigenesis, promoting angiogenesis, invasiveness, and tumor survival. Thus, MMP-7 may enhance CRC progression due to its proteolytic activity on several cell surface molecules as EGFR, Fas-L, etc. (Wang et al., 2006). MMP7 is abundantly synthesised by colon carcinoma cells (Zucker and Vacirca, 2004). A study of CRC patients demonstrated an increase in COX-2 and MMP-7 expression when compared to normal tissue and colon polyps sample (Bengi et al., 2015). High levels of MMP-7 together with PTEN down-regulation where detected in CRC and were related to tumor stage and progression (Bi et al., 2013). Moreover, COX-2 overexpression in carcinoma cells modulates the adhesive properties of MMPs (Tsujii and Dubois, 1995; Tsujii et al., 1997). PGE_2 can transactivate EGFR thereby inducing the proliferation of CRC cell lines and exerts its functions in part through molecules such as MMP-7 (Pai et al., 2002; Karpisheh et al., 2019).

Krüppel-Like Factor 4 (KLF4)

Krüppel-like factor 4 (KLF4) is a transcription factor that was firstly identified as a regulator of cell growth arrest being one of the most important factors in Cancer Stem Cells (CSC). It is expressed in differentiating cells and it is also known to have a role in apoptosis suppression (Ghaleb and Yang, 2017). KLF4 mRNA is consistently and strongly induced in our COX-2 but not in mPGES1 overexpressing colon carcinoma cells and it was shown that COX-2 and KLF4 colocalized in carcinoma cells of CRC tumor samples (Shao et al., 2008). Moreover, in HT-29 colon cancer cells, the expression of KLF4 was induced by 15-Deoxy-Delta-12, 14 PGJ_2, a downstream product of COX-2 signaling pathway (Chen and Tseng, 2005).

It has been described that the levels of high KLF4 mRNA in normal tissues can be used as a prognostic indicator of survival in CRC patients (Lee et al., 2014), suggesting a regulatory role in CRC progression. In contrast, other reports have indicated a protumoral role of KLF4 in CRC. Thus, KLF4 was found in colon adenocarcinoma metastasis to the liver (Humphries et al., 2018) and low KLF4 expression was found in poorly differentiated CRC tissues (Hu et al., 2011). A study performed in cancer stem cell (CSC)-enriched spheroid CRC cell lines revealed a role of KLF4 in

the invasiveness, migration, resistance to treatment, and ability to generate tumors as well as in the induction CSCs markers in those cells (Leng et al., 2013).

Programmed Cell Death 4 (PDCD4)

The expression of programmed cell death 4 (PDCD4), a tumor suppressor protein that inhibits tumor development in many cancer types, is decreased in CRC and this downregulation in colon tumors has been related to worse prognosis (Long et al., 2019). Several studies correlate PDCD4 overexpression with inhibition of cell proliferation and invasion in different tumor cancer cell types. In colon carcinoma cells, high levels of PDCD4 inhibited Akt signaling pathway, decreasing invasiveness (Wang et al., 2017). Moreover, a COX-2 inhibitor, NS-398, increases PDCD4 in HCA-7 cells, a colon cancer cell line (Zhang and Dubois, 2001), in agreement with our observation that is consistently downregulated by COX-2 overexpression in carcinoma cell lines. The overexpression of COX-2 in response to inflammation induces an increase in PGE_2 production and PGE_2 decreases PDCD4 protein levels through upregulation of miR-21 (Peacock et al., 2014). MiR-21 role in cancer has been extensively studied and its upregulation may explain the intrinsic resistance of some cancers to chemotherapy (Pfeffer et al., 2015), being miR-21 and PDCD4 inversely correlated in CRC (Chang et al., 2011; Horiuchi et al., 2012). Thus increasing PDCD4 levels through pharmacological manipulation of miR-21 could represent a novel therapeutic strategy in the treatment of CRC, as an alternative to COX-2/PGE_2 blockade in CRC.

Nuclear Factor of Activated T-Cells 5

Nuclear Factor of Activated T cells- 5 (NFAT5) is a transcription factor belonging to the NFAT family whose expression is upregulated by osmotic stress to difference with the other four members of this family (Qin et al., 2014). Dysregulation of NFAT signaling has been linked to tumor progression in several cancers (Qin et al., 2014). Interestingly, NFAT is a key transcription factor regulating COX-2 expression in CRC cell lines (Duque et al., 2005), suggesting the existence of a positive feedback loop between NFATs and COX-2. However, little is know on NFAT5 and CRC. We found NFAT5 is induced by COX-2 overexpression and it has been described that promotes metastasis through the induction of alpha(6)beta(4) integrin in human breast and colon tumors (Jauliac et al., 2002), and besides some associations between NAFT5 SNPs and CRC risk has been found (Slattery et al., 2011). NFAT5 expression has also been associated to metastasis in other cancer types and to proliferation of lung adenocarcinoma cells (Guo and Jin, 2015).

COX-2 AND THE TGF-β PATHWAY

Among the genes induced by COX2, we found in our arrays many genes of the TGF-β pathway, which we later confirmed by PCR (**Table 2**). Many of them are also induced by PGE_2 treatment although in a much lower extent, suggesting that other COX2 derived products, as $PGF_{2\alpha}$, are also involved. Apart from those, some other genes induced by TGF-β were also induced by COX-2/PGs.

One of them was Prostate Transmembrane Protein, Androgen Induced 1 (PMEPA1, also known as TMEPA1, transmembrane prostate androgen induced 1). PMEPA1 was first identified as being upregulated in renal cell carcinoma and was designated as solid tumor associated gene 1 (STAG1) (Rae et al., 2001). PMEPA1 is known to be induced by TGF-β and modulates TGF-β signaling by competing with SARA (SMAD Anchor for Receptor Activation) for R-SMAD binding to sequester R-SMAD phosphorylation and promoting lysosomal degradation of TGF-β receptor (Watanabe et al., 2010). PMEPA1, through a negative feedback loop, is described as the responsible to convert TGF-β from a tumor suppressor to a tumor promoter in breast cancer (Singha et al., 2010).

We have demonstrated that PMEPA1 is a COX-2, PGE_2, PGF2α, and calcium (through Ionophore treatment) -induced gene in colon and ovary cancer cells (Jimenez-Segovia et al., 2019). Furthermore, PMEPA1 is involved in differentiation of colon epithelial cells and high levels are also found in all phases of CRC development, including metastasis, in patients (Brunschwig et al., 2003). Furthermore, its high expression in CRC is related to poor prognosis and postulated as a predictor of relapse risk (Xu et al., 2017; Zhang et al., 2019). PMEPA1 is also overexpressed in intestinal tumors in Apc (Min) mice, which are prone to intestinal adenoma formation and is also dysregulated in human CRC adenomas (Reichling et al., 2005).

A recent study using colon cancer cell lines revealed the role of PMEPA1 in cell migration and invasion through bone morphogenetic proteins (BMP) signaling pathway activation and phosphorylation of the transcription factors Smad1 and Smad5 (Zhang et al., 2019), which also correlates with its described effect in ovarian cancer cells (Jimenez-Segovia et al., 2019).

TABLE 2 | Induction of TGFβ pathway or p53 pathway genes by COX-2 overexpression or PGE_2 treatment in HT-29 colon carcinoma cells.

TGFβ pathway	COX-2	PGE_2
TGFB1	+ 24 ± 4	+ 3 ± 1
TGFBR1	+ 18 ± 3	+ 3 ± 0.8
TGFBR2	+ 14 ± 3	+ 2 ± 0.4
SMAD1	+ 20 ± 8	+ 2 ± 0.6
SMAD3	+ 38 ± 3	+ 3 ± 0.5
SMAD5	+ 35 ± 6	+ 3 ± 0.5
SERPINE1	+ 7 ± 2	
EDN1	+ 25 ± 4	+ 2 ± 0.2
ID1		+ 2 ± 0.3
ENG	+ 36 ± 9	
CTGF	+ 2 ± 0.3	+ 3 ± 0.5
p53 pathway		
TP53	+ 10 ± 2	+ 4 ± 1
MDM2	+ 28 ± 3	+ 3 ± 1
CDKN1A	+ 7 ± 1	
TGFA	+ 46 ± 8	
GADD45	+ 20 ± 3	+ 2 ± 0.5
PCNA	+ 8 ± 1	+ 2 ± 0.3
BAX	+ 23 ± 4	+ 2 ± 0.8
FASL	+ 25 ± 2	
TSP1	+ 34 ± 2	+ 3 ± 0.7

Fold induction of indicated mRNA in HT-29 stably overexpressing COX-2 respect empty vector transfected cells or HT-29 treated with 1 μM PGE_2 for 24 h. Results shown are the mean ± SD of 3 independent experiments.

NEDD9 (Neural Precursor Cell Expressed, Developmentally Down-Regulated-9) or HEF1 (Human enhancer of filamentation 1) is a structural protein mainly found in epithelial cells, which is upregulated by TGF-β and expressed transiently during embryonic life in mice. Many functions have been attributed to NEDD9, among them, the transmission of growth control signals between focal adhesions. It has also been involved in melanoma tumorigenesis and, most notably, in metastasis (O'neill et al., 2007; Tikhmyanova et al., 2010; Lee et al., 2011).

NEDD9 was also identified as one of key genes in CRC, being upregulated in CRC tumors (Li et al., 2011; Cui et al., 2017). Interestingly, two independent groups have shown that NEDD9 overexpression elicited the same effects as PGE_2 treatment on cell proliferation, cell cycle progression, colony formation, migration, and xenograft tumor growth in colon carcinoma cell lines (Xia et al., 2010; Li et al., 2011). Interestingly, those PGE_2 effects were reversed by inhibition of NEDD9 expression, indicating that NEDD9/HEF-1 could be an important downstream mediator in the pro-tumoral activity of PGE_2 in CRC. In this sense, it would be possible to develop inhibitors to disrupt NEDD9 interactions with oncogenic partners (Tikhmyanova et al., 2010).

Cysteine Rich Angiogenic Inducer 61 (Cyr61/CNN1) is another TGF-β regulated gene (Han et al., 2016). It is a member of the CNN family of extracellular matrix associated proteins, which induces cell division and differentiation, chemotaxis, angiogenesis, and adhesion. In CRC, high levels of this protein induce migration and increase invasiveness (Chang et al., 2014). Cyr61/CNN1 has been described as potential serum marker for CRC (Song et al., 2017), whereas Cyr61 expression in the tumor tissue indicates poor prognosis in colon cancer patients being statistically associated with greater mortality (Jeong et al., 2014). Altered activation of CYR61 gene enhancers occurs during CRC development, being CYR61 expression induced by FOXA1 and CBP in colon cancer cells pointing at both transcription factors as targets for CRC treatment (Xie et al., 2019). Interestingly, PGF2α through FP receptors can upregulate the expression of CYR61 mRNA (Xu et al., 2009). We also found a significant positive correlation of COX2, DUSP10 with CYR61 in colon carcinoma cell lines (Jimenez-Martinez et al., 2019a).

Caldesmon (CaD), a major actin-associated protein, is found in smooth muscle and non-muscle cells with contractile function. In normal cells, CaD regulates contraction through the binding to two of the components of the thin filaments, actin, and tropomyosin, in the presence of Ca^{2+} (Wang and Coluccio, 2010). CaD expression was reported in both adenomas and adenocarcinomas (Porter et al., 1993). There are two alternatively spliced isoforms of CaD, high (h-CaD) and low (l-CaD), which differ in their molecular weight. H-CaD levels are lower in the late stages of colorectal adenocarcinoma while the low-molecular weight variant expression varies during tumor development and it is related to metastasis (Köhler, 2011; Kim et al., 2012). CaD is absent in the stroma in CRC but present in smooth muscle, thus it is a marker for desmoplasia and tumor invasiveness of the large intestine's muscularis propria (Roberts et al., 2014). It has been also described that CaD mRNA levels are higher in colon carcinoma cell lines than in non-transformed normal colon epithelial cells (Nimmrich et al., 2000).

However, little is known about the role of the protein in the carcinogenic process. CaD is an important regulator of podosome formation which is involved in the degradation of the extracellular matrix (ECM), promoting in this way cell invasion, that is required for metastasis (Yoshio et al., 2007). L-CaD overexpression promotes expression of the regulatory p21 and c-PARP and suppresses NF-κB and phosphorylated mTOR expression, whereas its suppression increases sensitivity to chemo- and radiotherapy in colon carcinoma lines (Kim et al., 2012). TGF-β1 regulates the expression and phosphorylation of CaD in epithelial cells modulating epithelial-mesenchymal (EMT) transition (Nalluri et al., 2018).

COX-2 AND THE p53 PATHWAY

The role of p53 in cancer in general and CRC in particular is well known [reviewed in (De Moraes et al., 2007; Watson and Collins, 2011)]. Mutations in the tumor suppressor gene TP53, which encodes the protein p53, are frequently found in human cancers. Mutations in K-ras, adenomatous polyposis coli (APC), and p53 induce the transition from healthy colonic epithelia to CRC (Dixon et al., 2013).

Among the genes induced by COX-2, we found in our arrays many genes of the p53 pathway, as it was found for the TGF pathway, that were also later confirmed by PCR (**Table 2**). Among those are TGFα, as well as others involved in cell cycle, apoptosis, DNA repair, and angiogenesis that my explain some of the diverse pro-tumoral activities of COX-2 in CRC. Again some of them were also induced by exogenous addition of PGE_2 but in lesser extent, further suggesting that other prostaglandins, likely $PGF_{2\alpha}$, may be involved.

In addition, there is a well-known crosstalk between p53 and COX-2, in which COX-2 decreases p53 transcription, and p53 also regulates COX-2 expression (De Moraes et al., 2007). Furthermore, many COX-2 inducers, such as hypoxia, cytokines, oncogene activation, carcinogens, and inflammation, can also activate p53. The role of p53 in the regulation of COX-2 expression and activity has been extensively described. Thus, it has been reported the increase in COX-2 transcript in human tumor cells expressing p53 (Han et al., 2002; De Moraes et al., 2007). COX-2 upregulation by p53 has been attributed to an increase on the binding of NF-κB to COX-2 promoter in response to p53 overexpression (Benoit et al., 2006). Conversely, COX-2 avoids the transcriptional activity of p53 on target genes (De Moraes et al., 2007). COX-2 is not only regulating p53 function but also its subcellular localization, as it was demonstrated in human colon cell lines treated with celecoxib, a COX-2 inhibitor, in which p53 nuclear location was promoted (Swamy et al., 2003). Interestingly, joint nuclear expression of COX-2 and p53 was significantly associated with adenoma recurrence in CRC (Brand et al., 2013).

Nonetheless there is less reported evidence of COX-2 effects on the p53 pathway. Interestingly our analyses have defined many p53 related genes that are modulated by COX-2 overexpression in colon carcinoma cells. Many of them were not described before as modulated by COX-2 and can provide clues in new therapeutic approaches.

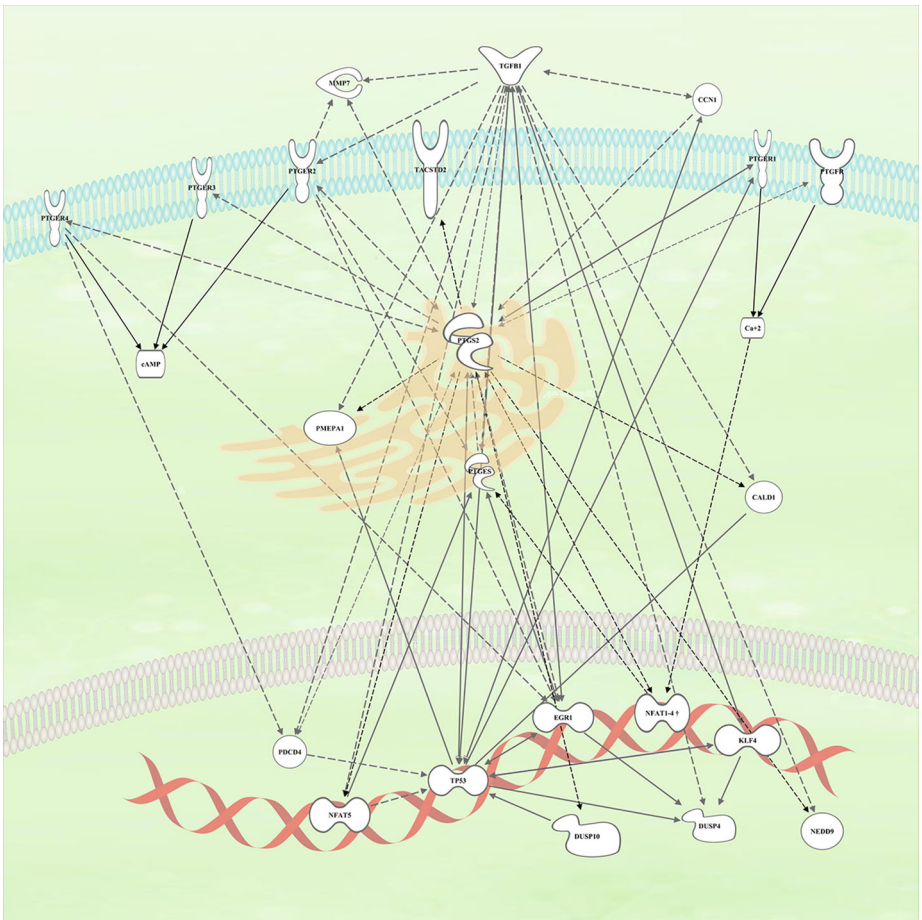


FIGURE 2 | Connection within the different genes upregulated by COX2 and likely involved in colorectal cancer (CRC) tumors. The continuous arrows are of direct interaction/activation while the discontinuous ones are indirect activation in which intermediaries participate.

CONCLUSION

COX-2 has been identified through clinical trials, epidemiological meta-analyses, and preclinical models and as a key molecule in the development of CRC. However, the use of specific COX-2 inhibitors in the clinic has been hampered by their side effects. The identification of genes regulated by COX-2 activity has allowed us to identify some genes that have been already implicated in several aspects of CRC development and therapy. The relationships among those genes and with the PG pathways are summarized in **Figure 2**.

Of all molecules described here, some of them are more amenable to drug discovery. Besides some molecules had already specific inhibitors developed, as the phosphatases DUSP10 and DUSP4, as well as monoclonal antibodies to TROP2. This, together with the clinical evidence available in CRC, pointed out to these 3 molecules as the most suitable to be considered as putative drug targets and to engage in clinical trials especially in drug resistant settings or advanced CRC stages most likely in combination therapy. Also the interaction of the TGF-β and COX2 pathway deserve further clinical exploration. In summary, drugs targeting the COX-2 downstream molecules described will likely lack the unwanted side effects of COX-2 pharmacological inhibitors, providing alternative approaches in colon cancer.

AUTHOR CONTRIBUTIONS

Writing—review and editing: MF, AH-E, KS, MJ-M, and RL-P. Review of published literature: MF, AH-E, KS, MJ-M, and RL-P. Analysis of the data in figures and tables: KS.

FUNDING

This research was funded by grants from "Ministerio de Ciencia e Innovación" (SAF2013-42850-R and SAF2016-75988-R) "Comunidad de Madrid (S2017/BMD-3671. INFLAMUNE-CM), Fondo de Investigaciones Sanitarias" (BIOIMID) to MF and Institutional grants from "Fundación Ramón Areces" and "Banco de Santander". KS was the recipient of a Spanish Association Against Cancer Oncology Investigator grant (AECC AIO).

REFERENCES

Agundez, J. A., Blanca, M., Cornejo-Garcia, J. A., and Garcia-Martin, E. (2015). Pharmacogenomics of cyclooxygenases. *Pharmacogenomics* 16, 501–522. doi: 10.2217/pgs.15.6

Alfranca, A., Iniguez, M. A., Fresno, M., and Redondo, J. M. (2006). Prostanoid signal transduction and gene expression in the endothelium: role in cardiovascular diseases. *Cardiovasc. Res.* 70, 446–456. doi: 10.1016/j.cardiores.2005.12.020

Arends, J. W. (2000). Molecular interactions in the Vogelstein model of colorectal carcinoma. *J. Pathol.* 190, 412–416. doi: 10.1002/(SICI)1096-9896(200003)190:4<412::AID-PATH533>3.0.CO;2-P

Backlund, M. G., Mann, J. R., Holla, V. R., Buchanan, F. G., Tai, H. H., Musiek, E. S., et al. (2005). 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. *J. Biol. Chem.* 280, 3217–3223. doi: 10.1074/jbc.M411221200

Bengi, G., Keles, D., Topalak, Ö., Yalçin, M., Kiyak, R., and Oktay, G. (2015). Expressions of TIMP-1, COX-2 and MMP-7 in Colon Polyp and Colon Cancer. *Euroasian J. Hepato-gastroenterol.* 5, 74–79. doi: 10.5005/jp-journals-10018-1138

Benoit, V., De Moraes, E., Dar, N. A., Taranchon, E., Bours, V., Hautefeuille, A., et al. (2006). Transcriptional activation of cyclooxygenase-2 by tumor suppressor p53 requires nuclear factor-kappaB. *Oncogene* 25, 5708–5718. doi: 10.1038/sj.onc.1209579

Bertagnolli, M. M. (2007). Chemoprevention of colorectal cancer with cyclooxygenase-2 inhibitors: two steps forward, one step back. *Lancet Oncol.* 8, 439–443. doi: 10.1016/S1470-2045(07)70139-0

Bi, Z., Dong, L.-D., and Gu, X.-M. (2013). Clinical significance of MMP-7 and PTEN expression in colorectal cancer. *Hepato-gastroenterology* 60, 32–36. doi: 10.5754/hge12537

Brand, L., Munding, J., Pox, C. P., Ziebarth, W., Reiser, M., Hüppe, D., et al. (2013). ß-Catenin, Cox-2 and p53 immunostaining in colorectal adenomas to predict recurrence after endoscopic polypectomy. *Int. J. Colorectal Dis.* 28, 1091–1098. doi: 10.1007/s00384-013-1667-z

Breyer, R. M., Bagdassarian, C. K., Myers, S. A., and Breyer, M. D. (2001). Prostanoid receptors: subtypes and signaling. *Annu. Rev. Pharmacol. Toxicol.* 41, 661–690. doi: 10.1146/annurev.pharmtox.41.1.661

Brunschwig, E. B., Wilson, K., Mack, D., Dawson, D., Lawrence, E., Willson, J. K., et al. (2003). PMEPA1, a transforming growth factor-beta-induced marker of terminal colonocyte differentiation whose expression is maintained in primary and metastatic colon cancer. *Cancer Res.* 63, 1568–1575.

Cassano, G., Gasparre, G., Susca, F., Lippe, C., and Guanti, G. (2000). Lack of effect by prostaglandin F2alpha on the proliferation of the HCT-8 and HT-29 human adenocarcinoma cell lines. *Oncol. Rep.* 7, 183–186. doi: 10.3892/or.7.1.183

Cathcart, M. C., Lysaght, J., and Pidgeon, G. P. (2011). Eicosanoid signalling pathways in the development and progression of colorectal cancer: novel approaches for prevention/intervention. *Cancer Metastasis Rev.* 30, 363–385. doi: 10.1007/s10555-011-9324-x

Cathcart, M.-C., O'byrne, K. J., Reynolds, J. V., O'sullivan, J., and Pidgeon, G. P. (2012). COX-derived prostanoid pathways in gastrointestinal cancer development and progression: Novel targets for prevention and intervention. *Biochim. Biophys. Acta (BBA) - Rev. Cancer* 1825, 49–63. doi: 10.1016/j.bbcan.2011.09.004

Chang, K. H., Miller, N., Kheirelseid, E. A., Ingoldsby, H., Hennessy, E., Curran, C. E., et al. (2011). MicroRNA-21 and PDCD4 expression in colorectal cancer. *Eur. J. Surg. Oncol.* 37, 597–603. doi: 10.1016/j.ejso.2011.04.001

Chang, C. C., Lin, B. R., Wu, T. S., Jeng, Y. M., and Kuo, M. L. (2014). Input of microenvironmental regulation on colorectal cancer: role of the CCN family. *World J. Gastroenterol.* 20, 6826–6831. doi: 10.3748/wjg.v20.i22.6826

Charames, G. S., and Bapat, B. (2006). Cyclooxygenase-2 knockdown by RNA interference in colon cancer. *Int. J. Oncol.* 28, 543–549. doi: 10.3892/ijo.28.2.543

Chen, Z. Y., and Tseng, C. C. (2005). 15-deoxy-Delta12,14 prostaglandin J2 up-regulates Kruppel-like factor 4 expression independently of peroxisome proliferator-activated receptor gamma by activating the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase signal transduction pathway in HT-29 colon cancer cells. *Mol. Pharmacol.* 68, 1203–1213. doi: 10.1124/mol.105.014944

Chen, W. S., Wei, S. J., Liu, J. M., Hsiao, M., Kou-Lin, J., and Yang, W. K. (2001). Tumor invasiveness and liver metastasis of colon cancer cells correlated with cyclooxygenase-2 (COX-2) expression and inhibited by a COX-2-selective inhibitor, etodolac. *Int. J. Cancer* 91, 894–899. doi: 10.1002/1097-0215(200102)9999:9999<894::AID-IJC1146>3.0.CO;2-#

Choi, S. H., Kim, B. G., Robinson, J., Fink, S., Yan, M., Sporn, M. B., et al. (2014). Synthetic triterpenoid induces 15-PGDH expression and suppresses inflammation-driven colon carcinogenesis. *J. Clin. Invest.* 124, 2472–2482. doi: 10.1172/JCI69672

Citterio, C., Menacho-Márquez, M., García-Escudero, R., Larive, R. M., Barreiro, O., Sánchez-Madrid, F., et al. (2012). The rho exchange factors vav2 and vav3 control a lung metastasis-specific transcriptional program in breast cancer cells. *Sci. Signaling* 5, ra71–ra71. doi: 10.1126/scisignal.2002962

Collins, D., Hogan, A. M., Skelly, M. M., Baird, A. W., and Winter, D. C. (2009). Cyclic AMP-mediated chloride secretion is induced by prostaglandin F2alpha in human isolated colon. *Br. J. Pharmacol.* 158, 1771–1776. doi: 10.1111/j.1476-5381.2009.00464.x

Corral, R. S., Iniguez, M. A., Duque, J., Lopez-Perez, R., and Fresno, M. (2007). Bombesin induces cyclooxygenase-2 expression through the activation of the nuclear factor of activated T cells and enhances cell migration in Caco-2 colon carcinoma cells. *Oncogene* 26, 958–969. doi: 10.1038/sj.onc.1209856

Cox, D. G., Pontes, C., Guino, E., Navarro, M., Osorio, A., Canzian, F., et al. (2004). Polymorphisms in prostaglandin synthase 2/cyclooxygenase 2 (PTGS2/COX2) and risk of colorectal cancer. *Br. J. Cancer* 91, 339–343. doi: 10.1038/sj.bjc.6601906

Cui, X., Shen, K., Xie, Z., Liu, T., and Zhang, H. (2017). Identification of key genes in colorectal cancer using random walk with restart. *Mol. Med. Rep.* 15, 867–872. doi: 10.3892/mmr.2016.6058

De Moraes, E., Dar, N. A., De Moura Gallo, C. V., and Hainaut, P. (2007). Cross-talks between cyclooxygenase-2 and tumor suppressor protein p53: Balancing life and death during inflammatory stress and carcinogenesis. *Int. J. Cancer* 121, 929–937. doi: 10.1002/ijc.22899

Dixon, D. A., Blanco, F. F., Bruno, A., and Patrignani, P. (2013). Mechanistic aspects of COX-2 expression in colorectal neoplasia. *Recent Results Cancer Res.* 191, 7–37. doi: 10.1007/978-3-642-30331-9_2

Doherty, G. A., Byrne, S. M., Austin, S. C., Scully, G. M., Sadlier, D. M., Neilan, T. G., et al. (2009). Regulation of the apoptosis-inducing kinase DRAK2 by cyclooxygenase-2 in colorectal cancer. *Br. J. Cancer* 101, 483–491. doi: 10.1038/sj.bjc.6605144

Duan, X., Gao, Y., Yang, H., Feng, T., Jin, T., Long, Y., et al. (2015). Polymorphisms in the DUSP10 gene are associated with sex-specific colorectal cancer risk in a Han population. *Int. J. Clin. Exp. Pathol.* 8, 2018–2025.

Duque, J., Fresno, M., and Iniguez, M. A. (2005). Expression and function of the nuclear factor of activated T cells in colon carcinoma cells: involvement in the regulation of cyclooxygenase-2. *J. Biol. Chem.* 280, 8686–8693. doi: 10.1074/jbc.M413076200

Ekambaram, P., Lambiv, W., Cazzolli, R., Ashton, A. W., and Honn, K. V. (2011). The thromboxane synthase and receptor signaling pathway in cancer: an emerging paradigm in cancer progression and metastasis. *Cancer Metastasis Rev.* 30, 397–408. doi: 10.1007/s10555-011-9297-9

Fang, Y. J., Lu, Z. H., Wang, G. Q., Pan, Z. Z., Zhou, Z. W., Yun, J. P., et al. (2009). Elevated expressions of MMP7, TROP2, and survivin are associated with survival, disease recurrence, and liver metastasis of colon cancer. *Int. J. Colorectal Dis.* 24, 875–884. doi: 10.1007/s00384-009-0725-z

Fearon, E. R., and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* 61, 759–767. doi: 10.1016/0092-8674(90)90186-I

Fitzgerald, G. A., and Patrono, C. (2001). The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl. J. Med.* 345, 433–442. doi: 10.1056/NEJM200108093450607

Fujino, H., and Regan, J. W. (2003). Prostaglandin F(2alpha) stimulation of cyclooxygenase-2 promoter activity by the FP(B) prostanoid receptor. *Eur. J. Pharmacol.* 465, 39–41. doi: 10.1016/S0014-2999(03)01485-7

Garavito, R. M., Malkowski, M. G., and Dewitt, D. L. (2002). The structures of prostaglandin endoperoxide H synthases-1 and -2. *Prostaglandins Other Lipid Mediat.* 68-69, 129–152. doi: 10.1016/S0090-6980(02)00026-6

Ghaleb, A. M., and Yang, V. W. (2017). Krüppel-like factor 4 (KLF4): What we currently know. *Gene* 611, 27–37. doi: 10.1016/j.gene.2017.02.025

Globocan (2018). https://gco.iarc.fr/today/data/factsheets/cancers/10_8_9-Colorectum-fact-sheet.pdf.

Greenhough, A., Smartt, H. J., Moore, A. E., Roberts, H. R., Williams, A. C., Paraskeva, C., et al. (2009). The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis* 30, 377–386. doi: 10.1093/carcin/bgp014

Gröschl, B., Bettstetter, M., Giedl, C., Woenckhaus, M., Edmonston, T., Hofstädter, F., et al. (2013). Expression of the MAP kinase phosphatase DUSP4 is associated with microsatellite instability in colorectal cancer (CRC) and causes increased cell proliferation. *Int. J. Cancer* 132, 1537–1546. doi: 10.1002/ijc.27834

Guo, K., and Jin, F. (2015). NFAT5 promotes proliferation and migration of lung adenocarcinoma cells in part through regulating AQP5 expression. *Biochem. Biophys. Res. Commun.* 465, 644–649. doi: 10.1016/j.bbrc.2015.08.078

Gustafsson, A., Hansson, E., Kressner, U., Nordgren, S., Andersson, M., Lonnroth, C., et al. (2007). Prostanoid receptor expression in colorectal cancer related to tumor stage, differentiation and progression. *Acta Oncol.* 46, 1107–1112. doi: 10.1080/02841860701403061

Han, J. A., Kim, J. I., Ongusaha, P. P., Hwang, D. H., Ballou, L. R., Mahale, A., et al. (2002). P53-mediated induction of Cox-2 counteracts p53- or genotoxic stress-induced apoptosis. *EMBO J.* 21, 5635–5644. doi: 10.1093/emboj/cdf591

Han, S., Bui, N. T., Ho, M. T., Kim, Y. M., Cho, M., and Shin, D. B. (2016). Dexamethasone Inhibits TGF-β1-Induced Cell Migration by Regulating the ERK and AKT Pathways in Human Colon Cancer Cells Via CYR61. *Cancer Res. Treat* 48, 1141–1153. doi: 10.4143/crt.2015.209

Hara, S., Kamei, D., Sasaki, Y., Tanemoto, A., Nakatani, Y., and Murakami, M. (2010). Prostaglandin E synthases: Understanding their pathophysiological roles through mouse genetic models. *Biochimie* 92, 651–659. doi: 10.1016/j.biochi.2010.02.007

Hennequart, M., Pilotte, L., Cane, S., Hoffmann, D., Stroobant, V., Plaen, E., et al. (2017). Constitutive IDO1 Expression in Human Tumors Is Driven by Cyclooxygenase-2 and Mediates Intrinsic Immune Resistance. *Cancer Immunol. Res.* 5, 695–709. doi: 10.1158/2326-6066.CIR-16-0400

Hommo, T., Pesu, M., Moilanen, E., and Korhonen, R. (2015). Regulation of Inflammatory Cytokine Production by MKP-5 in Macrophages. *Basic Clin. Pharmacol. Toxicol.* 117, 96–104. doi: 10.1111/bcpt.12380

Horiuchi, A., Iinuma, H., Akahane, T., Shimada, R., and Watanabe, T. (2012). Prognostic significance of PDCD4 expression and association with microRNA-21 in each Dukes' stage of colorectal cancer patients. *Oncol. Rep.* 27, 1384–1392. doi: 10.3892/or.2012.1648

Hu, R., Zuo, Y., Zuo, L., Liu, C., Zhang, S., Wu, Q., et al. (2011). KLF4 Expression Correlates with the Degree of Differentiation in Colorectal Cancer. *Gut liver* 5, 154–159. doi: 10.5009/gnl.2011.5.2.154

Humphries, H. N., Wickremesekera, S. K., Marsh, R. W., Brasch, H. D., Mehrotra, S., Tan, S. T., et al. (2018). Characterization of Cancer Stem Cells in Colon Adenocarcinoma Metastasis to the Liver. *Front. Surg.* 4, 76–76. doi: 10.3389/fsurg.2017.00076

Ichimanda, M., Hijiya, N., Tsukamoto, Y., Uchida, T., Nakada, C., Akagi, T., et al. (2018). Downregulation of dual-specificity phosphatase 4 enhances cell proliferation and invasiveness in colorectal carcinomas. *Cancer Sci.* 109, 250–258. doi: 10.1111/cas.13444

Imai, K., and Yamamoto, H. (2008). Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. *Carcinogenesis* 29, 673–680. doi: 10.1093/carcin/bgm228

Iniguez, M. A., Rodriguez, A., Volpert, O. V., Fresno, M., and Redondo, J. M. (2003). Cyclooxygenase-2: a therapeutic target in angiogenesis. *Trends Mol. Med.* 9, 73–78. doi: 10.1016/S1471-4914(02)00011-4

Iniguez, M. A., Cacheiro-Llaguno, C., Cuesta, N., Diaz-Munoz, M. D., and Fresno, M. (2008). Prostanoid function and cardiovascular disease. *Arch. Physiol. Biochem.* 114, 201–209. doi: 10.1080/13813450802180882

Jacoby, R. F., Seibert, K., Cole, C. E., Kelloff, G., and Lubet, R. A. (2000). The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. *Cancer Res.* 60, 5040–5044.

Jauliac, S., López-Rodriguez, C., Shaw, L. M., Brown, L. F., Rao, A., and Toker, A. (2002). The role of NFAT transcription factors in integrin-mediated carcinoma invasion. *Nat. Cell Biol.* 4, 540–544. doi: 10.1038/ncb816

Jeong, D., Heo, S., Sung Ahn, T., Lee, S., Park, S., Kim, H., et al. (2014). Cyr61 expression is associated with prognosis in patients with colorectal cancer. *BMC Cancer* 14, 164–164. doi: 10.1186/1471-2407-14-164

Jimenez-Martinez, M., Ostale, C. M., Van Der Burg, L. R., Galan-Martinez, J., Hardwick, J. C. H., Lopez-Perez, R., et al. (2019a). DUSP10 Is a Regulator of YAP1 Activity Promoting Cell Proliferation and Colorectal Cancer Progression. *Cancers (Basel)* 11 (11), 1767. doi: 10.3390/cancers11111767

Jimenez-Martinez, M., Stamatakis, K., and Fresno, M. (2019b). The Dual-Specificity Phosphatase 10 (DUSP10): Its Role in Cancer, Inflammation, and Immunity. *Int. J. Mol. Sci.* 20 (7), 1626. doi: 10.3390/ijms20071626

Jimenez-Segovia, A., Mota, A., Rojo-Sebastian, A., Barrocal, B., Rynne-Vidal, A., Garcia-Bermejo, M. L., et al. (2019). Prostaglandin F2alpha-induced Prostate Transmembrane Protein, Androgen Induced 1 mediates ovarian cancer progression increasing epithelial plasticity. *Neoplasia* 21, 1073–1084. doi: 10.1016/j.neo.2019.10.001

Kamei, D., Murakami, M., Nakatani, Y., Ishikawa, Y., Ishii, T., and Kudo, I. (2003). Potential role of microsomal prostaglandin E synthase-1 in tumorigenesis. *J. Biol. Chem.* 278, 19396–19405. doi: 10.1074/jbc.M213290200

Kang, X., Li, M., Zhu, H., Lu, X., Miao, J., Du, S., et al. (2017). DUSP4 promotes doxorubicin resistance in gastric cancer through epithelial-mesenchymal transition. *Oncotarget* 8, 94028–94039. doi: 10.18632/oncotarget.21522

Kapoor, S. (2013). TROP2 expression and its evolving role in tumor pathogenesis in systemic tumors. *Tumour. Biol.* 34, 1967–1968. doi: 10.1007/s13277-012-0586-x

Karpisheh, V., Nikkhoo, A., Hojjat-Farsangi, M., Namdar, A., Azizi, G., Ghalamfarsa, G., et al. (2019). Prostaglandin E2 as a potent therapeutic target for treatment of colon cancer. *Prostaglandins Other Lipid Mediat.* 144, 106338. doi: 10.1016/j.prostaglandins.2019.106338

Kawamori, T., Kitamura, T., Watanabe, K., Uchiya, N., Maruyama, T., Narumiya, S., et al. (2005). Prostaglandin E receptor subtype EP(1) deficiency inhibits colon cancer development. *Carcinogenesis* 26, 353–357. doi: 10.1093/carcin/bgh322

Keum, N., and Giovannucci, E. (2019). Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat. Rev. Gastroenterol. Hepatol.* 16, 713–732. doi: 10.1038/s41575-019-0189-8

Kim, K.-H., Yeo, S.-G., Kim, W. K., Kim, D. Y., Yeo, H. Y., Hong, J. P., et al. (2012). Up-regulated expression of l-caldesmon associated with malignancy of colorectal cancer. *BMC Cancer* 12, 601–601. doi: 10.1186/1471-2407-12-601

Köhler, C. (2011). Histochemical localization of caldesmon isoforms in colon adenocarcinoma and lymph node metastases. *Virchows Archiv.: an Int. J. Pathol.* 459, 81–89. doi: 10.1007/s00428-011-1091-0

Komoto, J., Yamada, T., Watanabe, K., Woodward, D. F., and Takusagawa, F. (2006). Prostaglandin F2alpha formation from prostaglandin H2 by prostaglandin F synthase (PGFS): crystal structure of PGFS containing bimatoprost. *Biochemistry* 45, 1987–1996. doi: 10.1021/bi051861t

Kuipers, E. J., Grady, W. M., Lieberman, D., Seufferlein, T., Sung, J. J., Boelens, P. G., et al. (2015). Colorectal cancer. *Nat. Rev. Dis. Primers* 1, 15065. doi: 10.1038/nrdp.2015.65

Lee, T. K., Murthy, S. R. K., Cawley, N. X., Dhanvantari, S., Hewitt, S. M., Lou, H., et al. (2011). An N-terminal truncated carboxypeptidase E splice isoform induces tumor growth and is a biomarker for predicting future metastasis in human cancers. *J. Clin. Invest.* 121, 880–892. doi: 10.1172/JCI40433

Lee, H.-Y., Ahn, J. B., Rha, S. Y., Chung, H. C., Park, K. H., Kim, T. S., et al. (2014). High KLF4 level in normal tissue predicts poor survival in colorectal cancer patients. *World J. Surg. Oncol.* 12, 232–232. doi: 10.1186/1477-7819-12-232

Leng, Z., Tao, K., Xia, Q., Tan, J., Yue, Z., Chen, J., et al. (2013). Krüppel-like factor 4 acts as an oncogene in colon cancer stem cell-enriched spheroid cells. *PloS One* 8, e56082. doi: 10.1371/journal.pone.0056082

Li, Y., Bavarva, J. H., Wang, Z., Guo, J., Qian, C., Thibodeau, S. N., et al. (2011). HEF1, a novel target of Wnt signaling, promotes colonic cell migration and cancer progression. *Oncogene* 30, 2633–2643. doi: 10.1038/onc.2010.632

Long, J., Yin, Y., Guo, H., Li, S., Sun, Y., Zeng, C., et al. (2019). The mechanisms and clinical significance of PDCD4 in colorectal cancer. *Gene* 680, 59–64. doi: 10.1016/j.gene.2018.09.034

Low, H. B., and Zhang, Y. (2016). Regulatory Roles of MAPK Phosphatases in Cancer. *Immune Netw.* 16, 85–98. doi: 10.4110/in.2016.16.2.85

Lu, Y., Shi, C., Qiu, S., and Fan, Z. (2016). Identification and validation of COX-2 as a co-target for overcoming cetuximab resistance in colorectal cancer cells. *Oncotarget* 7, 64766–64777. doi: 10.18632/oncotarget.8649

Ma, X., Aoki, T., Tsuruyama, T., and Narumiya, S. (2015). Definition of Prostaglandin E2-EP2 Signals in the Colon Tumor Microenvironment That Amplify Inflammation and Tumor Growth. *Cancer Res.* 75, 2822–2832. doi: 10.1158/0008-5472.CAN-15-0125

Ma, H., Brosens, L. A. A., Offerhaus, G. J. A., Giardiello, F. M., De Leng, W. W. J., and Montgomery, E. A. (2018). Pathology and genetics of hereditary colorectal cancer. *Pathology* 50, 49–59. doi: 10.1016/j.pathol.2017.09.004

Mcdougall, A. R. A., Tolcos, M., Hooper, S. B., Cole, T. J., and Wallace, M. J. (2015). Trop2: from development to disease. *Dev. Dynamics* 244, 99–109. doi: 10.1002/dvdy.24242

Moran, A., Ortega, P., De Juan, C., Fernandez-Marcelo, T., Frias, C., Sanchez-Pernaute, A., et al. (2010). Differential colorectal carcinogenesis: Molecular basis and clinical relevance. *World J. Gastrointest Oncol.* 2, 151–158. doi: 10.4251/wjgo.v2.i3.151

Mutoh, M., Watanabe, K., Kitamura, T., Shoji, Y., Takahashi, M., Kawamori, T., et al. (2002). Involvement of prostaglandin E receptor subtype EP(4) in colon carcinogenesis. *Cancer Res.* 62, 28–32.

Myung, S. J., Rerko, R. M., Yan, M., Platzer, P., Guda, K., Dotson, A., et al. (2006). 15-Hydroxyprostaglandin dehydrogenase is an *in vivo* suppressor of colon tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A* 103, 12098–12102. doi: 10.1073/pnas.0603235103

Nalluri, S. M., O'connor, J. W., Virgi, G. A., Stewart, S. E., Ye, D., and Gomez, E. W. (2018). TGFbeta1-induced expression of caldesmon mediates epithelial-mesenchymal transition. *Cytoskeleton (Hoboken)* 75, 201–212. doi: 10.1002/cm.21437

Nimmrich, I., Erdmann, S., Melchers, U., Finke, U., Hentsch, S., Moyer, M. P., et al. (2000). Seven genes that are differentially transcribed in colorectal tumor cell lines. *Cancer Lett.* 160, 37–43. doi: 10.1016/S0304-3835(00)00553-X

Nugent, K. P., Spigelman, A. D., and Phillips, R. K. (1996). Tissue prostaglandin levels in familial adenomatous polyposis patients treated with sulindac. *Dis. Colon Rectum* 39, 659–662. doi: 10.1007/BF02056946

O'mahony, C. A., Beauchamp, R. D., Albo, D., Tsujii, M., Sheng, H. M., Shao, J., et al. (1999). Cyclooxygenase-2 alters transforming growth factor-beta 1 response during intestinal tumorigenesis. *Surgery* 126, 364–370. doi: 10.1016/S0039-6060(99)70178-9

O'neill, G. M., Seo, S., Serebriiskii, I. G., Lessin, S. R., and Golemis, E. A. (2007). A new central scaffold for metastasis: parsing HEF1/Cas-L/NEDD9. *Cancer Res.* 67, 8975–8979. doi: 10.1158/0008-5472.CAN-07-1328

Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., et al. (1996). Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 87, 803–809. doi: 10.1016/S0092-8674(00)81988-1

Pai, R., Soreghan, B., Szabo, I. L., Pavelka, M., Baatar, D., and Tarnawski, A. S. (2002). Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat. Med.* 8, 289–293. doi: 10.1038/nm0302-289

Park, H., Jeon, T., Chien, P., Park, S., Oh, S., Kim, S., et al. (2014). Discovery of Novel DUSP4 Inhibitors through the Virtual Screening with Docking Simulations. *Bull. Korean Chem. Soc.* 35, 2655–2659. doi: 10.5012/bkcs.2014.35.9.2655

Park, S.-M., Hwang, C. Y., Cho, S.-H., Lee, D., Gong, J.-R., Lee, S., et al. (2019). Systems analysis identifies potential target genes to overcome cetuximab resistance in colorectal cancer cells. *FEBS J.* 286, 1305–1318. doi: 10.1111/febs.14773

Patrignani, P., and Patrono, C. (2016). Aspirin and Cancer. *J. Am. Coll. Cardiol.* 68, 967–976. doi: 10.1016/j.jacc.2016.05.083

Peacock, O., Lee, A. C., Cameron, F., Tarbox, R., Vafadar-Isfahani, N., Tufarelli, C., et al. (2014). Inflammation and MiR-21 pathways functionally interact to downregulate PDCD4 in colorectal cancer. *PloS One* 9, e110267. doi: 10.1371/journal.pone.0110267

Pfeffer, S. R., Yang, C. H., and Pfeffer, L. M. (2015). The Role of miR-21 in Cancer. *Drug Dev. Res.* 76, 270–277. doi: 10.1002/ddr.21257

Piazuelo, E., and Lanas, A. (2015). NSAIDS and gastrointestinal cancer. *Prostaglandins Lipid Mediat.* 120, 91–96. doi: 10.1016/j.prostaglandins.2015.06.001

Porter, R. M., Holme, T. C., Newman, E. L., Hopwood, D., Wilkinson, J. M., and Cuschieri, A. (1993). Monoclonal antibodies to cytoskeletal proteins: an immunohistochemical investigation of human colon cancer. *J. Pathol.* 170, 435–440. doi: 10.1002/path.1711700406

Pugh, S., and Thomas, G. A. (1994). Patients with adenomatous polyps and carcinomas have increased colonic mucosal prostaglandin E2. *Gut* 35, 675–678. doi: 10.1136/gut.35.5.675

Qin, J. J., Nag, S., Wang, W., Zhou, J., Zhang, W. D., Wang, H., et al. (2014). NFAT as cancer target: mission possible? *Biochim. Biophys. Acta* 1846, 297–311. doi: 10.1016/j.bbcan.2014.07.009

Qualtrough, D., Kaidi, A., Chell, S., Jabbour, H. N., Williams, A. C., and Paraskeva, C. (2007). Prostaglandin F(2alpha) stimulates motility and invasion in colorectal tumor cells. *Int. J. Cancer* 121, 734–740. doi: 10.1002/ijc.22755

Rae, F. K., Hooper, J. D., Nicol, D. L., and Clements, J. A. (2001). Characterization of a novel gene, STAG1/PMEPA1, upregulated in renal cell carcinoma and other solid tumors. *Mol. Carcinog* 32, 44–53. doi: 10.1002/mc.1063

Regan, J. W. (2003). EP2 and EP4 prostanoid receptor signaling. *Life Sci.* 74, 143–153. doi: 10.1016/j.lfs.2003.09.031

Reichling, T., Goss, K. H., Carson, D. J., Holdcraft, R. W., Ley-Ebert, C., Witte, D., et al. (2005). Transcriptional profiles of intestinal tumors in Apc(Min) mice are unique from those of embryonic intestine and identify novel gene targets dysregulated in human colorectal tumors. *Cancer Res.* 65, 166–176.

Rizzo, M. T. (2011). Cyclooxygenase-2 in oncogenesis. *Clin. Chim Acta* 412, 671–687. doi: 10.1016/j.cca.2010.12.026

Roberts, J. A., Waters, L., Ro, J. Y., and Zhai, Q. J. (2014). Smoothelin and caldesmon are reliable markers for distinguishing muscularis propria from desmoplasia: a critical distinction for accurate staging colorectal adenocarcinoma. *Int. J. Clin. Exp. Pathol.* 7, 792–796.

Rothwell, P. M. (2013). Aspirin in prevention of sporadic colorectal cancer: current clinical evidence and overall balance of risks and benefits. *Recent Results Cancer Res.* 191, 121–142. doi: 10.1007/978-3-642-30331-9_7

Saigusa, S., Inoue, Y., Tanaka, K., Toiyama, Y., Okugawa, Y., Shimura, T., et al. (2013). Decreased expression of DUSP4 is associated with liver and lung metastases in colorectal cancer. *Med. Oncol. (Northwood London Eng.)* 30, 620–620. doi: 10.1007/s12032-013-0620-x

Sancho, E., Batlle, E., and Clevers, H. (2004). Signaling pathways in intestinal development and cancer. *Annu. Rev. Cell Dev. Biol.* 20, 695–723. doi: 10.1146/annurev.cellbio.20.010403.092805

Sasaki, Y., Kamei, D., Ishikawa, Y., Ishii, T., Uematsu, S., Akira, S., et al. (2012). Microsomal prostaglandin E synthase-1 is involved in multiple steps of colon carcinogenesis. *Oncogene* 31, 2943–2952. doi: 10.1038/onc.2011.472

Sasaki, Y., Nakatani, Y., and Hara, S. (2015). Role of microsomal prostaglandin E synthase-1 (mPGES-1)-derived prostaglandin E2 in colon carcinogenesis. *Prostaglandins Other Lipid Mediat.* 121, 42–45. doi: 10.1016/j.prostaglandins.2015.06.006

Seo, T., Tatsuguchi, A., Shinji, S., Yonezawa, M., Mitsui, K., Tanaka, S., et al. (2009). Microsomal prostaglandin E synthase protein levels correlate with prognosis in colorectal cancer patients. *Virchows Arch.* 454, 667–676. doi: 10.1007/s00428-009-0777-z

Seternes, O. M., Kidger, A. M., and Keyse, S. M. (2019). Dual-specificity MAP kinase phosphatases in health and disease. *Biochim. Biophys. Acta Mol. Cell Res.* 1866, 124–143. doi: 10.1016/j.bbamcr.2018.09.002

Shao, J., Yang, V. W., and Sheng, H. (2008). Prostaglandin E2 and Krüppel-like transcription factors synergistically induce the expression of decay-accelerating factor in intestinal epithelial cells. *Immunology* 125, 397–407. doi: 10.1111/j.1365-2567.2008.02847.x

Shvartsur, A., and Bonavida, B. (2015). Trop2 and its overexpression in cancers: regulation and clinical/therapeutic implications. *Genes Cancer* 6, 84–105. doi: 10.18632/genesandcancer.40

Singha, P. K., Yeh, I. T., Venkatachalam, M. A., and Saikumar, P. (2010). Transforming growth factor-beta (TGF-beta)-inducible gene TMEPAI converts TGF-beta from a tumor suppressor to a tumor promoter in breast cancer. *Cancer Res.* 70, 6377–6383. doi: 10.1158/0008-5472.CAN-10-1180

Sinicrope, F. A. (2006). Targeting cyclooxygenase-2 for prevention and therapy of colorectal cancer. *Mol. Carcinog* 45, 447–454. doi: 10.1002/mc.20232

Slattery, M. L., Lundgreen, A., Bondurant, K. L., and Wolff, R. K. (2011). Tumor necrosis factor-related genes and colon and rectal cancer. *Int. J. Mol. Epidemiol. Genet.* 2, 328–338.

Song, Y. F., Xu, Z. B., Zhu, X. J., Tao, X., Liu, J. L., Gao, F. L., et al. (2017). Serum Cyr61 as a potential biomarker for diagnosis of colorectal cancer. *Clin. Trans. Oncol.* 19, 519–524. doi: 10.1007/s12094-016-1560-7

Sreeramkumar, V., Fresno, M., and Cuesta, N. (2012). Prostaglandin E(2) and T cells: friends or foes? *Immunol. Cell Biol.* 90, 579–586. doi: 10.1038/icb.2011.75

Stamatakis, K., Jimenez-Martinez, M., Jimenez-Segovia, A., Chico-Calero, I., Conde, E., Galan-Martinez, J., et al. (2015). Prostaglandins induce early growth response 1 transcription factor mediated microsomal prostaglandin E2 synthase up-regulation for colorectal cancer progression. *Oncotarget* 6, 39941–39959. doi: 10.18632/oncotarget.5402

Starodub, A. N., Ocean, A. J., Shah, M. A., Guarino, M. J., Picozzi, V. J.Jr., Vahdat, L. T., et al. (2015). First-in-Human Trial of a Novel Anti-Trop-2 Antibody-SN-38 Conjugate, Sacituzumab Govitecan, for the Treatment of Diverse Metastatic Solid Tumors. *Clin. Cancer Res.* 21, 3870–3878. doi: 10.1158/1078-0432.CCR-14-3321

Strillacci, A., Griffoni, C., Spisni, E., Manara, M. C., and Tomasi, V. (2006). RNA interference as a key to knockdown overexpressed cyclooxygenase-2 gene in tumour cells. *Br. J. Cancer* 94, 1300–1310. doi: 10.1038/sj.bjc.6603094

Subbaramaiah, K., and Dannenberg, A. J. (2003). Cyclooxygenase 2: a molecular target for cancer prevention and treatment. *Trends Pharmacol. Sci.* 24, 96–102. doi: 10.1016/S0165-6147(02)00043-3

Sun, Y., Tang, X. M., Half, E., Kuo, M. T., and Sinicrope, F. A. (2002). Cyclooxygenase-2 overexpression reduces apoptotic susceptibility by inhibiting the cytochrome c-dependent apoptotic pathway in human colon cancer cells. *Cancer Res.* 62, 6323–6328.

Swamy, M. V., Herzog, C. R., and Rao, C. V. (2003). Inhibition of COX-2 in colon cancer cell lines by celecoxib increases the nuclear localization of active p53. *Cancer Res.* 63, 5239–5242.

Takafuji, V., Cosme, R., Lublin, D., Lynch, K., and Roche, J. K. (2000). Prostanoid receptors in intestinal epithelium: selective expression, function, and change with inflammation. *Prostaglandins Leukot. Essent. Fatty Acids* 63, 223–235. doi: 10.1054/plef.2000.0144

Tanioka, T., Nakatani, Y., Semmyo, N., Murakami, M., and Kudo, I. (2000). Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. *J. Biol. Chem.* 275, 32775–32782. doi: 10.1074/jbc.M003504200

Thun, M. J., Jacobs, E. J., and Patrono, C. (2012). The role of aspirin in cancer prevention. *Nat. Rev. Clin. Oncol.* 9, 259–267. doi: 10.1038/nrclinonc.2011.199

Tikhmyanova, N., Little, J. L., and Golemis, E. A. (2010). CAS proteins in normal and pathological cell growth control. *Cell Mol. Life Sci.* 67, 1025–1048. doi: 10.1007/s00018-009-0213-1

Tsujii, M., and Dubois, R. N. (1995). Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 83, 493–501. doi: 10.1016/0092-8674(95)90127-2

Tsujii, M., Kawano, S., and Dubois, R. N. (1997). Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc. Natl. Acad. Sci. U. S. A.* 94, 3336–3340. doi: 10.1073/pnas.94.7.3336

Wang, C. L., and Coluccio, L. M. (2010). New insights into the regulation of the actin cytoskeleton by tropomyosin. *Int. Rev. Cell Mol. Biol.* 281, 91–128. doi: 10.1016/S1937-6448(10)81003-2

Wang, D., and Dubois, R. N. (2010). The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* 29, 781–788. doi: 10.1038/onc.2009.421

Wang, W. S., Chen, P. M., and Su, Y. (2006). Colorectal carcinoma: from tumorigenesis to treatment. *Cell. Mol. Life Sci.: CMLS* 63, 663–671. doi: 10.1007/s00018-005-5425-4

Wang, J., Day, R., Dong, Y., Weintraub, S. J., and Michel, L. (2008). Identification of Trop-2 as an oncogene and an attractive therapeutic target in colon cancers. *Mol. Cancer Ther.* 7, 280–285. doi: 10.1158/1535-7163.MCT-07-2003

Wang, Q., Zhu, J., Wang, Y. W., Dai, Y., Wang, Y. L., Wang, C., et al. (2017). Tumor suppressor Pdcd4 attenuates Sin1 translation to inhibit invasion in colon carcinoma. *Oncogene* 36, 6225–6234. doi: 10.1038/onc.2017.228

Wang, Y., Wang, W., Sanidad, K. Z., Shih, P. A., Zhao, X., and Zhang, G. (2018). Eicosanoid signaling in carcinogenesis of colorectal cancer. *Cancer Metastasis Rev.* 37, 257–267. doi: 10.1007/s10555-018-9739-8

Watanabe, Y., Itoh, S., Goto, T., Ohnishi, E., Inamitsu, M., Itoh, F., et al. (2010). TMEPAI, a transmembrane TGF-beta-inducible protein, sequesters Smad proteins from active participation in TGF-beta signaling. *Mol. Cell* 37, 123–134. doi: 10.1016/j.molcel.2009.10.028

Watson, A. J. M., and Collins, P. D. (2011). Colon cancer: a civilization disorder. *Digest. Dis. (Basel Switzerland)* 29, 222–228. doi: 10.1159/000323926

Williams, C. S., and Dubois, R. N. (1996). Prostaglandin endoperoxide synthase: why two isoforms? *Am. J. Physiol.* 270, G393–G400. doi: 10.1152/ajpgi.1996.270.3.G393

Williams, C. S., Luongo, C., Radhika, A., Zhang, T., Lamps, L. W., Nanney, L. B., et al. (1996). Elevated cyclooxygenase-2 levels in Min mouse adenomas. *Gastroenterology* 111, 1134–1140. doi: 10.1016/S0016-5085(96)70083-5

Xia, D., Holla, V. R., Wang, D., Menter, D. G., and Dubois, R. N. (2010). HEF1 is a crucial mediator of the proliferative effects of prostaglandin E(2) on colon cancer cells. *Cancer Res.* 70, 824–831. doi: 10.1158/0008-5472.CAN-09-2105

Xie, L., Song, X., Lin, H., Chen, Z., Li, Q., Guo, T., et al. (2019). Aberrant activation of CYR61 enhancers in colorectal cancer development. *J. Exp. Clin. Cancer Res.: CR* 38, 213–213. doi: 10.1186/s13046-019-1217-9

Xu, W., Chou, C.-L., Israel, D. D., Hutchinson, A. J., and Regan, J. W. (2009). PGF (2alpha) stimulates FP prostanoid receptor mediated crosstalk between Ras/Raf signaling and Tcf transcriptional activation. *Biochem. Biophys. Res. Commun.* 381, 625–629. doi: 10.1016/j.bbrc.2009.02.102

Xu, G., Zhang, M., Zhu, H., and Xu, J. (2017). A 15-gene signature for prediction of colon cancer recurrence and prognosis based on SVM. *Gene* 604, 33–40. doi: 10.1016/j.gene.2016.12.016

Yang, V. W., Shields, J. M., Hamilton, S. R., Spannhake, E. W., Hubbard, W. C., Hylind, L. M., et al. (1998). Size-dependent increase in prostanoid levels in adenomas of patients with familial adenomatous polyposis. *Cancer Res.* 58, 1750–1753.

Yoshimoto, T., Takahashi, Y., Kinoshita, T., Sakashita, T., Inoue, H., and Tanabe, T. (2002). Growth stimulation and epidermal growth factor receptor induction in cyclooxygenase-overexpressing human colon carcinoma cells. *Adv. Exp. Med. Biol.* 507, 403–407. doi: 10.1007/978-1-4615-0193-0_62

Yoshio, T., Morita, T., Kimura, Y., Tsujii, M., Hayashi, N., and Sobue, K. (2007). Caldesmon suppresses cancer cell invasion by regulating podosome/invadopodium formation. *FEBS Lett.* 581, 3777–3782. doi: 10.1016/j.febslet.2007.06.073

Zelenay, S., Van Der Veen, A. G., Bottcher, J. P., Snelgrove, K. J., Rogers, N., Acton, S. E., et al. (2015). Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. *Cell* 162, 1257–1270. doi: 10.1016/j.cell.2015.08.015

Zhang, Z., and Dubois, R. N. (2001). Detection of differentially expressed genes in human colon carcinoma cells treated with a selective COX-2 inhibitor. *Oncogene* 20, 4450–4456. doi: 10.1038/sj.onc.1204588

Zhang, L., Wang, X., Lai, C., Zhang, H., and Lai, M. (2019). PMEPA1 induces EMT *via a* non-canonical TGF-β signalling in colorectal cancer. *J. Cell. Mol. Med.* 23, 3603–3615. doi: 10.1111/jcmm.14261

Zhao, P., and Zhang, Z. (2018). TNF-alpha promotes colon cancer cell migration and invasion by upregulating TROP-2. *Oncol. Lett.* 15, 3820–3827. doi: 10.3892/ol.2018.7735

Zucker, S., and Vacirca, J. (2004). Role of matrix metalloproteinases (MMPs) in colorectal cancer. *Cancer Metastasis Rev.* 23, 101–117. doi: 10.1023/A:1025867130437

Zuo, X., and Shureiqi, I. (2013). Eicosanoid profiling in colon cancer: emergence of a pattern. *Prostaglandins Lipid Mediat.* 104-105, 139–143. doi: 10.1016/j.prostaglandins.2012.08.004

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

MINI REVIEW
published: 29 May 2020
doi: 10.3389/fphar.2020.00819

Eicosanoids in Cancer: Prostaglandin E_2 Receptor 4 in Cancer Therapeutics and Immunotherapy

Mc Millan Ching[1], *Jocelyn Reader*[2,3] *and Amy M. Fulton*[3,4,5]*

[1] Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2] Division of Gynecologic Oncology, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD, United States, [3] University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland, Baltimore, Baltimore, MD, United States, [4] Department of Pathology, University of Maryland School of Medicine, Baltimore, MD, United States, [5] Baltimore Veterans Administration Medical Center, Baltimore, MD, United States

The cyclooxygenase-2 (COX-2) enzyme is frequently overexpressed in epithelial malignancies including those of the breast, prostate, lung, kidney, ovary, and liver and elevated expression is associated with worse outcomes. COX-2 catalyzes the metabolism of arachidonic acid to prostaglandins. The COX-2 product prostaglandin E_2 (PGE_2) binds to four G-protein-coupled EP receptors designated EP1–EP4. EP4 is commonly upregulated in cancer and supports cell proliferation, migration, invasion, and metastasis through activation of multiple signaling pathways including ERK, cAMP/PKA, PI3K/AKT, and NF-κB. EP4 antagonists inhibit metastasis in preclinical models. Cancer stem cells, that underlie therapy resistance and disease relapse, are driven by the expression of EP4. Resistance to several chemotherapies is reversed in the presence of EP4 antagonists. In addition to tumor cell-autonomous roles of EP4, many EP4-positive host cells play a role in tumor behavior. Endothelial cell-EP4 supports tumor angiogenesis and lymphangiogenesis. Natural Killer (NK) cells are critical to the mechanism by which systemically administered EP4 antagonists inhibit metastasis. PGE_2 acts on EP4 expressed on the NK cell to inhibit tumor target cell killing, cytokine production, and chemotactic activity. Myeloid-derived suppressor cells (MDSCs), that inhibit the development of cytotoxic T cells, are induced by PGE_2 acting on myeloid-expressed EP2 and EP4 receptors. Inhibition of MDSC-EP4 leads to maturation of effector T cells and suppresses the induction of T regulatory cells. A number of EP4 antagonists have proven useful in dissecting these mechanisms. There is growing evidence that EP4 antagonism, particularly in combination with either chemotherapy, endocrine therapy, or immune-based therapies, should be investigated further as a promising novel approach to cancer therapy. Several EP4 antagonists have now progressed to early phase clinical trials and we eagerly await the results of those studies.

Keywords: EP4, microenvironment, prostaglandin E_2, immunotherapy, cancer

OPEN ACCESS

Edited by:
Emanuela Ricciotti, University of Pennsylvania, United States

Reviewed by:
Hiromichi Fujino, Tokushima University, Japan
Eva Maria Sturm, Medical University of Graz, Austria

***Correspondence:**
Amy M. Fulton
afulton@som.umaryland.edu

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *28 January 2020*
Accepted: *19 May 2020*
Published: *29 May 2020*

Citation:
Ching MM, Reader J and Fulton AM (2020) Eicosanoids in Cancer: Prostaglandin E_2 Receptor 4 in Cancer Therapeutics and Immunotherapy. Front. Pharmacol. 11:819. doi: 10.3389/fphar.2020.00819

EP4 IN THE MALIGNANT CELL

The cyclooxygenase-2 (COX-2) enzyme catalyzes the rate-limiting step in the metabolism of arachidonic acid to prostaglandins. COX-2 is frequently overexpressed in epithelial malignancies and is associated with poor clinical outcomes. In cancer, the most abundant COX-2 product is prostaglandin E_2 (PGE_2) which binds four G-protein-coupled EP receptors (EP1–EP4) to activate several intracellular signaling pathways. EP4 is upregulated and contributes to the pathology of many human malignancies including those of the breast, prostate, colon, ovary, and lung (Reader et al., 2011; Majumder et al., 2018; Take et al., 2020). EP4 receptor signaling is linked to many properties of malignant cells including proliferation, migration, invasion, metastasis, angiogenesis, immune evasion, epithelial to mesenchymal transition, and cancer stem cell (CSC) properties. This review will summarize some of the growing evidence that EP4 contributes to cancer progression both in a tumor cell-autonomous manner and by modulating the behavior of EP4-positive host cells in the tumor microenvironment.

The Role of EP4 in Cell Proliferation

Heightened EP4 expression supports the proliferation of some, but not all malignancies. Many mechanisms contribute to the growth-promoting properties of the PGE_2/EP4 axis. Exposure of cultured cancer cell lines to PGE_2 or specific EP4 agonists can lead to modest stimulation of cell growth; conversely, small molecule EP4 antagonists or EP4 gene targeting inhibits proliferation of some cell lines. For example, overexpression of EP4 in prostate cancer cells results in increased proliferation *in vitro* and in xenograft models (Terada et al., 2010). The pro-proliferative response is associated with the cAMP/PKA/PI3K-Akt signaling pathway (Xu et al., 2018). These findings support the continued investigation of EP4 as a potential target in castration-resistant prostate cancer. Mice transgenic for epithelial EP4 overexpression display more squamous cell carcinomas (Simper et al., 2014). An exception to the general conclusions regarding the tumor-promoting role of EP4 is in gastric carcinoma cell lines where administration of EP2 and EP4 *agonists* resulted in growth inhibition, decreased cell proliferation, and was accompanied by cAMP production. The possible role of EP antagonists was not investigated (Okuyama et al., 2002).

Elevated EP4 expression drives COX-2 expression and PGE_2 secretion in uterine cervical cancer tissue, promoting colony formation and VEGF expression (Oh et al., 2009). In colorectal cancer, EP4 occupation leads to ERK activation supporting anchorage-independent growth and resistance to apoptosis that is reversed by small molecule EP4 antagonists ONO-AE3-208 and AH23848 (Hawcroft et al., 2007). Likewise, inhibition of the EP2 and EP4 receptors (with AH6809 and GW627368X, respectively) represses IGF-1-induced proliferation of pancreatic BxPC-3 cancer cells (Takahashi et al., 2019) and is accompanied by increased phospho-PKC-θ and decreased phospho-ERK (Takahashi et al., 2015).

The Role of EP4 in Cell Migration/Invasion/Metastasis

Tumor dissemination is the chief cause of cancer mortality. Several early studies demonstrated that small molecule EP4 antagonists (AH23848; ONO-AE3-208) or EP4 gene silencing reduced metastatic potential *in vivo* in preclinical models of breast, prostate, colon, and lung cancer (Ma et al., 2006; Yang et al., 2006; Xu et al., 2018). The anti-metastatic activity is partially attributed to direct inhibition of tumor cell migration and invasion. For example, EP4-shRNA knockdown in lung cancer cells led to decreased cell migration *in vitro* by a β-arrestin1-dependent mechanism (Kim et al., 2010). EP4 blockade in prostate cancer cells overexpressing EP4 resulted in reduced migration, invasion, and metastasis. Down-regulation of EP4 and EP2 receptors or the EP4 antagonist AH23848 inhibit migration and invasion of human colorectal carcinoma cells (Jeong et al., 2018). Conversely, agonism of EP4 promoted *in vitro* lung cancer cell migration (Kim et al., 2010). EP4 is coupled to several downstream signaling pathways. In prostate, colon, and renal cell carcinomas, EP4 supports cell proliferation and invasion *via* the cAMP-PKA/PI3K-AKT signaling pathway and this response is inhibited by L161982 (Zhang et al., 2017) by ONO-AE3-208 or Cayman 10598 (Kashiwagi et al., 2018) or by RQ15986 (Majumder et al., 2018). EP4 regulates cell migration through Orai1 Ca^{2+} signaling in human oral squamous carcinoma cell lines that is blocked by ONO-AE3-208; cancer metastasis was inhibited when EP4 gene expression was reduced (Osawa et al., 2020). In melanoma, EP4 agonism induces cell migration accompanied by accumulation of β-catenin and decreased expression of several metalloproteinases (Vaid et al., 2015). Knockdown of EP4 abolished the transendothelial migration and metastatic intravasation capacity in metastatic renal carcinoma (Zhang et al., 2017). EP4 agonists can induce and the EP4 antagonist GW627368x blocks EGFR-dependent degradation of the extracellular matrix that, if left unchecked, facilitates breast cancer invasion (Tönisen et al., 2017).

The COX2/EP4 pathway is coupled to induction of several proinflammatory cytokines; several are tumor-promoting. In prostate cancer, the inhibitory effects of the EP4 antagonist AH23848 were linked to downregulation of several cytokines (CCL2, IL6, and CXCL8) (Han et al., 2019). In a murine model of bone metastatic prostate cancer, the EP4 antagonist ONO-AE3-208 abrogated inflammation-dependent bone metastasis and bone loss (Watanabe et al., 2016). EP4 antagonism with the same compound also prevents bone loss in malignant melanoma cells *via* the suppression of osteoclasts (Takita et al., 2007).

Cancer Stem Cells and EP4 in Chemoresistance

More recently, EP4 has been implicated in treatment resistance and as a driver of CSCs. Epigenetic activation of the EP4 receptor may support resistance to endocrine therapy in patients with breast cancer that is reversed by GW627368x (Hiken et al., 2017). Several labs have shown that EP4 is upregulated in malignant cells with stem-like properties and that treatment of tumor-bearing mice with EP4 antagonists (RQ15986, AH23848,

Frondoside A) reduces the number of CSC (Kundu et al., 2014; Majumder et al., 2014). A positive feedback loop involving EP4/miR-526b and miR-655/COX-2 is critical to the mechanism by which EP4 supports breast CSC (Majumder et al., 2018). Exposure of MCF7 cells to EP4 agonists upregulated both miR species and was accompanied by activation of PI3K/AKT, ERK, and NF-κB pathways leading to increased cell proliferation, migration, invasion, spheroid formation, and epithelial to mesenchymal transition. EP4 can mediate the effects of hypoxia-driven mesenchymal stem cells in the progression of hepatocellular carcinoma. This response is reversed in EP4 siRNA expressing cells or in the presence of GW627368x (Liu et al., 2019). The EP4 antagonist L161982 blocks the ability of enteric glial cells to stimulate colon CSCs *via* a PGE2/EP4/EGFR-dependent pathway (Valès et al., 2019). Although the exact mechanism by which EP4 antagonism allows for various cancer cells to transition from the mesenchymal/CSC state to an epithelial phenotype is not clear, one group has proposed that EP4 antagonism with GW627368x results in extracellular vesicle-mediated clearance of CSC markers, integrins, and drug transporters (Lin et al., 2018).

There is growing interest in the potential of EP4 antagonists in combination with other therapies, including chemotherapy. Gynecologic uterine smooth muscle tumors are sensitized to docetaxel in the presence of the EP4 antagonists AH23848 or RQ15986 (Reader et al., 2019). In a model of oxaliplatin-resistant colon cancer, EP4 blockade with L161982 inhibited CSC markers and tumorsphere formation (Huang et al., 2019). Likewise, cisplatin-resistant gastric cancer cells are sensitized to cisplatin *via* the suppression of COX-2/EP4/ERK1/2/P38 signaling by L161982 (Lin et al., 2019).

THE ROLE OF EP4 IN THE HOST RESPONSE TO MALIGNANCY

Regulation of Lymphangiogenesis

In addition to tumor cell-autonomous roles for EP4, many host cells express EP4 and should be considered in the context of systemic EP4 targeting. EP4 plays a role in activation of PGE_2 and COX-2 expression in rat mesenteric lymphatic endothelial cells (Nandi et al., 2017). EP4 antagonism with RQ15986 inhibits tumor growth and angiogenesis in a murine model of breast cancer (Majumder et al., 2014). In melanoma, PGE_2 acts on EP4 expressed on fibroblasts in the tumor microenvironment to regulate the release of angiogenic factors (Inada et al., 2015).

Regulatory Function of EP4 on NK Cells

Innate immunity plays a significant role in tumor control. Natural killer cells recognize and directly kill malignant cells and provide critical cytokines that promote the replication and activation of cytotoxic T lymphocytes. There are also critical interactions between NK and dendritic cells that further drive both innate and adaptive anti-tumor responses. Each of these functions is regulated by PGE_2 acting on EP receptors expressed on the NK cell. While NK cells express each EP, the negative regulation by PGE_2, in the context of malignancy, is through EP4 and EP2 (Kundu et al., 2009; Holt et al., 2012; Ma et al., 2013). EP4 antagonists AH23848, ONO-AE3-208, Frondoside A and RQ15986, or to a lesser extent, the EP2 antagonist AH6809, prevent PGE_2-mediated inhibition of NK cells.

In preclinical models of triple negative breast cancer, even when EP4 is directly inhibited in the malignant cell by shRNA targeting, the anti-metastatic activity is lost when NK cells are depleted in the host (Kundu et al., 2009; Ma et al., 2013). NK cells isolated from either patients or tumor-bearing mice are compromised regarding the ability to lysis target cells, produce critical cytokines and to migrate in response to chemotactic signals. NK recognition and lytic activity is triggered by the presence of activating ligands and the loss of inhibitory signals on the target cells; the latter are delivered chiefly through the expression of MHC class I antigens on the target cell. EP4 antagonists including Frondoside A, reduce surface expression of MHC class I antigens and, at the same time, increase sensitivity to NK-mediated lysis (Holt et al., 2012). PGE_2 prevents NKG2D, NCR, and CD16 activating receptors from triggering lysis (Martinet et al., 2010). Thyroid cancer-derived PGE_2 inhibits NK maturation as well as the expression of the NK receptors NK44, NK30, TRAIL, and NKG2D whereas NK inhibitory receptors are increased (Park et al., 2018). Either EP2 (AH6809) or EP4 (AH23848) antagonists block the inhibitory actions of PGE_2 on NK cells. Administration of EP4 antagonists (RQ15986, AH23848, Frondoside A) to tumor bearing mice restores the profound suppression of NK activity (cytotoxic functions, cytokine production, chemotactic activity), even in the context of progressive tumor growth (Holt et al., 2012; Ma et al., 2013). Thus, in both murine and human NK cells, the activating signals for lysis are reduced and the inhibitory signals are enhanced in response to PGE_2 and these inhibitory signals are reversed by EP4 blockade. Importantly, the ability of NK cells to control metastasis is restored.

In addition to direct inhibitory effects of tumor-PGE_2 on NK activities, myeloid-derived suppressor cells (MDSC) can also negatively regulate NK functions. MDSC derived from patients with advanced melanoma inhibit the activity of co-cultured NK cells (Mao et al., 2014). PGE_2, acting on EP2 and EP4 receptors on the MDSC, activates p38 MAPK/ERK pathways leading to secretion of TGFβ to inhibit NK cells. This mechanism of immune suppression is blocked by AH6809 or AH23848. The critical role of PGE_2 in regulating interactions between dendritic cells and NK cells was further elucidated in a recent study (Böttcher et al., 2018). In addition to MDSC regulating NK cells, NK cells can, in turn, recruit conventional type 1 dendritic cells that are critical to immune-mediated tumor control. cDC1 are recruited by the release of CCL5 and XCL1 from NK cells. The ability of PGE_2 to compromise NK functions also results in impaired recruitment of cDC1 to the tumor microenvironment in a COX-2 dependent manner.

MDSC and M2 Macrophages Are Induced Through PGE2/EP Activation

MDSC can become the dominant immune cell population in progressively growing tumors. PGE_2 induces bone marrow stem cells to differentiate into Gr1+CD11b+ MDSCs but either EP2 (AH6809) or EP4 (AH23848) antagonists block the induction (Sinha et al., 2007). In several tumor models, an EP4 antagonist (E7046) similarly blocked the induction of MDSC and M2 macrophages *in vivo* (Albu et al., 2017). Novel mechanisms by which PGE_2/EP4 induces MDSC have been reported (Rodríguez-Ubreva et al., 2017). PGE_2 upregulates DNA methyl transferase 3A (DNMT3A) resulting in hypermethylation and repression of immune-promoting genes, specifically in the MDSC population. Conversely, inhibition of DNMT3A results in loss of this pattern of hypermethylation and loss of the immune suppressive properties of MDSC. The ability of PGE_2 to induce DNMT3A and subsequent hypermethylation in MDSC was blocked in the presence of either EP2 (PF 04418948) or EP4 (L161982) inhibitors. Doxorubicin-resistant mammary tumor cells have higher levels of COX-2 and PGE_2 secretion than the Dox-sensitive parental cells (Rong et al., 2016). These resistant cells more effectively induce MDSC and this induction is blocked by EP4 (ONO-AE3-208) or EP2 (AH6809) inhibitors. The microRNA miR-10a is key to the mechanism by which PGE_2 induces MDSC through a PKA/AMPK-dependent signaling pathway.

PGE_2 exerts anti-inflammatory activity on macrophages and monocytes. The EP4 antagonists L161,982 or CJ042794 inhibit LPS-induced production of TNFα by lung macrophages (Gill et al., 2016) or by expression of EP4 siRNA in human monocytic cells (Kashmiry et al., 2018). The acute inflammatory response is supported by M1 macrophages whereas resolution of inflammation is driven by M2 phenotype macrophages. PGE_2 plays a key role in the polarization of macrophages to the M2 phenotype resulting in poor induction of anti-tumor immunity. MicroRNA-21 negatively regulates M2 polarization by PGE_2 (Wang et al., 2015).

Actions of EP Blockade on Cytotoxic T Cells and T Regulatory Cells

Mammary epithelial COX-2-derived mediators contribute to pro-tumor immune function, particularly through T lymphocyte and cytotoxic immune cell function (Markosyan et al., 2013). EP4 inhibitors, as monotherapy, do not typically result in marked inhibition of tumor growth in preclinical models of breast, colon, or pancreatic cancer, among others. The modest inhibition of growth of breast tumors is, however, dependent on CD8+ T cells (Albu et al., 2017). The mechanism is not by direct actions of the EP4 inhibitor E7046 on either CD8+ T cells or on T regulatory cells; rather it is likely explained by blockade of MDSC actions. The demonstration that EP4 blockade appears to synergize with checkpoint inhibitor blockade with anti-CTLA-4 antibody or, alternatively, with direct targeting of T regulatory cells with an IL-2/diphtheria toxin fusion product is very promising. The latter combination therapy resulted in an inflamed tumor microenvironment expressing T cell activating and T cell recruiting chemokines including CXCL9, CXCL10, CXCL11, and Ccl5/RANTES and MIP-1α.

FUTURE DIRECTIONS

There is growing evidence that EP4 represents a novel therapeutic target in cancer, both expressed on the malignant cell as well as on EP4-positive host cells. It is likely that EP4 antagonists would be used in combination with either cytotoxic therapies or with other immune-modulating strategies (for excellent reviews see Majumder et al., 2018; Take et al., 2020). There is growing evidence that EP4 blockade can reverse chemotherapy resistance in several tumor types and therefore combinations of EP4 antagonists with standard of care chemotherapy should be considered. Combinations of EP4 inhibitors with immune therapies are also being considered. NK cells are particularly important in the control of metastatic disease and, in preclinical models, EP4 antagonists reduce tumor dissemination by an NK-cell-dependent mechanism. Primary tumor control appears to be more dependent on CD8+ T cells. These studies and others suggest that EP4 inhibitors may be more effective when combined with immune based therapies rather than as monotherapy. In support of future combination clinical trials, Albu et al. showed that EP4 antagonism combined with either Treg depletion or with an antibody to CTLA-4 provided superior tumor control to either monotherapy (Albu et al., 2017). Cancer clinical trials to evaluate EP4 inhibitors are recruiting in trials of Grapiprant (ARY-007) in combination with pembrolizumab in subjects with either advanced MSS colorectal cancers (NCT03658772) or, in a separate trial in advanced NSCLC (NCT03696212) (Mizuno et al., 2019; Take et al., 2020). E7046 is being examined in a rectal cancer trial (NCT03152370) and BMS 986310 plus nivolumab is being investigated in a phase 1 trial in advanced solid tumors (NCT03661632). The possible role of EP4 in mediating resistance to endocrine therapies in breast cancer should also be explored further. Based on the ability of EP4 antagonists to inhibit CSCs and chemotherapy resistant cell lines, it is anticipated that clinical trials in these settings will also be forthcoming.

AUTHOR CONTRIBUTIONS

MC and AF drafted the manuscript. MC, AF, and JR contributed to manuscript revision, read and approved the submitted version.

FUNDING

Studies in AF's laboratory was supported by the Department of Health and Human Services (USA); U.S. Department of Defense; U.S. Veterans Administration and the Maryland Department of Health. While at the University of Maryland, Baltimore, MC was supported by the STAR-PREP program of the National Institute of General Medical Sciences.

REFERENCES

Albu, D. I., Wang, Z., Huang, K.-C., Wu, J., Twine, N., Leacu, S., et al. (2017). EP4 Antagonism by E7046 diminishes myeloid immunosuppression and synergizes with Treg-reducing IL-2-Diphtheria toxin fusion protein in restoring anti-tumor immunity. *Oncoimmunology* 6, e1338239. doi: 10.1080/2162402X.2017.1338239

Böttcher, J. P., Bonavita, E., Chakravarty, P., Blees, H., Cabeza-Cabrerizo, M., Sammicheli, S., et al. (2018). NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell* 172, 1022–1037.e14. doi: 10.1016/j.cell.2018.01.004

Gill, S. K., Yao, Y., Kay, L. J., Bewley, M. A., Marriott, H. M., and Peachell, P. T. (2016). The anti-inflammatory effects of PGE2 on human lung macrophages are mediated by the EP4 receptor. *Br. J. Pharmacol.* 173, 3099–3109. doi: 10.1111/bph.13565

Han, I.-H., Kim, J.-H., Jang, K.-S., and Ryu, J.-S. (2019). Inflammatory mediators of prostate epithelial cells stimulated with Trichomonas vaginalis promote proliferative and invasive properties of prostate cancer cells. *Prostate* 79, 1133–1146. doi: 10.1002/pros.23826

Hawcroft, G., Ko, C. W. S., and Hull, M. A. (2007). Prostaglandin E2-EP4 receptor signalling promotes tumorigenic behaviour of HT-29 human colorectal cancer cells. *Oncogene* 26, 3006–3019. doi: 10.1038/sj.onc.1210113

Hiken, J. F., McDonald, J. I., Decker, K. F., Sanchez, C., Hoog, J., VanderKraats, N. D., et al. (2017). Epigenetic activation of the prostaglandin receptor EP4 promotes resistance to endocrine therapy for breast cancer. *Oncogene* 36, 2319–2327. doi: 10.1038/onc.2016.397

Holt, D. M., Ma, X., Kundu, N., Collin, P. D., and Fulton, A. M. (2012). Modulation of host natural killer cell functions in breast cancer via prostaglandin E2 receptors EP2 and EP4. *J. Immunother.* 35, 179–188. doi: 10.1097/CJI.0b013e318247a5e9

Huang, H., Aladelokun, O., Ideta, T., Giardina, C., Ellis, L. M., and Rosenberg, D. W. (2019). Inhibition of PGE2/EP4 receptor signaling enhances oxaliplatin efficacy in resistant colon cancer cells through modulation of oxidative stress. *Sci. Rep.* 9, 4954. doi: 10.1038/s41598-019-40848-4

Inada, M., Takita, M., Yokoyama, S., Watanabe, K., Tominari, T., Matsumoto, C., et al. (2015). Direct melanoma cell contact induces stromal cell autocrine prostaglandin E2-EP4 receptor signaling that drives tumor growth, angiogenesis, and metastasis. *J. Biol. Chem.* 290, 29781–29793. doi: 10.1074/jbc.M115:669481

Jeong, J.-W., Park, C., Cha, H.-J., Hong, S. H., Park, S.-H., Kim, G.-Y., et al. (2018). Cordycepin inhibits lipopolysaccharide-induced cell migration and invasion in human colorectal carcinoma HCT-116 cells through down-regulation of prostaglandin E2 receptor EP4. *BMB Rep.* 51, 532–537. doi: 10.5483/BMBRep.2018.51.10.120

Kashiwagi, E., Inoue, S., Mizushima, T., Chen, J., Ide, H., Kawahara, T., et al. (2018). Prostaglandin receptors induce urothelial tumourigenesis as well as bladder cancer progression and cisplatin resistance presumably via modulating PTEN expression. *Br. J. Cancer* 118, 213–223. doi: 10.1038/bjc.2017.393

Kashmiry, A., Tate, R., Rotondo, G., Davidson, J., and Rotondo, D. (2018). The prostaglandin EP4 receptor is a master regulator of the expression of PGE2 receptors following inflammatory activation in human monocytic cells. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1863, 1297–1304. doi: 10.1016/j.bbalip.2018.07.003

Kim, J. I., Lakshmikanthan, V., Frilot, N., and Daaka, Y. (2010). Prostaglandin E2 promotes lung cancer cell migration via EP4-betaArrestin1-c-Src signalsome. *Mol. Cancer Res.* 8, 569–577. doi: 10.1158/1541-7786.MCR-09-0511

Kundu, N., Ma, X., Holt, D., Goloubeva, O., Ostrand-Rosenberg, S., and Fulton, A. M. (2009). Antagonism of the prostaglandin E receptor EP4 inhibits metastasis and enhances NK function. *Breast Cancer Res. Treat.* 117, 235–242. doi: 10.1007/s10549-008-0180-5

Kundu, N., Ma, X., Kochel, T., Goloubeva, O., Staats, P., Thompson, K., et al. (2014). Prostaglandin E receptor EP4 is a therapeutic target in breast cancer cells with stem-like properties. *Breast Cancer Res. Treat.* 143, 19–31. doi: 10.1007/s10549-013-2779-4

Lin, M.-C., Chen, S.-Y., He, P.-L., Herschman, H., and Li, H.-J. (2018). PGE2 /EP4 antagonism enhances tumor chemosensitivity by inducing extracellular vesicle-mediated clearance of cancer stem cells. *Int. J. Cancer* 143, 1440–1455. doi: 10.1002/ijc.31523

Lin, X.-M., Li, S., Zhou, C., Li, R.-Z., Wang, H., Luo, W., et al. (2019). Cisplatin induces chemoresistance through the PTGS2-mediated anti-apoptosis in gastric cancer. *Int. J. Biochem. Cell Biol.* 116, 105610. doi: 10.1016/j.biocel.2019.105610

Liu, Y., Ren, H., Zhou, Y., Shang, L., Zhang, Y., Yang, F., et al. (2019). The hypoxia conditioned mesenchymal stem cells promote hepatocellular carcinoma progression through YAP mediated lipogenesis reprogramming. *J. Exp. Clin. Cancer Res.* 38, 228. doi: 10.1186/s13046-019-1219-7

Ma, X., Kundu, N., Rifat, S., Walser, T., and Fulton, A. M. (2006). Prostaglandin E receptor EP4 antagonism inhibits breast cancer metastasis. *Cancer Res.* 66, 2923–2927. doi: 10.1158/0008-5472.CAN-05-4348

Ma, X., Holt, D., Kundu, N., Reader, J., Goloubeva, O., Take, Y., et al. (2013). A prostaglandin E (PGE) receptor EP4 antagonist protects natural killer cells from PGE2-mediated immunosuppression and inhibits breast cancer metastasis. *Oncoimmunology* 2, e22647. doi: 10.4161/onci.22647

Majumder, M., Xin, X., Liu, L., Girish, G. V., and Lala, P. K. (2014). Prostaglandin E2 receptor EP4 as the common target on cancer cells and macrophages to abolish angiogenesis, lymphangiogenesis, metastasis, and stem-like cell functions. *Cancer Sci.* 105, 1142–1151. doi: 10.1111/cas.12475

Majumder, M., Nandi, P., Omar, A., Ugwuagbo, K. C., and Lala, P. K. (2018). EP4 as a Therapeutic Target for Aggressive Human Breast Cancer. *Int. J. Mol. Sci.* 19, 1019–1038. doi: 10.3390/ijms19041019

Mao, Y., Sarhan, D., Steven, A., Seliger, B., Kiessling, R., and Lundqvist, A. (2014). Inhibition of tumor-derived prostaglandin-e2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity. *Clin. Cancer Res.* 20, 4096–4106. doi: 10.1158/1078-0432.CCR-14-0635

Markosyan, N., Chen, E. P., Evans, R. A., Ndong, V., Vonderheide, R. H., and Smyth, E. M. (2013). Mammary Carcinoma Cell Derived Cyclooxygenase 2 Suppresses Tumor Immune Surveillance by Enhancing Intratumoral Immune Checkpoint Activity. *Breast Cancer Res.* 15, 1186–1199. doi: 10.1186/bcr3469

Martinet, L., Jean, C., Dietrich, G., Fournié, J.-J., and Poupot, R. (2010). PGE2 inhibits natural killer and gamma delta T cell cytotoxicity triggered by NKR and TCR through a cAMP-mediated PKA type I-dependent signaling. *Biochem. Pharmacol.* 80, 838–845. doi: 10.1016/j.bcp.2010.05.002

Mizuno, R., Kawada, K., and Sakai, Y. (2019). Prostaglandin E2/EP signaling in the tumor microenvironment of colorectal cancer. *Int. J. Mol. Sci.* 20, 6254–6269. doi: 10.3390/ijms20246254

Nandi, P., Girish, G. V., Majumder, M., Xin, X., Tutunea-Fatan, E., and Lala, P. K. (2017). PGE2 promotes breast cancer-associated lymphangiogenesis by activation of EP4 receptor on lymphatic endothelial cells. *BMC Cancer* 17, 11. doi: 10.1186/s12885-016-3018-2

Oh, J.-M., Kim, S.-H., Lee, Y.-I., Seo, M., Kim, S.-Y., Song, Y.-S., et al. (2009). Human papillomavirus E5 protein induces expression of the EP4 subtype of prostaglandin E2 receptor in cyclic AMP response element-dependent pathways in cervical cancer cells. *Carcinogenesis* 30, 141–149. doi: 10.1093/carcin/bgn236

Okuyama, T., Ishihara, S., Sato, H., Rumi, M., Kawashima, K., Miyaoka, Y., et al. (2002). Activation of prostaglandin E2-receptor EP2 and EP4 pathways induces growth inhibition in human gastric carcinoma cell lines. *J. Lab. Clin. Med.* 140, 92–102. doi: 10.1067/mlc.2002.125784

Osawa, K., Umemura, M., Nakakaji, R., Tanaka, R., Islam, R. M., Nagasako, A., et al. (2020). Prostaglandin E2 receptor EP4 regulates cell migration through Orai1. *Cancer Sci.* 111, 160–174. doi: 10.1111/cas.14247

Park, A., Lee, Y., Kim, M. S., Kang, Y. J., Park, Y.-J., Jung, H., et al. (2018). Prostaglandin E2 secreted by thyroid cancer cells contributes to immune escape through the suppression of Natural Killer (NK) cell cytotoxicity and NK cell differentiation. *Front. Immunol.* 9, 1859. doi: 10.3389/fimmu.2018.01859

Reader, J., Holt, D., and Fulton, A. (2011). Prostaglandin E2 EP receptors as therapeutic targets in breast cancer. *Cancer Metastasis Rev.* 30, 449–463. doi: 10.1007/s10555-011-9303-2

Reader, J., Harper, A. K., Legesse, T., Staats, P. N., Goloubeva, O., Rao, G. G., et al. (2019). EP4 and Class III β-Tubulin expression in uterine smooth muscle tumors: Implications for prognosis and treatment. *Cancers (Basel)* 11, 1590–1606. doi: 10.3390/cancers11101590

Rodríguez-Ubreva, J., Català-Moll, F., Obermajer, N., Álvarez-Errico, D., Ramirez, R. N., Company, C., et al. (2017). Prostaglandin E2 Leads to the Acquisition of

DNMT3A-Dependent Tolerogenic Functions in Human Myeloid-Derived Suppressor Cells. *Cell Rep.* 21, 154–167. doi: 10.1016/j.celrep.2017.09.018

Rong, Y., Yuan, C.-H., Qu, Z., Zhou, H., Guan, Q., Yang, N., et al. (2016). Doxorubicin resistant cancer cells activate myeloid-derived suppressor cells by releasing PGE2. *Sci. Rep.* 6, 23824. doi: 10.1038/srep23824

Simper, M. S., Rundhaug, J. E., Mikulec, C., Bowen, R., Shen, J., Lu, Y., et al. (2014). The tumor promoting activity of the EP4 receptor for prostaglandin E2 in murine skin. *Mol. Oncol.* 8, 1626–1639. doi: 10.1016/j.molonc.2014.06.013

Sinha, P., Clements, V. K., Fulton, A. M., and Ostrand-Rosenberg, S. (2007). Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Res.* 67, 4507–4513. doi: 10.1158/0008-5472.CAN-06-4174

Tönisen, F., Perrin, L., Bayarmagnai, B., van den Dries, K., Cambi, A., and Gligorijevic, B. (2017). EP4 receptor promotes invadopodia and invasion in human breast cancer. *Eur. J. Cell Biol.* 96, 218–226. doi: 10.1016/j.ejcb.2016.12.005

Takahashi, T., Uehara, H., Ogawa, H., Umemoto, H., Bando, Y., and Izumi, K. (2015). Inhibition of EP2/EP4 signaling abrogates IGF-1R-mediated cancer cell growth: involvement of protein kinase C-θ activation. *Oncotarget* 6, 4829–4844. doi: 10.18632/oncotarget.3104

Takahashi, T., Ichikawa, H., Morimoto, Y., Tsuneyama, K., and Hijikata, T. (2019). Inhibition of EP2/EP4 prostanoid receptor-mediated signaling suppresses IGF-1-induced proliferation of pancreatic cancer BxPC-3 cells via upregulating γ-glutamyl cyclotransferase expression. *Biochem. Biophys. Res. Commun.* 516, 388–396. doi: 10.1016/j.bbrc.2019.06.054

Take, Y., Koizumi, S., and Nagahisa, A. (2020). Prostaglandin E receptor 4 antagonist in cancer immunotherapy: mechanisms of action. *Front. Immunol.* 11, 324. doi: 10.3389/fimmu.2020.00324

Takita, M., Inada, M., Maruyama, T., and Miyaura, C. (2007). Prostaglandin E receptor EP4 antagonist suppresses osteolysis due to bone metastasis of mouse malignant melanoma cells. *FEBS Lett.* 581, 565–571. doi: 10.1016/j.febslet.2007.01.005

Terada, N., Shimizu, Y., Kamba, T., Inoue, T., Maeno, A., Kobayashi, T., et al. (2010). Identification of EP4 as a potential target for the treatment of castration-resistant prostate cancer using a novel xenograft model. *Cancer Res.* 70, 1606–1615. doi: 10.1158/0008-5472.CAN-09-2984

Vaid, M., Singh, T., Prasad, R., Kappes, J. C., and Katiyar, S. K. (2015). Therapeutic intervention of proanthocyanidins on the migration capacity of melanoma cells is mediated through PGE2 receptors and β-catenin signaling molecules. *Am. J. Cancer Res.* 5, 3325–3338.

Valès, S., Bacola, G., Biraud, M., Touvron, M., Bessard, A., Geraldo, F., et al. (2019). Tumor cells hijack enteric glia to activate colon cancer stem cells and stimulate tumorigenesis. *EBioMedicine* 49, 172–188. doi: 10.1016/j.ebiom.2019.09.045

Wang, Z., Brandt, S., Medeiros, A., Wang, S., Wu, H., Dent, A., et al. (2015). MicroRNA 21 is a homeostatic regulator of macrophage polarization and prevents prostaglandin E2-mediated M2 generation. *PloS One* 10, e0115855. doi: 10.1371/journal.pone.0115855

Watanabe, K., Tominari, T., Hirata, M., Matsumoto, C., Maruyama, T., Murphy, G., et al. (2016). Abrogation of prostaglandin E-EP4 signaling in osteoblasts prevents the bone destruction induced by human prostate cancer metastases. *Biochem. Biophys. Res. Commun.* 478, 154–161. doi: 10.1016/j.bbrc.2016.07.075

Xu, S., Zhou, W., Ge, J., and Zhang, Z. (2018). Prostaglandin E2 receptor EP4 is involved in the cell growth and invasion of prostate cancer via the cAMP-PKA/PI3K-Akt signaling pathway. *Mol. Med. Rep.* 17, 4702–4712. doi: 10.3892/mmr.2018.8415

Yang, L., Huang, Y., Porta, R., Yanagisawa, K., Gonzalez, A., Segi, E., et al. (2006). Host and direct antitumor effects and profound reduction in tumor metastasis with selective EP4 receptor antagonism. *Cancer Res.* 66, 9665–9672. doi: 10.1158/0008-5472.CAN-06-1271

Zhang, Y., Thayele Purayil, H., Black, J. B., Fetto, F., Lynch, L. D., Masannat, J. N., et al. (2017). Prostaglandin E2 receptor 4 mediates renal cell carcinoma intravasation and metastasis. *Cancer Lett.* 391, 50–58. doi: 10.1016/j.canlet.2017.01.007

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 09 June 2020
doi: 10.3389/fphar.2020.00828

Expression and Function of Eicosanoid-Producing Cytochrome P450 Enzymes in Solid Tumors

Eric A. Evangelista[1], *Christi W. Cho*[2], *Theresa Aliwarga*[3] *and Rheem A. Totah*[2]*

[1] Department of Pharmacy, School of Pharmacy, University of Washington, Seattle, WA, United States, [2] Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, WA, United States, [3] Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, WA, United States

Oxylipins derived from the oxidation of polyunsaturated fatty acids (PUFAs) act as important paracrine and autocrine signaling molecules. A subclass of oxylipins, the eicosanoids, have a broad range of physiological outcomes in inflammation, the immune response, cardiovascular homeostasis, and cell growth regulation. Consequently, eicosanoids are implicated in the pathophysiology of various diseases, most notably cancer, where eicosanoid mediated signaling is involved in tumor development, progression, and angiogenesis. Cytochrome P450s (CYPs) are a superfamily of heme monooxygenases generally involved in the clearance of xenobiotics while a subset of isozymes oxidize PUFAs to eicosanoids. Several eicosanoid forming CYPs are overexpressed in tumors, elevating eicosanoid levels and suggesting a key function in tumorigenesis and progression of tumors in the lung, breast, prostate, and kidney. This review summarizes the current understanding of CYPs' involvement in solid tumor etiology and progression providing supporting public data for gene expression from The Cancer Genome Atlas.

Keywords: epoxygenases, hydroxylases, cytochrome P450, epoxyeicosatrienoic acids, hydroxyeicosatetraenoic acids, 20-HETE, tumor, angiogenesis

OPEN ACCESS

Edited by:
Emer Smyth,
University of Pennsylvania,
United States

Reviewed by:
Pallavi R. Devchand,
University of Calgary, Canada
Sara Bonafini,
University of Verona, Italy

***Correspondence:**
Rheem A. Totah
rtotah@uw.edu

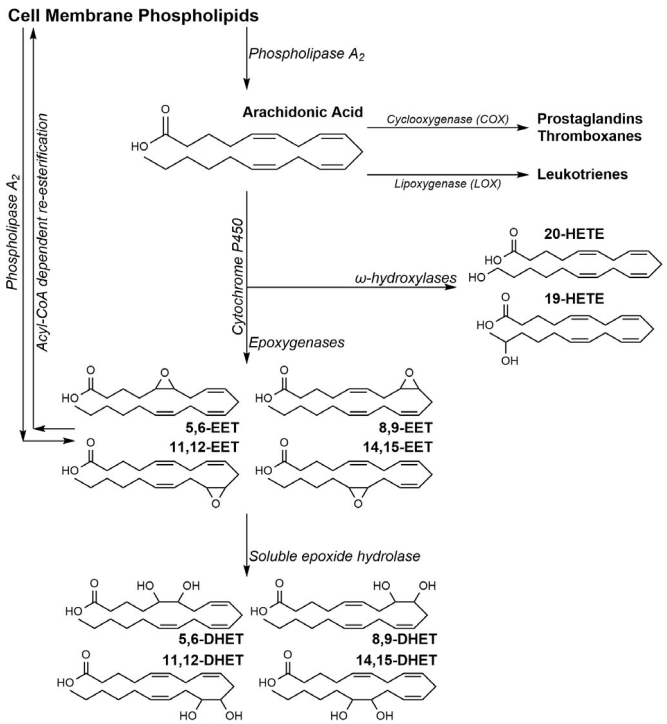
Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *25 February 2020*
Accepted: *20 May 2020*
Published: *09 June 2020*

Citation:
Evangelista EA, Cho CW, Aliwarga T and Totah RA (2020) Expression and Function of Eicosanoid-Producing Cytochrome P450 Enzymes in Solid Tumors. Front. Pharmacol. 11:828. doi: 10.3389/fphar.2020.00828

INTRODUCTION

Eicosanoids are a subset of physiologically active oxylipins derived from arachidonic acid (AA) oxidation. AA, esterified at the *sn2* position of lipid bilayer phospholipids, is released by phospholipase A_2 (PLA_2) in response to various cellular stimuli (Thomas et al., 1984). Upon release, AA can undergo oxidation *via* three pathways to produce multiple eicosanoids with distinct downstream effects (**Scheme 1**). The cyclooxygenase (COX) pathway results in prostaglandin H_2, a precursor to prostacyclins, prostaglandins, and thromboxanes (Otto and Smith, 1995); the lipoxygenase (LOX) pathway generates leukotrienes and mid-chain AA-hydroxylated metabolite (Brash, 1999). AA metabolized by the third pathway, undergoes oxidation by cytochrome P450 (CYP) isoforms to two distinct classes of eicosanoids; the epoxyeicosatrienoic acids (EETs) and the hydroxyeicosatetraenoic acids (HETEs) (Capdevila et al., 1981; Proctor et al., 1987). Despite a



SCHEME 1 | Arachidonic acid metabolism pathway.

common source, the numerous AA metabolites activate separate, sometimes overlapping, pathways to stimulate a broad array of physiological responses.

EETs are derived from the oxidation of AA's olefins by CYP epoxygenases to four possible regioisomers of *cis*-EETs (**Scheme 1**). EETs can stimulate a wide range of effects during inflammation, angiogenesis, and cardiovascular homeostasis (Node et al., 1999; Pozzi et al., 2005; Campbell and Fleming, 2010; Pfister et al., 2010). Due to their anti-inflammatory and pro-angiogenic activities, EETs have been investigated in recent years for their involvement in cancer progression and tumorigenesis (Spector et al., 2004; Panigrahy et al., 2011).

Another subset of CYPs, the hydroxylases, oxidize AA at various carbons to form many HETEs. (Powell et al., 1998; Choudhary et al., 2004). While CYP hydroxylases mostly prefer to oxidize AA at the terminal and penultimate carbons (ω and ω-1, respectively), metabolism on the mid-chain carbons has been observed. It is worth noting that some CYP isoforms function as both epoxygenases and hydroxylases generating a mixture of both EETs and HETEs (Choudhary et al., 2004).

CYP epoxygenases and hydroxylases are members of the larger CYP superfamily of membrane bound monooxygenases containing a heme prosthetic group in the active site (Ortiz de Montellano, 2005). Humans have 57 distinct CYP genes that encode for proteins and 46 pseudogenes (Ortiz de Montellano, 2005). Most research investigating changes in CYP expression during cancer progression is mainly focused on how these changes affect chemotherapeutic activation, clearance, or drug resistance. CYPs, specific to certain tumors, could potentially bioactivate agents as a novel targeted therapeutic approach, to concentrate the active, cytotoxic drug in tumor cells. Alternatively, CYPs could result in chemotherapeutic resistance due to their inherent ability to metabolize and often inactivate or expedite the clearance of xenobiotics thereby reducing their concentration in the tumor (Narjoz et al., 2014; van Eijk et al., 2019). CYP2J2, for example, is an isoform abundantly expressed in tumor tissues and metabolizes several antitumor tyrosine kinase inhibitors reducing their intracellular concentration (Narjoz et al., 2014). Similarly, CYP3A4 upregulation has been established in several breast cancer tumors and its expression levels are associated with docetaxel resistance (Miyoshi et al., 2002). Therefore, CYP expression in the context of cancer treatment is complex and CYP enzymes can act to enhance chemotherapeutic efficacy or diminish it.

This review compiles and summarizes the current status on the role of CYP epoxygenases and hydroxylases, and resulting EETs and HETEs, play in the development and progression of different sub-types of solid tumors. CYP gene expression data in tumor vs healthy tissue from The Cancer Genome Atlas (TCGA) database is analyzed and presented in figures to support findings. Where available, potential mechanisms and pathways involving CYPs, and their metabolites (**Table 1**), will be presented and novel chemotherapeutic strategies will be discussed for each tumor type.

TABLE 1 | Summary of key CYPs in solid tumors.

CYP450 Isoform	Role in AA metabolism	Tumors where expression is observed	Increase/decrease expression and potential mechanism	References
CYP1A2	Epoxygenase	Lung cancer Bladder cancer	Unknown	(Rifkind et al., 1995)
CYP1B1	Epoxygenase/ hydroxylase	Breast cancer Prostate cancer	Elevated enzyme levels -Increased EET/HETE production -Potential pro-angiogenic/pro-metastatic -Inhibits caspase-1 expression	(Finnström et al., 2001; Muskhelishvili et al., 2001; Chang et al., 2017)
CYP2C8	Major epoxygenase	Prostate cancer Renal cancer Bladder cancer	Potential increase in EET levels -promotes cell proliferation and survival -potential pro-angiogenic	(Sellmayer et al., 1991; Chen et al., 2001)
CYP2C9	Major epoxygenase	Lung cancer Prostate cancer Renal cancer Colorectal cancer Bladder cancer	Increased expression of CYP2C9, -increased EET levels -pro-inflammatory -pro-tumorigenic.	(Michaelis et al., 2003; Sausville et al., 2018; Wang et al., 2019)
CYP2C19	Epoxygenase	Breast cancer	Increased CYP2C19 -Increased EET levels -Increased cell proliferation -Metastasis Linked to FABP4/5, PPAY-γ, and SREBP-2	(Apaya et al., 2020)
CYP2J2	Major epoxygenase	Lung cancer Breast cancer Prostate cancer Renal cancer Colorectal cancer Ovarian cancer Bladder cancer	Increased CYP2J2 expression. -Increased EET levels -Increased tumor grade -Increased tumor size Potential therapy: dual-action sEH and COX-2 inhibitors showed increasing level of EETs and suppression of tumor growth in NDL/ FVB mouse model.	(Jiang et al., 2005; Jiang et al., 2007; Freedman et al., 2007; Jiang et al., 2009; Chen et al., 2012; Wei et al., 2014)
CYP3A5	Hydroxylase	Prostate cancer (adjacent normal tissue)	Increased production of 20-HETE in adjacent normal tissue -Induces growth and proliferation of tumor -Possibly through GPR75 pathway	(Leskelä et al., 2007; Garcia et al., 2017; Cárdenas et al., 2019)
CYP4A11	ω-Hydroxylases	Lung cancer Breast cancer	-Increased tumor growth -Increased tumor proliferation	(Leclerc et al., 2010; Yu et al., 2011)
CYP4F2	ω-Hydroxylases	Lung cancer Breast cancer Ovarian cancer	Increased CYP4F2 expression -Increased 20-HETE -Pro-inflammatory	(Leclerc et al., 2010; Alexanian et al., 2012)
CYP4A22 CYP4F3 CYP4F11 CYP4F12	ω-Hydroxylases	Lung cancer	Unknown	(Leclerc et al., 2010; Alexanian et al., 2012; Alexanian and Sorokin, 2013)
CYP4Z1	Epoxygenase	Breast cancer	-Pro-angiogenic	(Yu et al., 2012; McDonald et al., 2017)

Lung Cancer

In 2020, it is estimated that approximately 1,806,590 new cancer cases will be diagnosed in the United States. Of those, 228,820 cases of lung cancer (116,300 in men and 112,520 in women) will be diagnosed, representing roughly 13% of all new cancer diagnoses among adults in the United States (Siegel et al., 2020). Cigarette smoking is the most common, and major, known risk factor for lung cancer where epidemiologic trends strongly correlate with smoking patterns (Mao et al., 2016; Nasim et al., 2019). In the U.S. and most Western countries, the epidemic of cigarette smoking related lung cancer has declined steadily in recent years due to regulations and implementation of smoking cessation programs (Peto et al., 2000). However, in countries such as China, where smoking prevalence has yet to peak, significant increases in lung cancer cases will continue for the foreseeable future (Alberg et al., 2005). Other risk factors include exposure to environmental toxins that could potentially be bioactivated by CYPs to reactive intermediates affecting the lung (Hukkanen et al., 2002). Despite the declining trend in certain parts of the globe, lung cancer remains one of the leading causes of cancer related deaths worldwide and will be responsible for approximately 25% of all cancer related deaths in the U.S. in 2020 (Mao et al., 2016; Siegel et al., 2020).

Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) are two main types of lung cancer representing approximately 80% and 20% of all diagnoses, respectively. (Zheng, 2016). Several studies revealed that duration and number of cigarettes smoked significantly increased the likelihood of squamous cell carcinoma, a sub-type of NSCLC, and SCLC (Auerbach et al., 1975; Morabia and Wynder, 1991; Sobue et al., 2002). The treatment regimen for NSCLC generally

includes surgical resection followed by adjuvant therapy whereas the more aggressive SCLC treatments rely exclusively on chemotherapy and targeted immunotherapy (Zhao et al., 2018; Duma et al., 2019). Despite best efforts, mortality rates remain among the highest compared to other cancers. This is mainly because nearly 70% of patients have metastatic disease by the time of diagnosis. Lung cancer is inherently resistant to chemotherapeutics while safe resection of the tumor is often difficult and associated with a high propensity for recurrence (Molina et al., 2008; Leclerc et al., 2010).

The human lung consists of a diverse array of cell types, each with a unique expression pattern of CYPs, many of which are involved in AA metabolism (**Figure 1**). As a member of the CYP1 family, CYP1A2 is expressed in the lung (**Table 1**) and is highly induced by tobacco smoke (Zevin and Benowitz, 1999; Wei et al., 2001; Ding and Kaminsky, 2003) although there are conflicting reports on the level of basal expression of CYP1A2 in the lung (Shimada et al., 1996; Leclerc et al., 2010). CYP1A2 plays a key role in bioactivating inhaled environmental or tobacco smoke procarcinogens increasing susceptibility to lung cancer (Pavanello et al., 2012). In addition to xenobiotic metabolism, CYP1A2 oxidizes AA mainly to 8,9-EET (Rifkind et al., 1995). Currently, there are very few studies elucidating the role of CYP1A2 as an AA epoxygenase in cancer development or progression, however, it remains possible that the formation of the pro-angiogenic EETs by CYP1A2 can play a role in lung cancer progression.

CYP2J2 is another epoxygenase expressed in human lung (**Table 1**) (Zeldin et al., 1996a). It is known to generate all four regioisomers of *cis*-EETs, 14,15-, 11,12-, 8,9-, and 5,6-EET, and no hydroxylation products (Wu et al., 1996; Aliwarga et al., 2017). Jiang et al. reported that CYP2J2 mRNA and protein levels are upregulated, to varying degrees, in eight different tumor derived cell lines, including A549 from lung cancer, compared to non-cancerous cells (Jiang et al., 2005). When CYP2J2 is overexpressed in Tca8113 adenocarcinoma derived cells using an adeno-associated virus, apoptosis was attenuated, and cell migration was enhanced. Similar results were obtained when the Tca8113 cells were treated with EETs while silencing of *CYP2J2* expression resulted in the opposite effect implicating CYP2J2 and EETs in promoting a neoplastic phenotype. (Jiang et al., 2005). Findings from the same study show increases in CYP2J2 mRNA and protein levels in lung squamous cell carcinoma, lung adenocarcinoma, and small cell lung carcinoma tissues relative to adjacent normal tissue analyzed by RT-PCR, Western blotting, and immunohistochemistry, respectively. (Jiang et al., 2005).

CYP2J2 increased lung metastasis in mice xenograft models, independent of primary tumor size, (Jiang et al., 2007) while chemically inhibiting CYP2J2 activity, using compounds structurally related to terfenadine, significantly reduced the tumor cells' ability for adhesion and invasion (Chen et al., 2009). Another study demonstrated that reducing CYP2J2 protein by increasing miRNA let-7b levels resulted in reduced cell growth, increased apoptosis, and metastasis (Chen et al., 2012). Taken together, there is strong evidence that CYP2J2 alters the progression of lung cancer through EET formation and targeting CYP2J2 activity may be a useful therapeutic strategy.

CYP2C9 is also observed in the human lung (**Table 1**) and predominately forms 14,15-EET and 11,12-EET. (Rifkind et al., 1995; Shimada et al., 1996; Hukkanen et al., 2002). An increase in endothelial cell proliferation was observed in HUVEC cells overexpressing CYP2C9 compared to cells with CYP2C9 silenced or treated with sulfaphenazole, a specific CYP2C9 inhibitor (Michaelis et al., 2003). CYP2C9 is highly polymorphic with nearly 33 identified genetic variants (Wang et al., 2009). Compared to the wildtype *CYP2C9*1*, two extensively studied allelic variants *CYP2C9*2* and *CYP2C9*3* are known to have significantly reduced enzymatic activity (Zhou et al., 2010). Sausville et al. reported that mice injected with human NSCLC cells expressing *CYP2C9*2* and **3* variants

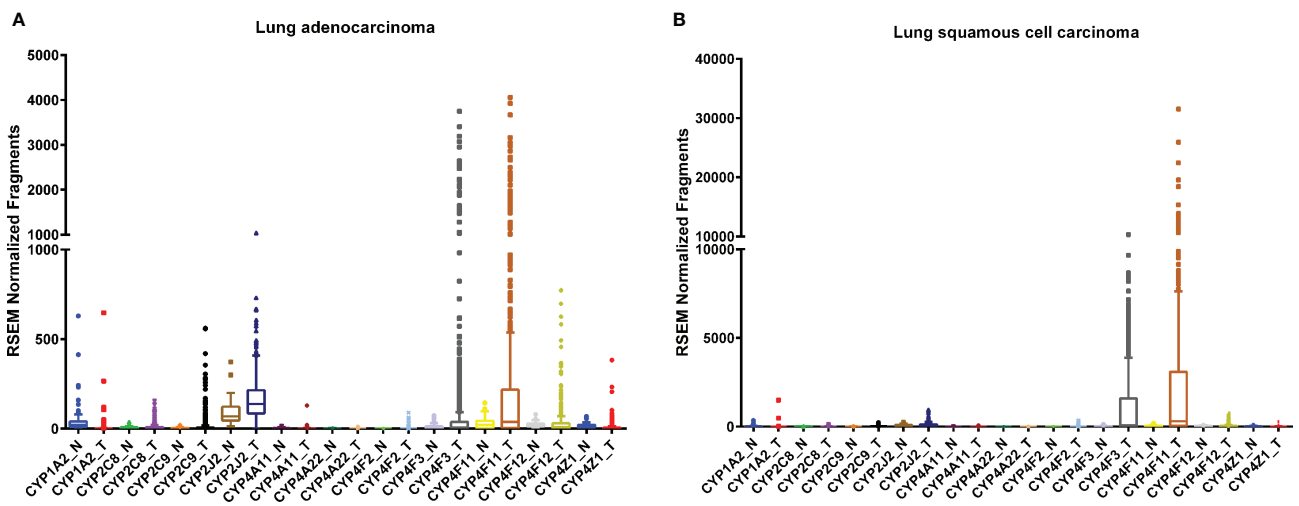


FIGURE 1 | RNA sequencing data obtained from the TCGA on the CYP expression in lung tumor (T) tissues and adjacent normal (N) tissue. Lung adenocarcinoma, **(A)** normal (n = 53) and tumor (n = 465); tissues and squamous cell carcinoma **(B)**, normal (n = 45) and tumor (n = 452) tissues.

produced significantly lower EETs resulting in smaller and less vascularized tumors compared with mice injected with human NSCLC cells expressing wildtype *CYP2C9*1* (Sausville et al., 2018). This study shows that CYP2C9 allelic variants with reduced enzymatic activity favorably alter susceptibility to lung cancer.

CYP4A and CYP4F sub-families are major ω-hydroxylases responsible for converting AA to several HETE regioisomers including 19- and 20-HETE (Powell et al., 1998; Gross et al., 2005; Kroetz and Xu, 2005). The expression of CYP4A and 4F families in the human lung has not been extensively studied and therefore, currently, the data are sparse. Trace levels of CYP4A11, CYP4A22, and CYP4F2 mRNA were detected in normal human lung tissue while low-to-moderate mRNA levels were detected for CYP4F3, CYP4F11, and CYP4F12 by RT-PCR (Leclerc et al., 2010). In a study comparing mRNA levels of major ω-hydroxylases in human cancers, upregulation of CYP4A and CYP4F enzymes in NSCLC tissue samples compared to matched normal tissue was observed (Alexanian et al., 2012; Alexanian and Sorokin, 2013). Overexpression of CYP4A11 in a NSCLC derived A549 cell line also increased 20-HETE production and significantly induced cell invasion (Yu et al., 2011). In a murine xenograft model, tumor size and angiogenesis were significantly potentiated in mice overexpressing CYP4A11 through upregulation of VEGF-A and matrix metallopeptidase-9 (MMP-9). Similar effects were observed with treatment of WIT003, a stable 20-HETE analog, and mitigated by administration of HET0016, a potent inhibitor of 20-HETE biosynthesis (Yu et al., 2011). Additionally, CYP4A expression in tumor-associated macrophages promotes pre-metastatic niche formation in lung which is attenuated in the presence of HET0016 strongly supporting the role of CYP4A and 20-HETE in cancer metastasis (Chen et al., 2017).

Breast Cancer

Breast cancer is one of the most prevalent cancers in women, with greater than 270,000 new cases and over 40,000 deaths predicted in the United States in 2020 (Siegel et al., 2020). Numerous studies have examined the expression of several CYP isoforms in breast cancer, including aromatase/CYP19A1 (Suzuki et al., 2009), CYP1A1 (Androutsopoulos et al., 2009; Rodriguez and Potter, 2013), CYP1B1 (McKay et al., 1995; Murray et al., 1997), CYP2J2 (Jiang et al., 2009; Murray et al., 2010), CYP2C (Huang et al., 1996; Murray et al., 2010), CYP3A (Huang et al., 1996), and the CYP4 family (Rieger et al., 2004; Alexanian et al., 2012). Differences in CYP expression in tumor tissue compared to adjacent normal tissue have also been catalogued in several repositories, such as the TCGA database (**Figure 2**).

All the CYPs mentioned above, apart from aromatase, can oxidize AA. CYP1A1 oxidizes AA to 19-HETE (Choudhary et al., 2004), 11,12- and 14,15-EET (Choudhary et al., 2004; Fer et al., 2008) as major products. Likewise, CYP1B1 also mediates both HETE and EET production (Choudhary et al., 2004), with preference for mid-chain HETEs with terminal HETEs and EETs production being relatively equal (Choudhary et al., 2004). CYP2J2, as mentioned above, is exclusively an

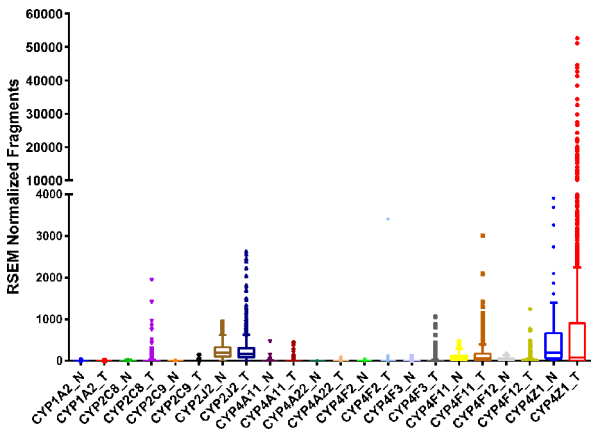


FIGURE 2 | RNA sequencing data obtained from the TCGA on the CYP expression in breast invasive adenocarcinoma tumor (T, n = 992) tissue and adjacent normal (N, n = 101) tissue.

epoxygenase expressed mostly in the heart, intestines, kidneys, and other tissues (Wu et al., 1996; Zeldin et al., 1997; Michaud et al., 2010; Xu et al., 2013) with increased, robust expression in multiple cancer cell lines and tissues including breast cancer (Jiang et al., 2005; Apaya et al., 2019). The CYP2C enzymes are also primarily AA-epoxygenases (Fer et al., 2008) but have been shown to act as hydroxylases as well (Rifkind et al., 1995). In contrast, CYP3A4/5 are primarily hydroxylases but epoxygenase activity has also been reported (Rifkind et al., 1995; Fer et al., 2008). Lastly, as previously mentioned, the CYP4 family are exclusively terminal chain hydroxylases.

In recent years, research has focused on the effects of eicosanoids on the progression of breast cancer tumors. Elevated EET levels in patients are linked to higher CYP2C and CYP2J2 mRNA and protein expression in tumor cells compared to adjacent normal cells (Wei et al., 2014; Apaya et al., 2019). In addition to elevated CYP epoxygenase expression, studies show reduced levels of soluble epoxide hydrolase (sEH), the enzyme responsible for the hydrolysis of EETs to the dihydroxyeicosatrienoic acids (DHETs), depicted in **Scheme 1** (Wei et al., 2014). The result is increased EET biosynthesis and reduced metabolism, and overall increase in tumor EET levels. Interestingly, Wei's study showed that the CYP2C enzymes were correlated with Ki67 status, a measure of the number of cells that are actively dividing, while CYP2J2 levels were correlated with tumor grade and size (Wei et al., 2014). This may indicate that CYP2C and CYP2J2 regulate distinct pathways necessary for cell proliferation and migration, summarized in **Table 1**. EETs have been shown to not only promote cell survival and growth but also promote angiogenesis, a process critical to sustain tumor growth.

Another study examining the effect of 14,15-EET in breast cancer demonstrated that 14,15-EET mediates epithelial-mesenchymal transition (EMT) in breast cancer cell lines (Luo et al., 2018). EMT of cells is indicative of their metastatic capability as cells become more migratory and invasive. Luo et al. identified FAK/PI3K/AKT activation in response to 14,15-

EET, through the upregulation of αvβ3 integrin, with the responses attenuated by 14,15-EEZE, a 14,15-EET antagonist. Lastly, the investigators observed that in addition to promoting mesenchymal properties, 14,15-EET also protected breast cancer cells from cisplatin toxicity *in vitro* and *in vivo* using a mouse xenograft model (Luo et al., 2018). This is significant because it provides evidence of a novel role for 14,15-EET in cancer progression. The other EETs (11,12- 8,9- and 5,6-EETs) were not investigated, and thus it is unclear if they also promote similar resistance to chemotherapeutic intervention. Unfortunately, Luo et al. specifically examined 14,15-EET and did not identify any specific epoxygenase responsible for its biosynthesis although CYP4Z1 is a possible candidate (McDonald et al., 2017). Studies by Luo et al. and Wei et al. both focused primarily on 14,15-EET and while the four isoforms of EETs are typically thought to elicit similar responses, there are no studies that identify definitive and distinct pathways for each regioisomer, if they exist. It is also important to note that different studies disagree on the ratios of EETs formed from individual CYP isoforms (Wu et al., 1996; Imaoka et al., 2005; Fer et al., 2008). Therefore, it is possible that specific EET regioisomers impact distinct aspects of breast cancer, from cell growth and division to overall tumor size, which may partially explain differences in correlation between CYP isoforms and either fraction of dividing cells or tumor grade and size. Nevertheless, the link between CYP epoxygenases, EETs and breast cancer growth and progression led many groups to propose CYP epoxygenases and sEH as potential therapeutic targets for new treatments (Jiang et al., 2007; Wei et al., 2014; Apaya et al., 2019).

A recent study by Apaya et al. examined if CYP2C19 and EET metabolites can be targeted to reduce breast cancer proliferation and prevent metastasis (Apaya et al., 2020). Their study demonstrated that elevated CYP2C19 leads to increased EET levels in a highly metastatic breast cancer cell line, LM6, and that using short hairpin RNA (shRNA) to silence *CYP2C19* expression reduces EET levels and decreases the cell's metastatic potential. Addition of external EETs negated the effects of *CYP2C19* silencing, indicating that the cells' invasive phenotype is, in part, EET-driven. The investigators also linked the pro-metastatic effects of EETs to fatty acid binding proteins (FABP), specifically FABP4 and FABP5, activating PPAR-γ and SREBP-2, resulting in downstream pro-oncogenic gene activation. Downregulation of FABP4/5 attenuated EET activation of pro-metastatic characteristics like cell migration, motility, invasiveness, and colony formation of cells *in vitro* and tumor growth and metastasis in an *in vivo* mouse xenograft model. Apaya et al.'s study provides important insight into the mechanistic cascade involved in EET-mediated metastasis in breast cancer. While their work focused solely on CYP2C19, it would be of interest to determine if inhibiting other CYP epoxygenases yield similar results.

CYP hydroxylases and 20-HETE have also been reported to affect breast cancer pathophysiology. Alexanian et al. demonstrated increased CYP4 enzyme levels in breast cancer tissue compared to normal mammary tissue (Alexanian et al., 2012). The CYP4 family are the primary source of 20-HETE, particularly CYP4F2 and CYP4A11. Borin et al. demonstrated that HET0016 decreases tumor growth and proliferation in a mouse xenograft model through reduction of angiogenic growth factors (Borin et al., 2014), indirectly suggesting that CYP4 enzymes, and other CYP hydroxylases, may be therapeutic targets through inhibition of AA metabolism to 20-HETE.

In a more recent study, Borin et al. showed that HET0016 also decreases tumor growth and metastasis *in vivo* (Borin et al., 2017), implying additional roles for CYP4 enzymes and their 20-HETE product in breast cancer metastasis. There were decreases observed in PI3K/AKT signaling and reduced levels of MMP2 and MMP9, proteins involved in the metastatic process, in mice treated with HET0016. In both their studies, while the investigators attributed the observed effects to inhibition of 20-HETE formation, they fell short of identifying the CYPs involved in their study nor did they measure changes in 20-HETE levels pre and post HET0016 treatment. It remains ambiguous whether the effects observed are due to inhibition of a CYP ω-hydroxylase or some off-target effect of HET0016. Further studies are needed in order to definitively link HET0016's anti-tumor and anti-metastatic effects to 20-HETE and identify the CYP ω-hydroxylases involved.

CYP4Z1 is an interesting isoform overexpressed in breast cancer. Despite being part of the CYP4 family, CYP4Z1 has recently been identified as an AA epoxygenase rather than a hydroxylase (McDonald et al., 2017), exclusively forming 14,15-EET. Previously, Yu et al. linked this enzyme to poor prognosis and worse tumor grades (Yu et al., 2012). The investigators demonstrated that CYP4Z1 promotes overexpression of the pro-angiogenic factor VEGF-A and the suppression of TIMP-2 (a tumor suppressor) in breast cancer cell lines. In *in vivo* models, the investigators also showed CYP4Z1 overexpression resulted in increased tumor size, weight, and vascularization, indicating a role for CYP4Z1 in breast cancer tumor growth and progression. These effects were surprisingly reversed by HET0016 (Yu et al., 2012). These results are puzzling since McDonald et al. convincingly demonstrated that CYP4Z1 lacks AA hydroxylase activity, instead producing only the 14,15-EET regioisomer using *S. cerevisiae* expressed human CYP4Z1. Unfortunately, Yu et al. did not examine directly which eicosanoid was responsible for the effects observed with CYP4Z1 overexpression. Further studies are therefore required to delineate the role CYP4Z1 plays in breast cancer, specifically identifying the metabolite responsible for inducing growth and angiogenesis, as well as how HET0016 is able to mitigate the effects of CYP4Z1 overexpression.

Prostate Cancer

The American Cancer Society estimates that the prostate will be the leading site of new cancer cases and the second leading cause of cancer deaths among men in the U.S. in 2020 (Siegel et al., 2020). Several studies have examined CYP expression in prostate tissue, both normal and cancerous. Like breast cancer, prostate cancer is sensitive to hormone signaling, specifically by androgens. Therefore, many early studies focused on CYPs known to play a role in hormone metabolism. For example, a

study by Finnstrom et al. (2001) investigated gene expression of a panel of drug metabolizing CYP isoforms in normal and cancer prostate tissue in 24 patients. The study is limited in its sample size and focused only on gene expression. CYP1B1 and CYP3A5 were predominant in this sample, both of which have been shown to metabolize hormones and known carcinogens. It should be noted that CYP3A5, as well as CYP3A4, are known AA hydroxylases (Rifkind et al., 1995). Additionally, Choudhary et al. demonstrated that CYP1B1 metabolizes AA, though with less specificity than the CYP3A isoforms, forming internal and terminal HETEs and EETs (Choudhary et al., 2004). A separate study observed CYP2C8, CYP2C9, and CYP2J2 mRNA and protein expression in prostate cancer derived cell lines (Nithipatikom et al., 2010). While this is of interest, studies demonstrating an overexpression of these isoforms in prostate cancer tissue compared to matched normal tissues are needed. Nevertheless, the presence of CYP epoxygenases in prostate cancer models suggests that CYP derived eicosanoids may be involved in prostate cancer progression.

Mid-chain HETEs are typically produced from AA by enzymes in the LOX pathway. Choudhary et al.'s findings, however, indicate that not only is CYP1B1 able to form internal and mid-chain HETEs, they are the preferred metabolites (Choudhary et al., 2004), comprising approximately 50% of metabolites produced. This is of particular interest as studies show that mid-chain HETE serum concentrations are elevated in patients with advanced prostate cancer (Rodríguez-Blanco et al., 2014), indicating that perhaps CYP1B1 and mid-chain HETEs contribute to prostate cancer progression. Rodriguez-Blanco et al.'s study specifically identified 5-, 8-, 11-, and 15-HETE (Rodríguez-Blanco et al., 2014). 5-HETE and 15-HETE, in particular have been shown to induce cell growth and proliferation *in vitro* in various tumor-derived cell lines (O'Flaherty et al., 2005; O'Flaherty et al., 2013; Cabral et al., 2013; Ma et al., 2013), implying a function for these compounds, and thus CYP1B1, in prostate tumor growth and proliferation.

CYP1B1 in prostate cancer was recently linked to caspase-1 activation by Chang et al. (Chang et al., 2017). Caspase-1 is a pro-inflammatory protease that induces programmed cell death (Zhang et al., 2003). The study by Chang et al. demonstrated an inverse relationship between CYP1B1 and caspase-1 expression. Silencing CYP1B1 expression *via* shRNA resulted in increased caspase-1 expression, and attenuated tumor growth and progression in an *in vivo* mouse model (Chang et al., 2017). Unfortunately, the investigators did not measure eicosanoid levels and the exact mechanism by which CYP1B1 regulates caspase-1 expression remains unknown. It is plausible that an eicosanoid product of CYP1B1 suppresses caspase 1 expression and when CYP1B1 is silenced, the eicosanoid levels drop, and caspase-1 expression is activated. It would be of interest in the future to determine if overexpression or silencing of CYP1B1 affects eicosanoid levels in prostate cancer tissue and how individual eicosanoids affect caspase-1 expression and activity *in vitro* and *in vivo*.

In addition to formation of mid-chain HETE metabolites of AA, CYP1B1 is also capable of generating both terminal HETE and EET metabolites, which make up the other 50% of CYP1B1 products (Choudhary et al., 2004). It is therefore possible that CYP1B1 contributes to tumor progression through similar mechanisms as CYP3A5 by producing terminal HETE products that act as autocrine signaling molecules by activating the 20-HETE/GPR75 signaling cascades previously mentioned. The effect of CYP1B1 on tumor formation and growth may be further exacerbated by the simultaneous production of EETs and mid-chain HETEs. EETs are known potent pro-angiogenic factors. This is important because rapidly growing solid tumors are limited by lack of new blood vessels and require angiogenesis to produce new vessels to sustain continuous growth and division (Folkman et al., 1971). The production of EETs within the tumor bed provides a mechanism for tumor cells to stimulate angiogenesis and a means to overcome this growth-limiting problem.

A study using immunohistochemistry observed CYP3A5 expression to be in adjacent non-tumor cells and not in the tumor itself (Leskelä et al., 2007). This is indicative of paracrine signaling that supports tumor growth, progression, and metastasis, where adjacent non-tumor cells produce 20-HETE with detrimental effects on tumor cells. A series of recent studies have identified some key roles for 20-HETE in prostate cancer progression. Garcia et al. first identified GPR75 as a receptor for 20-HETE (Garcia et al., 2017) and Cardenas et al. demonstrated that the 20-HETE/GPR75 signaling pathways contributed to metastatic prostate cancer progression (Cárdenas et al., 2019). Upon exposure to 20-HETE, the prostate cancer cell line PC-3 internalized GPR75, resulted in the phosphorylation of signaling molecules EGFR, NF-κB, and AKT, and altered cellular localization of AKT, NF-κB, and PKCα, all of which were reversed with the addition of GPR75 antagonist (Cárdenas et al., 2019). While several key players have been identified, the full signaling cascade remains to be elucidated. Cardenas et al. also looked at the downstream consequences of PC-3 cell exposure to 20-HETE, demonstrating that the cells exhibit pro-metastatic characteristics (Cárdenas et al., 2019). Their results offer promising insight into how 20-HETE might affect prostate cancer tumor growth and progression. Further studies are needed to determine whether these results can be replicated using animal models or if this is a general mechanism for 20-HETE function in other cell types.

To date, studies examining the direct effects of EETs on prostate cancer progression *in vivo* are absent. However, a study by Vanella et al. demonstrated that ellagic acid, an antioxidant with potential chemotherapeutic properties, may act to inhibit tumor growth by decreasing EET formation, specifically by downregulating CYP2J2 and upregulating soluble epoxide (sEH) expression in a prostate cancer cell line (Vanella et al., 2013). Their study also showed that ellagic acid also acts to decrease CYP4 isoform expression, and by extension, 20-HETE formation. While this study provides some experimental evidence that EETs and 20-HETE have significant roles in prostate cancer as pro-angiogenic factors, it is important to note that the investigators measured mRNA levels of these enzymes, both epoxygenases and hydroxylases, but

did not measure protein expression or eicosanoids. Nevertheless, this study marks a starting point for investigating a causal relationship between CYP-mediated AA metabolites and prostate cancer progression.

CYP2C9 expression has also been observed by Enayetallah et al. (Enayetallah et al., 2006) by immunohistochemistry in prostate tumors, however, the study design did not show CYP2C9 expression in adjacent normal tissue, therefore, it is unclear whether CYP2C9 is upregulated in this particular cancer. Regardless, CYP2C9 is an AA epoxygenase, producing all four EET isomers (Fer et al., 2008). As with the other epoxygenases that were previously discussed, CYP2C9 may be involved in tumor growth and progression through EET-mediated angiogenesis, but further studies are needed to provide experimental evidence for such a role.

Renal Cancer

Kidney cancer is among the top ten diagnosed cancers in the United States for men and women (Siegel et al., 2020). It is estimated that 73,750 adults (45,520 men and 28,230 women) will be diagnosed with kidney cancer in the U.S., of which approximately 85% to 90% of all kidney cancer will be renal cell carcinoma (RCC) (Nabi et al., 2018; Siegel et al., 2020). RCC develops from the proximal epithelial tubules in the renal cortex and is further divided into three histologically distinct subtypes with, the most common, clear cell RCC (ccRCC) representing 70% to 80% of all RCC cases, papillary RCC and chromophobe RCC representing 10% to 15% and 5% of all RCC cases, respectively (Rini et al., 2009; Saad et al., 2019). RCC neoplasms in the collecting duct have also been identified but these tumors are very rare (Sanchez and Simon, 2018; Capitanio et al., 2019; Saad et al., 2019). Among all subtypes of RCC, ccRCC is the most aggressive, and while prognosis for early stage disease is usually promising, advanced stage ccRCC is the most lethal urological malignancy (Rini et al., 2009; Saad et al., 2019).

The proximal tubules of the human kidney express drug metabolizing enzymes as well several CYPs involved in AA oxidation (Shah et al., 2017). Expression of CYP2C8, CYP2C9, and CYP2J2 in human kidney is contentious at best (Knights et al., 2013). Studies by Zeldin et al. did not detect CYP2C8 mRNA transcripts or protein in kidney microsomal fractions by Northern or western blot analyses, respectively (Zeldin et al., 1996b; Lasker et al., 2000). However, Enayetallah et al. reported that CYP2C8 is detected in one of the two normal kidney tissues they analyzed by immunohistochemistry (Enayetallah et al., 2006). Baker et al. reported inconclusive results of CYP2C9 in kidney due to very low abundance bands observed on the western blot suggesting that CYP2C9 is not robustly expressed in kidney. (Baker et al., 2001). In the same study by Enayetallah et al. above, positive immunohistochemical staining for CYP2C9 was observed in the two normal kidney tissue samples analyzed (Enayetallah et al., 2006). And finally, Wu et al. reported very low basal expression of CYP2J2 mRNA in the kidney (Wu et al., 1996).

Enayetallah et al. also examined the protein expression of three CYP epoxygenases in RCC tumor tissue, surrounding non-neoplastic tissue, and benign control tissue by immunohistochemistry. They observed that the staining intensities for CYP2C8, CYP2C9, and CYP2J2 decreased in RCC tissue and surrounding non-neoplastic tissue compared to normal control tissue suggesting a seminal role in carcinogenesis (Enayetallah et al., 2006). In the same study, an anomaly was observed in one of the ten RCC tissues analyzed where there was a significant increase in CYP2J2 staining intensity, relative to the control, and delineates a need for a larger sample size.

Compared to the CYP2C8 and CYP2C9 RNA sequencing data from The Protein Atlas database, CYP2J2 is highly expressed in renal cancers (https://www.proteinatlas.org/ENSG00000134716-CYP2J2/pathology/renal+cancer). Similarly, RNA sequencing data from the TCGA corroborates the Protein Atlas data and shows CYP2J2 to be overexpressed in ccRCC tumors, while changes in CYP2C8 or CYP2C9 are not significant (**Figure 3**). Overall, the expression of CYP epoxygenases in kidney remains equivocal and future investigations with a large sample size are needed.

EET derived metabolites of AA can promote kidney mesangial cell proliferation inferred from the significantly reduced cell growth following treatment with selective CYP epoxygenase inhibitors (Sellmayer et al., 1991). Yang et al. showed that overexpression of CYP epoxygenases, which in turn increase EET biosynthesis, attenuated TNF-α induced endothelial cell apoptosis (Yang et al., 2007). Further, 14,15-EET protects against serum-withdrawal induced apoptosis in an adherent proximal tubule-like epithelial cell line, LLCPKc14, derived from pig (Chen et al., 2001). The function of CYP epoxygenases, and individual EETs, in RCC has not been extensively studied. However, current studies indicate that EETs, especially 14,15-EET, may have the potential to alter the susceptibility to RCC progression.

CYP4A and CYP4F enzymes responsible for 20-HETE formation are highly expressed in normal renal epithelial cells (Simpson, 1997; Knights et al., 2013). Alexanian et al. showed that renal adenocarcinoma cells maintain the ability to generate 20-HETE through the expression of CYP4F isoforms (Alexanian et al., 2009). Upon inhibition of 20-HETE production with HET0016 and WIT002, cell proliferation is reduced *in vitro* in 786-O and 769-P cells, immortalized RCC derived cell lines, while minimal effects are observed in primary human proximal epithelial cells. Upon implantation of 786-O cells in nude mice, treatment with WIT002 reduced tumor growth by 84% compared to vehicle control (Alexanian et al., 2009). Together these studies indicate inhibition of 20-HETE synthesis by targeting CYP4s could be protective and reduce tumor growth in RCC.

Colorectal Cancer

The American Cancer Society estimated that there are approximately 147,950 new cases and 53,200 deaths associated with colorectal cancer in the United States in 2020 (Siegel et al., 2020). Although mortality rate has been gradually declining in the United States, colorectal cancer remains the second leading cause of cancer deaths worldwide (World Health Organization, 2018; Siegel et al., 2020). Depending on where the cancer

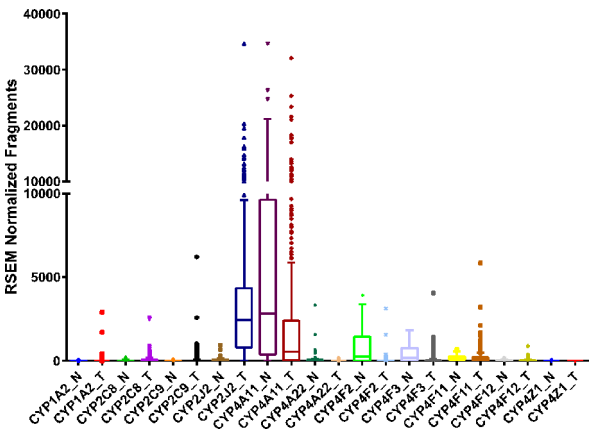


FIGURE 3 | RNA sequencing data obtained from the TCGA on the CYP expression in clear cell renal cell carcinoma tumor (T, n = 480) tissue and adjacent normal (N, n = 64) tissue.

originates, it is called colon or rectal cancer. However, due to the many common features between these cancers, they are often grouped together and referred to as colorectal cancer. Age, lifestyle, genetics, environmental, and dietary factors have been implicated in the etiology of colorectal cancer (Global Burden of Disease Cancer Collaboration et al., 2017; Siegel et al., 2020).

Most studies on eicosanoids involved in the growth and progression of colorectal cancer examined AA metabolites from the COX and LOX pathways. However, a handful of studies investigated epoxygenases and their metabolites. In an earlier study, both protein and transcript of CYP2J2 were observed in LS-174 cell line derived from human colon cancer (Jiang et al., 2005). Although mRNA transcripts of CYP epoxygenases (CYP2Cs and CYP2J2) in colorectal adenocarcinoma patients in TGCA database did not change compared with controls, transcripts of CYP epoxygenases and protein expression of CYP2C9 were significantly increased in primary human colorectal adenocarcinoma (Wang et al., 2019). In the same study, the investigators used azoxymethane/dextran sodium sulfate (AOM/DSS)-induced colon cancer mice to elucidate EETs involvement in colon tumorigenesis. EET levels in plasma and colon of AOM/DSS-induced colon cancer mice were significantly higher than their corresponding control untreated group. In addition, number of tumors and tumor size, expression of several pro-inflammatory and pro-tumorigenesis markers, and EET levels were significantly lower in *Cyp2c*$^{-/-}$ knockout mice treated with AOM/DSS to induce colon cancer. The results of Wang et al. contradict an earlier study by Zhang et al. (2013). Zhang et al. reported that homozygous sEH knockout mice exhibited significantly lower incidence of colorectal carcinoma and reduced tumor burden. Because sEH is responsible for EET hydrolysis to DHETs (**Scheme 1**), sEH knockout mice are expected to have higher levels of endogenous EETs which in turn promote pro-angiogenic activities, one would expect the observations reported by Wang et al. (2019) rather than by Zhang et al. (2013). It is possible that the phosphatase activity of sEH (Newman et al., 2003; Morisseau et al., 2012), also knocked down in these mice, is somehow responsible for the lower incidence of colorectal carcinoma and reduced tumor burden.

CYP2S1 is also overexpressed in colorectal cancer (Kumarakulasingham, 2005). Studies on CYP2S1, in general, are scarce. However, in the immunohistochemical studies, overexpression of CYP2S1 is associated with poor prognosis (Kumarakulasingham, 2005). In addition, Alexanian et al. reported that transcripts of CYP4F and CYP4A were significantly increased in two (stage IIA and stage IIIC) human colon cancer tissues (Alexanian et al., 2012). Unfortunately, they did not determine protein levels of either CYP4As, CYP4Fs or levels of 20-HETE in normal and cancerous colon tissues.

Several other hydroxylases, including CYP3A4/5 (McKay et al., 1993), CYP1B1 (Choudhary et al., 2004), and CYP2U1 (Chuang et al., 2004), were elevated relative to normal colon tissues (Kumarakulasingham, 2005). Because many of the cancer drugs are cleared by CYP3A, the presence of CYP3A could affect tumor sensitivity to treatments (Martínez et al., 2002). A study using human colorectal adenocarcinoma cell lines HCT116 and SW480 suggested that elevated interleukin-6 downregulated microRNA27b *via* DNA methylation which in turn upregulated the expression of CYP1B1 (Patel et al., 2014). Because of the overexpression of CYP1B1 in cancer tissue, it has been explored as a potential chemotherapeutic target (Li et al., 2017). No follow up studies, as of yet, reported on the importance of elevated levels of CYP2U1 in colorectal cancer (Kumarakulasingham, 2005).

Ovarian Cancers

The American Cancer Society estimated that there are approximately 21,750 new cases and 13,940 deaths associated with ovarian cancer in the U.S. in 2020 (Siegel et al., 2020). Age, history of breast or colorectal cancer, genetic, endometriosis, and nulliparity have been reported as potential risk factors for ovarian cancer (Kerlikowske et al., 1992; Li and Karlan, 2001; Risch et al., 2001; Watson et al., 2001; Boyd, 2003; Gates et al., 2010; Bonadona et al., 2011; Stewart et al., 2013; Wentzensen et al., 2016).

Differential gene analysis tumor-free specimens from malignant peritoneum and benign peritoneum from patients with benign pelvic disease and tumor specimens obtained from patients with advanced epithelial ovarian cancer showed a significant elevation in the expression of CYP2J2, but not CYP2C8 or CYP2C9, in tumors from patients with advanced epithelial ovarian cancer (Freedman et al., 2007). In addition, an immunohistochemistry study revealed that a minor and understudied CYP epoxygenase, CYP2S1, was elevated in metastatic ovarian cancer which correlated with poor prognosis (Downie et al., 2005). The significance of this finding is yet to be determined.

In a nested case–control study within the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, designed and sponsored by National Cancer Institute, EETs were not associated with increased risk of ovarian cancer (Hada et al.,

2019). This does not agree with previous reports that linked EETs to tumor growth and metastasis (Panigrahy et al., 2012; Skrypnyk et al., 2014). Since EETs are known as anti-inflammatory mediators (Node et al., 1999; Campbell, 2000), one would expect they will reduce inflammation that is widely associated with tumorigenesis and cancer progression (Multhoff et al., 2011; Greten and Grivennikov, 2019) thereby reducing the risk of developing ovarian cancer. Reducing inflammation proved protective in a population based, case-control study of women who previously had ovarian cancer and used aspirin and non-steroidal anti-inflammatory drugs continuously. In this population, risk of ovarian cancer was significantly reduced (Lo-Ciganic et al., 2012).

In a study using an array of human cancer cell lines, a combination of low-dose soluble epoxide hydrolase inhibitor and low-dose COX-2 inhibitor did not significantly inhibit cell proliferation (Zhang et al., 2014). In the same study, administration of the dual inhibitor to an *neu* deletion (NDL: $Her2^+$, $Ki67^+$, ER/PR negative) breast cancer Friend Virus B (FVB) female mouse model demonstrated elevated EETs while suppressing tumor growth (Zhang et al., 2014). Because of the discrepancy between in vitro and in vivo tumor models and the lack of EET levels measurement in the in vitro tumor model, functional characterization of EETs with respect to the anti-inflammatory and angiogenic properties need to be further explored in ovarian cancer.

Expression of several CYP hydroxylases, including CYP1B1 (Choudhary et al., 2004), CYP2U1 (Chuang et al., 2004), and CYP3A (Rifkind et al., 1995; Fer et al., 2008), along with the epoxygenase CYP4Z1 (Downie et al., 2005), were increased in primary ovarian cancer. Nonexpressers of CYP3A5 (carriers of *CYP3A5*3*) in Chinese epithelial ovarian cancer patients were at an increased risk of developing toxicity induced by the combination of paclitaxel/carboplatin (Hu et al., 2016). In human ovarian cancer tissue, elevated transcript and protein levels of CYP4F2, an ω-hydroxylase, were corroborated with higher arachidonic acid turnover relative to normal tissue (Alexanian et al., 2012). Considering that inflammation is critical in cancer development (Coussens and Werb, 2002) and the pro-inflammatory properties of 20-HETE and mid-chain HETEs (Cole et al., 2013; Hoopes et al., 2015; Powell and Rokach, 2015), elevated HETE levels in ovarian cancer could lead to poor prognosis and increase tumor progression.

Pancreatic Cancer

The American Cancer Society estimates that over 57,000 new cases of pancreatic cancer will be diagnosed in the United States, with approximately 47,000 deaths due to this cancer in 2020 (Siegel et al., 2020). Few studies examined changes in CYP expression levels in pancreatic cancer. To date, no studies have been conducted to assess the effect, if any, of CYP derived eicosanoids in the development and progression of pancreatic tumors. A single immunohistochemical study by Standop et al. (2003) demonstrated robust protein expression of CYP 1A1, 1A2, 2B6, 2C, 2D6, 2E1, and 3A4, as well as cytochrome P450 oxidoreductase (CPR) in normal pancreatic tissues from 24 individuals sourced from organ donors or autopsy. The investigators were also able to detect increased, though not statistically significant, expression of CYP 1A1, 2B6, 2C, 2D6, 2E1, 3A4 and CPR in pancreatic tumor tissues from 21 donors obtained from surgeries, indicating potential roles for these enzymes in cancer pathogenesis (Standop et al., 2003). There are several limitations associated with this study that need to be considered, primarily, it is unclear whether the increase in CYP expression was due to the cancer phenotype or to other covariates not considered in their study population (such as diabetic status, etc.). Normal and tumor tissue samples were not matched so there is no indication whether these enzymes increased in cancerous tissue relative to healthy tissue in the same patient. Finally, eicosanoid levels were not measured, and there is no indication that elevated CYPs correspond to elevated eicosanoids in pancreatic tissue. As with the other previously described solid tumors, it is likely that any CYP mediated eicosanoid involvement in pancreatic cancer would be due to their anti-apoptotic and pro-angiogenic properties. Currently, however, there is no evidence to suggest that CYP derived eicosanoids are involved in the initiation or progression of pancreatic cancer.

Bladder Cancer

Bladder cancer is the fourth most common cancer in men and significantly less common in women. It is estimated that 81,400 new cases (62,100 men and 19,300 women) of bladder cancer will be diagnosed in the United States in 2020 (Siegel et al., 2020). Several risk factors have been linked to bladder cancer with the most common bring cigarette smoking. Chemicals found in cigarettes, most notably arylamines including 2-naphthylamine and 4-aminobiphenyl (ABP), increase the susceptibility to bladder cancer by 2- to 4-fold and nearly half of all bladder cancer diagnosed is caused by cigarette smoking (Kirkali et al., 2005). Occupational exposure to these chemicals has traditionally put workers of the textile dye or rubber tire industry at a significantly higher risk for bladder cancer (Melick et al., 1955; Melick et al., 1971; You et al., 1990). CYP1A2 activity is associated with increased risk of bladder cancer due to its role in bioactivating arylamines in cigarette smoke but the role of CYP1A2 epoxygenase activity has not been studied (Lee et al., 1994; Tao et al., 2012). Enayetallah et al. reported expression of CYP2J2, CYP2C8, and CYP2C9 in urothelial cancer indicating a potential role for EETs in urinary tumorigenesis (Enayetallah et al., 2006). Wang et al. reported that CYP2J2 overexpression in human breast cancer cell line promoted metastasis in not only the lungs but also the liver and bladder as well (Panigrahy et al., 2011). However, there are very few studies investigating CYP mediated AA metabolites in bladder cancer or how these metabolites alter disease progression.

SUMMARY

Many eicosanoid forming CYPs are expressed in solid tumors, and each tumor has a unique expression profile of these isozymes. In general, CYP expression is usually studied in their

drug metabolizing capacity, reducing drug levels in tumors and potential contribution to chemotherapeutic resistance. However, due to the involvement of eicosanoids in cancer progression, CYPs acting as sources of HETEs and EETs and comparing eicosanoid levels in tumor compared to healthy tissue are also of interest. EET levels are further elevated as sEH is normally downregulated in cancerous tissue. Studies have linked elevated CYP levels with higher HETE/EET levels and demonstrated roles for these eicosanoids in tumor formation, growth, and metastasis. These studies highlight the potential of targeting CYP enzymes for novel cancer therapeutics.

In nonpathological settings, EETs and HETEs typically have opposing effects. For example, in the vasculature, EETs are potent vasodilators while 20-HETE is a vasoconstrictor. In cancer, both EETs and HETEs seem to have similar outcomes promoting cell growth and metastases. Studies comparing the effects of EETs vs. 20-HETE under comparable conditions are required to determine which eicosanoid is more potent. Similar studies comparing the potency of each EET isomer are also important to identify if they work through similar or different pathways. Most studies investigate, and establish, associations to a single isomer but studies investigating EETs individually and in combination are required to better understand how CYPs and their AA-derived metabolites are involved in tumor growth and progression. Despite the inhibition of these enzymes as promising mechanism for future therapeutic targets, there are no drugs in the clinic that target these pathways to halt or reverse tumor progression. This is likely due to the important physiological functions CYPs and eicosanoids play in non-cancer settings. Further understanding of the pathways triggered by EETs and 20-HETE in cancer could identify additional avenues for future novel therapeutic intervention.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception of this review. EE, CC, and TA wrote the first draft of the manuscript. EE and CC prepared schemes and figures. All authors contributed to manuscript revision, read and approved the submitted version.

FUNDING

This work was supported by the National Center for Advancing Translational Sciences grant TL1TR000422, and the National Institutes of General Medical Sciences grant T32GM007750.

ACKNOWLEDGMENTS

Data used to generate **Figures 1**–**3** are based on values deposited in the TCGA Research Network: https://www.cancer.gov/tcga. No additional analyses, including stratification by sex, age or tumor grade, were conducted and data were plotted as provided from the TCGA Research Network.

REFERENCES

Alberg, A. J., Brock, M. V., and Samet, J. M. (2005). Epidemiology of lung cancer: looking to the future. *J. Clin. Oncol.* 23, 3175–3185. doi: 10.1200/JCO.2005.10.462

Alexanian, A., and Sorokin, A. (2013). Targeting 20-HETE producing enzymes in cancer-rationale, pharmacology, and clinical potential. *Onco. Targets. Ther.* 6, 243–255. doi: 10.2147/OTT.S31586

Alexanian, A., Rufanova, V. A., Miller, B., Flasch, A., Roman, R. J., and Sorokin, A. (2009). Down-regulation of 20-HETE synthesis and signaling inhibits renal adenocarcinoma cell proliferation and tumor growth. *Anticancer Res.* 29, 3819–3824.

Alexanian, A., Miller, B., Roman, R. J., and Sorokin, A. (2012). 20-HETE-producing enzymes are up-regulated in human cancers. *Cancer Genomics Proteomics* 9, 163–169.

Aliwarga, T., Raccor, B. S., Lemaitre, R. N., Sotoodehnia, N., Gharib, S. A., Xu, L., et al. (2017). Enzymatic and free radical formation of cis- and trans-epoxyeicosatrienoic acids in vitro and in vivo. *Free Radic. Biol. Med.* 112, 131–140. doi: 10.1016/j.freeradbiomed.2017.07.015

Androutsopoulos, V. P., Tsatsakis, A. M., and Spandidos, D. A. (2009). Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. *BMC Cancer* 9, 187. doi: 10.1186/1471-2407-9-187

Apaya, M. K., Shiau, J.-Y., Liao, G.-S., Liang, Y.-J., Chen, C.-W., Yang, H.-C., et al. (2019). Integrated omics-based pathway analyses uncover CYP epoxygenase-associated networks as theranostic targets for metastatic triple negative breast cancer. *J. Exp. Clin. Cancer Res.* 38, 187. doi: 10.1186/s13046-019-1187-y

Apaya, M. K., Hsiao, P.-W., Yang, Y.-C., and Shyur, L.-F. (2020). Deregulating the CYP2C19/Epoxy-Eicosatrienoic Acid-Associated FABP4/FABP5 Signaling Network as a Therapeutic Approach for Metastatic Triple-Negative Breast Cancer. *Cancers (Basel).* 12. doi: 10.3390/cancers12010199

Auerbach, O., Garfinkel, L., and Parks, V. R. (1975). Histologic type of lung cancer in relation to smoking habits, year of diagnosis and sites of metastases. *Chest* 67, 382–387. doi: 10.1378/chest.67.4.382

Baker, J. R., Satarug, S., Reilly, P. E., Edwards, R. J., Ariyoshi, N., Kamataki, T., et al. (2001). Relationships between non-occupational cadmium exposure and expression of nine cytochrome P450 forms in human liver and kidney cortex samples. *Biochem. Pharmacol.* 62, 713–721. doi: 10.1016/s0006-2952(01)00716-x

Bonadona, V., Bonaïti, B., Olschwang, S., Grandjouan, S., Huiart, L., Longy, M., et al. (2011). Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA* 305, 2304–2310. doi: 10.1001/jama.2011.743

Borin, T. F., Zuccari, D. A. P. C., Jardim-Perassi, B. V., Ferreira, L. C., Iskander, A. S. M., Varma, N. R. S., et al. (2014). HET0016, a selective inhibitor of 20-HETE synthesis, decreases pro-angiogenic factors and inhibits growth of triple negative breast cancer in mice. *PloS One* 9, e116247. doi: 10.1371/journal.pone.0116247

Borin, T. F., Shankar, A., Angara, K., Rashid, M. H., Jain, M., Iskander, A., et al. (2017). HET0016 decreases lung metastasis from breast cancer in immune-competent mouse model. *PloS One* 12, e0178830. doi: 10.1371/journal.pone.0178830

Boyd, J. (2003). Specific keynote: hereditary ovarian cancer: what we know. *Gynecol. Oncol.* 88, S8–10; discussion S11-3. doi: 10.1006/gyno.2002.6674

Brash, A. R. (1999). Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. *J. Biol. Chem.* 274, 23679–23682. doi: 10.1074/jbc.274.34.23679

Cárdenas, S., Colombero, C., Panelo, L., Dakarapu, R., Falck John, R., Costas Monica, A., et al. (2019). GPR75 receptor mediates 20-HETE-signaling and metastatic features of androgen-insensitive prostate cancer cells. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1865, 158573. doi: 10.1016/j.bbalip.2019.158573

Cabral, M., Martín-Venegas, R., and Moreno, J. J. (2013). Role of arachidonic acid metabolites on the control ofnon-differentiated intestinal epithelial cell growth. *Int. J. Biochem. Cell Biol.* 45, 1620–1628. doi: 10.1016/j.biocel.2013.05.009

Campbell, W. B., and Fleming, I. (2010). Epoxyeicosatrienoic acids and endothelium-dependent responses. *Pflugers Arch.* 459, 881–895. doi: 10.1007/s00424-010-0804-6

Campbell, W. B. (2000). New role for epoxyeicosatrienoic acids as anti-inflammatory mediators. *Trends Pharmacol. Sci.* 21, 125–127. doi: 10.1016/s0165-6147(00)01472-3

Capdevila, J., Parkhill, L., Chacos, N., Okita, R., Masters, B. S., and Estabrook, R. W. (1981). The oxidative metabolism of arachidonic acid by purified cytochromes P-450. *Biochem. Biophys. Res. Commun.* 101, 1357–1363. doi: 10.1016/0006-291x(81)91597-7

Capitanio, U., Bensalah, K., Bex, A., Boorjian, S. A., Bray, F., Coleman, J., et al. (2019). Epidemiology of Renal Cell Carcinoma. *Eur. Urol.* 75, 74–84. doi: 10.1016/j.eururo.2018.08.036

Chang, I., Mitsui, Y., Kim, S. K., Sun, J. S., Jeon, H. S., Kang, J. Y., et al. (2017). Cytochrome P450 1B1 inhibition suppresses tumorigenicity of prostate cancer via caspase-1 activation. *Oncotarget* 8, 39087–39100. doi: 10.18632/oncotarget.16598

Chen, J. K., Capdevila, J., and Harris, R. C. (2001). Cytochrome p450 epoxygenase metabolism of arachidonic acid inhibits apoptosis. *Mol. Cell. Biol.* 21, 6322–6331. doi: 10.1128/MCB.21.18.6322-6331.2001

Chen, C., Li, G., Liao, W., Wu, J., Liu, L., Ma, D., et al. (2009). Selective inhibitors of CYP2J2 related to terfenadine exhibit strong activity against human cancers in vitro and in vivo. *J. Pharmacol. Exp. Ther.* 329, 908–918. doi: 10.1124/jpet.109.152017

Chen, F., Chen, C., Yang, S., Gong, W., Wang, Y., Cianflone, K., et al. (2012). Let-7b inhibits human cancer phenotype by targeting cytochrome P450 epoxygenase 2J2. *PloS One* 7, e39197. doi: 10.1371/journal.pone.0039197

Chen, X. W., Yu, T. J., Zhang, J., Li, Y., Chen, H. L., Yang, G. F., et al. (2017). CYP4A in tumor-associated macrophages promotes pre-metastatic niche formation and metastasis. *Oncogene* 36, 5045–5057. doi: 10.1038/onc.2017.118

Choudhary, D., Jansson, I., Stoilov, I., Sarfarazi, M., and Schenkman, J. B. (2004). Metabolism of retinoids and arachidonic acid by human and mouse cytochrome P450 1B1. *Drug Metab. Dispos.* 32, 840–847. doi: 10.1124/dmd.32.8.840

Chuang, S. S., Helvig, C., Taimi, M., Ramshaw, H. A., Collop, A. H., Amad, M., et al. (2004). CYP2U1, a Novel Human Thymus- and Brain-specific Cytochrome P450, Catalyzes ω- and (ω-1)-Hydroxylation of Fatty Acids. *J. Biol. Chem.* 279, 6305–6314. doi: 10.1074/jbc.M311830200

Cole, B. K., Lieb, D. C., Dobrian, A. D., and Nadler, J. L. (2013). 12- and 15-lipoxygenases in adipose tissue inflammation. *Prostaglandins Other Lipid Mediat.* 104–105, 84–92. doi: 10.1016/j.prostaglandins.2012.07.004

Coussens, L. M., and Werb, Z. (2002). Inflammation and cancer. *Nature* 420, 860–867. doi: 10.1038/nature01322

Ding, X., and Kaminsky, L. S. (2003). Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu. Rev. Pharmacol. Toxicol.* 43, 149–173. doi: 10.1146/annurev.pharmtox.43.100901.140251

Downie, D., McFadyen, M., Rooney, P., Cruickshank, M., Parkin, D., Miller, I., et al. (2005). Profiling Cytochrome P450 Expression in Ovarian Cancer: Identification of Prognostic Markers. *Clin. Cancer Res.* 11, 7369–7375. doi: 10.1158/1078-0432.CCR-05-0466

Duma, N., Santana-Davila, R., and Molina, J. R. (2019). Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. *Mayo Clin. Proc.* 94, 1623–1640. doi: 10.1016/j.mayocp.2019.01.013

Enayetallah, A. E., French, R. A., and Grant, D. F. (2006). Distribution of soluble epoxide hydrolase, cytochrome P450 2C8, 2C9 and 2J2 in human malignant neoplasms. *J. Mol. Histol.* 37, 133–141. doi: 10.1007/s10735-006-9050-9

Fer, M., Dréano, Y., Lucas, D., Corcos, L., Salaün, J.-P., Berthou, F., et al. (2008). Metabolism of eicosapentaenoic and docosahexaenoic acids by recombinant human cytochromes P450. *Arch. Biochem. Biophys.* 471, 116–125. doi: 10.1016/j.abb.2008.01.002

Finnström, N., Bjelfman, C., Söderström, T. G., Smith, G., Egevad, L., Norlén, B. J., et al. (2001). Detection of cytochrome P450 mRNA transcripts in prostate samples by RT-PCR. *Eur. J. Clin. Invest.* 31, 880–886. doi: 10.1046/j.1365-2362.2001.00893.x

Folkman, J., Merler, E., Abernathy, C., and Williams, G. (1971). Isolation of a tumor factor responsible for angiogenesis. *J. Exp. Med.* 133, 275–288. doi: 10.1084/jem.133.2.275

Freedman, R. S., Wang, E., Voiculescu, S., Patenia, R., Bassett, R. L., Deavers, M., et al. (2007). Comparative analysis of peritoneum and tumor eicosanoids and pathways in advanced ovarian cancer. *Clin. Cancer Res.* 13, 5736–5744. doi: 10.1158/1078-0432.CCR-07-0583

Garcia, V., Gilani, A., Shkolnik, B., Pandey, V., Fan Zhang, F., Dakarapu, R., et al. (2017). 20-HETE Signals Through G Protein-Coupled Receptor GPR75 (G q) to Affect Vascular Function and Trigger Hypertension HHS Public Access. *Circ. Res.* 120, 1776–1788. doi: 10.1161/CIRCRESAHA.116.310525

Gates, M. A., Rosner, B. A., Hecht, J. L., and Tworoger, S. S. (2010). Risk factors for epithelial ovarian cancer by histologic subtype. *Am. J. Epidemiol.* 171, 45–53. doi: 10.1093/aje/kwp314

Global Burden of Disease Cancer Collaboration, Fitzmaurice, C., Allen, C., Barber, R. M., Barregard, L., Bhutta, Z. A., et al. (2017). Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol.* 3, 524–548. doi: 10.1001/jamaoncol.2016.5688

Greten, F. R., and Grivennikov, S. I. (2019). Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity* 51, 27–41. doi: 10.1016/j.immuni.2019.06.025

Gross, G., Falck, J., Gross, E., Isbell, M., Moore, J., and Nithipatikom, K. (2005). Cytochrome and arachidonic acid metabolites: Role in myocardial ischemia/reperfusion injury revisited. *Cardiovasc. Res.* 68, 18–25. doi: 10.1016/j.cardiores.2005.06.007

Hada, M., Edin, M. L., Hartge, P., Lih, F. B., Wentzensen, N., Zeldin, D. C., et al. (2019). Prediagnostic Serum Levels of Fatty Acid Metabolites and Risk of Ovarian Cancer in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. *Cancer Epidemiol. Biomarkers Prev.* 28, 189–197. doi: 10.1158/1055-9965.EPI-18-0392

Hoopes, S. L., Garcia, V., Edin, M. L., Schwartzman, M. L., and Zeldin, D. C. (2015). Vascular actions of 20-HETE. *Prostaglandins Other Lipid Mediat.* 120, 9–16. doi: 10.1016/j.prostaglandins.2015.03.002

Hu, L., Lv, Q.-L., Guo, Y., Cheng, L., Wu, N.-Y., Qin, C.-Z., et al. (2016). Genetic variation of CYP3A5 influences paclitaxel/carboplatin-induced toxicity in Chinese epithelial ovarian cancer patients. *J. Clin. Pharmacol.* 56, 349–354. doi: 10.1002/jcph.587

Huang, Z., Fasco, M. J., Figge, H. L., Keyomarsi, K., and Kaminsky, L. S. (1996). Expression of Cvtochromes P450 in Human Breast Tissue and Tumors. *Drug Metab. Dispos.* 24, 899–905.

Hukkanen, J., Pelkonen, O., Hakkola, J., and Raunio, H. (2002). Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit. Rev. Toxicol.* 32, 391–411. doi: 10.1080/20024091064273

Imaoka, S., Hashizume, T., and Funae, Y. (2005). Localization of rat cytochrome P450 in various tissues and comparison of arachidonic acid metabolism by rat P450 with that by human P450 orthologs. *Drug Metab. Pharmacokinet.* 20, 478–484. doi: 10.2133/dmpk.20.478

Jiang, J.-G., Chen, C.-L., Card, J. W., Yang, S., Chen, J.-X., Fu, X.-N., et al. (2005). Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. *Cancer Res.* 65, 4707–4715. doi: 10.1158/0008-5472.CAN-04-4173

Jiang, J.-G., Ning, Y.-G., Chen, C., Ma, D., Liu, Z.-J., Yang, S., et al. (2007). Cytochrome p450 epoxygenase promotes human cancer metastasis. *Cancer Res.* 67, 6665–6674. doi: 10.1158/0008-5472.CAN-06-3643

Jiang, J.-G., Fu, X.-N., Chen, C.-L., and Wang, D.-W. (2009). Expression of cytochrome P450 arachidonic acid epoxygenase 2J2 in human tumor tissues and cell lines. *Ai Zheng* 28, 93–96.

Kerlikowske, K., Brown, J. S., and Grady, D. G. (1992). Should women with familial ovarian cancer undergo prophylactic oophorectomy? *Obstet. Gynecol.* 80, 700–707.

Kirkali, Z., Chan, T., Manoharan, M., Algaba, F., Busch, C., Cheng, L., et al. (2005). Bladder cancer: epidemiology, staging and grading, and diagnosis. *Urology* 66, 4–34. doi: 10.1016/j.urology.2005.07.062

Knights, K. M., Rowland, A., and Miners, J. O. (2013). Renal drug metabolism in humans: the potential for drug-endobiotic interactions involving cytochrome

P450 (CYP) and UDP-glucuronosyltransferase (UGT). *Br. J. Clin. Pharmacol.* 76, 587–602. doi: 10.1111/bcp.12086

Kroetz, D. L., and Xu, F. (2005). Regulation and inhibition of arachidonic acid omega-hydroxylases and 20-HETE formation. *Annu. Rev. Pharmacol. Toxicol.* 45, 413–438. doi: 10.1146/annurev.pharmtox.45.120403.100045

Kumarakulasingham, M. (2005). Cytochrome P450 Profile of Colorectal Cancer: Identification of Markers of Prognosis. *Clin. Cancer Res.* 11, 3758–3765. doi: 10.1158/1078-0432.CCR-04-1848

Lasker, J. M., Chen, W. B., Wolf, I., Bloswick, B. P., Wilson, P. D., and Powell, P. K. (2000). Formation of 20-hydroxyeicosatetraenoic acid, a vasoactive and natriuretic eicosanoid, in human kidney. Role of Cyp4F2 and Cyp4A11. *J. Biol. Chem.* 275, 4118–4126. doi: 10.1074/jbc.275.6.4118

Leclerc, J., Tournel, G., Courcot-Ngoubo Ngangue, E., Pottier, N., Lafitte, J.-J., Jaillard, S., et al. (2010). Profiling gene expression of whole cytochrome P450 superfamily in human bronchial and peripheral lung tissues: Differential expression in non-small cell lung cancers. *Biochimie* 92, 292–306. doi: 10.1016/j.biochi.2009.12.007

Lee, S. W., Jang, I. J., Shin, S. G., Lee, K. H., Yim, D. S., Kim, S. W., et al. (1994). CYP1A2 activity as a risk factor for bladder cancer. *J. Korean Med. Sci.* 9, 482–489. doi: 10.3346/jkms.1994.9.6.482

Leskelä, S., Honrado, E., Montero-Conde, C., Landa, I., Cascón, A., Letón, R., et al. (2007). Cytochrome P450 3A5 is highly expressed in normal prostate cells but absent in prostate cancer. *Endocr. Relat. Cancer* 14, 645–654. doi: 10.1677/ERC-07-0078

Li, A. J., and Karlan, B. Y. (2001). Genetic factors in ovarian carcinoma. *Curr. Oncol. Rep.* 3, 27–32. doi: 10.1007/s11912-001-0039-y

Li, F., Zhu, W., and Gonzalez, F. J. (2017). Potential role of CYP1B1 in the development and treatment of metabolic diseases. *Pharmacol. Ther.* 178, 18–30. doi: 10.1016/j.pharmthera.2017.03.007

Lo-Ciganic, W.-H., Zgibor, J. C., Bunker, C. H., Moysich, K. B., Edwards, R. P., and Ness, R. B. (2012). Aspirin, nonaspirin nonsteroidal anti-inflammatory drugs, or acetaminophen and risk of ovarian cancer. *Epidemiology* 23, 311–319. doi: 10.1097/EDE.0b013e3182456ad3

Luo, J., Yao, J.-F., Deng, X.-F., Zheng, X.-D., Jia, M., Wang, Y.-Q., et al. (2018). 14, 15-EET induces breast cancer cell EMT and cisplatin resistance by up-regulating integrin αvβ3 and activating FAK/PI3K/AKT signaling. *J. Exp. Clin. Cancer Res.* 37, 23. doi: 10.1186/s13046-018-0694-6

Ma, J., Zhang, L., Zhang, J., Liu, M., Wei, L., Shen, T., et al. (2013). 15-Lipoxygenase-1/15-hydroxyeicosatetraenoic acid promotes hepatocellular cancer cells growth through protein kinase B and heat shock protein 90 complex activation. *Int. J. Biochem. Cell Biol.* 45, 1031–1041. doi: 10.1016/j.biocel.2013.02.018

Mao, Y., Yang, D., He, J., and Krasna, M. J. (2016). Epidemiology of Lung Cancer. *Surg. Oncol. Clin. N. Am.* 25, 439–445. doi: 10.1016/j.soc.2016.02.001

Martínez, C., García-Martín, E., Pizarro, R. M., García-Gamito, F. J., and Agúndez, J. A. G. (2002). Expression of paclitaxel-inactivating CYP3A activity in human colorectal cancer: implications for drug therapy. *Br. J. Cancer* 87, 681–686. doi: 10.1038/sj.bjc.6600494

McDonald, M. G., Ray, S., Amorosi, C. J., Sitko, K. A., Kowalski, J. P., Paco, L., et al. (2017). Expression and Functional Characterization of Breast Cancer-Associated Cytochrome P450 4Z1 in Saccharomyces cerevisiae. *Drug Metab. Dispos.* 45, 1364–1371. doi: 10.1124/dmd.117.078188

McKay, J. A., Murray, G. I., Weaver, R. J., Ewen, S. W., Melvin, W. T., and Burke, M. D. (1993). Xenobiotic metabolising enzyme expression in colonic neoplasia. *Gut* 34, 1234–1239. doi: 10.1136/gut.34.9.1234

McKay, J. A., Melvin, W. T., Ah-See, A. K., Ewen, S. W., Greenlee, W. F., Marcus, C. B., et al. (1995). Expression of cytochrome P450 CYP1B1 in breast cancer. *FEBS Lett.* 374, 270–272. doi: 10.1016/0014-5793(95)01126-y

Melick, W. F., Escue, H. M., Naryka, J. J., Mezera, R. A., and Wheeler, E. P. (1955). The first reported cases of human bladder tumors due to a new carcinogen-xenylamine. *J. Urol.* 74, 760–766. doi: 10.1016/s0022-5347(17)67344-0

Melick, W. F., Naryka, J. J., and Kelly, R. E. (1971). Bladder cancer due to exposure to para-aminobiphenyl: a 17-year followup. *J. Urol.* 106, 220–226. doi: 10.1016/s0022-5347(17)61263-1

Michaelis, U. R., Fisslthaler, B., Medhora, M., Harder, D., Fleming, I., and Busse, R. (2003). Cytochrome P450 2C9-derived epoxyeicosatrienoic acids induce angiogenesis via cross-talk with the epidermal growth factor receptor (EGFR). *FASEB J.* 17, 770–772. doi: 10.1096/fj.02-0640fje

Michaud, V., Frappier, M., Dumas, M., and Turgeon, J. (2010). Metabolic Activity and mRNA Levels of Human Cardiac CYP450s Involved in Drug Metabolism. *PloS One* 5. doi: 10.1371/journal.pone.0015666

Miyoshi, Y., Ando, A., Takamura, Y., Taguchi, T., Tamaki, Y., and Noguchi, S. (2002). Prediction of response to docetaxel by CYP3A4 mRNA expression in breast cancer tissues. *Int. J. Cancer* 97, 129–132. doi: 10.1002/ijc.1568

Molina, J. R., Yang, P., Cassivi, S. D., Schild, S. E., and Adjei, A. A. (2008). Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship. in *Mayo Clinic Proceedings* (Elsevier Ltd), 584–594. doi: 10.4065/83.5.584

Morabia, A., and Wynder, E. L. (1991). Cigarette smoking and lung cancer cell types. *Cancer* 68, 2074–2078. doi: 10.1002/1097-0142(19911101)68:9<2074::aid-cncr2820680939>3.0.co;2-x

Morisseau, C., Schebb, N. H., Dong, H., Ulu, A., Aronov, P. A., and Hammock, B. D. (2012). Role of soluble epoxide hydrolase phosphatase activity in the metabolism of lysophosphatidic acids. *Biochem. Biophys. Res. Commun.* 419, 796–800. doi: 10.1016/j.bbrc.2012.02.108

Multhoff, G., Molls, M., and Radons, J. (2011). Chronic inflammation in cancer development. *Front. Immunol.* 2, 98. doi: 10.3389/fimmu.2011.00098

Murray, G. I., Taylor, M. C., Mcfadyen, M. C. E., Mckay, A., Greenlee, F., Burke, M. D., et al. (1997). Tumor-specific Expression of Cytochrome P450 CYP1B1 Tumor-specific. *Cancer Res.* 57, 3026–3031.

Murray, G. I., Patimalla, S., Stewart, K. N., Miller, I. D., and Heys, S. D. (2010). Profiling the expression of cytochrome P450 in breast cancer. *Histopathology* 57, 202–211. doi: 10.1111/j.1365-2559.2010.03606.x

Muskhelishvili, L., Thompson, P. A., Kusewitt, D. F., Wang, C., and Kadlubar, F. F. (2001). In situ hybridization and immunohistochemical analysis of cytochrome P450 1B1 expression in human normal tissues. *J. Histochem. Cytochem.* 49, 229–236. doi: 10.1177/002215540104900210

Nabi, S., Kessler, E. R., Bernard, B., Flaig, T. W., and Lam, E. T. (2018). Renal cell carcinoma: a review of biology and pathophysiology. *F1000Research* 7, 307. doi: 10.12688/f1000research.13179.1

Narjoz, C., Favre, A., McMullen, J., Kiehl, P., Montemurro, M., Figg, W. D., et al. (2014). Important Role of CYP2J2 in Protein Kinase Inhibitor Degradation: A Possible Role in Intratumor Drug Disposition and Resistance. *PloS One* 9, e95532. doi: 10.1371/journal.pone.0095532

Nasim, F., Sabath, B. F., and Eapen, G. A. (2019). Lung Cancer. *Med. Clin. North Am.* 103, 463–473. doi: 10.1016/j.mcna.2018.12.006

Newman, J. W., Morisseau, C., Harris, T. R., and Hammock, B. D. (2003). The soluble epoxide hydrolase encoded by EPXH2 is a bifunctional enzyme with novel lipid phosphate phosphatase activity. *Proc. Natl. Acad. Sci.* 100, 1558–1563. doi: 10.1073/pnas.0437724100

Nithipatikom, K., Brody, D. M., Tang, A. T., Manthati, V. L., Falck, J. R., Williams, C. L., et al. (2010). Inhibition of carcinoma cell motility by epoxyeicosatrienoic acid (EET) antagonists. *Cancer Sci.* 101, 2629–2636. doi: 10.1111/j.1349-7006.2010.01713.x

Node, K., Huo, Y., Ruan, X., Yang, B., Spiecker, M., Ley, K., et al. (1999). Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Sci. (80-)* 285, 1276–1279. doi: 10.1126/science.285.5431.1276

O'Flaherty, J. T., Rogers, L. A. C., Paumi, C. M., Hantgan, R. R., Thomas, L. R., Clay, C. E., et al. (2005). 5-Oxo-ETE analogs and the proliferation of cancer cells. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1736, 228–236. doi: 10.1016/j.bbalip.2005.08.009

O'Flaherty, J. T., Wooten, R. E., Samuel, M. P., Thomas, M. J., Levine, E. A., Case, L. D., et al. (2013). Fatty Acid Metabolites in Rapidly Proliferating Breast Cancer. *PloS One* 8, e63076. doi: 10.1371/journal.pone.0063076

Ortiz de Montellano, P. R. ed. (2005). *Cytochrome P450*. Boston, MA: Springer US. doi: 10.1007/b139087

Otto, J. C., and Smith, W. L. (1995). Prostaglandin endoperoxide synthases-1 and -2. *J. Lipid Mediat. Cell Signal.* 12, 139–156. doi: 10.1016/0929-7855(95)00015-i

Panigrahy, D., Greene, E. R., Pozzi, A., Wang, D. W., and Zeldin, D. C. (2011). EET signaling in cancer. *Cancer Metastasis Rev.* 30, 525–540. doi: 10.1007/s10555-011-9315-y

Panigrahy, D., Edin, M. L., Lee, C. R., Huang, S., Bielenberg, D. R., Butterfield, C. E., et al. (2012). Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. *J. Clin. Invest.* 122, 178–191. doi: 10.1172/JCI58128

Patel, S. A. A., Bhambra, U., Charalambous, M. P., David, R. M., Edwards, R. J., Lightfoot, T., et al. (2014). Interleukin-6 mediated upregulation of CYP1B1 and CYP2E1 in colorectal cancer involves DNA methylation, miR27b and STAT3. *Br. J. Cancer* 111, 2287–2296. doi: 10.1038/bjc.2014.540

Pavanello, S., Fedeli, U., Mastrangelo, G., Rota, F., Overvad, K., Raaschou-Nielsen, O., et al. (2012). Role of CYP1A2 polymorphisms on lung cancer risk in a prospective study. *Cancer Genet.* 205, 278–284. doi: 10.1016/j.cancergen.2012.02.004

Peto, R., Darby, S., Deo, H., Silcocks, P., Whitley, E., and Doll, R. (2000). Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. *BMJ* 321, 323–329. doi: 10.1136/bmj.321.7257.323

Pfister, S. L., Gauthier, K. M., and Campbell, W. B. (2010). Vascular pharmacology of epoxyeicosatrienoic acids. *Adv. Pharmacol.* 60, 27–59. doi: 10.1016/B978-0-12-385061-4.00002-7

Powell, W. S., and Rokach, J. (2015). Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1851, 340–355. doi: 10.1016/j.bbalip.2014.10.008

Powell, P. K., Wolf, I., Jin, R., and Lasker, J. M. (1998). Metabolism of arachidonic acid to 20-hydroxy-5,8,11, 14-eicosatetraenoic acid by P450 enzymes in human liver: involvement of CYP4F2 and CYP4A11. *J. Pharmacol. Exp. Ther.* 285, 1327–1336.

Pozzi, A., Macias-Perez, I., Abair, T., Wei, S., Su, Y., Zent, R., et al. (2005). Characterization of 5,6-and 8,9-Epoxyeicosatrienoic Acids (5,6-and 8,9-EET) as Potent in Vivo Angiogenic Lipids. *J Biol. Chem.* 280 (29), 27138–27146. doi: 10.1074/jbc.M501730200

Proctor, K. G., Falck, J. R., and Capdevila, J. (1987). Intestinal vasodilation by epoxyeicosatrienoic acids: arachidonic acid metabolites produced by a cytochrome P450 monooxygenase. *Circ. Res.* 60, 50–59. doi: 10.1161/01.res.60.1.50

Rieger, M. A., Ebner, R., Bell, D. R., Kiessling, A., Rohayem, J., Schmitz, M., et al. (2004). Identification of a novel mammary-restricted cytochrome P450, CYP4Z1, with overexpression in breast carcinoma. *Cancer Res.* 64, 2357–2364. doi: 10.1158/0008-5472.can-03-0849

Rifkind, A. B., Lee, C., Chang, T. K. H., and Waxman, D. J. (1995). Arachidonic acid metabolism by human cytochrome P450s 2C8, 2C9, 2E1, and 1A2: regioselective oxygenation and evidence for a role for CYP2C enzymes in arachidonic acid epoxygenation in human liver microsomes. *Arch. Biochem. Biophys.* 320, 380–389. doi: 10.1016/0003-9861(95)90023-3

Rini, B. I., Campbell, S. C., and Escudier, B. (2009). Renal cell carcinoma. *Lancet (London England)* 373, 1119–1132. doi: 10.1016/S0140-6736(09)60229-4

Risch, H. A., McLaughlin, J. R., Cole, D. E., Rosen, B., Bradley, L., Kwan, E., et al. (2001). Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. *Am. J. Hum. Genet.* 68, 700–710. doi: 10.1086/318787

Rodríguez-Blanco, G., Burgers, P. C., Dekker, L. J. M., IJzermans, J. J. N., Wildhagen, M. F., Schenk-Braat, E. A. M., et al. (2014). Serum levels of arachidonic acid metabolites change during prostate cancer progression. *Prostate* 74, 618–627. doi: 10.1002/pros.22779

Rodriguez, M., and Potter, D. A. (2013). CYP1A1 regulates breast cancer proliferation and survival. *Mol. Cancer Res.* 11, 780–792. doi: 10.1158/1541-7786.MCR-12-0675

Saad, A. M., Gad, M. M., Al-Husseini, M. J., Ruhban, I. A., Sonbol, M. B., and Ho, T. H. (2019). Trends in Renal-Cell Carcinoma Incidence and Mortality in the United States in the Last 2 Decades: A SEER-Based Study. *Clin. Genitourin. Cancer* 17, 46–57.e5. doi: 10.1016/j.clgc.2018.10.002

Sanchez, D. J., and Simon, M. C. (2018). Genetic and metabolic hallmarks of clear cell renal cell carcinoma. *Biochim. Biophys. Acta Rev. Cancer* 1870, 23–31. doi: 10.1016/j.bbcan.2018.06.003

Sausville, L. N., Gangadhariah, M. H., Chiusa, M., Mei, S., Wei, S., Zent, R., et al. (2018). The Cytochrome P450 Slow Metabolizers CYP2C9*2 and CYP2C9*3 Directly Regulate Tumorigenesis via Reduced Epoxyeicosatrienoic Acid Production. *Cancer Res.* 78, 4865–4877. doi: 10.1158/0008-5472.CAN-17-3977

Sellmayer, A., Uedelhoven, W. M., Weber, P. C., and Bonventre, J. V. (1991). Endogenous non-cyclooxygenase metabolites of arachidonic acid modulate growth and mRNA levels of immediate-early response genes in rat mesangial cells. *J. Biol. Chem.* 266, 3800–3807.

Shah, H., Patel, M., and Shrivastava, N. (2017). Gene expression study of phase I and II metabolizing enzymes in RPTEC/TERT1 cell line: application in in vitro nephrotoxicity prediction. *Xenobiotica* 47, 837–843. doi: 10.1080/00498254.2016.1236299

Shimada, T., Yamazaki, H., Mimura, M., Wakamiya, N., Ueng, Y. F., Guengerich, F. P., et al. (1996). Characterization of microsomal cytochrome P450 enzymes involved in the oxidation of xenobiotic chemicals in human fetal liver and adult lungs. *Drug Metab. Dispos.* 24, 515–522.

Siegel, R. L., Miller, K. D., and Jemal, A. (2020). Cancer statistics 2020. *CA Cancer J. Clin.* 70, 7–30. doi: 10.3322/caac.21590

Simpson, A. E. (1997). The cytochrome P450 4 (CYP4) family. *Gen. Pharmacol.* 28, 351–359. doi: 10.1016/s0306-3623(96)00246-7

Skrypnyk, N., Chen, X., Hu, W., Su, Y., Mont, S., Yang, S., et al. (2014). PPARα activation can help prevent and treat non-small cell lung cancer. *Cancer Res.* 74, 621–631. doi: 10.1158/0008-5472.CAN-13-1928

Sobue, T., Yamamoto, S., Hara, M., Sasazuki, S., Sasaki, S., Tsugane, S., et al. (2002). Cigarette smoking and subsequent risk of lung cancer by histologic type in middle-aged Japanese men and women: the JPHC study. *Int. J. Cancer* 99, 245–251. doi: 10.1002/ijc.10308

Spector, A. A., Fang, X., Snyder, G. D., and Weintraub, N. L. (2004). Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. *Prog. Lipid Res.* 43, 55–90. doi: 10.1016/S0163-7827(03)00049-3

Standop, J., Schneider, M., Ulrich, A., Büchler, M. W., and Pour, P. M. (2003). Differences in Immunohistochemical Expression of Xenobiotic-Metabolizing Enzymes Between Normal Pancreas, Chronic Pancreatitis and Pancreatic Cancer. *Toxicol. Pathol.* 31, 506–513. doi: 10.1080/01926230390226041

Stewart, L. M., Holman, C. D. J., Aboagye-Sarfo, P., Finn, J. C., Preen, D. B., and Hart, R. (2013). In vitro fertilization, endometriosis, nulliparity and ovarian cancer risk. *Gynecol. Oncol.* 128, 260–264. doi: 10.1016/j.ygyno.2012.10.023

Suzuki, M., Ishida, H., Shiotsu, Y., Nakata, T., Akinaga, S., Takashima, S., et al. (2009). Expression level of enzymes related to in situ estrogen synthesis and clinicopathological parameters in breast cancer patients. *J. Steroid Biochem. Mol. Biol.* 113, 195–201. doi: 10.1016/j.jsbmb.2008.12.008

Tao, L., Xiang, Y.-B., Chan, K. K., Wang, R., Gao, Y.-T., Yu, M. C., et al. (2012). Cytochrome P4501A2 phenotype and bladder cancer risk: The Shanghai bladder cancer study. *Int. J. Cancer* 130, 1174–1183. doi: 10.1002/ijc.26121

Thomas, J. M., Hullin, F., Chap, H., and Douste-Blazy, L. (1984). Phosphatidylcholine is the major phospholipid providing arachidonic acid for prostacyclin synthesis in thrombin-stimulated human endothelial cells. *Thromb. Res.* 34, 117–123. doi: 10.1016/0049-3848(84)90068-9

van Eijk, M., Boosman, R. J., Schinkel, A. H., Huitema, A. D. R., and Beijnen, J. H. (2019). Cytochrome P450 3A4, 3A5, and 2C8 expression in breast, prostate, lung, endometrial, and ovarian tumors: relevance for resistance to taxanes. *Cancer Chemother. Pharmacol.* 84, 487–499. doi: 10.1007/s00280-019-03905-3

Vanella, L., Di Giacomo, C., Acquaviva, R., Barbagallo, I., Li Volti, G., Cardile, V., et al. (2013). Effects of ellagic Acid on angiogenic factors in prostate cancer cells. *Cancers (Basel).* 5, 726–738. doi: 10.3390/cancers5020726

Wang, B., Wang, J., Huang, S.-Q., Su, H.-H., and Zhou, S.-F. (2009). Genetic polymorphism of the human cytochrome P450 2C9 gene and its clinical significance. *Curr. Drug Metab.* 10, 781–834. doi: 10.2174/138920009789895480

Wang, W., Yang, J., Edin, M. L., Wang, Y., Luo, Y., Wan, D., et al. (2019). Targeted metabolomics identifies the cytochrome P450 monooxygenase eicosanoid pathway as a novel therapeutic target of colon tumorigenesis. *Cancer Res.* 79, 1822–1830. doi: 10.1158/0008-5472.CAN-18-3221

Watson, P., Bützow, R., Lynch, H. T., Mecklin, J. P., Järvinen, H. J., Vasen, H. F., et al. (2001). The clinical features of ovarian cancer in hereditary nonpolyposis colorectal cancer. *Gynecol. Oncol.* 82, 223–228. doi: 10.1006/gyno.2001.6279

Wei, C., Caccavale, R. J., Kehoe, J. J., Thomas, P. E., and Iba, M. M. (2001). CYP1A2 is expressed along with CYP1A1 in the human lung. *Cancer Lett.* 171, 113–120. doi: 10.1016/s0304-3835(01)00529-8

Wei, X., Zhang, D., Dou, X., Niu, N., Huang, W., Bai, J., et al. (2014). Elevated 14,15- epoxyeicosatrienoic acid by increasing of cytochrome P450 2C8, 2C9 and 2J2 and decreasing of soluble epoxide hydrolase associated with aggressiveness of human breast cancer. *BMC Cancer* 14, 841. doi: 10.1186/1471-2407-14-841

Wentzensen, N., Poole, E. M., Trabert, B., White, E., Arslan, A. A., Patel, A. V., et al. (2016). Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium. *J. Clin. Oncol.* 34, 2888–2898. doi: 10.1200/JCO.2016.66.8178

World Health Organization (2018). *WHO | Cancer*, Available at: https://www.who.int/news-room/fact-sheets/detail/cancer.

Wu, S., Moomaw, C. R., Tomer, K. B., Falck, J. R., and Zeldin, D. C. (1996). Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. *J. Biol. Chem.* 271, 3460–3468. doi: 10.1074/jbc.271.7.3460

Xu, M., Ju, W., Hao, H., Wang, G., and Li, P. (2013). Cytochrome P450 2J2: distribution, function, regulation, genetic polymorphisms and clinical significance. *Drug Metab. Rev.* 45, 311–352. doi: 10.3109/03602532.2013.806537

Yang, S., Lin, L., Chen, J.-X., Lee, C. R., Seubert, J. M., Wang, Y., et al. (2007). Cytochrome P-450 epoxygenases protect endothelial cells from apoptosis induced by tumor necrosis factor-_ via MAPK and PI3K/Akt signaling pathways. *Am. J. Physiol. Hear. Circ. Physiol.* 293, H142–H151. doi: 10.1152/ajpheart.00783.2006

You, X. Y., Chen, J. G., and Hu, Y. N. (1990). Studies on the relation between bladder cancer and benzidine or its derived dyes in Shanghai. *Br. J. Ind. Med.* 47, 544–552. doi: 10.1136/oem.47.8.544

Yu, W., Chen, L., Yang, Y.-Q., Falck, J. R., Guo, A. M., Li, Y., et al. (2011). Cytochrome P450 ω-hydroxylase promotes angiogenesis and metastasis by upregulation of VEGF and MMP-9 in non-small cell lung cancer. *Cancer Chemother. Pharmacol.* 68, 619–629. doi: 10.1007/s00280-010-1521-8

Yu, W., Chai, H., Li, Y., Zhao, H., Xie, X., Zheng, H., et al. (2012). Increased expression of CYP4Z1 promotes tumor angiogenesis and growth in human breast cancer. *Toxicol. Appl. Pharmacol.* 264, 73–83. doi: 10.1016/j.taap.2012.07.019

Zeldin, D. C., Foley, J., Ma, J., Boyle, J. E., Pascual, J. M., Moomaw, C. R., et al. (1996a). CYP2J subfamily P450s in the lung: expression, localization, and potential functional significance. *Mol. Pharmacol.* 50, 1111–1117. doi: 10.1124/mol.51.6.931

Zeldin, D. C., Moomaw, C. R., Jesse, N., Tomer, K. B., Beetham, J., Hammock, B. D., et al. (1996b). Biochemical characterization of the human liver cytochrome P450 arachidonic acid epoxygenase pathway. *Arch. Biochem. Biophys.* 330, 87–96. doi: 10.1006/abbi.1996.0229

Zeldin, D. C., Foley, J., Goldsworthy, S. M., Cook, M. E., Boyle, J. E., Ma, J., et al. (1997). CYP2J subfamily cytochrome P450s in the gastrointestinal tract: expression, localization, and potential functional significance. *Mol. Pharmacol.* 51, 931–943. doi: 10.1124/mol.51.6.931

Zevin, S., and Benowitz, N. L. (1999). Drug interactions with tobacco smoking. An update. *Clin. Pharmacokinet.* 36, 425–438. doi: 10.2165/00003088-199936060-00004

Zhang, W. H., Wang, X., Narayanan, M., Zhang, Y., Huo, C., Reed, J. C., et al. (2003). Fundamental role of the Rip2/caspase-1 pathway in hypoxia and ischemia-induced neuronal cell death. *Proc. Natl. Acad. Sci. U. S. A.* 100, 16012–16017. doi: 10.1073/pnas.2534856100

Zhang, W., Li, H., Dong, H., Liao, J., Hammock, B. D., and Yang, G. Y. (2013). Soluble epoxide hydrolase deficiency inhibits dextran sulfate sodium-induced colitis and carcinogenesis in mice. *Anticancer Res.* 33, 5261–5272.

Zhang, G., Panigrahy, D., Hwang, S. H., Yang, J., Mahakian, L. M., Wettersten, H. I., et al. (2014). Dual inhibition of cyclooxygenase-2 and soluble epoxide hydrolase synergistically suppresses primary tumor growth and metastasis. *Proc. Natl. Acad. Sci. U. S. A.* 111, 11127–11132. doi: 10.1073/pnas.1410432111

Zhao, H., Ren, D., Liu, H., and Chen, J. (2018). Comparison and discussion of the treatment guidelines for small cell lung cancer. *Thorac. Cancer* 9, 769–774. doi: 10.1111/1759-7714.12765

Zheng, M. (2016). Classification and Pathology of Lung Cancer. *Surg. Oncol. Clin. N. Am.* 25, 447–468. doi: 10.1016/j.soc.2016.02.003

Zhou, S.-F., Zhou, Z.-W., and Huang, M. (2010). Polymorphisms of human cytochrome P450 2C9 and the functional relevance. *Toxicology* 278, 165–188. doi: 10.1016/j.tox.2009.08.013

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 19 August 2020
doi: 10.3389/fphar.2020.01289

Leukotrienes in Tumor-Associated Inflammation

Wen Tian[1,2†], *Xinguo Jiang*[1,2†], *Dongeon Kim*[1,2†], *Torrey Guan*[1,2], *Mark R. Nicolls*[1,2*] *and Stanley G. Rockson*[2*]

[1] Department of Medicine, VA Palo Alto Health Care System, Palo Alto, CA, United States, [2] Department of Medicine, Stanford University School of Medicine, Stanford, CA, United States

Leukotrienes are biologically active eicosanoid lipid mediators that originate from oxidative metabolism of arachidonic acid. Biosynthesis of leukotrienes involves a set of soluble and membrane-bound enzymes that constitute a machinery complex primarily expressed by cells of myeloid origin. Leukotrienes and their synthetic enzymes are critical immune modulators for leukocyte migration. Increased concentrations of leukotrienes are implicated in a number of inflammatory disorders. More recent work indicates that leukotrienes may also interact with a variety of tissue cells, contributing to the low-grade inflammation of cardiovascular, neurodegenerative, and metabolic conditions, as well as that of cancer. Leukotriene signaling contributes to the active tumor microenvironment, promoting tumor growth and resistance to immunotherapy. This review summarizes recent insights into the intricate roles of leukotrienes in promoting tumor growth and metastasis through shaping the tumor microenvironment. The emerging possibilities for pharmacological targeting of leukotriene signaling in tumor metastasis are considered.

Keywords: cancer, leukotrienes, inflammation, tumor microenvironment, LTB_4

OPEN ACCESS

Edited by:
Emanuela Ricciotti, University of Pennsylvania, United States

Reviewed by:
Dipak Panigrahy, Harvard Medical School, United States
Oliver Werz, Friedrich Schiller University Jena, Germany

***Correspondence:**
Mark R. Nicolls
mnicolls@stanford.edu
Stanley G. Rockson
rockson@stanford.edu

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *18 May 2020*
Accepted: *04 August 2020*
Published: *19 August 2020*

Citation:
Tian W, Jiang X, Kim D, Guan T, Nicolls MR and Rockson SG (2020) Leukotrienes in Tumor-Associated Inflammation. Front. Pharmacol. 11:1289. doi: 10.3389/fphar.2020.01289

INTRODUCTION

Low-grade inflammation and dysregulated immune responses are components of the tumor microenvironment (TME), pivotal for tumor growth and response to immunotherapies (Binnewies et al., 2018). While therapies that target the immune system, such as checkpoint inhibition, have significantly improved cancer prognosis, not all cancer patients respond to immunomodulatory treatments. Additionally, some who respond initially may develop treatment resistance and autoimmunity (Wei et al., 2018). A better understanding of the biology of TME may improve the efficacy of immunotherapies and reduce the potential adverse side-effects.

Leukotrienes are proinflammatory lipid mediators that initiate inflammation and mount adaptive immune responses for host defense (Peters-Golden et al., 2005; Peters-Golden and Henderson, 2007). Activated leukotriene signaling is implicated in inflammatory manifestations of a variety of pathologies. Recent studies have demonstrated that leukotrienes also play crucial roles in shaping the tumor microenvironment. This review summarizes recent efforts in elucidating how leukotrienes modulate tumor pathophysiology and discuss possible means to harness leukotriene signaling pathways in cancer therapeutics.

LEUKOTRIENE SYNTHESIS

There are two types of leukotrienes (LTs): the dihydroxy fatty acid leukotriene B_4 (LTB_4) and cysteinyl-leukotrienes (CysLTs), including the fatty acid-peptide conjugate LTC_4 and its metabolites, LTD_4 and LTE_4. Leukotrienes are mainly produced by myeloid cells, including, macrophages/monocytes, neutrophils, eosinophils, and mast cells (Peters-Golden et al., 2005; Peters-Golden and Henderson, 2007).

Leukotrienes biosynthesis starts from the production of polyunsaturated arachidonic acid (AA) from membrane phospholipids by phospholipase A2 (PLA2)s, especially the cytosolic form, PLA2α (cPLA2α) (Haeggstrom and Funk, 2011). 5-lipoxygenase (5-LO) is the most critical enzyme for leukotrienes production, which requires a set of stimulatory factors for its full activation, including 5-LO–activating protein (FLAP) and coactosin-like protein (Wan et al., 2017). Arachidonic acid is subsequently converted into leukotrienes in a concerted three-step reaction: first, AA is dioxygenated into 5-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid [5(S)-HpETE]; second, 5(S)-HpETE is dehydrated to yield the transient epoxide intermediate, LTA_4; and lastly, depending on the presence and functional coupling of 5-LO to its downstream enzymes, LTA_4 is further converted to LTB_4 by LTA_4 hydrolase (LTA_4H), or LTC_4 by LTC_4 synthase (LTC_4S), which conjugates LTA_4 with glutathione (Peters-Golden and Henderson, 2007; Haeggstrom and Funk, 2011; Wan et al., 2017) (**Figure 1**). Unlike many other enzymes, the catalytic activity of 5-LO and its contribution to inflammatory responses depend on its subcellular compartmentalization, phosphorylation state, and proximity to other eicosanoid-forming enzymes (Peters-Golden and Brock, 2001; Luo et al., 2003; Radmark and Samuelsson, 2009). Nuclear localized 5-LO translocates to the inner nuclear envelope and endoplasmic reticulum (ER)/Golgi membrane to facilitate the biosynthesis of LTB_4. 5-LO from the cytoplasm favors the production of CysLT and the anti-inflammatory eicosanoid lipid, such as lipoxin A_4 (LXA_4) (Peters-Golden and Brock, 2001; Luo et al., 2003; Mandal et al., 2008; Radmark and Samuelsson, 2009; Haeggstrom and Funk, 2011). Non-leukocyte cells generally do not contain the full spectrum of the synthetic enzymes of leukotrienes. However, these cells, including vascular endothelial cells, express LTA_4H and may convert LTA_4, generated by neighboring immune cells, to LTB_4, a mechanism known as transcellular LT synthesis (Gijon et al., 2007; Sala et al., 2010).

Leukotrienes signal through two sets of G-protein coupled receptors (GPCRs), with BLT1 (high affinity) and BLT2 (low affinity) serving as receptors for LTB_4, and CysLT1, CysLT2, and CysLTE (also known as gpr99) as receptors for CysLTs (Peters-Golden and Henderson, 2007; Nakamura and Shimizu, 2011). Leukotrienes act in a paracrine and cell type-dependent manner, exerting their functions at nanomolar concentrations (Peters-Golden and Henderson, 2007).

LEUKOTRIENES AND INFLAMMATORY DISORDERS

LTB_4 is noted to play essential roles in a variety of acute and chronic inflammatory diseases, including diabetes, obesity, Alzheimer's disease, myocardial infarction, asthma, idiopathic lung fibrosis, chronic obstructive pulmonary disease (COPD), pulmonary arterial hypertension (PAH), lymphedema, and cancer (Back, 2009; Drakatos et al., 2009; Izumo et al., 2009; Wang and Dubois, 2010; Price et al., 2011; Tian et al., 2013; Li et al., 2015; Qian et al., 2015; Wculek and Malanchi, 2015; Tian

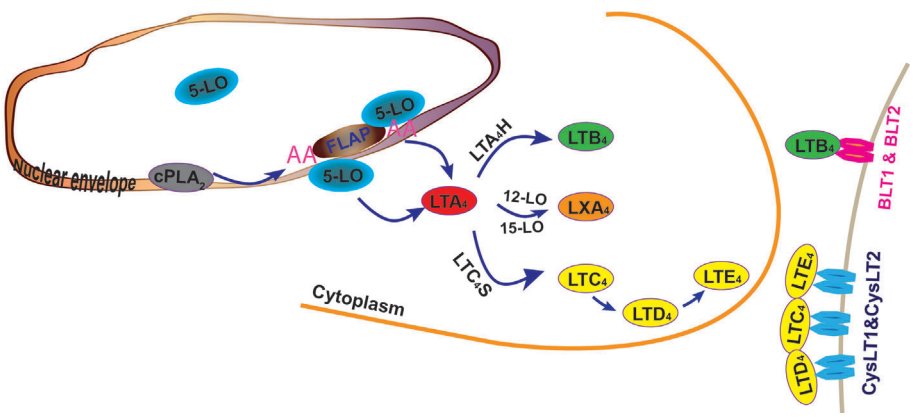


FIGURE 1 | Biosynthesis of leukotrienes. Arachidonic acid (AA) is first generated by cytoplasmic phospholipase A2 ($cPLA_2$) and converted into leukotriene A_4 (LTA_4) through the cooperative actions of 5-lipoxygenase (5-LO) and 5-LO–activating protein (FLAP). From the inner nuclear membrane, AA is metabolized into LTA_4 by inner nuclear membrane-localized 5-LO and is further converted into LTB_4 by LTA_4 hydrolase (LTA_4H). AA is also metabolized to LTA_4 by 5-LO located on the outer nuclear membrane and converted to LTC_4. LTC_4S (LTC_4 synthase) conjugates LTA_4 with glutathione to form LTC_4 and its metabolites, LTD_4 and LTE_4. (LTA_4 may also be metabolized into lipoxin A_4 [LXA_4] by 12-LO or 15-LO.) Leukotrienes signal through two sets of G-protein coupled receptors, which are receptors for LTB_4 (BLT1 and BLT2) and receptors for LTC_4, LTD_4, and LTE_4 (CysLT1 and CysLT2).

et al., 2017; Michael et al., 2019; Tian et al., 2019). Mechanisms of actions of LTB_4 in these conditions include chemotaxis for immune cell populations, facilitating endothelial adherence, and induction of blood and lymphatic vascular endothelial injury (Back, 2009; Drakatos et al., 2009; Izumo et al., 2009; Wang and Dubois, 2010; Price et al., 2011; Tian et al., 2013; Li et al., 2015; Qian et al., 2015; Wculek and Malanchi, 2015; Tian et al., 2017; Michael et al., 2019; Tian et al., 2019). LTB_4 is one of the most powerful identified chemotactic molecules (Subramanian et al., 2017). LTB_4 not only summons neutrophils, macrophages, mast cells, eosinophils, dendritic cells (DCs), T cells, and B cells to the site of tissue injury but also promotes the survival of these immune cells in lymphoid organs and peripheral tissues (Del Prete et al., 2007; Peters-Golden and Henderson, 2007; Subramanian et al., 2017). CysLTs is endowed with overlapping but distinct function in promoting inflammation responses when compared with LTB_4. The classical properties of CysLTs in the vasculature, LTD_4 in particular, are the regulation of smooth muscle contraction in the microcirculation and respiratory tract (Peters-Golden and Henderson, 2007; Araujo et al., 2018). CysLTs also induce pathological angiogenesis, maladaptive proliferative responses, expression of adhesion molecules (I-CAM-1 and VCAM-1), and loss of endothelial barrier function (Sjostrom et al., 2003; Uzonyi et al., 2006; Moos et al., 2008; Duah et al., 2013).

In the lung, 5-LO and LTB_4 are elevated in the airways of asthma and COPD patients, as well as in the pulmonary arterioles of PAH patients, where the concentrations of LTB_4 correlate with the severity of the disease. The upregulation of LTB_4 and subsequent interaction with its receptors stimulate the migration of immune cells into the lungs (Drakatos et al., 2009; Tian et al., 2013). Specifically, LTB_4 promotes the migration of immature and mature DCs along the gradient of CCL19 and CCL21 by increasing the membrane expression of CCR7 (Del Prete et al., 2007). LTB_4 induces the rapid integrin-mediated arrest of rolling of effector and memory $CD8^+$ cells and thereby mediates cytotoxic T cell trafficking (Goodarzi et al., 2003). Mast cells may secrete LTB_4 and recruit T cells in response to tissue inflammation and infection, including allergy, asthma, and rheumatoid arthritis (Goodarzi et al., 2003). Mast cells and macrophages promote the migration of neutrophils in the presence of LTB_4. Notably, 5-LO and LTB_4 also interact with a number of pulmonary proinflammatory signaling molecules, including NF-κB, TNF-α, MAPK, and IL-6/STAT3, suggesting that 5-LO/LTB_4 may further amplify the proinflammatory circuits. As an example, mice lacking 5-LO or BLT1 are impaired in TLR (toll-like receptor)-mediated NF-κB activation in pulmonary macrophages (Serezani et al., 2011).

In addition to being immunomodulatory in the lung, recent efforts also suggest that 5-LO and LTB_4 exert key roles in the pulmonary vasculature: LTB_4 promotes the growth and activation of pulmonary arterial smooth muscle cell (SMC) and adventitial fibroblasts. 5-LO and LTB_4 also induce early death of pulmonary arterial endothelial cells (ECs), cause the survival cells to become apoptosis-resistant and proliferative, change the properties of these ECs to acquire endogenous 5-LO expression, and transform them into proinflammatory phenotypes; responses are implicated in the PAH pathogenesis (Tian et al., 2013; Qian et al., 2015; Tian et al., 2019). Prominent expression of LTB_4 in the brain, adrenals, heart, adipose tissues, skin, as well as, in lymph fluid suggests an additional, less well-recognized function of this eicosanoid lipid. Emerging evidence indicates a pivotal role of LTB_4 in insulin resistance and hepatic steatosis, with LTB_4 directly enhancing macrophage chemotaxis, reducing insulin-stimulated glucose uptake in myocytes, and inhibiting insulin-mediated suppression of hepatic glucose output (Li et al., 2015). LTB_4 induces the migration and proliferation of coronary artery SMCs, and BLT1 is highly expressed in the human carotid artery atherosclerotic plaques (Back, 2009). Expression of 5-LO is significantly elevated in post-mortem brain tissues of neurodegenerative diseases; also, blocking 5-LO is beneficial to aged transgenic mice with pre-existing behavioral abnormality and tau neuropathology (Tian et al., 2017; Giannopoulos et al., 2019). 5-LO activation and increased LTB_4 concentrations are found in animal model and human lymphedema (Tian et al., 2017). LTB_4, at low physiological concentrations, is required for lymphangiogenesis and wound healing (Tian et al., 2017; Ramalho et al., 2018). Conversely, LTB_4, in high pathological concentrations, interferes with the protective VEGFR3 and Notch signaling in lymphatic endothelial cells, causing lymphatic vascular damage, abnormal lymphatic drainage, and lymphedema (Tian et al., 2017). The pivotal roles of LTB_4 signaling in lymphedema are further supported in two Phase II clinical studies, where pharmacological blockage of LTB_4 signaling is beneficial in restoring a failing lymphatic circulation (Rockson et al., 2018).

Cysteinyl leukotriene production has been directly linked to the pathogenesis of asthma (Peters-Golden and Henderson, 2007; Price et al., 2011). Extensive evidence shows that CysLTs regulate most of the key features of asthma, including airway SMC constriction, increased microvascular permeability, compromised respiratory cilia activities, airway remodeling, bronchial hyperresponsiveness, and maladaptive survival of immune cells (Price et al., 2011). Importantly, three selective antagonists of CysLT receptors (i.e., pranlukast, montelukast, zafirlukast) and the inhibitor of 5-LO (zileuton, zyflo^R) have been used in the clinical management of asthma for nearly 20 years (Price et al., 2011). These drugs are well-tolerated and efficient in restricting bronchoconstrictor challenge mediated by allergen and exercise. CysLTs are also key mediators in allergic rhinitis, acting on nasal vascular ECs, enhancing DC-stimulated antigen presentation, activating interstitial cells (eosinophils, mast cells, macrophages, and neutrophils), and modulating nasal allergic inflammation and clinical symptoms (Sousa et al., 2002).

INFLAMMATION IN CANCER

Chronic inflammation is a significant risk factor for the development of cancer (Coussens and Werb, 2002). Immune cells, orchestrating with cancer cells and surrounding stromal

cells, form an inflammatory TME (Wang et al., 2017). Cells within the TME are highly plastic. Inflammation may shape the TME towards a more malignant state and direct tumor-promoting signals (**Figure 2**). Recent transcriptomic and metabolic studies of the TME indicate enhanced expression of inflammatory cytokines and chemokines in primary tumors and metastatic lesions, which is correlated with an increased number of inflammatory infiltrates and poor clinical prognosis (Galon et al., 2006; Zhang et al., 2020). Neutralization or genetic silencing of inflammatory signaling in preclinical models diminishes tumor growth and progression. As an example, colitis-associated cancer is associated with IL-23 producing myeloid cells and IL-23-dependent TME (McGovern and Powrie, 2007). This review focuses on discussing the tumor-promoting functionality of inflammation.

Inflammation and Tumor Initiation

Over 15% of cancers are predisposed by infection, chronic inflammation, and autoimmunity (Abu-Shakra et al., 2001; Greten and Grivennikov, 2019; Valencia et al., 2019). The most prominent examples include inflammatory bowel disease that may risk patients for colon cancer, and chronic hepatitis increases the likelihood of developing liver cancer (Uko et al., 2012). A variety of proinflammatory environmental cues may prompt cancer development, including inhalation of fine particles and tobacco smoke. Obesity, hyperglycemia, and excessive lipid accumulation promote low-grade inflammation and increase the risk of liver, pancreatic, colon, breast cancer, and other malignancies (Abu-Shakra et al., 2001; Pietrzyk et al., 2015; Greten and Grivennikov, 2019; Valencia et al., 2019). Type II diabetes is increasingly recognized as a risk factor for cancer, promoting tumorigenesis through obesity-induced inflammation and obesity-related tissue injury (Giovannucci et al., 2010).

Two interdependent events are postulated to be required for tumor initiation: 1) genetic and epigenetic alterations of tumor-suppressive pathways and oncogenic signaling, and 2) generation and growth of transformed cell clones. Inflammation, driven by macrophages and neutrophils, potently produces reactive oxygen species, which may induce gene variants (Mittal et al., 2014). Inflammation may cause germline and somatic mutations in *Tp53* and other cancer-related genes and increase the tumor mutational burden (Werner et al., 2020). IL-22, IL-6, TNF-α, and IL-1β not only cause DNA damage but also activate epigenetic machinery in epithelial cells (Grivennikov et al., 2010). Inflammatory responses may trigger the de-differentiation of epithelial cells into tumor-initiating stem cell-like cells (Sainz et al., 2016; Ayob and Ramasamy, 2018). Notably, NF-κB and IL-6/STAT3 signaling increase the survival and proliferation of the transformed cells, so-called inflammation-driven cell survival (Grivennikov et al., 2010; Chaturvedi et al., 2011; Kumari et al., 2016). Evident in liver and skin cancers, inflammation-induced cell death is required for the growth of neighboring transformed tumor cells (i.e., autophagy) (Yun and Lee, 2018). While inflammation in early-stage tumors is localized, systemic inflammation prevails during the late stage of tumor invasion and magnify the cancer sequela, as exemplified by tobacco-smoke and obesity activating neutrophils to promote breast cancer metastasis into the lungs (Walser et al., 2008; Yu et al., 2018).

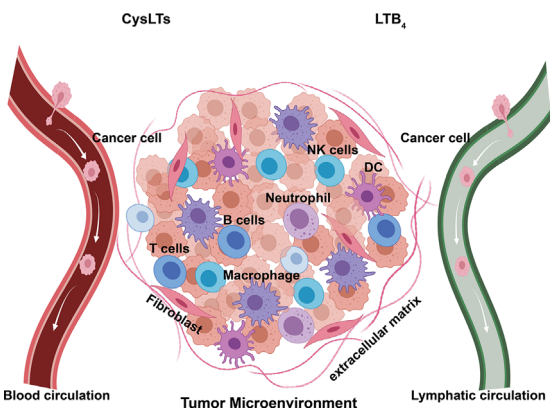


FIGURE 2 | Process of cancer metastasis. 1) Genetic risks and environmental factors (e.g., inflammation) cause epithelial cell transform into cancer cell phenotype. 2) Cancer cells and cancer stem cells proliferate to generate primary tumor. 3) The carcinoma cells recruit a variety of stromal cells and immune cells to form tumor microenvironment (TME). 4) Cancer cells, under the influence of TME, acquire an invasive phenotype through epithelial-to-mesenchymal-transition (EMT) and intravasate into blood vascular or lymphatic circulation. 5) The malignant cancer cells exit the circulation and develop into secondary tumor at the distant organs.

Inflammation and Tumor Progression

In a fashion similar to tumor initiation, inflammation provides direct growth signaling for tumor proliferation (Coussens and Werb, 2002). Additionally, inflammatory mediators may induce tumor cell plasticity within the TME by antagonizing potential anti-tumor immunity, stimulating angiogenesis, and recruiting fibroblasts and other stromal cells to support tumor metastasis. Furthermore, inflammatory molecules modify stromal and tumor cells metabolism and tissue stiffness by regulating the formation of extracellular matrix (Chaturvedi et al., 2011). For instance, IL-6, IL-17, and IL-11 may increase the proliferation of tumor cells, under conditions, such as chronic hypoxia, lack of nutrients, or insufficiency of anti-tumor immunity (Muz et al., 2015; Kumari et al., 2016; Zhong et al., 2016; McGeachy et al., 2019). IL-8 has been shown to recruit macrophages and neutrophils to the TME and to stimulate the angiogenic responses of vascular ECs in a paracrine manner. IL-8 may also induce carcinoma cells to acquire a mesenchymal-like phenotype (i.e., epithelial-to-mesenchymal transition, EMT) (Waugh and Wilson, 2008).

Inflammation and Tumor Metastasis

Majority of tumor-related death is due to cancer metastasis. Growing number of studies support the role of inflammation in cancer mortality (Coussens and Werb, 2002). The migration of primary cancer cells away from the epithelium into the neighboring tissues depends on EMT (Brabletz et al., 2018; Pastushenko and Blanpain, 2019). EMT enhances the mobility of cancer cells and allows them to break from the basal membrane and enter into lymphatic and blood circulation for

further dissemination (Brabletz et al., 2018; Pastushenko and Blanpain, 2019). Cancer stem cells adapt transcriptional and functional similarities to mesenchymal cells in motility (Brabletz et al., 2018; Pastushenko and Blanpain, 2019). TNF-α and IL-1β directly induce the expression of key transcription factors for EMT, SLUG, SNAIL, and Twist (Brabletz et al., 2018; Pastushenko and Blanpain, 2019). IL-11 is associated with the recruitment of fibroblasts, supporting tumor invasion, immune escape, and selection of malignant cancerous cells; increased IL-11 expression is correlated with worse clinical prognosis (Zhong et al., 2016). IL-17 activates neutrophils and drives breast cancer metastasis by facilitating the formation of a pre-metastatic niche (Gu et al., 2015). Inflammatory signals also prompt the expression of tissue-specific adhesion molecules and integrins, thereby aid the generation of tropism of metastasis (Bendas and Borsig, 2012). In malignant tissue, tumor-associated macrophages (TAMs) are the most abundant cell types of TME; these polarized macrophages mediate tumor growth and angiogenesis, secrete pro-tumor signaling molecules, and suppressing anti-tumor adaptive immune responses (Noy and Pollard, 2014; Che et al., 2017).

Therapy-Induced Inflammation in Cancer

Chemotherapy, radiotherapy, and surgical interventions may generate inflammatory responses as well. Damage-associated molecular patterns (DAMPs) released from dying tumor cells may regulate the synthesis of IL-1β and other cytokines; DAMPs also induce and sustain *de novo* anti-tumor T cell responses (Hernandez et al., 2016). In contrast, dying tumor cells can stimulate the production of TNF-α, epidermal growth factor (EGF), IL-6, and Wnt ligands, which in turn recruit myeloid cells and fibroblasts to the local TME, serving as anti-cell death signals and decreasing the efficacy of anti-tumor therapies (Labi and Erlacher, 2015). For example, paracrine EGFs, which are secreted from macrophages or fibroblasts, are major factors causing cancer therapy resistance (Srivatsa et al., 2017).

LEUKOTRIENES IN CANCER

Elevated 5-LO and leukotriene signaling is reported in various forms of cancers, including cancer of the pancreas, colon, stomach, prostate, ovaries, and lungs (Wang and Dubois, 2010; Jala et al., 2017). These proinflammatory mediators may modulate the initiation, progression, and metastasis of tumors through regulating the proliferation, apoptosis, migration, and invasion of cancer cells. 5-LO and leukotriene signaling may also be capable of shaping the TME through inducing the migration and activation of immune cells, production of growth factors, secretion of proinflammatory mediators and angiogenic factors; they may also directly interact with blood and lymphatic endothelium and influence the migration of cancer cells (**Figure 3**). The remainder of this review discusses the specific links between 5-LO and leukotriene-mediated signaling in cancer by summarizing and speculating the roles of 5-LO and leukotriene in promoting tumorigenesis and tumor microenvironment.

5-LO and Leukotriene Signaling and Cell Growth

Western/Northern blotting and histology are not able to detect 5-LO expression in healthy pulmonary ECs (Walker et al., 2002; Zhang et al., 2002; Porter et al., 2014). However, antagonizing 5-LO signaling arrests ECs from dividing in culture and chronic hypoxia induces abnormal EC proliferation in a 5-LO-dependent fashion, implicating that 5-LO expression is required for EC mitosis (Walker et al., 2002; Porter et al., 2014). Additionally, 5-LO is expressed in the nuclei of a number of cancer stem cells; 5-LO causes ECs lack of BMPR2 signaling to transform into a cancer stem cell-like phenotype, and targeting 5-LO suppresses the adverse growth responses (Romano et al., 2001; Chen et al., 2009a; Chen et al., 2009b; Roos et al., 2014; Tian et al., 2019). The mechanisms by which 5-LO signaling regulate cell proliferation is not understood but is postulated to be related to the nuclear localization of this eicosanoid enzyme. Consistently, inhibition of LTA_4H impedes the growth of colon cancer cells, suggesting that LTB_4 is essential for cancer cell growth (Jeong et al., 2009; Lin et al., 2016; Zhao et al., 2019). LTB_4 directly stimulates colon cancer cell growth and survival through a BLT1/ERK-dependent pathway *in vitro*; it induces the proliferation of human pancreatic cancer cells through MAPK/ERK and PI3K/Akt-dependent pathways; both LTB_4 and CysLTs prompt the expansion of $CD24^+CD90^+$ metastasis-initiating cells (Tong et al., 2005; Ihara et al., 2007; Wculek and Malanchi, 2015). Activation of LTD_4 promotes the growth and survival of human intestinal epithelial cells through multiple parallel pathways, including GSK3β/β-catenin, PKC/Raf/ERK1, and ERK2 signaling (Paruchuri et al., 2002; Mezhybovska et al., 2006). By contrast, inhibition of LTD_4 signaling by a CysLT1 antagonist causes apoptosis of prostate carcinoma cells (Matsuyama et al., 2007; Larre et al., 2008). Increased CysLT1 and CysLT2 expression are associated with LTD_4-induced apoptosis-resistance, hyper-proliferation of colorectal cancer cells, and poor clinical prognosis (Magnusson et al., 2007; Jeong et al., 2009). A prominent role of leukotrienes in the regulation of stem cell homeostasis is established, with LTB_4 and LTD_4 known to stimulate the proliferation and maturation of several types of stem cells, progenitor cells, and cancer stem cells (Chung et al., 2005; Paruchuri et al., 2006; Wada et al., 2006).

Leukotrienes and Epithelial (Endothelial)-to-Mesenchymal Transition

Alterations in cell phenotypes, EMT in particular, have been shown to play an important role in tumorigenic processes (Brabletz et al., 2018; Pastushenko and Blanpain, 2019). Complete and partial EMT in cancer is executed by EMT-activating transcription factors, including SNAIL, TWIST, and ZEB families, which regulate all stages of cancer progression from initiation, primary tumor growth, invasion, metastasis, to colonization, as well as resistance to therapy (Brabletz et al., 2018; Pastushenko and Blanpain, 2019). In addition, EMT-activating transcription factors have been shown to be critical for the maintenance of cancer stemness (Pastushenko and Blanpain, 2019). Specifically, studies focusing on IL-6, IL-8, and TNF-α

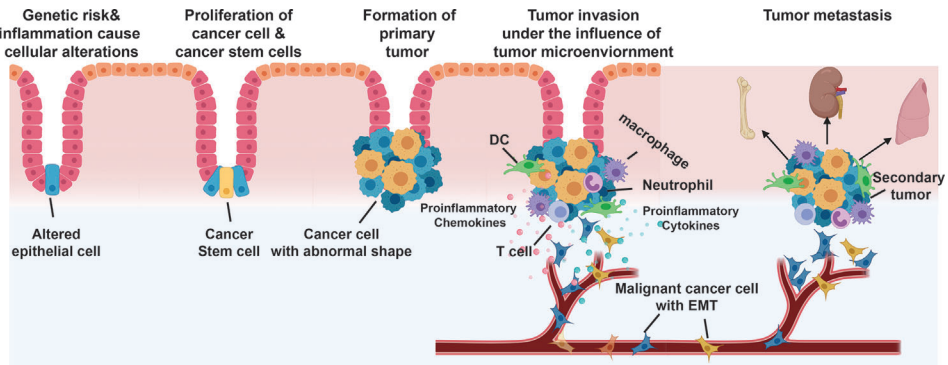


FIGURE 3 | Components of tumor microenvironment (TME). Established primary tumor consists of a wide array of cellular infiltrates, including immune cells of both innate and adaptive immunity. These cells secrete proinflammatory cytokines, chemokines, and leukotrienes, as well as form a complex regulatory network that fosters tumor metastasis by creating an environment enabling cancer to evade immune surveillance and destruction. DC, dendritic cells; NK cells, natural killing cells; CysLTs, LTC_4, LTD_4, and LTE_4.

demonstrate that expression of these inflammatory mediators is associated with clinical occurrences of EMT and resistance to EGFR inhibition (Singh et al., 2013; Thompson et al., 2015).

In culture, LTB_4, *via* BLT2, promotes EMT and the expression of vimentin in some human cancer cell lines through activation of reactive oxygen species, NF-κB, TGF-β, and ERK (Kim et al., 2014). LTB_4 also upregulates the production of IL-8 in breast cancer cells, while blocking BLT2 suppresses the formation of metastatic lung nodules in animal models of breast cancer metastasis (Kim et al., 2012). In coordination with NF-κB signaling, LTB_4 mediates the synthesis of both IL-6 and IL-8 to increase the invasiveness of cancer cells (Thompson et al., 2015). Exogenous LTB_4 or 5-LO causes pulmonary arterial EC, derived from patients with BMPR2 mutations, to undergo endothelial-to-mesenchymal-transition (EndMT) through activated TGF-β signaling; these cells lose the typical endothelial cobblestone appearance and, instead, acquires an elongated mesenchymal cell shape with increased expression of mesenchymal markers (vimentin, SM-actin, and SLUG) and inflammatory molecules (IL-6, IL-1β, and TNF-α), a process resembling EMT (Tian et al., 2019).

Leukotrienes and Tumor Microenvironment

A growing body of literature indicates that 5-LO and leukotrienes are critical components TME, mediating the crosstalk between epithelial cells, stromal cells, and immune cells. In lung cancer, a complex molecular interaction initiated by mast cell-derived LTB_4 has been described, in which mast cells orchestrate with tumor-promoting neutrophils through production of LTB_4 (Malaviya and Abraham, 2000; Satpathy et al., 2015). Neutrophils support the metastatic transformation and colonization of breast cancer cells through leukotriene-mediated ERK activation (Wculek and Malanchi, 2015). Treatment with zileuton reduces systemic inflammation, blocks macrophage infiltrates, and decreases polyp burden of the small intestine and colon in a murine model of polyposis (Gounaris et al., 2015). Genetic deletion or pharmacological ablation of 5-LO or LTA_4H significantly reduces the tumor burden in K-ras–driven pancreatic ductal adenocarcinoma and in xenograft mouse model of human pancreatic cancer, through reduction of TNF-α secretion (Oi et al., 2010; Knab et al., 2015). TNF-α, one of the main cytokines in the TME, has a context-dependent role in tumor growth (Balkwill, 2009). In a cooperative manner with TNF-α, LTB_4 may influence the growth, survival, invasion, and metastasis of tumor cells.

Leukotrienes and Tumor Suppression

Human and animal studies suggest a positive correlation of prolonged survival with the presence of tumor-infiltrating $CD8^+$ T cells (Galon et al., 2006; Kmiecik et al., 2013). LTB_4/BLT1 signaling is required for cytotoxic T cells accumulation during allergic inflammation (Miyahara et al., 2005). A recent study demonstrates a pivotal function of LTB_4/BLT1 signaling for the tumor immune suppression of $CD8^+$ T cells: $CD8^+$ T cells depletion enhances tumor growth in wild-type but not in *BLT1*$^{-/-}$ mice, implicating the importance of BLT1 in $CD8^+$ T cells cancer immunity (Sharma et al., 2013). The presence of tumor-infiltrating lymphocytes correlates with the responsiveness to PD-1 (programmed cell death protein 1)-targeting cancer therapies (Yao et al., 2018). Notably, PD-1 blockade fails to reduce melanoma growth in *BLT1*$^{-/-}$ mice due to deficiency in T cell infiltrations (Chheda et al., 2016). Collectively, these findings suggest an important role of LTB_4 signaling to facilitate the migration of tumor-infiltrating lymphocytes in anti-tumor immunity in cancer.

Leukotrienes in Angiogenesis and Lymphangiogenesis

To initiate metastasis, cancer cells disseminate from the primary tumor either through blood (hematogenous spread after angiogenesis) or through the lymphatic (lymphogenous spread after lymphangiogenesis) circulation (Christiansen and Detmar, 2011; Ziyad and Iruela-Arispe, 2011). It is generally believed that alterations in the primary TME and EMT of tumor cells facilitate

the migration towards blood or lymphatic vessels (Christiansen and Detmar, 2011; Ziyad and Iruela-Arispe, 2011). Although not fully understood how the route of intravasation is determined (i.e., hematogenous or lymphogenous), inflammatory signaling appears to be the key regulator attracting tumor cells towards circulation (Noonan et al., 2008; Zuazo-Gaztelu and Casanovas, 2018). Blocking 5-LO or LTB_4/BLT2 signaling significantly inhibits the VEGF-A-mediated angiogenic responses (Tsopanoglou et al., 1994). LTC_4 and LTD_4, working through CysLT2, enhance angiogenesis and the permeability of blood vessels, independent of VEGF-A signaling, and thereby, contribute to tumor metastasis (Kim et al., 2009). LTB_4 poses bimodal effects on lymphatic vessel health: at low concentrations, LTB_4 promotes lymphatic EC growth and sprouting, while high concentrations of LTB_4 inhibit lymphangiogenesis and induce apoptosis of lymphatic ECs (Tian et al., 2017). Recent studies have suggested that a viable lymphatic vasculature promotes the efficacy of immunotherapy (Fankhauser et al., 2017; Jiang, 2020). Therefore, LTB_4 may also influence immunotherapeutics by affecting the growth and survival of lymphatic vessels.

THERAPEUTIC INTERVENTIONS TARGETING LEUKOTRIENES AND 5-LO

Many anti-inflammatory agents, including NSAIDs (nonsteroidal anti-inflammatory drugs) which inhibits cyclooxygenase (COX-1, COX-2, or both) or both COX and 5-LO, as well as, specific inhibitors of 5-LO and leukotriene signaling, have demonstrated promising results in interfering with the tumor microenvironment (Cruz-Correa et al., 2002; Baron, 2003; Rayburn et al., 2009; Wong, 2019). In particular, a meta-analysis of 16 independent studies, including 202,780 patients with a diagnosis of prostate, lung, colorectal, and breast cancers, suggests the potential of NSAID in reducing tumor incidence and mortality (Cruz-Correa et al., 2002). Specifically, familial adenomatous polyposis (FAP) patients receiving NSAID (celecoxib or sulindac), dual blockers of COX-2 and 5-LO, display a decreased recurrence, lower polyp number, and a regression of existing adenomas (Steinbach et al., 2000; Cruz-Correa et al., 2002; Maier et al., 2008; Steinbrink et al., 2010; Lynch et al., 2016). Celecoxib was approved by FDA as adjuvant therapy for FAP in 2011. The potential usage of rofecoxib or valdecoxib, two other dual inhibitors of COX-2 and 5-LO, as adjuvant therapy for tumor metastasis, is still under investigation. Nevertheless, the application of NSAIDs as anticancer agents remains controversial because of their possible gastrointestinal and cardiovascular toxicity.

Antagonists specific for 5-LO and leukotrienes are well-tolerated and confer no adverse effect in the gastrointestinal and cardiovascular systems. Despite numerous reports of the anti-tumor properties of these agents in preclinical and cell culture studies, only a few clinical trials have been conducted to evaluate their potential in cancer treatment. The 5-LO inhibitor, zileuton, has shown positive results in treating experimental models of colon, lung, and pancreatic cancers (Rioux and Castonguay, 1998; Wenger et al., 2002; Chen et al., 2004; Wculek and Malanchi, 2015). Blocking FLAP also indicates therapeutic benefit in the hamster model of pancreatic cancer. A few clinical trials have been conducted using LY293111, a well-tolerated inhibitor of BLT1, in patients with pancreatic cancer and non-small cell lung cancer; no significant difference in short-term survival was noted from these human studies (Ding et al., 2005; Janne et al., 2014). Notably, LTA_4H inhibitor, ubenimex, has been marketed in Japan for over 30 years as an adjunct therapy for adult acute leukemia and lung cancers.

Various natural compounds, found in food and plants, may inhibit COX-2 and 5-LO pathways. Resveratrol (enriched in grapes and red wine), ginsenosides (found in ginseng), s-allylmercaptocysteine (purified from garlic), and turmeric curcumin (present in curry) are among these categories (Das and Das, 2007; Park et al., 2007; Li et al., 2014; Jeong et al., 2016). A number of recent clinical studies demonstrate encouraging results in the usage of these natural compounds in combination with conventional cancer therapies, possibly through arresting cancer cell growth, potentiating cellular apoptosis, inhibiting NF-κB, MAPK, JNK, and VEGF pathways and sequestering reactive oxygen species (Hassan, 2004; Park et al., 2007; Hofseth and Wargovich, 2007; Howard et al., 2008).

CONCLUDING REMARKS

Promising preclinical results have motivated the pharmaceutical industry to develop anti-5-LO and anti-leukotriene drugs to treat a wide range of cancers (Orafaie et al., 2020). To date, most of the small molecule inhibitors in these classes failed to merit clinical application. Leukotrienes are typically generated by activated leukocytes; therefore, the impact of these mediators depends on the temporal accumulation and composition of immune cells at various stages of cancer. Leukotrienes fall into a big category of eicosanoid metabolites which are interconnected in a complex manner. Modulating one pathway may likely cause shunting the eicosanoid synthesis to lipids with opposing bioaction [e.g., blocking macrophage production of LTB_4 causes increased expression of PGE_2, which is implicated in multiple pro-tumor responses (Nakanishi and Rosenberg, 2013)]. Inflammation is the primary risk factor for the development of certain types of cancers. The proinflammatory tumor microenvironment is pivotal to the spread of cancer. However, immunotherapies, such as checkpoint inhibition and adoptive cell transfer, may benefit from a fine-tuned and tumor-specific T cell response. Understanding the communications between cancer and inflammation will facilitate the discovery of the next breakthrough in cancer management. 5-LO and leukotriene signalling is a critical component of the inflammatory tumor microenvironment: leukotrienes are strong chemoattractants for leukocytes; 5-LO and leukotrienes may elicit robust immune reactions by promoting the growth and survival of immune cells; 5-LO and leukotrienes participate in the maladaptive growth response of cancer cells and may modulate the expansion of

blood and lymphatic vessels around primary tumor (i.e., angiogenesis and lymphangiogenesis); and last but not least, interactions between 5-LO and leukotriene signaling and a number of inflammatory pathways (e.g., COX, IL-1, IL-6, IL-17, NF-κB, and TNF-α) are identified in various inflammatory conditions, including cancers. Elucidating the biology of eicosanoids in tumorigenesis, profiling these biologically active lipids and their associated enzymes and receptors in cancer, and gaining structural insight into the eicosanoid proteins are crucial steps to inform future efforts in developing biomarkers and designing drug targets.

AUTHOR CONTRIBUTIONS

Conceived and participated in the writing of this manuscript (WT, XJ SR). Assisted in the preparation and editing of the manuscript (DK, TG, MN).

FUNDING

This work was supported by Stanford Endowed Chair funds to (MN and SR), the NIH K12 HL120001-05 (to XJ) and the NIH HL095686, HL141105, HL138473 to (MN).

REFERENCES

Abu-Shakra, M., Buskila, D., Ehrenfeld, M., Conrad, K., and Shoenfeld, Y. (2001). Cancer and autoimmunity: autoimmune and rheumatic features in patients with malignancies. *Ann. Rheum. Dis.* 60 (5), 433–441. doi: 10.1136/ard.60.5.433

Araujo, A. C., Wheelock, C. E., and Haeggstrom, J. Z. (2018). The Eicosanoids, Redox-Regulated Lipid Mediators in Immunometabolic Disorders. *Antioxid. Redox Signal* 29 (3), 275–296. doi: 10.1089/ars.2017.7332

Ayob, A. Z., and Ramasamy, T. S. (2018). Cancer stem cells as key drivers of tumour progression. *J. BioMed. Sci.* 25 (1), 20. doi: 10.1186/s12929-018-0426-4

Back, M. (2009). Leukotriene signaling in atherosclerosis and ischemia. *Cardiovasc. Drugs Ther.* 23 (1), 41–48. doi: 10.1007/s10557-008-6140-9

Balkwill, F. (2009). Tumour necrosis factor and cancer. *Nat. Rev. Cancer* 9 (5), 361–371. doi: 10.1038/nrc2628

Baron, J. A. (2003). Epidemiology of nonsteroidal anti-inflammatory drugs and cancer. *Prog. Exp. Tumor Res.* 37, 1–24. doi: 10.1159/000071364

Bendas, G., and Borsig, L. (2012). Cancer cell adhesion and metastasis: selectins, integrins, and the inhibitory potential of heparins. *Int. J. Cell Biol.* 2012, 676731. doi: 10.1155/2012/676731

Binnewies, M., Roberts, E. W., Kersten, K., Chan, V., Fearon, D. F., Merad, M., et al. (2018). Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat. Med.* 24 (5), 541–550. doi: 10.1038/s41591-018-0014-x

Brabletz, T., Kalluri, R., Nieto, M. A., and Weinberg, R. A. (2018). EMT in cancer. *Nat. Rev. Cancer* 18 (2), 128–134. doi: 10.1038/nrc.2017.118

Chaturvedi, M. M., Sung, B., Yadav, V. R., Kannappan, R., and Aggarwal, B. B. (2011). NF-kappaB addiction and its role in cancer: 'one size does not fit all'. *Oncogene* 30 (14), 1615–1630. doi: 10.1038/onc.2010.566

Che, D., Zhang, S., Jing, Z., Shang, L., Jin, S., Liu, F., et al. (2017). Macrophages induce EMT to promote invasion of lung cancer cells through the IL-6-mediated COX-2/PGE2/beta-catenin signalling pathway. *Mol. Immunol.* 90, 197–210. doi: 10.1016/j.molimm.2017.06.018

Chen, X., Wang, S., Wu, N., Sood, S., Wang, P., Jin, Z., et al. (2004). Overexpression of 5-lipoxygenase in rat and human esophageal adenocarcinoma and inhibitory effects of zileuton and celecoxib on carcinogenesis. *Clin. Cancer Res.* 10 (19), 6703–6709. doi: 10.1158/1078-0432.CCR-04-0838

Chen, Y., Hu, Y., Zhang, H., Peng, C., and Li, S. (2009a). Loss of the Alox5 gene impairs leukemia stem cells and prevents chronic myeloid leukemia. *Nat. Genet.* 41 (7), 783–792. doi: 10.1038/ng.389

Chen, Y., Li, D., and Li, S. (2009b). The Alox5 gene is a novel therapeutic target in cancer stem cells of chronic myeloid leukemia. *Cell Cycle* 8 (21), 3488–3492. doi: 10.4161/cc.8.21.9852

Chheda, Z. S., Sharma, R. K., Jala, V. R., Luster, A. D., and Haribabu, B. (2016). Chemoattractant Receptors BLT1 and CXCR3 Regulate Antitumor Immunity by Facilitating CD8+ T Cell Migration into Tumors. *J. Immunol.* 197 (5), 2016–2026. doi: 10.4049/jimmunol.1502376

Christiansen, A., and Detmar, M. (2011). Lymphangiogenesis and cancer. *Genes Cancer* 2 (12), 1146–1158. doi: 10.1177/1947601911423028

Chung, J. W., Kim, G. Y., Mun, Y. C., Ahn, J. Y., Seong, C. M., and Kim, J. H. (2005). Leukotriene B4 pathway regulates the fate of the hematopoietic stem cells. *Exp. Mol. Med.* 37 (1), 45–50. doi: 10.1038/emm.2005.6

Coussens, L. M., and Werb, Z. (2002). Inflammation and cancer. *Nature* 420 (6917), 860–867. 10.1038/nature01322

Cruz-Correa, M., Hylind, L. M., Romans, K. E., Booker, S. V., and Giardiello, F. M. (2002). Long-term treatment with sulindac in familial adenomatous polyposis: a prospective cohort study. *Gastroenterology* 122 (3), 641–645. doi: 10.1053/gast.2002.31890

Das, S., and Das, D. K. (2007). Anti-inflammatory responses of resveratrol. *Inflammation Allergy Drug Targets* 6 (3), 168–173. doi: 10.2174/187152807781696464

Del Prete, A., Shao, W. H., Mitola, S., Santoro, G., Sozzani, S., and Haribabu, B. (2007). Regulation of dendritic cell migration and adaptive immune response by leukotriene B4 receptors: a role for LTB4 in up-regulation of CCR7 expression and function. *Blood* 109 (2), 626–631. doi: 10.1182/blood-2006-02-003665

Ding, X. Z., Talamonti, M. S., Bell, R. H., Jr., and Adrian, T. E. (2005). A novel anti-pancreatic cancer agent, LY293111. *Anticancer Drugs* 16 (5), 467–473. doi: 10.1097/00001813-200506000-00001

Drakatos, P., Lykouras, D., Sampsonas, F., Karkoulias, K., and Spiropoulos, K. (2009). Targeting leukotrienes for the treatment of COPD? *Inflammation Allergy Drug Targets* 8 (4), 297–306. doi: 10.2174/187152809789352177

Duah, E., Adapala, R. K., Al-Azzam, N., Kondeti, V., Gombedza, F., Thodeti, C. K., et al. (2013). Cysteinyl leukotrienes regulate endothelial cell inflammatory and proliferative signals through CysLT(2) and CysLT(1) receptors. *Sci. Rep.* 3, 3274. doi: 10.1038/srep03274

Fankhauser, M., Broggi, M. A. S., Potin, L., Bordry, N., Jeanbart, L., Lund, A. W., et al. (2017). Tumor lymphangiogenesis promotes T cell infiltration and potentiates immunotherapy in melanoma. *Sci. Transl. Med.* 9 (407). doi: 10.1126/scitranslmed.aal4712

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 313 (5795), 1960–1964. doi: 10.1126/science.1129139

Giannopoulos, P. F., Chiu, J., and Pratico, D. (2019). Learning Impairments, Memory Deficits, and Neuropathology in Aged Tau Transgenic Mice Are Dependent on Leukotrienes Biosynthesis: Role of the cdk5 Kinase Pathway. *Mol. Neurobiol.* 56 (2), 1211–1220. doi: 10.1007/s12035-018-1124-7

Gijon, M. A., Zarini, S., and Murphy, R. C. (2007). Biosynthesis of eicosanoids and transcellular metabolism of leukotrienes in murine bone marrow cells. *J. Lipid Res.* 48 (3), 716–725. doi: 10.1194/jlr.M600508-JLR200

Giovannucci, E., Harlan, D. M., Archer, M. C., Bergenstal, R. M., Gapstur, S. M., Habel, L. A., et al. (2010). Diabetes and cancer: a consensus report. *Diabetes Care* 33 (7), 1674–1685. doi: 10.2337/dc10-0666

Goodarzi, K., Goodarzi, M., Tager, A. M., Luster, A. D., and von Andrian, U. H. (2003). Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. *Nat. Immunol.* 4 (10), 965–973. doi: 10.1038/ni972

Gounaris, E., Heiferman, M. J., Heiferman, J. R., Shrivastav, M., Vitello, D., Blatner, N. R., et al. (2015). Zileuton, 5-lipoxygenase inhibitor, acts as a chemopreventive agent in intestinal polyposis, by modulating polyp and systemic inflammation. *PLoS One* 10 (3), e0121402. doi: 10.1371/journal.pone.0121402

Greten, F. R., and Grivennikov, S. I. (2019). Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity* 51 (1), 27–41. doi: 10.1016/j.immuni.2019.06.025

Grivennikov, S. I., Greten, F. R., and Karin, M. (2010). Immunity, inflammation, and cancer. *Cell* 140 (6), 883–899. doi: 10.1016/j.cell.2010.01.025

Gu, K., Li, M. M., Shen, J., Liu, F., Cao, J. Y., Jin, S., et al. (2015). Interleukin-17-induced EMT promotes lung cancer cell migration and invasion via NF-kappaB/ZEB1 signal pathway. *Am. J. Cancer Res.* 5 (3), 1169–1179. doi: 2156-6976/ajcr0005648

Haeggstrom, J. Z., and Funk, C. D. (2011). Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease. *Chem. Rev.* 111 (10), 5866–5898. doi: 10.1021/cr200246d

Hassan, H. T. (2004). Ajoene (natural garlic compound): a new anti-leukaemia agent for AML therapy. *Leuk. Res.* 28 (7), 667–671. doi: 10.1016/j.leukres.2003.10.008

Hernandez, C., Huebener, P., and Schwabe, R. F. (2016). Damage-associated molecular patterns in cancer: a double-edged sword. *Oncogene* 35 (46), 5931–5941. doi: 10.1038/onc.2016.104

Hofseth, L. J., and Wargovich, M. J. (2007). Inflammation, cancer, and targets of ginseng. *J. Nutr.* 137 (1 Suppl), 183S–185S. doi: 10.1093/jn/137.1.183S

Howard, E. W., Lee, D. T., Chiu, Y. T., Chua, C. W., Wang, X., Wong, Y. C., et al. (2008). Evidence of a novel docetaxel sensitizer, garlic-derived S-allylmercaptocysteine, as a treatment option for hormone refractory prostate cancer. *Int. J. Cancer* 122 (9), 1941–1948. doi: 10.1002/ijc.23355

Ihara, A., Wada, K., Yoneda, M., Fujisawa, N., Takahashi, H., and Nakajima, A. (2007). Blockade of leukotriene B4 signaling pathway induces apoptosis and suppresses cell proliferation in colon cancer. *J. Pharmacol. Sci.* 103 (1), 24–32. doi: 10.1254/jphs.FP0060651

Izumo, T., Kondo, M., and Nagai, A. (2009). Effects of a leukotriene B4 receptor antagonist on bleomycin-induced pulmonary fibrosis. *Eur. Respir. J.* 34 (6), 1444–1451. doi: 10.1183/09031936.00143708

Jala, V. R., Bodduluri, S. R., Satpathy, S. R., Chheda, Z., Sharma, R. K., and Haribabu, B. (2017). The yin and yang of leukotriene B4 mediated inflammation in cancer. *Semin. Immunol.* 33, 58–64. doi: 10.1016/j.smim.2017.09.005

Janne, P. A., Paz-Ares, L., Oh, Y., Eschbach, C., Hirsh, V., Enas, N., et al. (2014). Randomized, double-blind, phase II trial comparing gemcitabine-cisplatin plus the LTB4 antagonist LY293111 versus gemcitabine-cisplatin plus placebo in first-line non-small-cell lung cancer. *J. Thorac. Oncol.* 9 (1), 126–131. doi: 10.1097/JTO.0000000000000037

Jeong, C. H., Bode, A. M., Pugliese, A., Cho, Y. Y., Kim, H. G., Shim, J. H., et al. (2009). [6]-Gingerol suppresses colon cancer growth by targeting leukotriene A4 hydrolase. *Cancer Res.* 69 (13), 5584–5591. doi: 10.1158/0008-5472.CAN-09-0491

Jeong, Y. Y., Ryu, J. H., Shin, J. H., Kang, M. J., Kang, J. R., Han, J., et al. (2016). Comparison of Anti-Oxidant and Anti-inflammatory Effects between Fresh and Aged Black Garlic Extracts. *Molecules* 21 (4), 430. doi: 10.3390/molecules21040430

Jiang, X. (2020). Lymphatic vasculature in tumor metastasis and immunobiology. *J. Zhejiang Univ. Sci. B.* 21 (1), 3–11. 10.1631/jzus.B1800633

Kim, G. Y., Lee, J. W., Cho, S. H., Seo, J. M., and Kim, J. H. (2009). Role of the low-affinity leukotriene B4 receptor BLT2 in VEGF-induced angiogenesis. *Arterioscler. Thromb. Vasc. Biol.* 29 (6), 915–920. doi: 10.1161/ATVBAHA.109.185793

Kim, H., Choi, J. A., Park, G. S., and Kim, J. H. (2012). BLT2 upregulates interleukin-8 production and promotes the invasiveness of breast cancer cells. *PLoS One* 7 (11), e49186. doi: 10.1371/journal.pone.0049186

Kim, H., Choi, J. A., and Kim, J. H. (2014). Ras promotes transforming growth factor-beta (TGF-beta)-induced epithelial-mesenchymal transition via a leukotriene B4 receptor-2-linked cascade in mammary epithelial cells. *J. Biol. Chem.* 289 (32), 22151–22160. doi: 10.1074/jbc.M114.556126

Kmiecik, J., Poli, A., Brons, N. H., Waha, A., Eide, G. E., Enger, P. O., et al. (2013). Elevated CD3+ and CD8+ tumor-infiltrating immune cells correlate with prolonged survival in glioblastoma patients despite integrated immunosuppressive mechanisms in the tumor microenvironment and at the systemic level. *J. Neuroimmunol.* 264 (1-2), 71–83. doi: 10.1016/j.jneuroim.2013.08.013

Knab, L. M., Schultz, M., Principe, D. R., Mascarinas, W. E., Gounaris, E., Munshi, H. G., et al. (2015). Ablation of 5-lipoxygenase mitigates pancreatic lesion development. *J. Surg. Res.* 194 (2), 481–487. doi: 10.1016/j.jss.2014.10.021

Kumari, N., Dwarakanath, B. S., Das, A., and Bhatt, A. N. (2016). Role of interleukin-6 in cancer progression and therapeutic resistance. *Tumour Biol.* 37 (9), 11553–11572. doi: 10.1007/s13277-016-5098-7

Labi, V., and Erlacher, M. (2015). How cell death shapes cancer. *Cell Death Dis.* 6, e1675. doi: 10.1038/cddis.2015.20

Larre, S., Tran, N., Fan, C., Hamadeh, H., Champigneulles, J., Azzouzi, R., et al. (2008). PGE2 and LTB4 tissue levels in benign and cancerous prostates. *Prostaglandins Other Lipid Mediat.* 87 (1-4), 14–19. doi: 10.1016/j.prostaglandins.2008.05.001

Li, X., Lu, Y., Jin, Y., Son, J. K., Lee, S. H., and Chang, H. W. (2014). Curcumin inhibits the activation of immunoglobulin e-mediated mast cells and passive systemic anaphylaxis in mice by reducing serum eicosanoid and histamine levels. *Biomol. Ther. (Seoul)* 22 (1), 27–34. doi: 10.4062/biomolther.2013.092

Li, P., Oh, D. Y., Bandyopadhyay, G., Lagakos, W. S., Talukdar, S., Osborn, O., et al. (2015). LTB4 promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes. *Nat. Med.* 21 (3), 239–247. doi: 10.1038/nm.3800

Lin, Y. L., Tsai, N. M., Hsieh, C. H., Ho, S. Y., Chang, J., Wu, H. Y., et al. (2016). In vivo amelioration of endogenous anti-tumor autoantibodies via low-dose P4N through the LTA4H/activin A/BAFF pathway. *Proc. Natl. Acad. Sci. U. S. A.* 113 (48), E7798–E7807. doi: 10.1073/pnas.1604752113

Luo, M., Jones, S. M., Peters-Golden, M., and Brock, T. G. (2003). Nuclear localization of 5-lipoxygenase as a determinant of leukotriene B4 synthetic capacity. *Proc. Natl. Acad. Sci. U. S. A.* 100 (21), 12165–12170. doi: 10.1073/pnas.2133253100

Lynch, P. M., Burke, C. A., Phillips, R., Morris, J. S., Slack, R., Wang, X., et al. (2016). An international randomised trial of celecoxib versus celecoxib plus difluoromethylornithine in patients with familial adenomatous polyposis. *Gut* 65 (2), 286–295. doi: 10.1136/gutjnl-2014-307235

Magnusson, C., Ehrnstrom, R., Olsen, J., and Sjolander, A. (2007). An increased expression of cysteinyl leukotriene 2 receptor in colorectal adenocarcinomas correlates with high differentiation. *Cancer Res.* 67 (19), 9190–9198. doi: 10.1158/0008-5472.CAN-07-0771

Maier, T. J., Tausch, L., Hoernig, M., Coste, O., Schmidt, R., Angioni, C., et al. (2008). Celecoxib inhibits 5-lipoxygenase. *Biochem. Pharmacol.* 76 (7), 862–872. doi: 10.1016/j.bcp.2008.07.009

Malaviya, R., and Abraham, S. N. (2000). Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis. *J. Leukoc. Biol.* 67 (6), 841–846. doi: 10.1002/jlb.67.6.841

Mandal, A. K., Jones, P. B., Bair, A. M., Christmas, P., Miller, D., Yamin, T. T., et al. (2008). The nuclear membrane organization of leukotriene synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 105 (51), 20434–20439. doi: 10.1073/pnas.0808211106

Matsuyama, M., Hayama, T., Funao, K., Kawahito, Y., Sano, H., Takemoto, Y., et al. (2007). Overexpression of cysteinyl LT1 receptor in prostate cancer and CysLT1R antagonist inhibits prostate cancer cell growth through apoptosis. *Oncol. Rep.* 18 (1), 99–104. doi: 10.3892/or.18.1.99

McGeachy, M. J., Cua, D. J., and Gaffen, S. L. (2019). The IL-17 Family of Cytokines in Health and Disease. *Immunity* 50 (4), 892–906. doi: 10.1016/j.immuni.2019.03.021

McGovern, D., and Powrie, F. (2007). The IL23 axis plays a key role in the pathogenesis of IBD. *Gut* 56 (10), 1333–1336. doi: 10.1136/gut.2006.115402

Mezhybovska, M., Wikstrom, K., Ohd, J. F., and Sjolander, A. (2006). The inflammatory mediator leukotriene D4 induces beta-catenin signaling and its association with antiapoptotic Bcl-2 in intestinal epithelial cells. *J. Biol. Chem.* 281 (10), 6776–6784. doi: 10.1074/jbc.M509999200

Michael, J., Marschallinger, J., and Aigner, L. (2019). The leukotriene signaling pathway: a druggable target in Alzheimer's disease. *Drug Discovery Today* 24 (2), 505–516. doi: 10.1016/j.drudis.2018.09.008

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., and Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxid. Redox Signal* 20 (7), 1126–1167. doi: 10.1089/ars.2012.5149

Miyahara, N., Takeda, K., Miyahara, S., Taube, C., Joetham, A., Koya, T., et al. (2005). Leukotriene B4 receptor-1 is essential for allergen-mediated recruitment of CD8+ T cells and airway hyperresponsiveness. *J. Immunol.* 174 (8), 4979–4984. doi: 10.4049/jimmunol.174.8.4979

Moos, M. P., Mewburn, J. D., Kan, F. W., Ish, S., Abe, M., Sakimura, K., et al. (2008). Cysteinyl leukotriene 2 receptor-mediated vascular permeability via transendothelial vesicle transport. *FASEB J.* 22 (12), 4352–4362. doi: 10.1096/fj.08-113274

Muz, B., de la Puente, P., Azab, F., and Azab, A. K. (2015). The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia (Auckl)* 3, 83–92. doi: 10.2147/HP.S93413

Nakamura, M., and Shimizu, T. (2011). Leukotriene receptors. *Chem. Rev.* 111 (10), 6231–6298. doi: 10.1021/cr100392s

Nakanishi, M., and Rosenberg, D. W. (2013). Multifaceted roles of PGE2 in inflammation and cancer. *Semin. Immunopathol.* 35 (2), 123–137. doi: 10.1007/s00281-012-0342-8

Noonan, D. M., De Lerma Barbaro, A., Vannini, N., Mortara, L., and Albini, A. (2008). Inflammation, inflammatory cells and angiogenesis: decisions and indecisions. *Cancer Metastasis Rev.* 27 (1), 31–40. doi: 10.1007/s10555-007-9108-5

Noy, R., and Pollard, J. W. (2014). Tumor-associated macrophages: from mechanisms to therapy. *Immunity* 41 (1), 49–61. doi: 10.1016/j.immuni.2014.06.010

Oi, N., Jeong, C. H., Nadas, J., Cho, Y. Y., Pugliese, A., Bode, A. M., et al. (2010). Resveratrol, a red wine polyphenol, suppresses pancreatic cancer by inhibiting leukotriene A(4)hydrolase. *Cancer Res.* 70 (23), 9755–9764. doi: 10.1158/0008-5472.CAN-10-2858

Orafaie, A., Mousavian, M., Orafaie, H., and Sadeghian, H. (2020). An overview of lipoxygenase inhibitors with approach of in vivo studies. *Prostaglandins Other Lipid Mediat.* 148, 106411. doi: 10.1016/j.prostaglandins.2020.106411

Park, S., Yeo, M., Jin, J. H., Lee, K. M., Kim, S. S., Choi, S. Y., et al. (2007). Inhibitory activities and attenuated expressions of 5-LOX with red ginseng in Helicobacter pylori-infected gastric epithelial cells. *Dig. Dis. Sci.* 52 (4), 973–982. doi: 10.1007/s10620-006-9440-6

Paruchuri, S., Hallberg, B., Juhas, M., Larsson, C., and Sjolander, A. (2002). Leukotriene D(4) activates MAPK through a Ras-independent but PKCepsilon-dependent pathway in intestinal epithelial cells. *J. Cell Sci.* 115 (Pt 9), 1883–1893.

Paruchuri, S., Mezhybovska, M., Juhas, M., and Sjolander, A. (2006). Endogenous production of leukotriene D4 mediates autocrine survival and proliferation via CysLT1 receptor signalling in intestinal epithelial cells. *Oncogene* 25 (50), 6660–6665. doi: 10.1038/sj.onc.1209666

Pastushenko, I., and Blanpain, C. (2019). EMT Transition States during Tumor Progression and Metastasis. *Trends Cell Biol.* 29 (3), 212–226. doi: 10.1016/j.tcb.2018.12.001

Peters-Golden, M., and Brock, T. G. (2001). Intracellular compartmentalization of leukotriene synthesis: unexpected nuclear secrets. *FEBS Lett.* 487 (3), 323–326. doi: 10.1016/S0014-5793(00)02374-7

Peters-Golden, M., and Henderson, W. R. Jr. (2007). Leukotrienes. *N Engl. J. Med.* 357 (18), 1841–1854. doi: 10.1056/NEJMra071371

Peters-Golden, M., Canetti, C., Mancuso, P., and Coffey, M. J. (2005). Leukotrienes: underappreciated mediators of innate immune responses. *J. Immunol.* 174 (2), 589–594. doi: 10.4049/jimmunol.174.2.589

Pietrzyk, L., Torres, A., Maciejewski, R., and Torres, K. (2015). Obesity and Obese-related Chronic Low-grade Inflammation in Promotion of Colorectal Cancer Development. *Asian Pac. J. Cancer Prev.* 16 (10), 4161–4168. doi: 10.7314/APJCP.2015.16.10.4161

Porter, K. M., Kang, B. Y., Adesina, S. E., Murphy, T. C., Hart, C. M., Sutliff, R. L., et al. (2014). Chronic hypoxia promotes pulmonary artery endothelial cell proliferation through H2O2-induced 5-lipoxygenase. *PLoS One* 9 (6), e98532. doi: 10.1371/journal.pone.0098532

Price, D., Musgrave, S. D., Shepstone, L., Hillyer, E. V., Sims, E. J., Gilbert, R. F., et al. (2011). Leukotriene antagonists as first-line or add-on asthma-controller therapy. *N Engl. J. Med.* 364 (18), 1695–1707. doi: 10.1056/NEJMoa1010846

Qian, J., Tian, W., Jiang, X., Tamosiuniene, R., Sung, Y. K., Shuffle, E. M., et al. (2015). Leukotriene B4 Activates Pulmonary Artery Adventitial Fibroblasts in Pulmonary Hypertension. *Hypertension* 66 (6), 1227–1239. doi: 10.1161/HYPERTENSIONAHA.115.06370

Radmark, O., and Samuelsson, B. (2009). 5-Lipoxygenase: mechanisms of regulation. *J. Lipid Res.* 50 (Suppl), S40–S45. doi: 10.1194/jlr.R800062-JLR200

Ramalho, T., Filgueiras, L., Silva-Jr, I. A., Pessoa, A. F. M., and Jancar, S. (2018). Impaired wound healing in type 1 diabetes is dependent on 5-lipoxygenase products. *Sci. Rep.* 8 (1), 14164. doi: 10.1038/s41598-018-32589-7

Rayburn, E. R., Ezell, S. J., and Zhang, R. (2009). Anti-Inflammatory Agents for Cancer Therapy. *Mol. Cell Pharmacol.* 1 (1), 29–43. doi: 10.4255/mcpharmacol.09.05

Rioux, N., and Castonguay, A. (1998). Inhibitors of lipoxygenase: a new class of cancer chemopreventive agents. *Carcinogenesis* 19 (8), 1393–1400. doi: 10.1093/carcin/19.8.1393

Rockson, S. G., Tian, W., Jiang, X., Kuznetsova, T., Haddad, F., Zampell, J., et al. (2018). Pilot studies demonstrate the potential benefits of anti-inflammatory therapy in human lymphedema. *JCI Insight* 3 (20). doi: 10.1172/jci.insight.123775

Romano, M., Catalano, A., Nutini, M., D'Urbano, E., Crescenzi, C., Claria, J., et al. (2001). 5-lipoxygenase regulates malignant mesothelial cell survival: involvement of vascular endothelial growth factor. *FASEB J.* 15 (13), 2326–2336. doi: 10.1096/fj.01-0150com

Roos, J., Oancea, C., Heinssmann, M., Khan, D., Held, H., Kahnt, A. S., et al. (2014). 5-Lipoxygenase is a candidate target for therapeutic management of stem cell-like cells in acute myeloid leukemia. *Cancer Res.* 74 (18), 5244–5255. doi: 10.1158/0008-5472.CAN-13-3012

Sainz, B. Jr., Carron, E., Vallespinos, M., and Machado, H. L. (2016). Cancer Stem Cells and Macrophages: Implications in Tumor Biology and Therapeutic Strategies. *Mediators Inflammation* 2016, 9012369. doi: 10.1155/2016/9012369

Sala, A., Folco, G., and Murphy, R. C. (2010). Transcellular biosynthesis of eicosanoids. *Pharmacol. Rep.* 62 (3), 503–510. doi: 10.1016/S1734-1140(10)70306-6

Satpathy, S. R., Jala, V. R., Bodduluri, S. R., Krishnan, E., Hegde, B., Hoyle, G. W., et al. (2015). Crystalline silica-induced leukotriene B4-dependent inflammation promotes lung tumour growth. *Nat. Commun.* 6, 7064. doi: 10.1038/ncomms8064

Serezani, C. H., Lewis, C., Jancar, S., and Peters-Golden, M. (2011). Leukotriene B4 amplifies NF-kappaB activation in mouse macrophages by reducing SOCS1 inhibition of MyD88 expression. *J. Clin. Invest.* 121 (2), 671–682. doi: 10.1172/JCI43302

Sharma, R. K., Chheda, Z., Jala, V. R., and Haribabu, B. (2013). Expression of leukotriene B(4) receptor-1 on CD8(+) T cells is required for their migration into tumors to elicit effective anti-tumor immunity. *J. Immunol.* 191 (6), 3462–3470. doi: 10.4049/jimmunol.1300967

Singh, J. K., Simoes, B. M., Howell, S. J., Farnie, G., and Clarke, R. B. (2013). Recent advances reveal IL-8 signaling as a potential key to targeting breast cancer stem cells. *Breast Cancer Res.* 15 (4), 210. doi: 10.1186/bcr3436

Sjostrom, M., Johansson, A. S., Schroder, O., Qiu, H., Palmblad, J., and Haeggstrom, J. Z. (2003). Dominant expression of the CysLT2 receptor accounts for calcium signaling by cysteinyl leukotrienes in human umbilical vein endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 23 (8), e37–e41. doi: 10.1161/01.ATV.0000082689.46538.DF

Sousa, A. R., Parikh, A., Scadding, G., Corrigan, C. J., and Lee, T. H. (2002). Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. *N Engl. J. Med.* 347 (19), 1493–1499. doi: 10.1056/NEJMoa013508

Srivatsa, S., Paul, M. C., Cardone, C., Holcmann, M., Amberg, N., Pathria, P., et al. (2017). EGFR in Tumor-Associated Myeloid Cells Promotes Development of Colorectal Cancer in Mice and Associates With Outcomes of Patients. *Gastroenterology* 153 (1), 178–190 e10. doi: 10.1053/j.gastro.2017.03.053

Steinbach, G., Lynch, P. M., Phillips, R. K., Wallace, M. H., Hawk, E., Gordon, G. B., et al. (2000). The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl. J. Med.* 342 (26), 1946–1952. doi: 10.1056/NEJM200006293422603

Steinbrink, S. D., Pergola, C., Buhring, U., George, S., Metzner, J., Fischer, A. S., et al. (2010). Sulindac sulfide suppresses 5-lipoxygenase at clinically relevant concentrations. *Cell Mol. Life Sci.* 67 (5), 797–806. doi: 10.1007/s00018-009-0206-0

Subramanian, B. C., Majumdar, R., and Parent, C. A. (2017). The role of the LTB4-BLT1 axis in chemotactic gradient sensing and directed leukocyte migration. *Semin. Immunol.* 33, 16–29. doi: 10.1016/j.smim.2017.07.002

Thompson, P. A., Khatami, M., Baglole, C. J., Sun, J., Harris, S. A., Moon, E. Y., et al. (2015). Environmental immune disruptors, inflammation and cancer risk. *Carcinogenesis* 36 (Suppl 1), S232–S253. doi: 10.1093/carcin/bgv038

Tian, W., Jiang, X., Sung, Y. K., Shuffle, E., Wu, T. H., Kao, P. N., et al. (2013). Blocking macrophage leukotriene b4 prevents endothelial injury and reverses pulmonary hypertension. *Sci. Transl. Med.* 5 (200), 200ra117. doi: 10.1126/scitranslmed.3006674

Tian, W., Rockson, S. G., Jiang, X., Kim, J., Begaye, A., Shuffle, E. M., et al. (2017). Leukotriene B4 antagonism ameliorates experimental lymphedema. *Sci. Transl. Med.* 9 (389). doi: 10.1126/scitranslmed.aal3920

Tian, W., Jiang, X., Tamosiuniene, R., Sung, Y. K., Qian, J., Dhillon, G., et al. (2019). Phenotypically Silent Bone Morphogenetic Protein Receptor 2 Mutations Predispose Rats to Inflammation-Induced Pulmonary Arterial Hypertension by Enhancing the Risk for Neointimal Transformation. *Circulation* 140 (17), 1409–1425. doi: 10.1161/CIRCULATIONAHA.119.040629

Tong, W. G., Ding, X. Z., Talamonti, M. S., Bell, R. H., and Adrian, T. E. (2005). LTB4 stimulates growth of human pancreatic cancer cells via MAPK and PI-3

kinase pathways. *Biochem. Biophys. Res. Commun.* 335 (3), 949–956. doi: 10.1016/j.bbrc.2005.07.166

Tsopanoglou, N. E., Pipili-Synetos, E., and Maragoudakis, M. E. (1994). Leukotrienes C4 and D4 promote angiogenesis via a receptor-mediated interaction. *Eur. J. Pharmacol.* 258 (1-2), 151–154. doi: 10.1016/0014-2999(94)90068-X

Uko, V., Thangada, S., and Radhakrishnan, K. (2012). Liver disorders in inflammatory bowel disease. *Gastroenterol. Res. Pract.* 2012, 642923. doi: 10.1155/2012/642923

Uzonyi, B., Lotzer, K., Jahn, S., Kramer, C., Hildner, M., Bretschneider, E., et al. (2006). Cysteinyl leukotriene 2 receptor and protease-activated receptor 1 activate strongly correlated early genes in human endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 103 (16), 6326–6331. doi: 10.1073/pnas.0601223103

Valencia, J. C., Egbukichi, N., and Erwin-Cohen, R. A. (2019). Autoimmunity and Cancer, the Paradox Comorbidities Challenging Therapy in the Context of Preexisting Autoimmunity. *J. Interferon Cytokine Res.* 39 (1), 72–84. doi: 10.1089/jir.2018.0060

Wada, K., Arita, M., Nakajima, A., Katayama, K., Kudo, C., Kamisaki, Y., et al. (2006). Leukotriene B4 and lipoxin A4 are regulatory signals for neural stem cell proliferation and differentiation. *FASEB J.* 20 (11), 1785–1792. doi: 10.1096/fj.06-5809com

Walker, J. L., Loscalzo, J., and Zhang, Y. Y. (2002). 5-Lipoxygenase and human pulmonary artery endothelial cell proliferation. *Am. J. Physiol. Heart Circ. Physiol.* 282 (2), H585–H593. doi: 10.1152/ajpheart.00003.2001

Walser, T., Cui, X., Yanagawa, J., Lee, J. M., Heinrich, E., Lee, G., et al. (2008). Smoking and lung cancer: the role of inflammation. *Proc. Am. Thorac. Soc.* 5 (8), 811–815. doi: 10.1513/pats.200809-100TH

Wan, M., Tang, X., Stsiapanava, A., and Haeggstrom, J. Z. (2017). Biosynthesis of leukotriene B4. *Semin. Immunol.* 33, 3–15. doi: 10.1016/j.smim.2017.07.012

Wang, D., and Dubois, R. N. (2010). Eicosanoids and cancer. *Nat. Rev. Cancer* 10 (3), 181–193. doi: 10.1038/nrc2809

Wang, M., Zhao, J., Zhang, L., Wei, F., Lian, Y., Wu, Y., et al. (2017). Role of tumor microenvironment in tumorigenesis. *J. Cancer* 8 (5), 761–773. doi: 10.7150/jca.17648

Waugh, D. J., and Wilson, C. (2008). The interleukin-8 pathway in cancer. *Clin. Cancer Res.* 14 (21), 6735–6741. doi: 10.1158/1078-0432.CCR-07-4843

Wculek, S. K., and Malanchi, I. (2015). Neutrophils support lung colonization of metastasis-initiating breast cancer cells. *Nature* 528 (7582), 413–417. doi: 10.1038/nature16140

Wei, S. C., Duffy, C. R., and Allison, J. P. (2018). Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. *Cancer Discovery* 8 (9), 1069–1086. doi: 10.1158/2159-8290.CD-18-0367

Wenger, F. A., Kilian, M., Bisevac, M., Khodadayan, C., von Seebach, M., Schimke, I., et al. (2002). Effects of Celebrex and Zyflo on liver metastasis and lipidperoxidation in pancreatic cancer in Syrian hamsters. *Clin. Exp. Metastasis* 19 (8), 681–687. doi: 10.1023/A:1021387826867

Werner, M., Pace, S., Czapka, A., Jordan, P. M., Gerstmeier, J., Koeberle, A., et al. (2020). Communication between human macrophages and epithelial cancer cell lines dictates lipid mediator biosynthesis. *Cell Mol. Life Sci*. doi: 10.1007/s00018-019-03413-w

Wong, R. S. Y. (2019). Role of Nonsteroidal Anti-inflammatory Drugs (NSAIDs) in Cancer Prevention and Cancer Promotion. *Adv. Pharmacol. Sci.* 2019, 3418975. doi: 10.1155/2019/3418975

Yao, H., Wang, H., Li, C., Fang, J. Y., and Xu, J. (2018). Cancer Cell-Intrinsic PD-1 and Implications in Combinatorial Immunotherapy. *Front. Immunol.* 9, 1774. doi: 10.3389/fimmu.2018.01774

Yu, D., Zheng, W., Johansson, M., Lan, Q., Park, Y., White, E., et al. (2018). Overall and Central Obesity and Risk of Lung Cancer: A Pooled Analysis. *J. Natl. Cancer Inst.* 110 (8), 831–842. doi: 10.1093/jnci/djx286

Yun, C. W., and Lee, S. H. (2018). The Roles of Autophagy in Cancer. *Int. J. Mol. Sci.* 19 (11), 3466. doi: 10.3390/ijms19113466

Zhang, Y. Y., Song, J., Zhao, Z., Yang, M., Chen, M., Liu, C., et al. (2002). Expression of 5-lipoxygenase in pulmonary artery endothelial cells. *Biochem. J.* 361 (Pt 2), 267–276. doi: 10.1042/0264-6021:3610267

Zhang, Y., Walker, J.L., Huang, A., Keaney, J.F., Clish, C.B., Serhan, C.N, et al. (2020). Single-cell transcriptome analysis reveals tumor immune microenvironment heterogenicity and granulocytes enrichment in colorectal cancer liver metastases. *Cancer Lett.* 470, 84–94. doi: 10.1016/j.canlet.2019.10.016

Zhao, S., Yao, K., Li, D., Liu, K., Jin, G., Yan, M., et al. (2019). Inhibition of LTA4H by bestatin in human and mouse colorectal cancer. *EBioMedicine* 44, 361–374. doi: 10.1016/j.ebiom.2019.05.008

Zhong, Z., Hu, Z., Jiang, Y., Sun, R., Chen, X., Chu, H., et al. (2016). Interleukin-11 promotes epithelial-mesenchymal transition in anaplastic thyroid carcinoma cells through PI3K/Akt/GSK3beta signaling pathway activation. *Oncotarget* 7 (37), 59652–59663. doi: 10.18632/oncotarget.10831

Ziyad, S., and Iruela-Arispe, M. L. (2011). Molecular mechanisms of tumor angiogenesis. *Genes Cancer* 2 (12), 1085–1096. doi: 10.1177/1947601911432334

Zuazo-Gaztelu, I., and Casanovas, O. (2018). Unraveling the Role of Angiogenesis in Cancer Ecosystems. *Front. Oncol.* 8, 248. doi: 10.3389/fonc.2018.00248

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 26 August 2020
doi: 10.3389/fphar.2020.01233

The Role of Eicosanoids in Gynecological Malignancies

Paige G. Smith[1], *Dana Roque*[1,2], *Mc Millan Ching*[3], *Amy Fulton*[2,4,5], *Gautam Rao*[1,2] *and Jocelyn C. Reader*[1,2]*

[1] Department of Obstetrics, Gynecology and Reproductive Medicine, University of Maryland School of Medicine, Baltimore, MD, United States, [2] Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, MD, United States, [3] Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [4] Department of Pathology, University of Maryland School of Medicine, Baltimore, MD, United States, [5] Baltimore Veterans Administration Medical Center, Baltimore, MD, United States

Eicosanoids, bio-active lipid molecules, evoke a multitude of biological effects that directly affect cancer cells and indirectly affect tumor microenvironment. An emerging role has been shown for eicosanoids in the pathogenesis of gynecological malignancies which include cancers of the vulva, vagina, cervix, uterine, and ovary. Eicosanoid biosynthesis pathways start at the metabolism of phospholipids by phospholipase A2 then proceeding to one of three pathways: the cyclooxygenase (COX), lipoxygenase (LOX), or P450 epoxygenase pathways. The most studied eicosanoid pathways include COX and LOX; however, more evidence is appearing to support further study of the P450 epoxygenase pathway in gynecologic cancers. In this review, we present the current knowledge of the role of COX, LOX and P450 pathways in the pathogenesis of gynecologic malignancies. Vulvar and vaginal cancer, the rarest subtypes, there is association of COX-2 expression with poor disease specific survival in vulvar cancer and, in vaginal cancer, COX-2 expression has been found to play a role in mucosal inflammation leading to disease susceptibility and transmission. Cervical cancer is associated with COX-2 levels 7.4 times higher than in healthy tissues. Additionally, HPV elevates COX-2 levels through the EGFR pathway and HIV promotes elevated COX-2 levels in cervical tissue as well as increases PGE_2 levels eliciting inflammation and progression of cancer. Evidence supports significant roles for both the LOX and COX pathways in uterine cancer. In endometrial cancer, there is increased expression of 5-LOX which is associated with adverse outcomes. Prostanoids in the COX pathway PGE_2 and $PGF_{2\alpha}$ have been shown to play a significant role in uterine cancer including alteration of proliferation, adhesion, migration, invasion, angiogenesis, and the inflammatory microenvironment. The most studied gynecological malignancy in regard to the potential role of eicosanoids in tumorigenesis is ovarian cancer in which all three pathways have shown to be associated or play a role in ovarian tumorigenesis directly on the tumor cell or through modulation of the tumor microenvironment. By identifying the gaps in knowledge, additional pathways and targets could be identified in order to obtain a better understanding of eicosanoid signaling in gynecological malignancies and identify potential new therapeutic approaches.

Keywords: gynecological cancers, eicosanoid, lipoxygenase, epoxygenase, cyclooxygenase

OPEN ACCESS

Edited by:
Paola Patrignani,
University of Studies G. d'Annunzio Chieti and Pescara, Italy

Reviewed by:
Melania Dovizio,
University of Studies G. d'Annunzio Chieti and Pescara, Italy
Pallavi R. Devchand,
University of Calgary, Canada

***Correspondence:**
Jocelyn C. Reader
jreader@som.umaryland.edu

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *13 April 2020*
Accepted: *28 July 2020*
Published: *26 August 2020*

Citation:
Smith PG, Roque D, Ching MM, Fulton A, Rao G and Reader JC (2020) The Role of Eicosanoids in Gynecological Malignancies. Front. Pharmacol. 11:1233. doi: 10.3389/fphar.2020.01233

INTRODUCTION

Eicosanoids, bio-active lipid molecules, elicit a wide range of biological effects that plays a role in physiological and pathophysiological conditions (Harizi et al., 2008; Buczynski et al., 2009; Chhonker et al., 2018). Eicosanoid driven processes have been shown to have increasing importance in the development, progression and metastasis of gynecological malignancies. Gynecological malignancies are cancers that initiate in the reproductive organs of women that include vulvar, vaginal, cervical, uterine, and ovarian cancers. The eicosanoid pathway in cancer has been reviewed in detail (Panigrahy et al., 2010; Wang and DuBois, 2010; Gomes et al., 2018; Umamaheswaran et al., 2018); briefly, arachidonic acid (AA) is liberated from membrane phospholipids by phospholipase A2 (PLA2) and metabolized by one of three pathways cyclooxygenase (COX), lipoxygenase (LOX) and P450 epoxygenase which then produce a wide range of prostanoids, leukotrienes, epoxyeicosatrienoic acids (EETs), and hydroxyeicosatetraenoic acids (HETEs) (Wang and DuBois, 2010; Gomes et al., 2018). The most prominent eicosanoids to be identified to play a role in cancer are those involved in the COX and LOX pathways (Jones et al., 2019); however, products of the cytochrome P450 epoxygenase pathway are shown to play an emerging role in angiogenesis, inflammation and cancer [as reviewed by (Panigrahy et al., 2010)]. In gynecological malignancies, eicosanoids can act directly on the cancer cells, indirectly in the tumor microenvironment and, in many cases, at the confluence of infectious diseases such as HIV and HPV, inflammation and carcinogenesis (De Nola et al., 2019). This review will highlight the role of eicosanoids in the development and progression of gynecological malignancies.

The cyclooxygenase (COX) pathway converts arachidonic acid to PGH_2 which is then further converted to 5 different eicosanoids, prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin D_2 (PGD_2), Prostaglandin I_2 (PGI_2) (prostacyclin), and thromboxane (TXA_2) by specific PG synthases which are then exported to signal through their cognate receptors (Wang and DuBois, 2010; Reader et al., 2011) (**Figure 1**). The COX pathway has been the main focus of research especially concerning PGE_2. PGE_2 binds to four different G protein-coupled receptors EP1-4 which signal to different downstream signaling pathways (Reader et al., 2011). In the lipoxygenase pathway both HETEs and leukotrienes are produced by multiple subfamilies of LOX enzymes: 5-, 8-, 12-, 15-LOX. 5- (ALOX5) and 12-LOX (ALOX12) have been reported to have pro-carcinogenic roles, whereas 15-LOX-2 (ALOX15B) may have an anticancer effect (Guo et al., 2011).

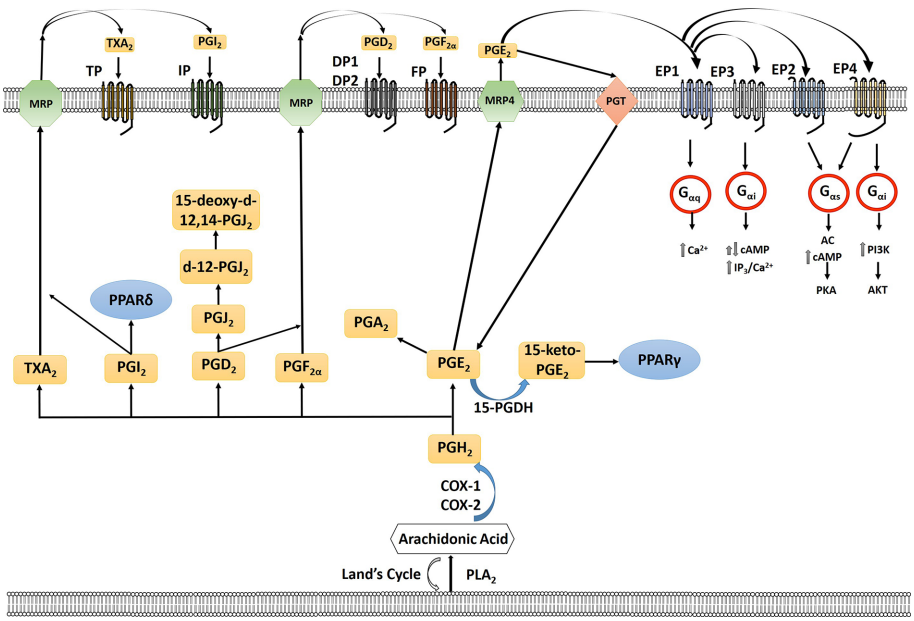


FIGURE 1 | Arachidonic acid (AA) is liberated from the plasma membrane *via* phospholipase A2 (PLA2). AA can be recycled by the Lands cycle, which is a reacylation/deacylation cycle, that serves to keep the concentration of free AA at a low level. AA is converted by cyclooxygenase 1 or 2 (COX-1/COX-2) to PGH_2 which is then converted to PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, or TXA_2 by prostaglandin specific synthases. PGE_2 is exported out of the cell by multidrug resistance-associated protein four (MRP4) where it can bind to its receptors, the E series of prostaglandin receptors on the plasma membrane, EP1-4. Each of the G-protein-coupled- receptors signal through a different intracellular pathway: EP1 leads to elevation of intracellular calcium through Gαq, EP3, which exists in multiple isoforms, can lead to different responses with the majority acting to inhibit cAMP through Gαi as well as an increase in IP3/intracellular calcium; EP2 and EP4 cause stimulation of cyclic AMP (cAMP) production and protein kinase A (PKA) by sequential activation of Gαs and adenylate cyclase (AC); EP4 can also activate phosphoinositide-3-kinase (PI3K) through Gαi. PGE_2 is imported back into the cell through prostaglandin transporter (PGT) where it can either be re-exported or inactivated by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) to 15-keto-PGE_2. 15-keto-PGE_2 can signal through PPARγ. PGE_2 can be converted to PGA_2 through a dehydration reaction. PGD_2 through a series of dehydrogenation reactions creates PGJ_2, delta-12-prostaglandin J2 (d-12-PGJ_2) and 15-deoxy-delta12,14-prostaglandin J2 (15-d-PGJ_2). PGI_2 can either signal through PPARδ intracellularly or exported *via* multidrug resistance protein (MRP) to signal through the IP receptor. TXA_2 is exported out of the cell *via* MRP to signal through the TP receptor. $PGF_{2\alpha}$ is exported out of the cell *via* MRP where it can signal *via* the FP receptor on the cell surface.

The role of 15-LOX-1 (ALOX15) is controversial (as reviewed in Guo et al., 2011). The two major isoforms of 12-LOX are platelet (p12-LOX) and leukocyte (l12-LOX) LOX with pLOX defined as the main 12-LOX in humans. 12-LOX catalyzes the stereospecific oxygenation of AA to form 12(S)-hydroperoxyeicosatetraenoic acid (HPETE), which is converted to 12(S)-hydroxyeicosatetraenoic acid (12-HETE) (Wang and DuBois, 2010; Guo et al., 2011) (**Figure 2**). It has been hypothesized that HETEs can bind to GPCRs, with 3 identified thus far, and at higher concentrations they can also activate nuclear transcription factors peroxisome proliferator-activated receptors (PPARs) (Panigrahy et al., 2010; Choi and Bothwell, 2012; Powell and Rokach, 2015; Garcia et al., 2017; Hoxha and Zappacosta, 2020). In addition to HETEs, 5-LOX, and 5-LOX activating protein (FLAP) convert AA to a series of leukotrienes including LTA_4, LTB_4, LTC_4, LTD_4, and LTE_4 that when exported can signal through cognate receptors including leukotriene B4 receptors (BLT) and cysteinyl leukotriene receptors (CysLTR) (Wang and DuBois, 2010).

The cytochrome P450 (CYP) epoxygenase pathway, a largely neglected pathway in cancer, leads to the creation of regioisomeric epoxyeicosatrienoic acids (EETs) or HETEs (Panigrahy et al., 2010; Panigrahy et al., 2011). EETs are then metabolized by soluble epoxide hydrolase (sEH) to form the corresponding fatty acid diols (Wang et al., 2019) (**Figure 3**). Arachidonic acid is metabolized by the CYP ω-hydroxylases to 7-, 10-, 12-, 13-, 15-, 16-, 17-, 18-, 19-, and 20-HETEs, the principal metabolite being the pro-inflammatory 20-HETE (Panigrahy et al., 2010). Although EETs are primarily metabolized by sEH, a few studies have observed that the 5,6-EET and 8,9-EET are but substrates for COX-1 and COX-2 (Zhang et al., 1992; Carroll et al., 1993; Moreland et al., 2007; Rand et al., 2017). Three EET regioisomers were found to be substrates for COX, and EET substrate preference for both COX-1 and COX-2 were estimated as 8,9-EET > 5,6-EET > 11,12-EET, whereas 14,15-EET was inactive. 8,9-EET is metabolized by COX to form ct-8,9-E-11-HET (8,9,11-EHET) and ct-8,9-E-15-HET (8,9,15-EHET) (Choi and Bothwell, 2012; Rand et al., 2017; Rand et al., 2019). Receptors for EETs have only recently been identified. A low affinity receptor for 11,12-EET, GPR40, has been identified in vascular cells that upon stimulation can lead to increase in Cx43 and COX-2 expression in endothelial cells *via* ERK phosphorylation (Park et al., 2018). A high affinity binding protein that may be a receptor was identified in smooth muscle, endothelial and U937 cells that can bind 8,9-EET, 11,12-EET, and 14,15-EET (Chen et al., 2011). Is it not yet known if these receptors play a role in gynecological malignancies.

EICOSANOIDS IN OVARIAN CANCER

In the United States, ovarian cancer is the second most common gynecologic cancer and leads to more deaths than any other cancer of the female reproductive tract (Desai et al., 2014; Torre et al., 2018). Epithelial ovarian cancer encompasses a

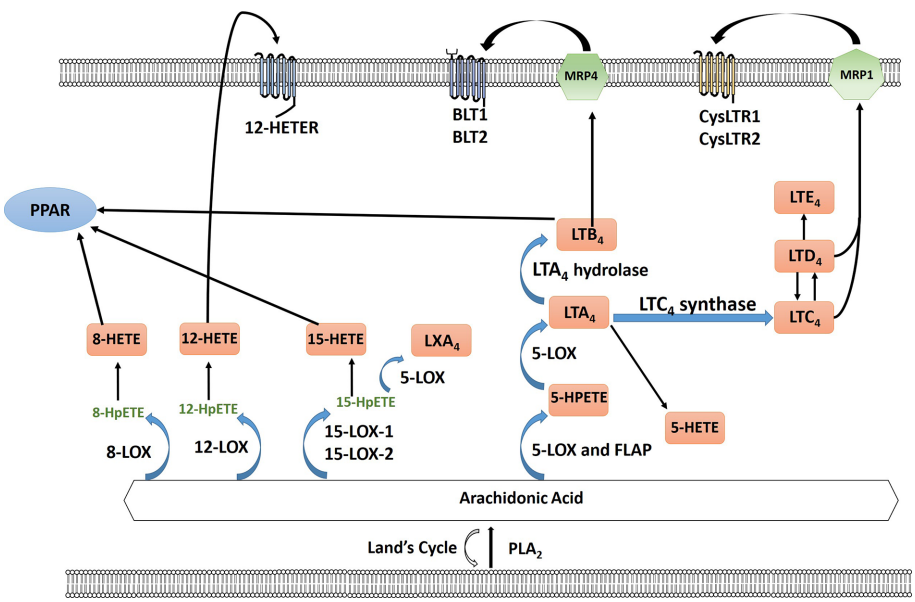


FIGURE 2 | Arachidonic acid (AA) is liberated from the plasma membrane *via* phospholipase A2 (PLA_2). AA can be recycled by the Lands cycle, which is a reacylation/deacylation cycle, that serves to keep the concentration of free AA at a low level. AA is converted by different lipoxygenase enzymes to form either hydroperoxyeicosatetraenoic acid (HPETEs) which is then reduced to the corresponding hydroxy compound. 12-HETE can signal through the G-protein-coupled receptor 12-HETE (12-HETER). 8-HETE, 15-HETE and leukotriene LTB_4 can signal through PPAR. 5-LOX and 5-lipoxygenase activating protein (FLAP) convert AA to leukotrienes through a series of reactions using 5-LOX and as well as LTA_4 hydrolase and LTC_4 synthase including LTA_4, LTB_4, LTC_4, LTD_4, and LTE_4. LTB_4 is exported *via* multidrug resistance protein 4 (MRP4) to signal through leukotriene B4 receptor BLT1/2. LTC_4 and LTD_4 is exported *via* multidrug resistance protein 1 (MRP1) to bind to cysteinyl leukotriene receptor 1 or 2 (CysLTR1/2).

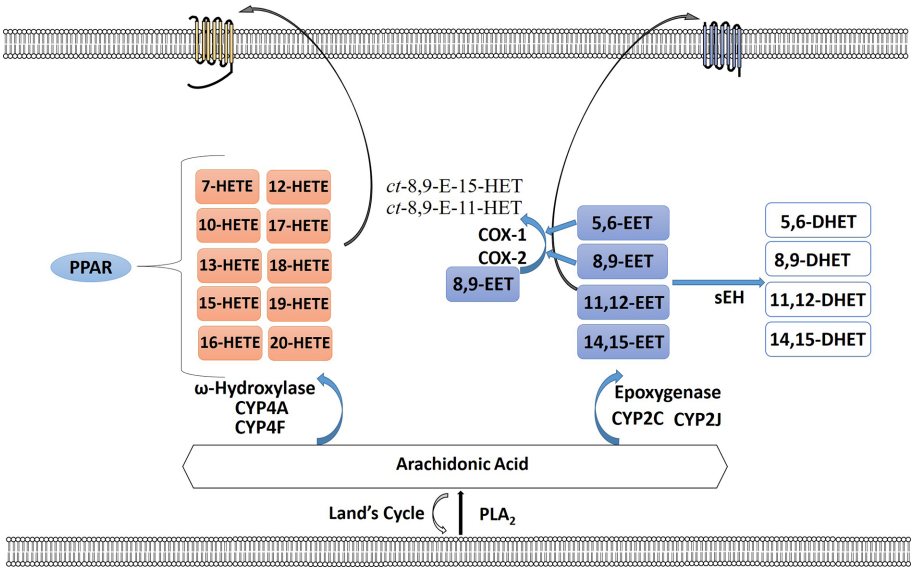


FIGURE 3 | The cytochrome P450 epoxygenase pathway creates a series of regioisomeric epoxyeicosatrienoic acids (EETs) or hydroperoxyeicosatetraenoic (HETEs). Arachidonic acid (AA) is metabolized by the CYP ω-hydroxylases to 7-, 10-, 12-, 13-, 15-, 16-, 17-, 18-, 19-, and 20-HETEs. HETEs can bind to PPAR intracellularly or bind their receptor on the plasma membrane surface. EETs are metabolized by soluble epoxide hydrolase (sEH) to form the corresponding fatty acid diols. 5,6-EET and 8,9-EET are also substrates for COX-1 and COX-2 forming *ct*-8,9-E-11-HET and *ct*-8,9-E-15-HET. EETs can also signal through their cognate receptors.

heterogenous group that is differentiated by cell, site of origin, pathological grade, risk factors, prognosis, and treatment (Torre et al., 2018). And of those, epithelial ovarian cancer accounts for 90% of all ovarian cancer cases (Torre et al., 2018). Epithelial malignancies are further subdivided into type I or type II based on clinicopathologic features and molecular features. Type I ovarian cancers, with the exception of clear cell, are considered low grade and are thought to usually develop from extraovarian benign lesions that embed within the ovary. Type II ovarian cancers are high grade characterized by aggressive behavior, late stage at diagnosis and low survival (Prahm et al., 2015; Torre et al., 2018). Clear cell, mucinous, low grade endometrioid and low grade serous cancers are type I, whereas high grade serous, high grade endometrioid, carcinosarcomas, and undifferentiated cancers are type II tumors (Jayson et al., 2014; Nezhat et al., 2015; Beeghly-Fadiel et al., 2018).

Epoxygenase and Lipoxygenase Pathways in Ovarian Cancer

While the cyclooxygenase pathway is the most studied pathway in ovarian cancer, several studies have identified a role for the CYP P450 epoxygenase pathway. HETEs produced by CYP mono-oxygenases have been implicated in cancer development, particularly 20-HETE (Chen et al., 2005; Guo et al., 2006; Guo et al., 2007; Alexanian et al., 2009; Alexanian et al., 2012). Alexanian and colleagues have shown that CYP4A/4F genes were increased in ovarian cancer tissue compared to normal tissues and further more CYP4F2 protein and 20-HETE were also higher in ovarian cancer samples (Alexanian et al., 2012) thus demonstrating a potential role for 20-HETE in ovarian cancer which warrant additional mechanistic studies for this pathway.

The lipoxygenase pathway has been identified to play a role in ovarian cancer growth and progression (**Figure 2**). Women with increased levels of 8-HETE produced by 8-LOX and other free fatty acid metabolites were at higher risk of developing ovarian cancer in the ensuing decade implicating a role for inflammation in the initiation and promotion of ovarian cancer (Hada et al., 2019). 12-LOX expression is higher in high grade serous ovarian cancer compared to normal ovarian tissue. Ovarian cancer cell lines with increased expression of 12-LOX had increased production of 12-HETE. The addition of 12-HETE to ovarian cancer cells leads to an increase in cellular proliferation (Guo et al., 2011). 12-LOX-12-HETE pathway also inhibits apoptosis in ovarian cancer through activation of NF-kB pathway (Liu Q. et al., 2018). The 5-LOX enzyme leads to the creation of both 5-HETE and several leukotrienes. Wen and colleagues reported that hypoxia leads to an increase in 5-LOX metabolites and promotes migration and invasion of tumor associated macrophages and upregulation of MMP-7 (Wen et al., 2015). In cisplatin-resistant ovarian cancer cell line SKOV-3, upregulation of leukotriene B4 and its receptor leukotriene B4 receptor-2 (BLT2) leads to activation of STAT-3 and IL-6 and depletion of BLT2 increased cell sensitivity to cisplatin chemotherapy (Park et al., 2016). In addition, BLT2 also plays a key role in ovarian cancer invasiveness and metastasis (Seo et al., 2012). Transcriptomic analysis of tumor cells and tumor-associated macrophages (TAMs) from ascites identified a synergistic relationship between

arachidonic acid, cytokines and disease recurrence (Reinartz et al., 2016). A network of lipid mediators between tumor cells and TAMs were identified which includes products of phospholipid hydrolysis, prostanoids and products of lipoxygenase pathway (Reinartz et al., 2016). These studies highlight the lipoxygenase pathway in modulation of both the tumor microenvironment as well as directly affecting the ovarian tumor cells in the progression of ovarian tumorigenesis.

Cyclooxygenase Pathway in Ovarian Cancer

The cyclooxygenase pathway is the most investigated pathway in ovarian cancers with studies identifying both COX-1 and COX-2 as potential mediators of pro-tumorigenic prostaglandin production. There has been conflicting evidence as to whether COX-1 or COX-2 plays a larger role in ovarian cancer tumorigenesis. Denkert et al. (2002) found that COX-2 expression was an independent prognostic factor for poor survival in ovarian surface epithelial tumors. Various groups have reported differences in COX-1 vs COX-2 expression in different ovarian cancer histological subtypes. Epithelial ovarian cancer demonstrates a high rate of COX-1 and COX-2 expression especially in non-mucinous tumors and a combination of high COX-1 but low COX-2 was associated with poor progression-free and overall survival (Khunnarong et al., 2010). COX-1 was found to be overexpressed in high grade serous ovarian tumors; whereas, COX-2 was highly expressed in endometrioid and mucinous tumors (Wilson et al., 2015). In a follow up study, COX-1 expression was higher in low and high grade serous tumors and type II tumors in comparison to type I tumors while COX-2 was more highly expressed in non-serous and type I tumors (Beeghly-Fadiel et al., 2018).

The association of elevated prostaglandins in the tissues and ascites from ovarian cancer was first reported almost 40 years ago. Prostaglandins PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (a degradation product of PGI_2) were measured in advanced human ovarian cancer tissues (Bauknecht et al., 1985). In tumors that were resistant to chemotherapy, higher levels of these prostaglandins were detected compared to chemotherapy responsive tumors (Bauknecht et al., 1985). Ascitic fluids from ovarian cancer patients were compared to patients with benign gynecologic conditions and higher amounts of fatty acid palmitoleic acid were measured in cancer patients (Punnonen et al., 1986). Concentrations of PGE_2, 6-keto-$PGF_{1\alpha}$, TxB_2, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in ovarian tumor tissue were measured in 38 post-menopausal women with malignant or benign ovarian tumors and in six women without ovarian neoplasms (Heinonen and Metsä-Ketelä, 1988). PGE_2 and TXB_2 contents in ovarian cancer tissue were significantly ($P < 0.05$) higher than in normal ovarian tissue (Heinonen and Metsä-Ketelä, 1988). Ovarian cancer is more common in patients with elevated gonadotropins such as FSH and LH and they are detectable in ovarian tumor fluid. FSH/LH lead to increased PGE_2 production through upregulation of the expression of COX-1 and COX-2 and activation of the PI3K/AKT pathway that ultimately leads to tumor cell migration and invasion (Lau et al., 2010).

Phospholipase A_2 in Ovarian Cancer

Phospholipase A_2 enzymes, which convert lipids to arachidonic acid, the substrate for the COX enzyme, have been implicated in several cancers. EOC ascites contains high levels of oncogenic lipid growth factors including lipid products produced by PLA_2 such as lysophosphatidic acid (LPA) and lysophosphatidylcholine (LPC) (Cai et al., 2012). Human EOC ascites contained microvesicle-free $cPLA_2$ and $iPLA_2$ that were secreted in an ABC transporter dependent mechanism. Chen et al. (2018) reported that the ABCC1 inhibitor MK571 was the most effective inhibitor of PLA_2 activity. However, ABCC4 is also inhibited by MK571 and we have found increased expression of ABCC4 (MRP4), the exporter for PGE_2, to be overexpressed in multiple ovarian cancer cell lines and tumor tissue; therefore, it is possible that ABCC4 could also play a role in PLA_2 secretion (Kochel and Fulton, 2015; Kochel et al., 2017; Ching et al., 2018). Treatment of ovarian cancer cells with chemotherapy drug VP16 causes release of AA resulting in elevation of PGE_2 levels and enhancing the repopulation of ovarian cancer cells (Zhao et al., 2017). Berberine can block the caspase 3-$iPLA_2$-AA-COX-2-PGE_2 pathway by inhibiting the expression of $iPLA_2$ and COX-2. PGE_2 can lead to increased phosphorylation of FAK. Treatment with berberine reverse PGE_2 induced FAK phosphorylation thereby leading to inhibition of VP16 induced ovarian cancer cell repopulation (Cui et al., 2017; Zhao et al., 2017). Targeting of cytosolic phospholipase A_2 can increase efficacy of low-dose radiotherapy in ovarian cancer (Schulte et al., 2011). These studies demonstrate the role of PLA_2 in treatment response and tumor promoting activities such as proliferation, migration and invasion and tumor growth *in vivo* (Schulte et al., 2011; Cai et al., 2012; Chen et al., 2018).

Prostanoids in Ovarian Cancer

PGE_2 signals through four receptors of which two have been identified to play a role in ovarian cancer (Spinella et al., 2004a; Ching et al., 2018; Zhang et al., 2019). There is increased expression of EP4 in ovarian cancer cell lines and primary tumor tissue compared to normal ovarian cell lines and tissue (Ching et al., 2018; Fan et al., 2018). Treatment of SKOV-3 and MDAH-2774 with $dmPGE_2$ lead to a dose dependent increase in COX-2, Bcl-2 and bax expression as well as an increase in proliferation and decrease in apoptosis (Munkarah et al., 2002). Bagnato's group studied the relationship between endothelin-1, COX and EP receptors in ovarian cancer growth and invasion (Spinella et al., 2004b; Spinella et al., 2004a; Spinella et al., 2004c). Activation of the endothelian A receptor (ET_AR) by endothelin-1 (ET-1) leads to an increase in COX-1 and COX-2 expression as well as PGE_2 production (Spinella et al., 2004b). ET-1 induced PGE_2 production then signals through EP2 and EP4 to stimulate VEGF production. Additionally, signaling through the EP4 receptor leads to an increase in ovarian cancer cell invasiveness (Spinella et al., 2004a). Signaling through the EP2 receptor leads to an increase in proliferation and invasion through the NFkB pathway (Zhang et al., 2019).

There is conflicting evidence on whether PGI_2 is anti- or pro-tumoral prostanoid. PGI_2 analog iloprost has been shown to have anti-inflammatory and anti-tumoral effects in lung cancer

(Lo et al., 1998; Tennis et al., 2010). In ovarian cancer, iloprost inhibited migration and invasion through downregulation of MMP-2 expression (Ahn et al., 2018). In study using engineered OSE cells to form ovarian tumors, tumors demonstrated an increase in COX-1 expression but not COX-2 (Daikoku et al., 2005). Additionally, PGI1, not PGE2, was the major prostaglandin produced by these tumors (Daikoku et al., 2005). Treatment with COX-1 inhibitor SC-560 reduced cell proliferation and accelerated apoptosis; whereas, celecoxib had little effect on tumor growth. SC-560 also decreased PGI_2 production by OSE cells and tumors. In a follow up study, Daikoku et al. (2007) demonstrated that aspirin (Cox-1 inhibitor) not only lowered PGI_2 levels in T2 and OVCAR-3 cells, but it also attenuates the transactivation of PPARδ, which indicates the inhibition of PG production and PPARδ are downstream targets of COX-1 PGs. This suppression of COX-1- PPARδ signaling by aspirin lead to compromised tumor growth in mice; this result suggests the importance of this pathway to stimulate tumor growth and offers a promising target for ovarian cancer treatment (Daikoku et al., 2007).

Lipid mediators play an important role in both platelet and ovarian cancer functions. Tumor cells can act as agonists for platelets leading to an increase in platelet adhesion, aggregation and degranulation (Serhan et al., 2018). Meanwhile platelets can protect tumor cells from host defenses and increase tumor cell invasion and extravasation (Serhan et al., 2018). Lipid mediators that act as platelet agonists are often produced by tumor cells and are found in the tumor microenvironment. TXA_2, PGD_2, PGE_2, and PGI_2 are all important prostanoids for platelet function including activation of platelets, TXA_2 and PGE_2, or inhibiting platelet aggregation, PGI_2 or PGD_2 (Friedman et al., 2015; Contursi et al., 2017; Serhan et al., 2018). PGE_2 and prostacyclin are found in the ovarian tumor microenvironment linking lipid signaling and platelets in ovarian cancer (Reinartz et al., 2016). In ovarian cancer patients with residual disease, plasma 6-keto-PGF1α (a metabolite of PGI_2) in cancer patients (146.7 +/− 14.7 pg/ml, mean +/− SE) was higher (P less than 0.02) than that in the controls (85.3 +/− 9.2 pg/ml, n = 17). Also, the release of TXB_2 (a metabolite of TXA_2) during spontaneous clotting of the blood samples was greater in the patients than controls (Ylikorkala et al., 1983).

Cyclopentenone prostaglandins of the A and J series, PGA_2, PGA_1, and PGJ_2, which are produced by dehydration of PGE_2, PGE_1, and PGD_2, respectively, have various biological activities including both pro- (Oliva et al., 2003) and anti-tumoral effects (Sasaki and Fukushima, 1994; Straus and Glass, 2001). Sasaki demonstrated that ovarian cancer cell lines resistant to cisplatin, doxorubicin and L-phenylalanine mustard were sensitive to antitumor prostaglandins delta-7-prostaglandin A1 (d7-PGA_1) and delta-12-prostaglandin J2 (d-12-PGJ_2) (Sasaki et al., 1991) and a derivative of d7-PGA_1, 13,14-dihydro-15-deoxy-delta7-prostaglandin-A1-methyl ester, exhibits antitumor activities against cisplatin resistant ovarian cancer cells (Sasaki et al., 1999). McClay and colleagues showed that d-12-PGJ_2 can synergize with cisplatin and radiation thus increasing the efficacy of these treatments against ovarian cancer (McClay et al., 1996). 15-deoxy-delta12,14-prostaglandin J2 (15-d-PGJ_2) has been shown to strongly induce apoptosis and autophagic cell death pathways and inhibit sirtuin in chemotherapy resistant cell lines (Jong et al., 2011; Tae et al., 2018). Interestingly, expression of PGD_2 in high-grade serous ovarian cancer is an independent marker of good prognosis and is associated with increase in disease-free survival, the absence of relapse and sensitivity to platinum-based therapy (Alves et al., 2019) which further links the antitumor properties associated with the PGJ prostaglandins which are derived from PGD_2. Given the activity of these metabolites against drug resistant ovarian cancer cell lines, which is what ultimately leads to mortality in patients, warrants additional studies on these compounds.

COX Enzymes in Ovarian Cancer

Several studies have been published supporting the role of COX-1 in ovarian cancer development and progression. Gupta et al. (2003) found increased expression of COX-1 mRNA and protein in ovarian cancer tissues compared to normal ovarian tissue. They also examined the link between the COX pathway and angiogenesis; ovarian epithelial cells with high COX-1 expression exhibit high levels of transcription factors including HIF-1α, VEGF, VEGF receptor: Flk-1. In a follow up study, a genetically engineered mouse model of ovarian EOC was used to evaluate whether COX-1 expression is linked to mutations in tumor suppressors *p53* and *Rb* (Daikoku et al., 2006). In mice with these mutations, high COX-1 mRNA levels were detected in well differentiated serous epithelial neoplasms; COX-2 levels remain to be undetectable and this is consistent *in situ* tumor samples (Daikoku et al., 2006). In a follow up study, peroxisome proliferator-activated receptor δ (PPARδ), a downstream target of COX-1, is directly activated by PGI_2 and transactivated by PGE_2, stimulates cell proliferation and tumor growth in cancers like mammary and hepatocellular, and was found to be overexpressed in mouse ovarian cancer cell lines (Daikoku et al., 2007). COX-1 has been identified to also produce prostaglandins in ovarian cancer cell lines. Increased levels of COX-1 were identified in 3 out of 10 ovarian cancer cell lines, PGE_2 production was also elevated in those cell lines (Kino et al., 2005). Non-selective COX inhibitor (indomethacin) and a selective COX-1 inhibitor (SC-569) significantly decreased PGE_2 production; however, COX-2 inhibitors, NS-398 and rofecoxib, did not affect PGE_2 production which suggests that COX-1 is regulating the production of PGE_2 in these cell lines (Gupta et al., 2003; Kino et al., 2005; Rask et al., 2006).

In addition to COX-1, COX-2 has also been shown to play a role in ovarian cancer progression. COX-2 is constitutively expressed in various EOC cell lines; introduction of PGE_2 increases COX-2 expression, increases proliferation as well as reduces apoptosis (Munkarah et al., 2002). COX-2 derived PGE_2 also promotes ovarian cancer cell invasion through an epidermal growth factor (EGF) signaling mechanism in which a positive feedback loop leads to increases in COX-2, PGE_2 and EGF (Qiu et al., 2014). COX-2 can also lead to an increase in pro-angiogenic proteins. The expression of nerve growth factor (NGF) is frequently overexpressed in EOC leading to an increase in VEGF, β-catenin/TCF-Lef, survivin, and

MYC. Garrido et al. (2019) identified that this was due to a NGF-regulated increase in COX-2/PGE_2 signaling. Dursun et al. (2005) hypothesize that the difference between invasive ovarian tumors and borderline ovarian tumors, which are less threatening in terms of survival, is the level of COX-2 expression. Overexpression of COX-2 stimulates prostaglandin production and cellular growth, reduces apoptosis enzymes, promotes mutagenesis, tumorigenesis, and angiogenesis (Dursun et al., 2005). COX-2 positive ovarian cancer cases also have higher expression of MDR1/P-glycoprotein (P-gp). P-gp is a drug efflux pump whose presence is associated with unfavorable significance in ovarian cancer treated with chemotherapy and may be upregulated by COX-2. Previously, Denkert et al. (2002) demonstrated that expression of COX-2 is an independent unfavorable prognostic factor in ovarian cancers. In a follow up study, they have extended these findings to demonstrate a positive correlation between COX-2 and P-gp expression in human ovarian carcinomas and unfavorable prognostic and predictive significance in tumors expressing COX-2 and/or P-gp (Surowiak et al., 2006). Combination treatment with cisplatin or docetaxel with NSAID, NS-398 or sulindac, can lead to dose-dependent enhancement of cytotoxicity which could be enhanced under hyperthermic conditions (41°C) (Barnes et al., 2007). Denkert et al. (2003) investigated COX enzyme dependent vs independent effects of the NSAID NS-398 on ovarian cancer cell lines that are either positive for COX-1/2, OVCAR-3, or negative for COX-1/2 expression, SKOV-3. They demonstrated that NS-398 could inhibit proliferation through induction of cell cycle arrest that is independent of COX-2 inhibition and the inhibitory effect could not be rescued with the addition of PGE_2 (Denkert et al., 2003).

COX inhibitors have been explored as both a monotherapy and as well in combination with chemotherapeutics. Li and colleagues investigated the effect of COX-1 and COX-2 inhibitors as a monotherapy and as well as in combination with taxol on SKOV-3 xenograft mouse model (Li et al., 2008; Li et al., 2010; Li et al., 2011; Li et al., 2012a; Li et al., 2012b; Li et al., 2013). Nimesulide (COX-2 inhibitor) treatment of mice with SKOV-3 xenograft tumors leads to a reduction in microvessel density (MVD) and PGE_2 levels but did not significantly decrease tumor growth (Li et al., 2008). This led Li to examine the effects of COX-1 inhibitor, SC-560, and COX-2 inhibitor, celecoxib, or dual inhibitor, indomethacin, on xenograft tumor growth. Monotherapy treatment was not effective in decreasing tumor volume; however, dual SC-560 and celecoxib inhibitor significantly reduced relative tumor volume (Li et al., 2012a). Single treatment with SC-560 or celecoxib also significantly prolonged survival of the mice compared to control. Previously, Gupta et al. (2003) reported that COX-1 and pro-angiogenic proteins were overexpressed in ovarian tumors and treatment with COX-1 inhibitor SC-560 but not COX-2 inhibitor celecoxib lead to a decrease in secretion of VEGF by OVCAR-3 cells *in vitro*. Li found similar results in their *in vivo* studies (Li et al., 2012a). Li and colleagues also investigated the combination of SC-560 and taxol as well as celecoxib and taxol on the effect of SKOV-3 tumor MVD, apoptosis and VEGF transcript levels (Li et al., 2012a). Taxol in combination with SC-560 was superior to taxol with celecoxib and while the authors did not provide an explanation for the observed differences in the two inhibitors, the potential remains for the possibility of off target or non-COX related effects of the inhibitors (Li et al., 2012a; Li et al., 2013). Using a combination of siRNAs against COX-1/COX-2, Denkert et al. (2003) examined the role COX-2-dependent and COX-2 independent effects of COX inhibitors on ovarian cancer proliferation. As opposed to the predominant role of COX-1 in SKOV-3 cells, in OVCAR-3 cells, COX-2 was responsible for PGE_2 production and production could be inhibited with COX-2 inhibitor NS-398. COX-1/COX-2 inhibitors lead to a decrease in proliferation that was independent of COX activity and expression through induction of Go/G1 cell cycle arrest. Uddin et al. (2010) examined the molecular pathways involved in COX-2 inhibition in ovarian cancer. COX-2, but not COX-1, expression was associated with expression of pAKT in ovarian cancer tumor tissue. In MDAH2774 and SKOV3 cell lines, aspirin and NS-398 also lead to a decrease in proliferation and induction of apoptosis. While the authors did not explore specific EP receptors in this study, EP4 has been shown to activate PI3K/AKT (Hsu et al., 2017). Given the results of these studies the possibility exists that COX independent effects play a significant role in the function of COX inhibitors.

Cyclooxygenase in Ovarian Cancer Tumor Microenvironment

The innate and adaptive immune system plays an essential role in the progression and spread of cancer. The ovarian cancer microenvironment is highly immune suppressive (Preston et al., 2011). Natural killer cells (NK), part of the innate immune system, can lyse cells without first having to recognize specific antigens and have been reported to play an essential role in controlling metastasis in breast cancer with contradictory roles in ovarian cancer (Dong et al., 2006; Holt et al., 2012; Sun et al., 2018). Myeloid-derived suppressor cells (MDSC), which are a heterogenous population of macrophages, DCs and granulocytes at early stages of differentiation, is another driver of immune suppression with the ability to block local and systemic immune activation (Preston et al., 2011). TAMs are one of the most abundant immune cells in the tumor microenvironment (Colvin, 2014). These heterogenous cells can act to either stimulate or suppress the immune response and these opposing behaviors have been classified as either classically activated, M1, or alternatively activated, M2 (Colvin, 2014). M1-polarized macrophages produce pro-inflammatory and immunostimulatory cytokines, are involved in T_H1 responses and are considered cancer killing; whereas, M2-polarized macrophages are immunosuppressive, associated with the T_H2 response and are considered cancer promoting (Colvin, 2014). Dendritic cells (DC) can either activate adaptive immunity or tolerize T cells specific for tumor-associated antigens (Pardoll, 2015). Cytotoxic T cells (CTL) exhibit antitumor immune functions with CD8+ cytotoxic T cells generally are the primary mediators of antitumor immune response. Helper T cells, which are required to activate cytotoxic T cells, are functionally divided into subsets including T_H1 and T_H2 according to secretory cytokines and immunologic roles (Lee et al., 2019). The T_H1/T_H2 balance plays

an essential role in immune response to cancer with T_H1 eliciting an anti-tumor response whereas T_H2 is pro-tumorigenic (Burkholder et al., 2014).

Mucin molecules (MUCs) are aberrantly secreted from ovarian cancer cells. There is a positive correlation with five year survival rate in patients with high density of M1 TAMS and a high M1/M2 ratio and therapeutic targeting against M2 inhibits disease progression (Luo et al., 2006; Mantovani et al., 2006; He et al., 2013). He et al. showed that an increased concentration of COX-2+ cancer cells, decreased ratio of M1/M2 TAMs, and an increased concentration of COX-2+ TAMs was associated with poor prognosis (He et al., 2013). Populations of M2 TAMs and COX-2+ TAMs frequently converged. There was also an association between COX-2+ cancer cells and COX-2+ TAMs (He et al., 2013). Expression of MUC2 can result in M2-polarization and induce TAM COX-2 expression. The polarization of M2 TAMs and expression of COX-2 can lead to an increase in PGE_2 in the microenvironment by TAMs and cancer cells which can lead to an acceleration of ovarian cancer progression (He et al., 2013).

MDSCs expand during cancer and can suppress T-cell facilitated responses (Gabrilovich and Nagaraj, 2009; Ostrand-Rosenberg and Sinha, 2009; Rodríguez-Ubreva et al., 2017). Myeloid cell differentiation is highly dependent on DNA methylation changes and pro-inflammatory mediators such as PGE_2 regulate MDSC accumulation (Sinha et al., 2007; Álvarez-Errico et al., 2015; Rodríguez-Ubreva et al., 2017). Rodríguez-Ubreva et al. (2017) demonstrated that ovarian cancer cells can induce differentiation of monocytes to MDSCs by causing hypermethylation of MDSC specific genes by signaling through the PGE_2 EP2 and EP4 receptors leading to upregulation of DNMT3A. DCs and MDSCs show opposing roles in the immune system, suppression of DCs contributes to cancer progression while MDSCs suppress the ability of CD8+ T cells to mediate effective anti-tumor response (Rodríguez-Ubreva et al., 2017). COX-2-PGE_2 leads to positive feedback signaling that results in blocking CD1α+ DC differentiation and leading to induction of CD14+CD33+CD34+ monocytic MDSCs (Obermajer et al., 2011). The frequency of CD11b(+)CD33(+) MDSCs in ovarian cancer are closely correlated with local PGE_2 production. Inhibition of EP2 and EP4 or interruption of COX-2-PGE_2 feedback using COX-2 inhibitors decreases the production of suppressive mediators and inhibitory functions of MDSCs from cancer patients.

Wong et al. (2016) observed an unexpected increase in both type-1 mediators, IFNγ and TNFα, induction of immune suppressive COX-2 and hyperactivation of MDSCs in the tumor microenvironment of ovarian cancer patients. The synergistic action of both IFNγ and TNFα which lead to increased MDSC activity and expression of IDO1, NOX2, IL-10 and COX-2 mRNA and protein was dependent on COX-2-PGE_2 such that blockade of COX-2 was able to completely reverse enhanced suppressive ability of the hyperactivated MDSCs (Wong et al., 2016). Blockade of COX-2 or the EP4 receptor reduces expression of IDO1, an immunosuppressive enzyme, leading to an increase in CD3+ and CD8+ cells thus increasing T cells and control of tumor growth (Hennequart et al., 2017). While IL-10 and PGE_2 were identified as having immunosuppressive capabilities in ascites from ovarian cancer patients, PGE_2 was found to be the more important of the two that leads to impaired T cell stimulatory capacity of DC cells (Brencicova et al., 2017). Cytotoxic T cells directed against endogenously expressed antigens are key for antigen-specific cancer immunotherapy. COX-2-PGE_2 was identified as resistance factors for suppression of antigen-induced interferon-gamma secretion of T cells and generation of factors that suppress the immune system leading to escape from immune surveillance and resistance to cellular immunotherapy (Göbel et al., 2014).

Maintenance of CTL, T_H1 and NK cell-mediated type I immunity is key for effective antitumor responses. Liu et al. (2009) analyzed the pattern of CD8+ (CTL), CD57+ (NK), and CD1α+ (DC) infiltrating immune cells in intraepithelial or stromal spaces with survival and COX-1 and COX-2 expression. PGE_2 can alter the T_H1/T_H2 balance, suppress DC function, and increase the number of tumor-infiltrating regulatory T cells (Liu et al., 2009). Higher levels of COX-2 expression occurred more frequently in serous ovarian carcinoma compared to other types ($P<0.05$) and higher levels of COX-1 or COX-2 tended to correlate with poor prognosis (n.s.) (Liu et al., 2009). COX may impact ovarian cancer prognosis and the pattern of tumor-infiltrating immune cells. Ovarian cancers were analyzed by hierarchical cluster analysis based on the number of tumor-infiltering immune cells and three different clusters were generated (Liu et al., 2009). Cluster 1 was characterized with the worst progression free survival and overall survival. Cluster 1 was characterized by higher expression of COX-1 and COX-2 compared to cluster 2 ($P<0.05$, respectively). The presence of intraepithelial CD8+ cells was negatively correlated with COX-1 and COX-2 expression ($P<0.05$ for both) (Liu et al., 2009). It is clear that PGE_2 *via* COX-1/COX-2 expression plays a critical role in the ovarian cancer tumor microenvironment leading to immune suppression through multiple mechanisms. The COX/PGE2 pathway warrants additional studies including combining PGE_2 EP4 inhibitors with immunotherapy in ovarian cancer.

EICOSANOIDS IN UTERINE CANCER

Uterine cancer is the fourth most common malignancy as well as the most common gynecologic malignancy (Siegel et al., 2019). There are two subgroups, Type I, endometrioid cancer, which is the most common, is associated with expression of hormonal receptors and, Type 2, unrelated to estrogen, and includes histological types such as serous, clear cell, mucinous and uterine sarcomas (Creutzberg and Fleming, 2016; Di Tucci et al., 2019).

Lipoxygenase in Uterine Cancer

The majority of the research published to date discusses the role of prostanoids in uterine cancer; however, the lipoxygenase pathway has been shown to be involved in type 2 endometrial cancer. ALOX5 (5-LOX) is generally reported to be absent in normal epithelial but is induced by pro-inflammatory stimuli and can be overexpressed in various epithelial cancers

(Pidgeon et al., 2007; Wang and DuBois, 2010). In a study performed by Cummings et al. (2019), there is a significant increase in ALOX5 (5-LOX) mRNA expression in type II endometrial cancer compared to normal endometrium and elevated ALOX5 expression is associated with adverse outcomes. Given the limited number of studies available on the role of lipoxygenase as well as the lack of studies in the cytochrome P450 pathway in uterine cancer, these pathways are ones that should be evaluated in uterine cancer in the future.

Cyclooxygenase in Uterine Cancer

Uterine leiomyosarcoma (LMS) is the second most common subtype of uterine sarcoma with uterine leiomyomas comprising the most common benign pelvic tumors in women (Sabry and Al-Hendy, 2012; Kobayashi et al., 2013). Leiomyomas release prostaglandins including 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 (Rees and Turnbull, 1985). Endothelin (ET)-1 exerts survival effects in uterine leiomyoma cells and leads to increased protein expression of COX-2 and PGE_2 production (Oyeniran and Tanfin, 2011). COX-2 expression was higher in uterine fibroids compared to healthy smooth muscle cells and selective COX-2 inhibitor celecoxib decreased fibroid cell proliferation and PGE_2 secretion (Ke et al., 2013). Recently, in a study published by Reader et al. (2019), the role of EP4 was evaluated in LMS proliferation and migration. While minimal effects in proliferation was observed with monotherapy treatment of LMS with an EP4 inhibitor when combined with docetaxel treatment, we saw a sensitization of LMS cells to chemotherapeutic treatment (Reader et al., 2019). In addition to sensitization to chemotherapy, we also demonstrated a significant decrease in LMS cell migration. PI3K has been shown to play a significant role in the development of uterine cancer and activation of EP4 can lead to activation of the PI3K pathway (**Figure 1**) (Naumann, 2011).

One common inflammatory disease that affects approximately 10% of reproductive aged women is endometriosis, which causes chronic pain and infertility, and is defined as the presence of endometrium-like tissue outside the uterus (As-Sanie et al., 2019). Epidemiological, biological, and molecular data all indirectly suggest links between the endometriosis and endometrial cancer (Painter et al., 2018; As-Sanie et al., 2019). In 1992, Koike et al. (1992) identified increased production of 6-keto-$PGF_{1\alpha}$, TXB_2, and PGE_2 in endometrial cysts compared to non-endometrial cysts and normal ovaries. Additionally, the COX-2 pathway promotes survival, migration, and invasion of endometriotric cells and promotes angiogenesis during endometriosis progression (Banu et al., 2008; Jana et al., 2016). Inhibition of EP2 and EP4 leads to apoptosis of endometriotic cells as well as a decrease of human endometriotic epithelial and stromal cells through integrin-mediated mechanisms (Banu et al., 2009; Lee et al., 2013). As indicated in these studies a relationship exists between COX eicosanoids in the pathogenesis of endometriosis and therefore endometrial cancer.

COX-1 and COX-2 are important in normal reproduction as reviewed in (Sales and Jabbour, 2003). COX enzymes convert arachidonic acid to PGH_2 which is then metabolized by specific synthases to thromboxanes and prostaglandins including PGE_2 and $PGF_{2\alpha}$ (Wang and DuBois, 2010). PGE_2, EP2 and EP4 receptors work in an autocrine/paracrine role in the epithelial/endothelial cell function in normal human endometrium (Milne et al., 2001). During the menstrual cycle, signaling of EP2/EP4 receptors produced a greater response in proliferative tissue compared with early and midsecretory stage tissue. COX-2 expression is up-regulated in neoplastic epithelial and endothelial cells of endometrial carcinomas (Tong et al., 2000; Jabbour et al., 2001; Ferrandina et al., 2002; Uotila et al., 2002; Jabbour et al., 2006). Lower risk of endometrial cancer was reported when aspirin and other nonsteroidal anti-inflammatory agents were used in patients which could block production of prostaglandins (Black et al., 2014). The importance of COX-2 in the early stages of endometrial cancer development was shown using a conditional endometrial-specific phosphatase and tensin homologue gene (PTEN) knockout mouse model (Daikoku et al., 2008). Dual inhibition of COX-2 and mTORC1 signaling markedly reduces endometrial cancer progression (Daikoku et al., 2014). Paclitaxel resistance may be associated with COX-2 and multidrug resistance 1 (MDR1) expression, an efflux pump that can transport a wide range of compounds including chemotherapy out of the cell, in endometrial cancer (Hasegawa et al., 2013). Co-administration of COX-2 inhibitor etodolac with paclitaxel leads to a decrease in MDR1 expression which may enhance accumulation of MDR1 substrates such as paclitaxel thus leading to an increase in paclitaxel sensitivity (Hasegawa et al., 2013). However, recently Cummings, et al. using laser capture microdissection, whole genome expression analysis and liquid chromatography-tandem mass spectrometry, did not show an increase in COX isoform expression nor $PGE_2/PGF_{2\alpha}$ levels in neoplastic endometrium compared to sample matched epithelial tissue (Cummings et al., 2019). The authors do suggest that an increase in local levels of PGE_2 and $PGF_{2\alpha}$ could occur due to decreased catabolism, *via* downregulation of HPGD (**Figure 1**). There was an increase in EP3 (PTGER3) and EP4 (PTGER4) receptor gene expression in type I and type II endometrial cancers which could lead to an increase in local PGE_2-EP3/EP4 mediated signaling. In addition, our group has observed an increase in EP4 expression in primary endometriod cancer tissue (Reader et al., 2016). The differences could be due to the fact that the tissues used for this study were microdissected instead using the entire tissue for analysis as well as the use of fewer clinical samples in previous studies.

Prostanoids in Uterine Cancer

The F and E prostanoid pathways have been shown to contribute to endometrial cancer phenotypes including proliferation, adhesion, migration, invasion, angiogenesis, and inflammatory microenvironment by binding with their respective G-protein coupled receptors (Sales et al., 2004b; Sales et al., 2005; Sales et al., 2008a; Keightley et al., 2010b; Wallace et al., 2010; Zhu et al., 2017). $PGF_{2\alpha}$ exerts its action *via* FP receptors of which two isoforms exists generated *via* alternative mRNA splicing designated FPA and FPB. These isoforms are coupled to $G_{\alpha q}$ and can produce IP_3 through activation of phospholipase C, intracellular calcium flux and activation of protein kinase C (Sales and Jabbour, 2003). $PGF_{2\alpha}$

plays an essential role in parturition and regulation of expression of the oxytocin receptor. PGE_2 can be converted to $PGF_{2\alpha}$ adding a layer of crosstalk and complexity (Dozier et al., 2008) (**Figure 4**). Downstream signaling of FP and EP receptors leads to an increase in COX-2 expression (Fujino and Regan, 2003; Sales et al., 2008b). PGE_2 can act as an agonist for FP receptors thus PGE_2 and $PGF_{2\alpha}$ when locally produced by endometrial adenocarcinomas can regulate tumor cell function in an autocrine/paracrine manner by the FP receptor leading to an induction of COX-2 expression (Sales et al., 2008b). $PGF_{2\alpha}$ and FP receptor interaction enhances proliferation of endometrial epithelial cells (Sales et al., 2005; Sales et al., 2007). There is co-localization of COX-2 and the FP receptor within neoplastic epithelial cells of endometrial adenocarcinoma and activation of $PGF_{2\alpha}$-FP receptor lead to an increase in COX-2 expression through activation of ERK1/2 pathway which leads to a concomitant increase in $PGF_{2\alpha}$ creating a positive feedback loop (Jabbour et al., 2005). Another positive feedback involving FGF2-FGFR1 interaction that results in elevated FGF2 and COX-2 expression and enhanced proliferation *via* the FGFR1 and ERK pathways (Sales et al., 2007). In addition to proliferative changes, activation of the FP receptor can lead to an increase in adhesion, migration and invasion of endometrial adenocarcinoma cells. Alterations in cell adhesion and increased migration occur through a αVβ3-ECM dependent mechanism after $PGF_{2\alpha}$-FP activation (Sales et al., 2008a) and regulation of endometrial adenocarcinoma cell invasion occurs *via* increased expression of disintegrin and metalloprotease with a thrombospondin repeat 1 (ADAMTS1) (Keightley et al., 2010b). Chemokines play an important role in normal uterine function; however, dysregulated chemokines can contribute to uncontrolled proliferation, migration and invasion and contribute to an inflammatory microenvironment (Kayisli et al., 2002). Chemokines CXCL8 and CXCL1 are both targets of $PGF_{2\alpha}$ (Sales et al., 2009; Wallace et al., 2009). CXCL8 leads to increased proliferation of Ishikawa cells *in vivo* (Sales et al., 2009) while CXCL1induces neutrophil chemotaxis *in vitro* and *in vivo* (Wallace et al., 2009). The effects of these chemokines presumably also involve activation of the $PGF_{2\alpha}$ receptor. A pro-angiogenic and anti-angiogenic relationship has been described as a result of $PGF_{2\alpha}$ and FP receptor interactions (Sales et al., 2005; Keightley et al., 2010b; Keightley et al., 2010a). $PGF_{2\alpha}$ and FP receptor activation enables endothelial cell network formation and proliferation *via* FGF2-FGFR1 signaling *in vitro* (Keightley et al., 2010a) and through transactivation of EGFR and secretion of VEGF leads to an increase in angiogenesis *in vivo* (Sales et al., 2005). Elevated COX-2 is associated with increased VEGF, PGE_2 and angiogenesis in endometrial cancer and can be negatively regulated by miR-101 and while a specific receptor was not identified in this study it is feasible that FP and $PGF_{2\alpha}$ could play a role by conversion of PGE_2 to $PGF_{2\alpha}$ (Dozier et al., 2008; Liu Y. et al., 2018).

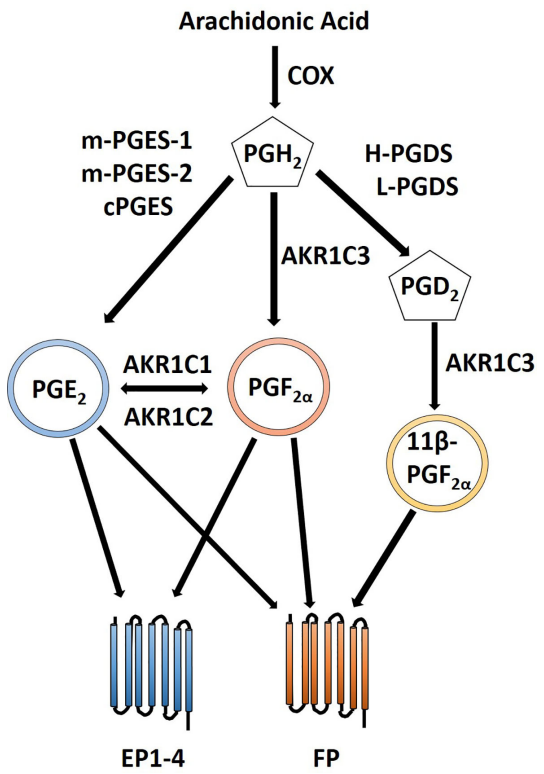


FIGURE 4 | Crosstalk between PGE_2 and $PGF_{2\alpha}$ prostanoid pathways. PGE_2 is created *via* conversion of PGH_2 by PGE synthases including m-PGES-1, m-PGES-2 and cPGES. Likewise, $PGF_{2\alpha}$ is formed by conversion of PGH_2 by AKR1C3. Additionally, $PGF_{2\alpha}$ can be created from PGE_2 by AKR1C1 or AKR1C2. 11β-PGF2α, a stereoisomer of $PGF_{2\alpha}$, can also signal *via* the FP receptor and is created by the PGD_2 prostanoid pathway with an additional conversion by AKR1C3. PGE_2 can signal through its cognate PGE_2 EP receptors as well as the through $PGF_{2\alpha}$ FP receptor. Similarly, $PGF_{2\alpha}$ can signal through its FP receptor as well as through PGE_2 EP receptors.

Cyclooxygenase in Uterine Tumor Microenvironment

The production of PGE_2 by malignant endometrial epithelial cells in the tumor microenvironment can cause an increase in COX-2 expression. In normal endometrial cells, activation of transcription *via* NF-kB and stabilization of COX-2 mRNA leads to production of PGE_2 (Tamura et al., 2002). PGE_2 signals through four different E series receptors EP1-EP4 which are linked to different intracellular signaling pathways (**Figure 1**). The expression of the EP1 receptor was only increased in endometriotic tissue compared to healthy endometrium and tumor tissue; and in the tumor stroma, the expression of EP1 in the tumor was lower than in both normal tissue and endometriosis (Zhu et al., 2018). There was no significant differences in EP1 staining in either epithelium or in stroma within histological stage, grading, metastatic and recurrent subtypes (Zhu et al., 2018). High expression of EP3 was associated with impaired progression free survival and inhibition of EP3 signaling lead to a decrease in proliferation and migration in RL95-2 endometrial cancer cells (Zhu et al., 2017). Compared to EP1 and EP3, the EP2 and EP4 receptors are the most prominent receptors in regard to playing a role in uterine cancer pathogenesis. Jabbour et al. (2001) demonstrated an increase in expression of EP2 and EP4 receptors in endometrial adenocarcinomas and signaling through these

receptors resulted in significantly higher cAMP production in response to PGE_2. Signaling of the EP2 receptor in endometrial adenocarcimoma leads to transactivation of EGFR through PKA and c-Src and induction of VEGF (Sales et al., 2004b). Abera et al. (2010) reported on crosstalk between FP and EP2 receptors that results in increased cAMP release *via* the $G\alpha_q$-Ca^{2+}-calmodulin pathway. Endogenous PGE_2 and exogenous PGE_2, through exposure of seminal plasma, leads to expression of FGF2, a potent mitogenic and angiogenic factor, though activation of EP_2 (Battersby et al., 2007). Exposure to PGE_2 leads to an increase in phosphorylation of Akt and tuberin, a tumor suppressor that negatively regulates cellular proliferation, with a concomitant decrease in total tuberin protein expression (Sales et al., 2004a). In this study, the authors used inhibitors to block PI3K/Akt signaling and while involvement of a specific EP receptor was not identified (Sales et al., 2004a) several studies have determined that the EP4 receptor can lead to activation of PI3K pathway (Xu et al., 2018; Lu et al., 2019; Osawa et al., 2020).

Based on the studies presented in this section, there is a significant role for prostanoid signaling in uterine cancer development and progression. It would be interesting to see if other prostanoids as well as eicosanoids in LOX and P450 pathways also play a role in uterine cancer. It would also be beneficial to see additional studies combining inhibition of specific prostanoid receptors with first-line therapies.

EICOSANOIDS IN CERVICAL CANCER

Cervical cancer is one of the foremost contributors to worldwide gynecological malignancies and the top contributor to that in Southern Africa (Sales and Katz, 2012; Adefuye et al., 2014). In 2008, as reported by the International Agency for Research on Cancer, an estimated 493,243 women were diagnosed with cervical cancer annually, contributing to approximately 27,300 deaths (Sales and Katz, 2012). Cervical cancer primarily arises as a neoplasm that's initiated by oncogenic variations of human papillomavirus (HPV) and of the 180 different genotypes of the infectious disease, 40 can be accredited to cause infection of the anogenital tract (Adefuye et al., 2014). The most common mode of transmission of HPV is through the exchanging of bodily fluids (primarily seminal fluid) during sexual intercourse (Adefuye et al., 2014). Not only does seminal fluid contribute to the transmission of HPV, but the presence of prominent concentrations of prostaglandins associated with seminal plasma (including PGE_2) is likely to regulate inflammatory and tumorigenic pathways due to elevated expression of PGE_2 receptors in cervical cancers (Adefuye et al., 2014). Increased binding of PGE_2 to EP receptors can increase COX-2 expression as well as regulate target genes such as growth factors, angiogenic factors, prostaglandins, chemokines, and cytokines, which supports tumorigenesis, inflammation, and local changes in tissue architecture (Adefuye et al., 2014). Since COX-2 is controlled by growth factors, tumor promoters, oncogenes, and carcinogens, it is speculated that COX-2 expression is increased in a variety of cancers, including cervical (Saldivar et al., 2007). By producing prostaglandins, COX-2 is responsible for suppressing apoptosis and promoting tumor invasion (Saldivar et al., 2007). Colposcopically, within the same patient, COX-2 expression levels were measured at 83.1± 30.1 ng COX-2/mg protein in normal tissues versus 403.7± 175.6 in abnormal biopsies (CIN tissues). Inflammation is associated with carcinogenesis and in line with this association, Saldivar found that COX-2 levels were 3.7 times higher in specimen that were inflammation positive and of those specimens, COX-2 protein levels were 7.4 times higher than that in control biopsies (Saldivar et al., 2007). These data provide evidence that COX-2 may play an integral role in cervical cancer inflammation and tumorigenesis.

Upregulation of COX-2 can be attributed to HPV oncoproteins playing a role in cervical carcinogenesis. Kim and colleagues investigated the role of HPV 16, the most frequent genotype detected in cervical cancer tissue, E5 oncoprotein in cervical cancer development (Kim et al., 2009b). One-way E5 affects COX-2 levels is through EGFR activation; in HaCa T cells transfected with E5, there is activation of the EGFR pathway (Kim et al., 2009b). Transactivation of EGFR by protease-activated receptors are also known to have an effect on COX-2 upregulation (Almeida et al., 2018). Inhibition of EGFR activation lead to a decrease in COX-2 expression (Kim et al., 2009b). E5 also induces COX-2 mRNA expression; assessment of transcriptional activity determined that the COX-2 gene expression was regulated through binding of NF-κB and AP-1 to the COX-2 promoter (Kim et al., 2009b). Inhibition of NF- κB or AP-1 lead to significant decrease in COX-2 expression (Kim et al., 2009b). Bcl proteins are essential for cell clearance and when defective, can promote cancer (Alibek et al., 2014).

Sales and Katz (2012) have shown that PGE_2 biosynthesized by COX-1 and COX-2 in HeLa cells, increases expression of pro-angiogenic factors that can exert paracrine activity on endothelial cells to promote blood supply for tumor growth and alter vascular permeability for the release and distribution of leucocytes and macrophages to surrounding tissues. The production of prostaglandins *via* elevated COX enzyme activity, act on G-protein couple receptors (GPCRs) to encourage tissue remodeling for tumors, angiogenesis, apoptosis inhibition, cell proliferation, and altered vascular permeability (Adefuye et al., 2014). Peng et al. (2019) examines anti-cancer activity of medicinal plant, *Conyza blinii*, through the downregulation of COX-2 and decrease in PGE_2 levels. *C. blinii* acts as a NF-κB pathway inhibitor and it inhibits downstream gene expression of COX-2, which in turn decreases PGE_2 production (Peng et al., 2019). In *C. blinii* treated mice, PGE_2 levels in both serum and tumors were significantly lower. This decrease in prostaglandin activity has promising implication in inflammation and immunomodulation (Peng et al., 2019).

The Bcl family is important in regulating apoptosis according to environmental cues. Bcl-2 homologues act as oncoproteins that are anti-apoptotic and thus, integral for cancer initiation (Alibek et al., 2014). Viruses have been known to have developed a mechanism of apoptosis prevention in host cells through

targeting Bcl-2 homologues. HPV protein E6 interacts with p53 in infected cells in order to prevent p53 from blocking the anti-apoptotic Bcl-2 protein (Alibek et al., 2014). It is unknown how viral protein E5 interacts with the Bcl family directly; however, it is known that as pro-apoptotic Bak and Bax proteins are decreased, Bcl-2 expression levels are increased thus inhibition of Bak and Bax is facilitated by E5 (Alibek et al., 2014). This inhibition is performed through ubiquitin-proteasome-dependent degradation, involving EGFR activation and subsequently COX-2 upregulation (Alibek et al., 2014).

In addition to the contribution of HPV in cervical cancer, HIV also poses a role in infection and inflammation through elevation of COX-2 expression as well as elevated PGE_2 levels Fitzgerald and colleagues (Fitzgerald et al., 2012). HIV infected women are five times more likely to develop cervical cancer than HIV-negative women. HIV induces high COX-2 levels in a number of immune cells circulating and in tissue (Fitzgerald et al., 2012). Fitzgerald and colleagues hypothesized that HIV promotes COX-2 levels in cervical tissue and in turn increases systemic levels of PGE_2 leading to inflammation and cancer (Fitzgerald et al., 2012). Women positive for HIV and negative HPV have significantly higher levels of COX-2 mRNA (+/−) ($P<0.001$) with additional increase in COX-2 observed in women positive for both HIV and HPV (+/+)($P<0.001$). Similarly, urinary PGE-M levels were elevated in HIV positive women; HPV positive women did not exhibit significantly higher levels of PGE-M meaning HIV status was the only statistical predictor of PGE-M levels. Through this observation, it was concluded that COX-2 and PGE-M levels were positively correlated ($P= 0.005$) (Fitzgerald et al., 2012). This study confirms association of HIV with development of cervical cancer. The data presented in this section demonstrates a significant role for COX-2 and PGE_2 in the pathogenesis of cervical cancer alone and in conjunction with infection by HPV or HIV.

EICOSANOIDS IN VULVAR CANCER

Vulvar cancers are rare, accounting for only an estimated 6,070 cases and 1,280 deaths in the United States (US) in 2019 (Siegel et al., 2019). The majority are squamous cell carcinomas (Moroney et al., 2013). Vulvar cancer arises from two pathways (Zarcone et al., 1997). The most common mechanism involves chronic inflammation in older women from vulvar dystrophies such as lichen sclerosus with development of differentiated vulvar intraepithelial neoplasia (VIN) then keratinizing squamous cell carcinoma. In 25–30% of cases and often in younger women, pathogenesis is driven by human papillomavirus (HPV) leading to usual VIN or high-grade squamous intraepithelial neoplasia (HSIL) and basaloid or warty squamous cell carcinoma. HPV-related vulvar cancers may exhibit a predilection for localization to the perineum and carry a favorable prognosis (Hinten et al., 2018) compared to vulvar cancers that arise independent of HPV.

COX-2 expression has clinicopathologic relevance in vulvar cancer. Lee et al. (2007) found that COX-2 expression by immunohistochemistry and adjacent inflammatory cell infiltrate was higher in older compared to younger vulvar cancer patients ($P = 0.002$) (Lee et al., 2007). Interestingly, COX-2 expression was inversely correlated with differentiation. Fons et al. (2007) found that strong COX-2 expression was an independent predictor of poor disease-specific survival (HR 4.01, 95% CI 1.10-14.63, $P = 0.035$), as was lymphovascular space invasion, lymph node involvement, tumor size > 4 cm, and absence of apoptotic caspase-3.

Inhibition of COX-2 may potentiate the effects of chemotherapies used to treat vulvar cancer. Dual treatment of COX-2-expressing vulvar squamous cell carcinoma cell lines (A431 and SW962) with the COX-2 inhibitor celecoxib and cisplatin inhibited 49% of growth, compared to 25% with cisplatin monotherapy alone after 48 h (Kim et al., 2009a). Interestingly, exposure of the cells to either agent at 10 micromol/L or in combination increased COX-2 expression, putatively through downregulation of its negative regulator PI3K. Inhibition of PI3K has been shown to block activation of mitogen-activated kinase (MAPK) independent of EGFR in A431 cells (Graness et al., 2000), suggesting a prominent role for therapies targeting PI3K in vulvar cancer and as mentioned previously signaling through PGE_2 receptor EP4 can lead to activation of PI3K. In squamous cell carcinoma of the head and neck, agonists of the PGE_2 receptor EP4 promote migration through PI3K activation and Ca^{2+} influx (Osawa et al., 2020). EP4 antagonists are currently under study in phase IB trials of rectal cancer in conjunction with pre-operative chemoradiation (Adlai Nortye Biopharma CO, Ltd). Based on molecular mechanisms, it may be hypothesized that EP4 inhibition may one day merit study in vulvar cancers and other gynecologic malignancies treated with chemoradiation.

EICOSANOIDS IN VAGINAL CANCER

Vaginal cancer represents only 1–2% of gynecologic malignancies and is under-studied due to its rarity. There were only 5,350 US cases and 1,430 deaths from this disease in 2019 (Siegel et al., 2019). Approximately 10–50% of patients with vaginal cancer or its precursor lesion [high grade vaginal intraepithelial neoplasia (VAIN)] have a history of hysterectomy or radiation for cervical cancer. HPV infection and immunosuppression are strong risk factors for development. The majority arise in the upper third of the canal and involve the posterior wall and are squamous in histology. Less common subtypes include clear cell adenocarcinoma, which is characteristically associated with *in utero* diethylstilbestrol (DES) exposure or malignant degeneration of endometriosis, and embryonal rhabdomyosarcoma, the most common vaginal malignancy in children (Cardenes et al., 2013).

COX-2 expression has been found to be a prominent feature in mucosal inflammation of the vagina leading to disease susceptibility and transmission (Joseph et al., 2012), as well as rectovaginal endometriotic implants (Fagotti et al., 2004). Recently, we have observed lower expression of EP4 in vaginal tissues of patients with pelvic organ prolapse and higher expression in women with radiation-induced vaginal stenosis after treatment for gynecologic malignancy compared to healthy controls (Santayana et al., 2018). There also appears to be

differential expression between luminal, basal, and intermediate (smooth muscle) layers of the vagina. Additional studies are underway to better elucidate the role of EP4 in both alterations of tissue integrity as well as its relevance in gynecologic malignancy, but this preliminary data raises interest in a possible pharmacologic role for use of EP4 inhibitors to modify a number of biologic processes in vaginal cancer.

CONCLUSION

This manuscript aimed to provide a comprehensive review of the role of eicosanoids in gynecological malignancies. With increasing evidence, it is demonstrated that eicosanoid driven processes are associated with the progression and spread of gynecological malignancies. These bio-active lipids have direct effects on cancer cells as well as indirect effects on the tumor microenvironment. Most of the studies reported in gynecological malignancies focused on the cyclooxygenase pathway especially the role of PGE_2 and $PGF_{2\alpha}$ and their receptors. The least reported pathway was the P450 cytochrome epoxygenase pathway in which only ovarian cancer had associative studies involving the pathway but with a lack of mechanistic investigation; thus, this pathway is one that should be examined more in the future for all gynecological malignancies.

Given the rare nature of vaginal and vulvar cancer, there is not an abundance of research available; however, the studies performed are directed towards the cyclooxygenase pathway and exploring all of the eicosanoid pathways would be of benefit in order to find much needed treatments and information related to tumorigenesis of these malignancies. It was demonstrated that the COX pathway plays a significant role in the pathogenesis of cervical cancer alone and in conjunction with HPV and HIV; however, there was a lack of mechanistic studies in interrogating which receptors are involved in the tumorigenic signaling pathways. In uterine cancer, it would be of benefit to see more studies performed on the potential for PGE_2 receptors EP1 and EP3 in uterine cancer given that to date most of the investigations have been correlative.

In ovarian cancer, the data presented for the potential role for the lipoxygenase pathway in ovarian cancer tumorigenesis and modulation of the tumor microenvironment supports the need for additional studies including antagonizing the receptors for both leukotrienes and HETEs. Given the potential anti-neoplastic effects on some of the prostaglandins such as the cyclopentenone prostaglandins as well as the association of PGD_2 with good prognosis in high grade serous ovarian cancer (Alves et al., 2019) as well as the positive effects of blocking PGE_2 EP4 in modulating the tumor microenvironment (Hennequart et al., 2017), additional mechanistic studies exploring modulation of COX signaling downstream of the COX enzymes as a monotherapy and in combination with additional therapeutic interventions is warranted.

Overall there is a theme that inhibition of COX pathways leads to potentiation of chemotherapeutic treatment in multiple gynecological malignancies including ovarian (Li et al., 2012a; Li et al., 2013), uterine (Hasegawa et al., 2013; Reader et al., 2019) and vulvar (Kim et al., 2009a). Additional studies are warranted to explore the role of and the mechanism behind adding inhibition of prostanoid receptors to chemotherapy and other treatment modalities in order to conserve potential anti-neoplastic eicosanoid activities for development of novel treatments for gynecologic malignancies.

AUTHOR CONTRIBUTIONS

PS, DR, GR, and JR contributed written sections and figures. MC and AF contributed edits. All authors contributed to the article and approved the submitted version.

FUNDING

The work presented has been supported by Department of Obstetrics, Gynecology, and Reproductive Medicine (JR, GR, DR), the Kaleidoscope of Hope Ovarian Cancer Foundation (JR), The Foundation for Women's Cancer Research (DR), Maryland Department of Health's Cigarette Restitution Fund Program (AF, JR), Baltimore Veterans Affairs Administration (AF) the STAR-PREP program of the National Institute of General Medical Sciences at the University of Maryland School of Medicine (MMC).

REFERENCES

Abera, A. B., Sales, K. J., Catalano, R. D., Katz, A. A., and Jabbour, H. N. (2010). EP2 receptor mediated cAMP release is augmented by PGF 2 alpha activation of the FP receptor *via the* calcium-calmodulin pathway. *Cell. Signal.* 22, 71–79. doi: 10.1016/j.cellsig.2009.09.012

Adefuye, A., Katz, A. A., and Sales, K. J. (2014).The regulation of inflammatory pathways and infectious disease of the cervix by seminal fluid. Available at: https://www.hindawi.com/journals/pri/2014/748740/ (Accessed January 8, 2020).

Adlai Nortye Biopharma CO, Ltd *NCT03152370: Preoperative Radiotherapy and E7046 in Rectum Cancer (PRAER 1)*. Available at: www.clinicaltrials.gov/ct2/show/NCT3152370 (Accessed November 23, 2018).

Ahn, J.-H., Lee, K.-T., Choi, Y. S., and Choi, J.-H. (2018). Iloprost, a prostacyclin analog, inhibits the invasion of ovarian cancer cells by downregulating matrix metallopeptidase-2 (MMP-2) through the IP-dependent pathway. *Prostaglandins Other Lipid Mediat.* 134, 47–56. doi: 10.1016/j.prostaglandins.2017.12.002

Alexanian, A., Rufanova, V. A., Miller, B., Flasch, A., Roman, R. J., and Sorokin, A. (2009). Down-regulation of 20-HETE synthesis and signaling inhibits renal Adenocarcinoma cell proliferation and tumor growth. Available at: http://ar.iiarjournals.org/content/29/10/3819 (Accessed February 25, 2020).

Alexanian, A., Miller, B., Roman, R. J., and Sorokin, A. (2012). 20-HETE-producing enzymes are up-regulated in human cancers. *Cancer Genomics Proteomics* 9, 163–169.

Alibek, K., Irving, S., Sautbayeva, Z., Kakpenova, A., Bekmurzayeva, A., Baiken, Y., et al. (2014). Disruption of Bcl-2 and Bcl-xL by viral proteins as a possible cause of cancer. *Infect. Agents Cancer* 9, 44. doi: 10.1186/1750-9378-9-44

Almeida, V. H., dos, S. I., Almendra, L. R., Rondon, A. M. R., Tilli, T. M., de, A. C., et al. (2018). Positive crosstalk between EGFR and the TF-PAR2 pathway

mediates resistance to cisplatin and poor survival in cervical cancer. *Oncotarget* 9, 30594–30609. doi: 10.18632/oncotarget.25748

Álvarez-Errico, D., Vento-Tormo, R., Sieweke, M., and Ballestar, E. (2015). Epigenetic control of myeloid cell differentiation, identity and function. *Nat. Rev. Immunol.* 15, 7–17. doi: 10.1038/nri3777

Alves, M. R., Do Amaral, N. S., Marchi, F. A., Silva, F.II, Da Costa, A. A. B. A., Carvalho, K. C., et al. (2019). Prostaglandin D2 expression is prognostic in high –grade serous ovarian cancer. *Oncol. Rep.* 41, 2254–2264. doi: 10.3892/or.2019.6984

As-Sanie, S., Black, R., Giudice, L. C., Gray Valbrun, T., Gupta, J., Jones, B., et al. (2019). Assessing research gaps and unmet needs in endometriosis. *Am. J. Obstetrics Gynecol.* 221, 86–94. doi: 10.1016/j.ajog.2019.02.033

Banu, S. K., Lee, J., Speights, V. O., Starzinski-Powitz, A., and Arosh, J. A. (2008). Cyclooxygenase-2 regulates survival, migration, and invasion of human endometriotic cells through multiple mechanisms. *Endocrinology* 149, 1180–1189. doi: 10.1210/en.2007-1168

Banu, S. K., Lee, J., Speights, V. O., Starzinski-Powitz, A., and Arosh, J. A. (2009). Selective inhibition of prostaglandin E2 receptors EP2 and EP4 induces apoptosis of human endometriotic cells through suppression of ERK1/2, AKT, NFkappaB, and beta-catenin pathways and activation of intrinsic apoptotic mechanisms. *Mol. Endocrinol.* 23, 1291–1305. doi: 10.1210/me.2009-0017

Barnes, A. P., Miller, B. E., and Kucera, G. L. (2007). Cyclooxygenase inhibition and hyperthermia for the potentiation of the cytotoxic response in ovarian cancer cells. *Gynecol. Oncol.* 104, 443–450. doi: 10.1016/j.ygyno.2006.08.008

Battersby, S., Sales, K. J., Williams, A. R., Anderson, R. A., Gardner, S., and Jabbour, H. N. (2007). Seminal plasma and prostaglandin E2 up-regulate fibroblast growth factor 2 expression in endometrial adenocarcinoma cells *via* E-series prostanoid-2 receptor-mediated transactivation of the epidermal growth factor receptor and extracellular signal-regulated kinase pathway. *Hum. Reprod.* 22, 36–44. doi: 10.1093/humrep/del328

Bauknecht, T., Siegel, A., Meerpohl, H. G., and Zahradnik, H. P. (1985). Formation of prostaglandins by ovarian carcinomas. *Prostaglandins* 29, 665–672. doi: 10.1016/0090-6980(85)90128-5

Beeghly-Fadiel, A., Wilson, A. J., Keene, S., El Ramahi, M., Xu, S., Marnett, L. J., et al. (2018). Differential cyclooxygenase expression levels and survival associations in type I and type II ovarian tumors. *J. Ovarian Res.* 11, 17. doi: 10.1186/s13048-018-0389-9

Black, J. D., English, D. P., Roque, D. M., and Santin, A. D. (2014). Targeted therapy in uterine serous carcinoma: an aggressive variant of endometrial cancer. *Womens Health (Lond)* 10, 45–57. doi: 10.2217/whe.13.72

Brencicova, E., Jagger, A. L., Evans, H. G., Georgouli, M., Laios, A., Attard Montalto, S., et al. (2017). Interleukin-10 and prostaglandin E2 have complementary but distinct suppressive effects on Toll-like receptor-mediated dendritic cell activation in ovarian carcinoma. *PloS One* 12, e0175712. doi: 10.1371/journal.pone.0175712

Buczynski, M. W., Dumlao, D. S., and Dennis, E. A. (2009). Thematic review series: proteomics. an integrated omics analysis of eicosanoid biology. *J. Lipid Res.* 50, 1015–1038. doi: 10.1194/jlr.R900004-JLR200

Burkholder, B., Huang, R.-Y., Burgess, R., Luo, S., Jones, V. S., Zhang, W., et al. (2014). Tumor-induced perturbations of cytokines and immune cell networks. *Biochim. Biophys. Acta (BBA) - Rev. Cancer* 1845, 182–201. doi: 10.1016/j.bbcan.2014.01.004

Cai, Q., Zhao, Z., Antalis, C., Yan, L., Del Priore, G., Hamed, A. H., et al. (2012). Elevated and secreted phospholipase A2 activities as new potential therapeutic targets in human epithelial ovarian cancer. *FASEB J.* 26, 3306–3320. doi: 10.1096/fj.12-207597

Cardenes, H. R., Schiler, J. M., and Emerson, R. (2013). "Vagina," in *Principles and Practice of Gynecologic Oncology*. Eds. R. Barakat, A. Berchuck, M. Markman and M. Randall (Lippincott Williams & Wilkins), 557–597.

Carroll, M. A., Balazy, M., Margiotta, P., Falck, J. R., and McGiff, J. C. (1993). Renal vasodilator activity of 5,6-epoxyeicosatrienoic acid depends upon conversion by cyclooxygenase and release of prostaglandins. (Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, ©2013) *J. Biol. Chem.* 268, 12260–12266.

Chen, P., Guo, M., Wygle, D., Edwards, P. A., Falck, J. R., Roman, R. J., et al. (2005). Inhibitors of Cytochrome P450 4A Suppress Angiogenic Responses. *Am. J. Pathol.* 166, 615–624. doi: 10.1016/S0002-9440(10)62282-1

Chen, Y., Falck, J. R., Manthati, V. L., Jat, J. L., and Campbell, W. B. (2011). 20-Iodo-14,15-Epoxyeicosa-8Z-enoyl-3-azidophenylsulfonamide: Photoaffinity Labeling of a 14,15-Epoxyeicosatrienoic Acid Receptor. *Biochemistry* 50, 3840–3848. doi: 10.1021/bi102070w

Chen, Y., Yuan, X., Xiao, Z., Jin, H., Zhang, L., and Liu, Z. (2018). Discovery of novel multidrug resistance protein 4 (MRP4) inhibitors as active agents reducing resistance to anticancer drug 6-Mercaptopurine (6-MP) by structure and ligand-based virtual screening. *PloS One* 13. doi: 10.1371/journal.pone.0205175

Chhonker, Y. S., Bala, V., and Murry, D. J. (2018). Quantification of eicosanoids and their metabolites in biological matrices: a review. *Bioanalysis* 10, 2027–2046. doi: 10.4155/bio-2018-0173

Ching, M. M., Fan, C., Roque, D., Rao, G., Staats, P., Fulton, A., et al. (2018). Functional analysis of PGE2 pathway members EP4 and MRP4 in ovarian cancer. [abstract]. In: Proceedings of the AACR Conference: Addressing Critical Questions in Ovarian Cancer Research and Treatment; Oct 1-4, 2017. (Pittsburgh, PA. Philadelphia (PA): AACR), *Clin. Cancer Res.* 2018, 24 (15_Suppl:Abstract nr A22).

Choi, J.-M., and Bothwell, A. L. M. (2012). The Nuclear Receptor PPARs as important regulators of T-cell functions and autoimmune diseases. *Mol. Cells* 33, 217–222. doi: 10.1007/s10059-012-2297-y

Colvin, E. K. (2014). Tumor-associated macrophages contribute to tumor progression in ovarian cancer. *Front. Oncol.* 4, 137. doi: 10.3389/fonc.2014.00137

Contursi, A., Sacco, A., Grande, R., Dovizio, M., and Patrignani, P. (2017). Platelets as crucial partners for tumor metastasis: from mechanistic aspects to pharmacological targeting. *Cell. Mol. Life Sci.* 74, 3491–3507. doi: 10.1007/s00018-017-2536-7

Creutzberg, C. L., and Fleming, G. F. (2016). "Chapter 59 - Endometrial Cancer," in *Clinical Radiation Oncology*, *4th ed*. Eds. L. L. Gunderson and J. E. Tepper (Philadelphia: Elsevier), 1203–1229.e7. doi: 10.1016/B978-0-323-24098-7.00059-9

Cui, L., Zhao, Y., Pan, Y., Zheng, X., Shao, D., Jia, Y., et al. (2017). Chemotherapy induces ovarian cancer cell repopulation through the caspase 3-mediated arachidonic acid metabolic pathway. *Oncol. Targets Ther.* 10, 5817–5826. doi: 10.2147/OTT.S150456

Cummings, M., Massey, K. A., Mappa, G., Wilkinson, N., Hutson, R., Munot, S., et al. (2019). Integrated eicosanoid lipidomics and gene expression reveal decreased prostaglandin catabolism and increased 5-lipoxygenase expression in aggressive subtypes of endometrial cancer. *J. Pathol.* 247, 21–34. doi: 10.1002/path.5160

Daikoku, T., Wang, D., Tranguch, S., Morrow, J. D., Orsulic, S., DuBois, R. N., et al. (2005). Cyclooxygenase-1 is a potential target for prevention and treatment of ovarian epithelial cancer. *Cancer Res.* 65, 3735–3744. doi: 10.1158/0008-5472.CAN-04-3814

Daikoku, T., Tranguch, S., Trofimova, I. N., Dinulescu, D. M., Jacks, T., Nikitin, A. Y., et al. (2006). Cyclooxygenase-1 Is Overexpressed in Multiple Genetically Engineered Mouse Models of Epithelial Ovarian Cancer. *Cancer Res.* 66, 2527–2531. doi: 10.1158/0008-5472.CAN-05-4063

Daikoku, T., Tranguch, S., Chakrabarty, A., Wang, D., Khabele, D., Orsulic, S., et al. (2007). Extracellular Signal-Regulated Kinase Is a Target of Cyclooxygenase-1-Peroxisome Proliferator-Activated Receptor-δ Signaling in Epithelial Ovarian Cancer. *Cancer Res.* 67, 5285–5292. doi: 10.1158/0008-5472.CAN-07-0828

Daikoku, T., Hirota, Y., Tranguch, S., Joshi, A. R., DeMayo, F. J., Lydon, J. P., et al. (2008). Conditional loss of uterine Pten unfailingly and rapidly induces endometrial cancer in mice. *Cancer Res.* 68, 5619–5627. doi: 10.1158/0008-5472.CAN-08-1274

Daikoku, T., Terakawa, J., Hossain, M. M., Yoshie, M., Cappelletti, M., Yang, P., et al. (2014). Mammalian target of rapamycin complex 1 and cyclooxygenase 2 pathways cooperatively exacerbate endometrial cancer. *Am. J. Pathol.* 184, 2390–2402. doi: 10.1016/j.ajpath.2014.05.023

De Nola, R., Menga, A., Castegna, A., Loizzi, V., Ranieri, G., Cicinelli, E., et al. (2019). The Crowded Crosstalk between Cancer Cells and Stromal Microenvironment in Gynecological Malignancies: Biological Pathways and Therapeutic Implication. *Int. J. Mol. Sci.* 20. doi: 10.3390/ijms20102401

Denkert, C., Köbel, M., Pest, S., Koch, I., Berger, S., Schwabe, M., et al. (2002). Expression of Cyclooxygenase 2 Is an Independent Prognostic Factor in Human Ovarian Carcinoma. *Am. J. Pathol.* 160, 893–903. doi: 10.1016/S0002-9440(10)64912-7

Denkert, C., Fürstenberg, A., Daniel, P. T., Koch, I., Köbel, M., Weichert, W., et al. (2003). Induction of G0/G1 cell cycle arrest in ovarian carcinoma cells by the anti-inflammatory drug NS-398, but not by COX-2-specific RNA interference. *Oncogene* 22, 8653–8661. doi: 10.1038/sj.onc.1206920

Desai, A., Xu, J., Aysola, K., Qin, Y., Okoli, C., Hariprasad, R., et al. (2014). Epithelial ovarian cancer: An overview. *World J. Transl. Med.* 3, 1–8. doi: 10.5528/wjtm.v3.i1.1

Di Tucci, C., Capone, C., Galati, G., Iacobelli, V., Schiavi, M. C., Di Donato, V., et al. (2019). Immunotherapy in endometrial cancer: new scenarios on the horizon. *J. Gynecol. Oncol.* 30:e46. doi: 10.3802/jgo.2019.30.e46

Dong, H. P., Elstrand, M. B., Holth, A., Silins, I., Berner, A., Trope, C. G., et al. (2006). NK- and B-cell infiltration correlates with worse outcome in metastatic ovarian carcinoma. *Am. J. Clin. Pathol.* 125, 451–458. doi: 10.1309/15B66DQMFYYM78CJ

Dozier, B. L., Watanabe, K., and Duffy, D. M. (2008). Two Pathways for Prostaglandin F2α (PGF2α) Synthesis by the Primate Periovulatory Follicle. *Reproduction* 136, 53–63. doi: 10.1530/REP-07-0514

Dursun, P., Gultekin, M., Yuce, K., and Ayhan, A. (2005). Lower expression of cyclooxygenase-2: is it associated with the development of borderline ovarian tumors? *Med. Hypotheses* 64, 273–278. doi: 10.1016/j.mehy.2004.06.031

Fagotti, A., Ferrandina, G., Fanfani, F., Legge, F., Lauriola, L., Gessi, M., et al. (2004). Analysis of cyclooxygenase-2 (COX-2) expression in different sites of endometriosis and correlation with clinico-pathological parameters. *Hum. Reprod.* 19, 393–397. doi: 10.1093/humrep/deh054

Fan, C., Reader, J., Rao, G., Staats, P., Ching, M. M., Fulton, A., et al. (2018). Abstract A50: EP4 receptor antagonism in paclitaxel-resistant ovarian clear cell carcinomas. *Clin. Cancer Res.* 24, A50–A50. doi: 10.1158/1557-3265.OVCA17-A50

Ferrandina, G., Legge, F., Ranelletti, F. O., Zannoni, G. F., Maggiano, N., Evangelisti, A., et al. (2002). Cyclooxygenase-2 expression in endometrial carcinoma. *Cancer* 95, 801–807. doi: 10.1002/cncr.10736

Fitzgerald, D. W., Bezak, K., Ocheretina, O., Riviere, C., Wright, T. C., Milne, G. L., et al. (2012). The effect of HIV and HPV coinfection on Cervical COX-2 expression and systemic Prostaglandin E2 Levels. *Cancer Prev. Res.* 5, 34–40. doi: 10.1158/1940-6207.CAPR-11-0496

Fons, G., Burger, M. P., Ten Kate, F. J., and van der Velden, J. (2007). Identification of potential prognostic markers for vulvar cancer using immunohistochemical staining of tissue microarrays. *Int. J. Gynecol. Pathol.* 26, 188–193. doi: 10.1097/01.pgp.0000228140.94339.db

Friedman, E. A., Ogletree, M. L., Haddad, E. V., and Boutaud, O. (2015). Understanding the role of prostaglandin E2 in regulating human platelet activity in health and disease. *Thromb. Res.* 136, 493–503. doi: 10.1016/j.thromres.2015.05.027

Fujino, H., and Regan, J. W. (2003). Prostaglandin F(2alpha) stimulation of cyclooxygenase-2 promoter activity by the FP(B) prostanoid receptor. *Eur. J. Pharmacol.* 465, 39–41. doi: 10.1016/s0014-2999(03)01485-7

Gabrilovich, D.II, and Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol.* 9, 162–174. doi: 10.1038/nri2506

Garcia, V., Gilani, A., Shkolnik, B., Pandey, V., Zhang, F. F., Dakarapu, R., et al. (2017). 20-HETE signals through G-Protein-Coupled Receptor GPR75 (Gq) to affect vascular function and trigger hypertension. *Circ. Res.* 120, 1776–1788. doi: 10.1161/CIRCRESAHA.116.310525

Garrido, M. P., Hurtado, I., Valenzuela-Valderrama, M., Salvatierra, R., Hernández, A., Vega, M., et al. (2019). NGF-enhanced vasculogenic properties of epithelial ovarian cancer cells is reduced by inhibition of the COX-2/PGE2 Signaling Axis. *Cancers (Basel)* 11. doi: 10.3390/cancers11121970

Göbel, C., Breitenbuecher, F., Kalkavan, H., Hähnel, P. S., Kasper, S., Hoffarth, S., et al. (2014). Functional expression cloning identifies COX-2 as a suppressor of antigen-specific cancer immunity. *Cell Death Dis.* 5, e1568. doi: 10.1038/cddis.2014.531

Gomes, R. N., Felipe da Costa, S., and Colquhoun, A. (2018). Eicosanoids and cancer. *Clinics (Sao Paulo)* 73. doi: 10.6061/clinics/2018/e530s

Graness, A., Hanke, S., Boehmer, F. D., Presek, P., and Liebmann, C. (2000). Protein-tyrosine-phosphatase-mediated epidermal growth factor (EGF) receptor transinactivation and EGF receptor-independent stimulation of mitogen-activated protein kinase by bradykinin in A431 cells. *Biochem. J.* 347, 441–447. doi: 10.1042/0264-6021:3470441

Guo, M., Roman, R. J., Fenstermacher, J. D., Brown, S. L., Falck, J. R., Arbab, A. S., et al. (2006). 9L Gliosarcoma cell proliferation and tumor growth in rats are suppressed by N-Hydroxy-N′-(4-butyl-2-methylphenol) Formamidine (HET0016), a selective inhibitor of CYP4A. *J. Pharmacol. Exp. Ther.* 317, 97–108. doi: 10.1124/jpet.105.097782

Guo, A. M., Arbab, A. S., Falck, J. R., Chen, P., Edwards, P. A., Roman, R. J., et al. (2007). Activation of vascular Endothelial growth factor through reactive Oxygen species mediates 20-Hydroxyeicosatetraenoic acid-induced endothelial cell Proliferation. *J. Pharmacol. Exp. Ther.* 321, 18–27. doi: 10.1124/jpet.106.115360

Guo, A. M., Liu, X., Al-Wahab, Z., Maddippati, K. R., Ali-Fehmi, R., Scicli, A. G., et al. (2011). Role of 12-lipoxygenase in regulation of ovarian cancer cell proliferation and survival. *Cancer Chemother. Pharmacol.* 68, 1273–1283. doi: 10.1007/s00280-011-1595-y

Gupta, R. A., Tejada, L. V., Tong, B. J., Das, S. K., Morrow, J. D., Dey, S. K., et al. (2003).Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. Available at: http://cancerres.aacrjournals.org/content/63/5/906 (Accessed May 25, 2015).

Hada, M., Edin, M. L., Hartge, P., Lih, F. B., Wentzensen, N., Zeldin, D. C., et al. (2019). Prediagnostic serum levels of fatty acid metabolites and risk of ovarian cancer in the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial. *Cancer Epidemiol. Biomarkers Prev.* 28, 189–197. doi: 10.1158/1055-9965.EPI-18-0392

Harizi, H., Corcuff, J.-B., and Gualde, N. (2008). Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. *Trends Mol. Med.* 14, 461–469. doi: 10.1016/j.molmed.2008.08.005

Hasegawa, K., Ishikawa, K., Kawai, S., Torii, Y., Kawamura, K., Kato, R., et al. (2013). Overcoming paclitaxel resistance in uterine endometrial cancer using a COX-2 inhibitor. *Oncol. Rep.* 30, 2937–2944. doi: 10.3892/or.2013.2790

He, Y., Zhang, M., Wu, X., Sun, X., Xu, T., He, Q., et al. (2013). High MUC2 expression in ovarian cancer is inversely associated with the M1/M2 ratio of tumor-associated Macrophages and patient survival time. *PloS One* 8. doi: 10.1371/journal.pone.0079769

Heinonen, P. K., and Metsä-Ketelä, T. (1988). Prostanoids and cyclic nucleotides in malignant and benign ovarian tumors. *Med. Oncol. Tumor Pharmacother.* 5, 11. doi: 10.1007/BF03003177

Hennequart, M., Pilotte, L., Cane, S., Hoffmann, D., Stroobant, V., Plaen, E. D., et al. (2017). Constitutive IDO1 expression in human tumors is driven by Cyclooxygenase-2 and mediates intrinsic immune resistance. *Cancer Immunol. Res.* 5, 695–709. doi: 10.1158/2326-6066.CIR-16-0400

Hinten, F., Molijn, A., Eckhardt, L., Massuger, L. F., Quint, W., Bult, P., et al. (2018). Vulvar cancer: Two pathways with different localization and prognosis. *Gynecol. Oncol.* 149, 310–317. doi: 10.1016/j.ygyno.2018.03.003

Holt, D. M., Ma, X., Kundu, N., Collin, P. D., and Fulton, A. M. (2012). Modulation of host natural killer cell functions in breast cancer *via* prostaglandin E2 receptors EP2 and EP4. *J. Immunother.* 35, 179–188. doi: 10.1097/CJI.0b013e318247a5e9

Hoxha, M., and Zappacosta, B. (2020). CYP-derived eicosanoids: implications for rheumatoid arthritis. *Prostaglandins Other Lipid Mediators* 146:106405. doi: 10.1016/j.prostaglandins.2019.106405

Hsu, H.-H., Lin, Y.-M., Shen, C.-Y., Shibu, M. A., Li, S.-Y., Chang, S.-H., et al. (2017). Prostaglandin E2-induced COX-2 expressions *via* EP2 and EP4 signaling pathways in human LoVo colon cancer cells. *Int. J. Mol. Sci.* 18. doi: 10.3390/ijms18061132

Jabbour, H. N., Milne, S. A., Williams, A. R., Anderson, R. A., and Boddy, S. C. (2001). Expression of COX-2 and PGE synthase and synthesis of PGE(2)in endometrial adenocarcinoma: a possible autocrine/paracrine regulation of neoplastic cell function *via* EP2/EP4 receptors. *Br. J. Cancer* 85, 1023–1031. doi: 10.1054/bjoc.2001.2033

Jabbour, H. N., Sales, K. J., Boddy, S. C., Anderson, R. A., and Williams, A. R. W. (2005). A positive feedback loop that regulates cyclooxygenase-2 expression and prostaglandin F2alpha synthesis *via the* F-series-prostanoid receptor and

extracellular signal-regulated kinase 1/2 signaling pathway. *Endocrinology* 146, 4657–4664. doi: 10.1210/en.2005-0804

Jabbour, H. N., Sales, K. J., Smith, O. P. M., Battersby, S., and Boddy, S. C. (2006). Prostaglandin receptors are mediators of vascular function in endometrial pathologies. *Mol. Cell. Endocrinol.* 252, 191–200. doi: 10.1016/j.mce.2006.03.025

Jana, S., Chatterjee, K., Ray, A. K., DasMahapatra, P., and Swarnakar, S. (2016). Regulation of Matrix Metalloproteinase-2 Activity by COX-2-PGE2-pAKT Axis promotes Angiogenesis in Endometriosis. *PloS One* 11, e0163540. doi: 10.1371/journal.pone.0163540

Jayson, G. C., Kohn, E. C., Kitchener, H. C., and Ledermann, J. A. (2014). Ovarian cancer. *Lancet* 384, 1376–1388. doi: 10.1016/S0140-6736(13)62146-7

Jones, R. D., Liao, J., Tong, X., Xu, D., Sun, L., Li, H., et al. (2019). Epoxy-Oxylipins and Soluble Epoxide Hydrolase metabolic pathway as targets for NSAID-induced Gastroenteropathy and inflammation-associated Carcinogenesis. *Front. Pharmacol.* 10, 731. doi: 10.3389/fphar.2019.00731

Jong, E., Winkel, P., Poelstra, K., and Prakash, J. (2011). Anticancer effects of 15d-Prostaglandin-J2 in Wild-Type and Doxorubicin-resistant ovarian cancer cells: novel actions on SIRT1 and HDAC. *PloS One* 6, e25192. doi: 10.1371/journal.pone.0025192

Joseph, T., Zalenskaya, I. A., Yousefieh, N., Schriver, S. D., Cote, L. C., Chandra, N., et al. (2012). Induction of cyclooxygenase (COX)-2 in human vaginal epithelial cells in response to TLR ligands and TNF-α. *Am. J. Reprod. Immunol.* 67, 482–490. doi: 10.1111/j.1600-0897.2011.01099.x

Kayisli, U. A., Mahutte, N. G., and Arici, A. (2002). Uterine chemokines in reproductive physiology and pathology. *Am. J. Reprod. Immunol.* 47, 213–221. doi: 10.1034/j.1600-0897.2002.01075.x

Ke, X., Dou, F., Cheng, Z., Dai, H., Zhang, W., Qu, X., et al. (2013). High expression of cyclooxygenase-2 in uterine fibroids and its correlation with cell proliferation. *Eur. J. Obstetrics Gynecol. Reprod. Biol.* 168, 199–203. doi: 10.1016/j.ejogrb.2013.01.006

Keightley, M. C., Brown, P., Jabbour, H. N., and Sales, K. J. (2010a). F-Prostaglandin receptor regulates endothelial cell function *via* fibroblast growth factor-2. *BMC Cell Biol.* 11:8. doi: 10.1186/1471-2121-11-8

Keightley, M. C., Sales, K. J., and Jabbour, H. N. (2010b). PGF2α-F-prostanoid receptor signalling *via* ADAMTS1 modulates epithelial cell invasion and endothelial cell function in endometrial cancer. *BMC Cancer* 10, 488. doi: 10.1186/1471-2407-10-488

Khunnarong, J., Tangjitgamol, S., Manusirivithaya, S., Pataradool, K., Thavaramara, T., and Leelahakorn, S. (2010). Expression of Cyclooxygenase-1 and 2 in Epithelial ovarian cancer: a Clinicopathologic study. *World J. Oncol.* 1, 19–27. doi: 10.4021/wjon2010.02.190w

Kim, S.-H., Kim, S.-H., Song, Y.-C., and Song, Y.-S. (2009a). Celecoxib potentiates the anticancer effect of cisplatin on vulvar cancer cells independently of cyclooxygenase. *Ann. N. Y. Acad. Sci.* 1171 (1). doi: 10.1111/j.1749-6632.2009.04888.x

Kim, S.-H., Oh, J.-M., No, J.-H., Bang, Y.-J., Juhnn, Y.-S., and Song, Y.-S. (2009b). Involvement of NF-κB and AP-1 in COX-2 upregulation by human papillomavirus 16 E5 oncoprotein. *Carcinogenesis* 30, 753–757. doi: 10.1093/carcin/bgp066

Kino, Y., Kojima, F., Kiguchi, K., Igarashi, R., Ishizuka, B., and Kawai, S. (2005). Prostaglandin E2 production in ovarian cancer cell lines is regulated by cyclooxygenase-1, not cyclooxygenase-2. *Prostaglandins Leukotrienes Essent. Fatty Acids* 73, 103–111. doi: 10.1016/j.plefa.2005.04.014

Kobayashi, H., Uekuri, C., Akasaka, J., Ito, F., Shigemitsu, A., Koike, N., et al. (2013). The biology of uterine sarcomas: a review and update. *Mol. Clin. Oncol.* 1, 599–609. doi: 10.3892/mco.2013.124

Kochel, T. J., and Fulton, A. M. (2015). Multiple drug resistance-associated protein 4 (MRP4), Prostaglandin Transporter (PGT), and 15-Hydroxyprostaglandin Dehydrogenase (15-PGDH) as determinants of PGE2 levels in cancer. *Prostaglandins Other Lipid Mediat* 0, 99–103. doi: 10.1016/j.prostaglandins.2014.11.003

Kochel, T. J., Reader, J. C., Ma, X., Kundu, N., and Fulton, A. M. (2017). Multiple drug resistance-associated protein (MRP4) exports prostaglandin E2 (PGE2) and contributes to metastasis in basal/triple negative breast cancer. *Oncotarget* 8, 6540–6554. doi: 10.18632/oncotarget.14145

Koike, H., Egawa, H., Ohtsuka, T., Yamaguchi, M., Ikenoue, T., and Mori, N. (1992). Eicosanoids production in endometriosis. *Prostaglandins Leukot. Essent. Fatty Acids* 45, 313–317. doi: 10.1016/0952-3278(92)90089-2

Lau, M.-T., Wong, A. S. T., and Leung, P. C. K. (2010). Gonadotropins induce tumor cell migration and invasion by increasing cyclooxygenases expression and prostaglandin E(2) production in human ovarian cancer cells. *Endocrinology* 151, 2985–2993. doi: 10.1210/en.2009-1318

Lee, T. S., Jeon, Y. T., Kim, J.-W., Park, N., and Park, I. (2007). Increased cyclooxygenase-2 expression associated with inflammatory cellular infiltration in elderly patient with vulvar cancer. *Signal Transduction Pathways, Part C: Cell Signaling in Health and Disease*, 143–153. doi: 10.1196/annals.1397.018

Lee, J., Banu, S. K., Burghardt, R. C., Starzinski-Powitz, A., and Arosh, J. A. (2013). Selective inhibition of prostaglandin E2 receptors EP2 and EP4 inhibits adhesion of human endometriotic epithelial and stromal cells through suppression of integrin-mediated mechanisms. *Biol. Reprod.* 88, 77. doi: 10.1095/biolreprod.112.100883

Lee, H. L., Jang, J. W., Lee, S. W., Yoo, S. H., Kwon, J. H., Nam, S. W., et al. (2019). Inflammatory cytokines and change of Th1/Th2 balance as prognostic indicators for hepatocellular carcinoma in patients treated with transarterial chemoembolization. *Sci. Rep.* 9, 1–8. doi: 10.1038/s41598-019-40078-8

Li, W., Zhang, H., Xu, R., Zhuo, G., Hu, Y., and Li, J. (2008). Effects of a selective cyclooxygenase-2 inhibitor, nimesulide, on the growth of ovarian carcinoma *in vivo*. *Med. Oncol.* 25, 172–177. doi: 10.1007/s12032-007-9016-0

Li, W., Ji, Z., Zhuo, G., Xu, R., Wang, J., and Jiang, H. (2010). Effects of a selective cyclooxygenase-1 inhibitor in SKOV-3 ovarian carcinoma xenograft-bearing mice. *Med. Oncol.* 27, 98–104. doi: 10.1007/s12032-009-9179-y

Li, W., Wang, J., Jiang, H.-R., Xu, X.-L., Zhang, J., Liu, M.-L., et al. (2011). Combined Effects of Cyclooxygenase-1 and Cyclooxygenase-2 Selective Inhibitors on Ovarian Carcinoma in Vivo. *Int. J. Mol. Sci.* 12, 668–681. doi: 10.3390/ijms12010668

Li, W., Liu, M.-L., Cai, J.-H., Tang, Y.-X., Zhai, L.-Y., and Zhang, J. (2012a). Effect of the combination of a cyclooxygenase-1 selective inhibitor and taxol on proliferation, apoptosis and angiogenesis of ovarian cancer *in vivo*. *Oncol. Lett.* 4, 168–174. doi: 10.3892/ol.2012.688

Li, W., Xu, X.-L., Zhang, J., Cai, J.-H., and Tang, Y.-X. (2012b). Effects of cyclooxygenase inhibitors on survival time in ovarian cancer xenograft-bearing mice. *Oncol. Lett.* 4, 1269–1273. doi: 10.3892/ol.2012.929

Li, W., Tang, Y.-X., Wan, L., Cai, J.-H., and Zhang, J. (2013). Effects of combining Taxol and cyclooxygenase inhibitors on the angiogenesis and apoptosis in human ovarian cancer xenografts. *Oncol. Lett.* 5, 923–928. doi: 10.3892/ol.2012.1086

Liu, M., Matsumura, N., Mandai, M., Li, K., Yagi, H., Baba, T., et al. (2009). Classification using hierarchical clustering of tumor-infiltrating immune cells identifies poor prognostic ovarian cancers with high levels of COX expression. *Modern Pathol.* 22, 373–384. doi: 10.1038/modpathol.2008.187

Liu, Q., Tan, W., Che, J., Yuan, D., Zhang, L., Sun, Y., et al. (2018). 12-HETE facilitates cell survival by activating the integrin-linked kinase/NF-κB pathway in ovarian cancer. *Cancer Manag. Res.* 10, 5825–5838. doi: 10.2147/CMAR.S180334

Liu, Y., Li, H., Zhao, C., and Jia, H. (2018). MicroRNA-101 inhibits angiogenesis *via* COX-2 in endometrial carcinoma. *Mol. Cell. Biochem.* 448, 61–69. doi: 10.1007/s11010-018-3313-0

Lo, C.-J., Fu, M., and Lo, F. R. (1998). Prostaglandin I2Analogue, Iloprost, down regulates Mitogen-activated protein kinases of macrophages. *J. Surg. Res.* 76, 159–164. doi: 10.1006/jsre.1998.5312

Lu, J.-W., Wang, W.-S., Zhou, Q., Gan, X.-W., Myatt, L., and Sun, K. (2019). Activation of prostaglandin EP4 receptor attenuates the induction of cyclooxygenase-2 expression by EP2 receptor activation in human amnion fibroblasts: implications for parturition. *FASEB J.* 33, 8148–8160. doi: 10.1096/fj.201802642R

Luo, Y., Zhou, H., Krueger, J., Kaplan, C., Lee, S.-H., Dolman, C., et al. (2006). Targeting tumor-associated macrophages as a novel strategy against breast cancer. *J. Clin. Invest.* 116, 2132–2141. doi: 10.1172/JCI27648

Mantovani, A., Porta, C., Rubino, L., Allavena, P., and Sica, A. (2006). Tumor-associated macrophages (TAMs) as new target in anticancer therapy. *Drug Discovery Today: Ther. Strategies* 3, 361–366. doi: 10.1016/j.ddstr.2006.07.001

McClay, E., Winski, P., Jones, J., Jennerette, J., and Gattoni-Celli, S. (1996). Δ12-Prostaglandin-J2 is cytotoxic in human malignancies and synergizes with both cisplatin and radiation. *Cancer Res.* 56, 3866–3869.

Milne, S. A., Perchick, G. B., Boddy, S. C., and Jabbour, H. N. (2001). Expression, localization, and signaling of PGE(2) and EP2/EP4 receptors in human nonpregnant endometrium across the menstrual cycle. *J. Clin. Endocrinol. Metab.* 86, 4453–4459. doi: 10.1210/jcem.86.9.7856

Moreland, K. T., Procknow, J. D., Sprague, R. S., Iverson, J. L., Lonigro, A. J., and Stephenson, A. H. (2007). Cyclooxygenase (COX)-1 and COX-2 participate in 5,6-epoxyeicosatrienoic acid-induced contraction of rabbit intralobar pulmonary arteries. *J. Pharmacol. Exp. Ther.* 321, 446–454. doi: 10.1124/jpet.106.107904

Moroney, J. W., Kunos, C., Wilkinson, E. J., and Levenback, C. (2013). "Vulva," in *Principles and Practive of Gynecologic Oncology* (Lippincott Williams & Wilkins).

Munkarah, A. R., Morris, R., Baumann, P., Deppe, G., Malone, J., Diamond, M. P., et al. (2002). Effects of prostaglandin E(2) on proliferation and apoptosis of epithelial ovarian cancer cells. (Wolters Kluwer Health/Lippincott Williams & Wilkins, ©2013) *J. Soc Gynecol. Investig.* 9, 168–173. doi: 10.1177/1071557602009003 09

Naumann, R. W. (2011). The role of the phosphatidylinositol 3-kinase (PI3K) pathway in the development and treatment of uterine cancer. *Gynecol. Oncol.* 123, 411–420. doi: 10.1016/j.ygyno.2011.08.002

Nezhat, F. R., Apostol, R., Nezhat, C., and Pejovic, T. (2015). New insights in the pathophysiology of ovarian cancer and implications for screening and prevention. *Am. J. Obstet. Gynecol.* 213, 262–267. doi: 10.1016/j.ajog.2015.03.044

Obermajer, N., Muthuswamy, R., Lesnock, J., Edwards, R. P., and Kalinski, P. (2011). Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. *Blood* 118, 5498–5505. doi: 10.1182/blood-2011-07-365825

Oliva, J. L., Pérez-Sala, D., Castrillo, A., Martínez, N., Cañada, F. J., Boscá, L., et al. (2003). The cyclopentenone 15-deoxy-Δ12,14-prostaglandin J2 binds to and activates H-Ras. *PNAS* 100, 4772–4777. doi: 10.1073/pnas.0735842100

Osawa, K., Umemura, M., Nakakaji, R., Tanaka, R., Islam, R. M., Nagasako, A., et al. (2020). Prostaglandin E2 receptor EP4 regulates cell migration through Orai1. *Cancer Sci.* 111, 160–174. doi: 10.1111/cas.14247

Ostrand-Rosenberg, S., and Sinha, P. (2009). Myeloid-derived suppressor cells: linking inflammation and cancer. *J. Immunol.* 182, 4499–4506. doi: 10.4049/jimmunol.0802740

Oyeniran, C., and Tanfin, Z. (2011). MAPK14 cooperates with MAPK3/1 to regulate endothelin-1-mediated prostaglandin synthase 2 induction and survival in leiomyoma but not in normal myometrial cells. *Biol. Reprod.* 84, 495–504. doi: 10.1095/biolreprod.110.089011

Painter, J. N., O'Mara, T. A., Morris, A. P., Cheng, T. H. T., Gorman, M., Martin, L., et al. (2018). Genetic overlap between endometriosis and endometrial cancer: evidence from cross-disease genetic correlation and GWAS meta-analyses. *Cancer Med.* 7, 1978–1987. doi: 10.1002/cam4.1445

Panigrahy, D., Kaipainen, A., Greene, E. R., and Huang, S. (2010). Cytochrome P450-derived eicosanoids: the neglected pathway in cancer. *Cancer Metastasis Rev.* 29, 723–735. doi: 10.1007/s10555-010-9264-x

Panigrahy, D., Greene, E. R., Pozzi, A., Wang, D. W., and Zeldin, D. C. (2011). EET signaling in cancer. *Cancer Metastasis Rev.* 30, 525–540. doi: 10.1007/s10555-011-9315-y

Pardoll, D. (2015). Cancer and the Immune System: Basic Concepts and Targets for Intervention. *Semin. Oncol.* 42, 523–538. doi: 10.1053/j.seminoncol.2015.05.003

Park, J., Park, S. Y., and Kim, J.-H. (2016). Leukotriene B4 receptor-2 contributes to chemoresistance of SK-OV-3 ovarian cancer cells through activation of signal transducer and activator of transcription-3-linked cascade. *Biochim. Biophys. Acta* 1863, 236–243. doi: 10.1016/j.bbamcr.2015.11.011

Park, S.-K., Herrnreiter, A., Pfister, S. L., Gauthier, K. M., Falck, B. A., Falck, J. R., et al. (2018). GPR40 is a low-affinity epoxyeicosatrienoic acid receptor in vascular cells. *J. Biol. Chem.* 293, 10675–10691. doi: 10.1074/jbc.RA117.001297

Peng, L., Hu, C., Zhang, C., Lu, Y., Man, S., and Ma, L. (2019). Anti-cancer activity of Conyza blinii saponin against cervical carcinoma through MAPK/TGF-β/Nrf2 signaling pathways. *J. Ethnopharmacol.* 112503. doi: 10.1016/j.jep.2019.112503

Pidgeon, G. P., Lysaght, J., Krishnamoorthy, S., Reynolds, J. V., O'Byrne, K., Nie, D., et al. (2007). Lipoxygenase metabolism: roles in tumor progression and survival. *Cancer Metastasis Rev.* 26, 503–524. doi: 10.1007/s10555-007-9098-3

Powell, W. S., and Rokach, J. (2015). Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid. *Biochim. Biophys. Acta* 1851, 340–355. doi: 10.1016/j.bbalip.2014.10.008

Prahm, K. P., Karlsen, M. A., Høgdall, E., Scheller, N. M., Lundvall, L., Nedergaard, L., et al. (2015). The prognostic value of dividing epithelial ovarian cancer into type I and type II tumors based on pathologic characteristics. *Gynecol. Oncol.* 136, 205–211. doi: 10.1016/j.ygyno.2014.12.029

Preston, C. C., Goode, E. L., Hartmann, L. C., Kalli, K. R., and Knutson, K. L. (2011). Immunity and immune suppression in human ovarian cancer. *Immunotherapy* 3, 539–556. doi: 10.2217/imt.11.20

Punnonen, R., Seppälä, E., Punnonen, K., and Heinonen, P. K. (1986). Fatty acid composition and arachidonic acid metabolites in ascitic fluid of patients with ovarian cancer. *Prostaglandins Leukot. Med.* 22, 153–158. doi: 10.1016/0262-1746(86)90084-3

Qiu, X., Cheng, J.-C., Chang, H.-M., and Leung, P. C. K. (2014). COX2 and PGE2 mediate EGF-induced E-cadherin-independent human ovarian cancer cell invasion. *Endocrine-Related Cancer* 21, 533–543. doi: 10.1530/ERC-13-0450

Rand, A. A., Barnych, B., Morisseau, C., Cajka, T., Lee, K. S. S., Panigrahy, D., et al. (2017). Cyclooxygenase-derived proangiogenic metabolites of epoxyeicosatrienoic acids. *Proc. Natl. Acad. Sci. U.S.A.* 114, 4370–4375. doi: 10.1073/pnas.1616893114

Rand, A., Rajamani, A., Kodani, S., Harris, T., Schlatt, L., Barnych, B., et al. (2019). Epoxyeicosatrienoic acid (EET)-stimulated angiogenesis is mediated by epoxy hydroxyeicosatrienoic acids (EHETs) formed from COX-2. *J Lipid Res* 60 (12), 1996–2005. doi: 10.1194/jlr.M094219

Rask, K., Zhu, Y., Wang, W., Hedin, L., and Sundfeldt, K. (2006). Ovarian epithelial cancer: a role for PGE2-synthesis and signalling in malignant transformation and progression. *Mol. Cancer* 5, 62. doi: 10.1186/1476-4598-5-62

Reader, J., Holt, D., and Fulton, A. (2011). Prostaglandin E2 EP receptors as therapeutic targets in breast cancer. *Cancer Metastasis Rev.* 30, 449–463. doi: 10.1007/s10555-011-9303-2

Reader, J. C., Staats, P., Goloubeva, O., Jian, N., Roque, D. M., Matheny, M. E., et al. (2016). Abstract 5169: Functional studies of the prostaglandin E2 receptor EP4 in ovarian cancer. *Cancer Res.* 76, 5169–5169. doi: 10.1158/1538-7445.AM2016-5169

Reader, J., Harper, A. K., Legesse, T., Staats, P. N., Goloubeva, O., Rao, G. G., et al. (2019). EP4 and Class III β-Tubulin expression in uterine smooth muscle tumors: implications for prognosis and treatment. *Cancers (Basel)* 11. doi: 10.3390/cancers11101590

Rees, M. C., and Turnbull, A. C. (1985). Leiomyomas release prostaglandins. *Prostaglandins Leukot. Med.* 18, 65–68. doi: 10.1016/0262-1746(85)90051-4

Reinartz, S., Finkernagel, F., Adhikary, T., Rohnalter, V., Schumann, T., Schober, Y., et al. (2016). A transcriptome-based global map of signaling pathways in the ovarian cancer microenvironment associated with clinical outcome. *Genome Biol.* 17, 108. doi: 10.1186/s13059-016-0956-6

Rodríguez-Ubreva, J., Català-Moll, F., Obermajer, N., Álvarez-Errico, D., Ramirez, R. N., Company, C., et al. (2017). Prostaglandin E2 leads to the acquisition of DNMT3A-dependent tolerogenic functions in human Myeloid-derived suppressor cells. *Cell Rep.* 21, 154–167. doi: 10.1016/j.celrep.2017.09.018

Sabry, M., and Al-Hendy, A. (2012). Medical treatment of Uterine Leiomyoma. *Reprod. Sci.* 19, 339–353. doi: 10.1177/1933719111432867

Saldivar, J. S., Lopez, D., Feldman, R. A., Tharappel-Jacob, R., de la Rosa, A., Terreros, D., et al. (2007). COX-2 overexpression as a biomarker of early cervical carcinogenesis: a pilot study. *Gynecol. Oncol.* 107, S155–S162. doi: 10.1016/j.ygyno.2007.07.023

Sales, K. J., and Jabbour, H. N. (2003). Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium. *Reproduction* 126, 559–567. doi: 10.1530/rep.0.1260559

Sales, K. J., and Katz, A. A. (2012). Inflammatory pathways in cervical cancer – the UCT contribution. *South Afr. Med. J.* 102, 493-496–496. doi: 10.7196/SAMJ.5532

Sales, K. J., Battersby, S., Williams, A. R. W., Anderson, R. A., and Jabbour, H. N. (2004a). Prostaglandin E2 mediates phosphorylation and down-regulation of the tuberous sclerosis-2 tumor suppressor (tuberin) in human endometrial adenocarcinoma cells *via the* Akt signaling pathway. *J. Clin. Endocrinol. Metab.* 89, 6112–6118. doi: 10.1210/jc.2004-0892

Sales, K. J., Maudsley, S., and Jabbour, H. N. (2004b). Elevated prostaglandin EP2 receptor in endometrial adenocarcinoma cells promotes vascular endothelial

growth factor expression *via* cyclic 3',5'-adenosine monophosphate-mediated transactivation of the epidermal growth factor receptor and extracellular signal-regulated kinase 1/2 signaling pathways. *Mol. Endocrinol.* 18, 1533–1545. doi: 10.1210/me.2004-0022

Sales, K. J., List, T., Boddy, S. C., Williams, A. R. W., Anderson, R. A., Naor, Z., et al. (2005). A novel angiogenic role for prostaglandin F2alpha-FP receptor interaction in human endometrial adenocarcinomas. *Cancer Res.* 65, 7707–7716. doi: 10.1158/0008-5472.CAN-05-0101

Sales, K. J., Boddy, S. C., Williams, A. R. W., Anderson, R. A., and Jabbour, H. N. (2007). F-prostanoid receptor regulation of fibroblast growth factor 2 signaling in endometrial adenocarcinoma cells. *Endocrinology* 148, 3635–3644. doi: 10.1210/en.2006-1517

Sales, K. J., Boddy, S. C., and Jabbour, H. N. (2008a). F-prostanoid receptor alters adhesion, morphology and migration of endometrial adenocarcinoma cells. *Oncogene* 27, 2466–2477. doi: 10.1038/sj.onc.1210883

Sales, K. J., Grant, V., and Jabbour, H. N. (2008b). Prostaglandin E2 and F2alpha activate the FP receptor and up-regulate cyclooxygenase-2 expression *via the* cyclic AMP response element. *Mol. Cell. Endocrinol.* 285, 51–61. doi: 10.1016/j.mce.2008.01.016

Sales, K. J., Maldonado-Pérez, D., Grant, V., Catalano, R. D., Wilson, M. R., Brown, P., et al. (2009). Prostaglandin F2α-F-prostanoid receptor regulates CXCL8 expression in endometrial adenocarcinoma cells *via the* calcium–calcineurin–NFAT pathway. *Biochim. Biophys. Acta (BBA) - Mol. Cell Res.* 1793, 1917–1928. doi: 10.1016/j.bbamcr.2009.09.018

Santayana, C., Sun, W., Yang, P., Dick-Biascoechea, M., Johnson, H. W., Fan, C., et al. (2018). EP4 Receptor Expression in Pelvic Organ Prolapse and Radiation-Induced Vaginal Stenosis: A Novel Therapeutic Target. American Urogyencologic Society 39th Annual Scientific Meeting, October 9-13, 2018. (Chicago: IL Female Pelvic Medicine & Reconstructive Surgery: 9/10 2018) 24 (5S), S138–S157. doi: 10.1097/SPV.0000000000000616

Sasaki, H., and Fukushima, M. (1994). Prostaglandins in the treatment of cancer. *Anticancer Drugs* 5, 131–138. doi: 10.1097/00001813-199404000-00002

Sasaki, H., Takada, K., Terashima, Y., Ekimoto, H., Takahashi, K., Tsuruo, T., et al. (1991). Human ovarian cancer cell lines resistant to cisplatin, doxorubicin, and L-phenylalanine mustard are sensitive to delta 7-prostaglandin A1 and delta 12-prostaglandin J2. *Gynecol. Oncol.* 41, 36–40. doi: 10.1016/0090-8258(91)90251-y

Sasaki, H., Niimi, S., Akiyama, M., Tanaka, T., Hazato, A., Kurozumi, S., et al. (1999). Antitumor activity of 13,14-dihydro-15-deoxy-delta7-prostaglandin-A1-methyl ester integrated into lipid microspheres against human ovarian carcinoma cells resistant to cisplatin *in vivo*. *Cancer Res.* 59, 3919–3922.

Schulte, R. R., Linkous, A. G., Hallahan, D. E., and Yazlovitskaya, E. M. (2011). Cytosolic phospholipase A2 as a molecular target for the radiosensitization of ovarian cancer. *Cancer Lett.* 304, 137–143. doi: 10.1016/j.canlet.2011.02.015

Seo, J.-M., Park, S., and Kim, J.-H. (2012). Leukotriene B4 Receptor-2 promotes invasiveness and metastasis of ovarian cancer cells through signal transducer and activator of transcription 3 (STAT3)-dependent Up-regulation of Matrix Metalloproteinase 2. *J. Biol. Chem.* 287, 13840–13849. doi: 10.1074/jbc.M111.317131

Serhan, K., Gartung, A., and Panigrahy, D. (2018). Drawing a link between the thromboxane A2 pathway and the role of platelets and tumor cells in ovarian cancer. *Prostaglandins Other Lipid Mediat.* 137, 40–45. doi: 10.1016/j.prostaglandins.2018.06.001

Siegel, R. L., Miller, K. D., and Jemal, A. (2019). Cancer statistic. *CA: A Cancer J. Clin.* 69, 7–34. doi: 10.3322/caac.21551

Sinha, P., Clements, V. K., Fulton, A. M., and Ostrand-Rosenberg, S. (2007). Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Res.* 67, 4507–4513. doi: 10.1158/0008-5472.CAN-06-4174

Spinella, F., Rosanò, L., Di Castro, V., Natali, P. G., and Bagnato, A. (2004a). Endothelin-1-induced prostaglandin E2-EP2, EP4 signaling regulates vascular endothelial growth factor production and ovarian carcinoma cell invasion. *J. Biol. Chem.* 279, 46700–46705. doi: 10.1074/jbc.M408584200

Spinella, F., Rosanò, L., Di Castro, V., Nicotra, M. R., Natali, P. G., and Bagnato, A. (2004b). Inhibition of cyclooxygenase-1 and -2 expression by targeting the endothelin a receptor in human ovarian carcinoma cells. *Clin. Cancer Res.* 10, 4670–4679. doi: 10.1158/1078-0432.CCR-04-0315

Spinella, F., Rosanò, L., Elia, G., Di Castro, V., Natali, P. G., and Bagnato, A. (2004c). Endothelin-1 stimulates cyclooxygenase-2 expression in ovarian cancer cells through multiple signaling pathways: evidence for involvement of transactivation of the epidermal growth factor receptor. *J. Cardiovasc. Pharmacol.* 44 Suppl 1, S140–S143. doi: 10.1097/01.fjc.0000166255.12229.0d

Straus, D. S., and Glass, C. K. (2001). Cyclopentenone prostaglandins: new insights on biological activities and cellular targets. *Med. Res. Rev.* 21, 185–210. doi: 10.1002/med.1006

Sun, Y., Yao, Z., Zhao, Z., Xiao, H., Xia, M., Zhu, X., et al. (2018). Natural killer cells inhibit metastasis of ovarian carcinoma cells and show therapeutic effects in a murine model of ovarian cancer. *Exp. Ther. Med.* 16, 1071–1078. doi: 10.3892/etm.2018.6342

Surowiak, P., Materna, V., Denkert, C., Kaplenko, I., Spaczyński, M., Dietel, M., et al. (2006). Significance of cyclooxygenase 2 and MDR1/P-glycoprotein coexpression in ovarian cancers. *Cancer Lett.* 235, 272–280. doi: 10.1016/j.canlet.2005.04.035

Tae, I. H., Park, E. Y., Dey, P., Son, J. Y., Lee, S.-Y., Jung, J. H., et al. (2018). Novel SIRT1 inhibitor 15-deoxy-Δ12,14-prostaglandin J2 and its derivatives exhibit anticancer activity through apoptotic or autophagic cell death pathways in SKOV3 cells. *Int. J. Oncol.* 53, 2518–2530. doi: 10.3892/ijo.2018.4561

Taek, S. L., Yong, T. J., Kim, J.-W., Won, J. K., Park, N., Park, I., et al. (2007). Increased cyclooxygenase-2 expression associated with inflammatory cellular infiltration in elderly patients with vulvar cancer. *Signal Transduction Pathways Part C: Cell Signaling Health Dis.* 1095, 143–153. doi: 10.1196/annals.1397.018

Tamura, M., Sebastian, S., Yang, S., Gurates, B., Ferrer, K., Sasano, H., et al. (2002). Up-regulation of cyclooxygenase-2 expression and prostaglandin synthesis in endometrial stromal cells by malignant endometrial epithelial cells. A paracrine effect mediated by prostaglandin E2 and nuclear factor-kappa B. *J. Biol. Chem.* 277, 26208–26216. doi: 10.1074/jbc.M201347200

Tennis, M. A., Vanscoyk, M., Keith, R. L., and Winn, R. A. (2010). The role of prostacyclin in lung cancer. *Trans. Res.* 155, 57–61. doi: 10.1016/j.trsl.2009.06.010

Tong, B. J., Tan, J., Tajeda, L., Das, S. K., Chapman, J. A., DuBois, R. N., et al. (2000). Heightened expression of Cyclooxygenase-2 and Peroxisome proliferator-activated receptor-δ in human Endometrial Adenocarcinoma. *Neoplasia* 2, 483–490. doi: 10.1038/sj.neo.7900119

Torre, L. A., Trabert, B., DeSantis, C. E., Miller, K. D., Samimi, G., Runowicz, C. D., et al. (2018). Ovarian cancer statistics 2018. *CA: A Cancer J. Clin.* 68, 284–296. doi: 10.3322/caac.21456

Uddin, S., Ahmed, M., Hussain, A., Assad, L., Al-Dayel, F., Bavi, P., et al. (2010). Cyclooxygenase-2 inhibition inhibits PI3K/AKT kinase activity in epithelial ovarian cancer. *Int. J. Cancer* 126, 382–394. doi: 10.1002/ijc.24757

Umamaheswaran, S., Dasari, S. K., Yang, P., Lutgendorf, S. K., and Sood, A. K. (2018). Stress, inflammation, and eicosanoids: an emerging perspective. *Cancer Metastasis Rev.* 37, 203–211. doi: 10.1007/s10555-018-9741-1

Uotila, P. J., Erkkola, R. U., and Klemi, P. J. (2002). The expression of cyclooxygenase-1 and -2 in proliferative endometrium and endometrial adenocarcinoma. *Ann. Med.* 34, 428–433. doi: 10.1080/07853890232101 2379

Wallace, A. E., Sales, K. J., Catalano, R. D., Anderson, R. A., Williams, A. R. W., Wilson, M. R., et al. (2009). Prostaglandin F2alpha-F-prostanoid receptor signaling promotes neutrophil chemotaxis *via* chemokine (C-X-C motif) ligand 1 in endometrial adenocarcinoma. *Cancer Res.* 69, 5726–5733. doi: 10.1158/0008-5472.CAN-09-0390

Wallace, A. E., Gibson, D. A., Saunders, P. T. K., and Jabbour, H. N. (2010). Inflammatory events in endometrial adenocarcinoma. *J. Endocrinol.* 206, 141–157. doi: 10.1677/JOE-10-0072

Wang, D., and DuBois, R. N. (2010). Eicosanoids and cancer. *Nat. Rev. Cancer* 10, 181–193. doi: 10.1038/nrc2809

Wang, W., Zhang, J., and Zhang, G. (2019). Cytochrome P450 monooxygenase-mediated eicosanoid pathway: a potential mechanistic linkage between dietary fatty acid consumption and colon cancer risk. *Food Sci. Hum. Wellness* 8, 337–343. doi: 10.1016/j.fshw.2019.11.002

Wen, Z., Liu, H., Li, M., Li, B., Gao, W., Shao, Q., et al. (2015). Increased metabolites of 5-lipoxygenase from hypoxic ovarian cancer cells promote tumor-associated macrophage infiltration. *Oncogene* 34, 1241–1252. doi: 10.1038/onc.2014.85

Wilson, A. J., Fadare, O., Beeghly-Fadiel, A., Son, D.-S., Liu, Q., Zhao, S., et al. (2015). Aberrant over-expression of COX-1 intersects multiple pro-

tumorigenic pathways in high-grade serous ovarian cancer. *Oncotarget* 6, 21353–21368. doi: 10.18632/oncotarget.3860

Wong, J. L., Obermajer, N., Odunsi, K., Edwards, R. P., and Kalinski, P. (2016). Synergistic COX2 induction by IFNγ and TNFα self-limits Type-1 immunity in the human tumor microenvironment. *Cancer Immunol. Res.* 4, 303–311. doi: 10.1158/2326-6066.CIR-15-0157

Xu, S., Zhou, W., Ge, J., and Zhang, Z. (2018). Prostaglandin E2 receptor EP4 is involved in the cell growth and invasion of prostate cancer *via the* cAMP–PKA/PI3K–Akt signaling pathway. *Mol. Med. Rep.* 17, 4702–4712. doi: 10.3892/mmr.2018.8415

Ylikorkala, O., Kauppila, A., and Viinikka, L. (1983). Prostacyclin and thromboxane in ovarian cancer: effect of cytostatics and prostaglandin synthesis inhibitors. *Gynecol. Oncol.* 16, 340–345. doi: 10.1016/0090-8258(83)90160-9

Zarcone, R., Mainini, G., Carfora, E., and Cardone, A. (1997). Current etiopathogenetic views in vulvar cancer. *Panminerva Med.* 39, 30–34.

Zhang, J. Y., Prakash, C., Yamashita, K., and Blair, I. A. (1992). Regiospecific and enantioselective metabolism of 8,9-epoxyeicosatrienoic acid by cyclooxygenase. *Biochem. Biophys. Res. Commun.* 183, 138–143. doi: 10.1016/0006-291x(92)91619-2

Zhang, X., Yan, K., Deng, L., Liang, J., Liang, H., Feng, D., et al. (2019). Cyclooxygenase 2 promotes proliferation and invasion in ovarian cancer cells *via the* PGE2/NF-κB pathway. *Cell Transplant.* 28, 1S–13S. doi: 10.1177/0963689719890597

Zhao, Y., Cui, L., Pan, Y., Shao, D., Zheng, X., Zhang, F., et al. (2017). Berberine inhibits the chemotherapy-induced repopulation by suppressing the arachidonic acid metabolic pathway and phosphorylation of FAK in ovarian cancer. *Cell Prolif.* 50, e12393. doi: 10.1111/cpr.12393

Zhu, J., Trillsch, F., Mayr, D., Kuhn, C., Rahmeh, M., Hofmann, S., et al. (2017). Prostaglandin receptor EP3 regulates cell proliferation and migration with impact on survival of endometrial cancer patients. *Oncotarget* 9, 982–994. doi: 10.18632/oncotarget.23140

Zhu, J., Mayr, D., Kuhn, C., Mahner, S., Jeschke, U., and von Schönfeldt, V. (2018). Prostaglandin E2 receptor EP1 in healthy and diseased human endometrium. *Histochem. Cell Biol.* 149, 153–160. doi: 10.1007/s00418-017-1616-y

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 03 September 2020
doi: 10.3389/fphar.2020.01297

The Emerging Therapeutic Potential of Nitro Fatty Acids and Other Michael Acceptor-Containing Drugs for the Treatment of Inflammation and Cancer

Matthias Piesche[1,2], Jessica Roos[3,4]*, Benjamin Kühn[5], Jasmin Fettel[5], Nadine Hellmuth[4], Camilla Brat[4], Isabelle V. Maucher[5], Omar Awad[3], Carmela Matrone[6], Simon Gabriel Comerma Steffensen[7,8], Georg Manolikakes[9], Ulrike Heinicke[4], Kai D. Zacharowski[4], Dieter Steinhilber[5] and Thorsten J. Maier[3,4]*

[1] Biomedical Research Laboratories, Medicine Faculty, Catholic University of Maule, Talca, Chile, [2] Oncology Center, Medicine Faculty, Catholic University of Maule, Talca, Chile, [3] Department of Safety of Medicinal Products and Medical Devices, Paul-Ehrlich-Institut (Federal Institute for Vaccines and Biomedicines), Langen, Germany, [4] Department of Anesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt, Goethe University Frankfurt, Frankfurt, Germany, [5] Institute of Pharmaceutical Chemistry, Goethe-University, Frankfurt am Main, Germany, [6] Division of Pharmacology, Department of Neuroscience, School of Medicine, University of Naples Federico II, Naples, Italy, [7] Department of Biomedicine, Medicine Faculty, Aarhus University, Aarhus, Denmark, [8] Animal Physiology, Department of Biomedical Sciences, Veterinary Faculty, Central University of Venezuela, Maracay, Venezuela, [9] Department of Organic Chemistry, Technical University Kaiserslautern, Kaiserslautern, Germany

OPEN ACCESS

Edited by:
Emanuela Ricciotti, University of Pennsylvania, United States

Reviewed by:
Francisco J. Schopfer, University of Pittsburgh, United States
Homero Rubbo, Universidad de la República, Uruguay

***Correspondence:**
Matthias Piesche
mpiesche@ucm.cl
Jessica Roos
Jessica.Roos@pei.de

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *03 April 2020*
Accepted: *05 August 2020*
Published: *03 September 2020*

Citation:
Piesche M, Roos J, Kühn B, Fettel J, Hellmuth N, Brat C, Maucher IV, Awad O, Matrone C, Comerma Steffensen SG, Manolikakes G, Heinicke U, Zacharowski KD, Steinhilber D and Maier TJ (2020) The Emerging Therapeutic Potential of Nitro Fatty Acids and Other Michael Acceptor-Containing Drugs for the Treatment of Inflammation and Cancer. Front. Pharmacol. 11:1297. doi: 10.3389/fphar.2020.01297

Nitro fatty acids (NFAs) are endogenously generated lipid mediators deriving from reactions of unsaturated electrophilic fatty acids with reactive nitrogen species. Furthermore, Mediterranean diets can be a source of NFA. These highly electrophilic fatty acids can undergo Michael addition reaction with cysteine residues, leading to post-translational modifications (PTM) of selected regulatory proteins. Such modifications are capable of changing target protein function during cell signaling or in biosynthetic pathways. NFA target proteins include the peroxisome proliferator-activated receptor γ (PPAR-γ), the pro-inflammatory and tumorigenic nuclear factor-κB (NF-κB) signaling pathway, the pro-inflammatory 5-lipoxygenases (5-LO) biosynthesis pathway as well as soluble epoxide hydrolase (sEH), which is essentially involved in the regulation of vascular tone. In several animal models of inflammation and cancer, the therapeutic efficacy of well-tolerated NFA has been demonstrated. This has already led to clinical phase II studies investigating possible therapeutic effects of NFA in subjects with pulmonary arterial hypertension. Albeit Michael acceptors feature a broad spectrum of bioactivity, they have for a rather long time been avoided as drug candidates owing to their presumed unselective reactivity and toxicity. However, targeted covalent modification of regulatory proteins by Michael acceptors became recognized as a promising approach to drug discovery with the recent FDA approvals of the cancer therapeutics, afatanib (2013), ibrutinib (2013), and osimertinib (2015). Furthermore, the Michael acceptor, neratinib, a

dual inhibitor of the human epidermal growth factor receptor 2 and epidermal growth factor receptor, was recently approved by the FDA (2017) and by the EMA (2018) for the treatment of breast cancer. Finally, a number of further Michael acceptor drug candidates are currently under clinical investigation for pharmacotherapy of inflammation and cancer. In this review, we focus on the pharmacology of NFA and other Michael acceptor drugs, summarizing their potential as an emerging class of future antiphlogistics and adjuvant in tumor therapeutics.

Keywords: covalent drugs, electrophilic fatty acids, Michael acceptor, nitroalkylation, post-translational modifications

INTRODUCTION

Compounds possessing Michael acceptor units feature a broad spectrum of bioactivity. However, they have been largely excluded from drug discovery endeavors because of their presumed unselective reactivity and toxicity. Nevertheless, the recent FDA approval of several cancer drugs has demonstrated that covalent modifications *via* Michael addition can be a powerful tool to develop new drugs (Bauer, 2015; Ghosh et al., 2019).

Covalent modifications of proteins *via* post-translational modifications (PTMs) are a rather effective strategy to modulate protein function and activity. Such modifications include phosphorylation, acetylation, glycosylation, oxidation, and hydroxylation. Among all amino acids, cysteine plays a particularly important role in covalent modifications and is susceptible to phosphorylation, acetylation as well as oxidation. Such modifications can affect the cellular localization of the protein, its interaction with other binding partners as well as its function or activity. PTMs of proteins are of regulatory significance in almost all cell types and functional systems, including the immune system, the cardiovascular system, and the gastrointestinal system (Bürkle, 2002; Ehrentraut and Colgan, 2012; Liu et al., 2016; Fert-Bober et al., 2018).

The majority of research studies had focus on protein phosphorylation. Methylation of lysine or arginine residues, acetylation, nitrosation of thiol groups and tyrosine residues as well as alkylation of cysteines or other nucleophilic amino acids have received less attention. Alkylation of nucleophilic amino acids, including cysteine, is achieved either by reaction with alpha-halocarbonylation, aminoethylation, or by Michael addition to a molecule containing a Michael acceptor. In this review, we will focus on the Michael addition as an important reaction of approved drugs or drug candidates to induce PTMs that alter protein function.

The Michael reaction is defined as a conjugate addition of a nucleophile (Michael donor) to an electron-deficient olefin, such as an α,β-unsaturated carbonyl compound (Michael acceptor) (**Figures 1A, B**). However, instead of the carbonyl group, the substituent can also be a nitro group or another strongly electron-withdrawing group. Cellular nucleophiles, *e.g.* the thiol group of cysteine, the imidazole of histidine, or the ϵ-amino group of the amino acid lysine have also been described to be Michael donors. Well-recognized Michael acceptors that play a major part in the resolution process of inflammation are endogenously generated anti-inflammatory electrophilic lipids called nitro fatty acids (NFAs) (Rubbo, 2013). Other electrophilic species, which are formed during inflammatory reactions, are cyclopentenone prostaglandins (*i.e.*, 15Δ-PGJ2). In this review, we will focus on NFA as representatives of lipid-derived electrophilic species, discuss other Michael acceptor-containing drugs engaged in clinical trials or already approved, and show the emerging therapeutic potential of this class of drugs.

NITRO FATTY ACIDS AS NATURALLY OCCURRING MEDIATORS CONTAINING A MICHAEL ACCEPTOR MOIETY

Unsaturated fatty acids can be metabolized under inflammatory conditions to reactive products to act as pro- or anti-inflammatory mediators (Grimble and Tappia, 1998) (**Figure 1C**). A special group of those lipid mediators are electrophilic alkenes, like NFA. They are generated endogenously and can be detected in the plasma of human blood. Besides their endogenous generation, NFAs can also be dietary supplemented as natural ingredients of olives or native olive oil (Fazzari et al., 2014). Moreover, evidence has shown that dietary supplementation with nitrate (NO_3^-), nitrite (NO_2^-), and conjugated linoleic acid (cLA) can have an obvious effect on NFA plasma levels (Delmastro-Greenwood et al., 2015). High concentrations of reactive oxygen and nitrogen-derived species generated within inflamed tissue promote the formation of NFA. Hereafter, the nitrogen-derived species react with unsaturated fatty acids, yielding electrophilic NFA (Freeman et al., 2008). NFAs engage in cell signaling, among others, through Michael addition reactions showing distinct anti-inflammatory actions (Rubbo, 2013). The most studied NFAs are nitro-oleic acid (NO_2-OA), nitro-linoleic acid (NO_2-LA), nitro-conjugated linoleic acid (NO_2-cLA), and nitro-arachidonic acid (NO2-AA). Through Michael addition, NFAs can adduct intracellular glutathione (GSH) as well as susceptible protein cysteine and histidine residues, inducing changes in protein structure, functionality, and subcellular distribution. PTM of cysteine residues by NFA has been shown to be reversible (Batthyany et al., 2006; Baker et al., 2007).

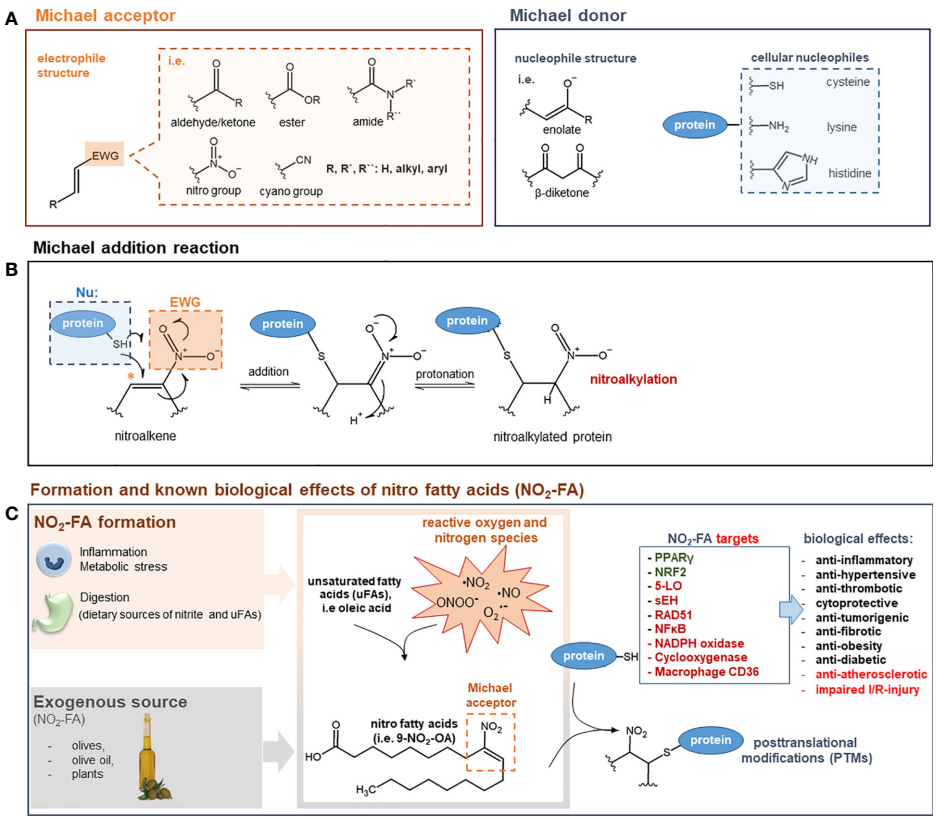


FIGURE 1 | (A) General structure of Michael acceptors and Michael donors. Michael acceptor moiety: electron withdrawing group (EWG) adjacent to an olefin structure forming an electrophilic, electron-deficient olefin. Examples of EWGs: aldehyde, keto, ester, amide, cyano, or nitro groups. Michael donor: nucleophiles such as enolates, β-diketones, thiols of cysteines, imidazoles of histidines, or ϵ-amino groups of lysines. **(B)** Mechanism of Michael addition reaction. The Michael addition reaction is exemplified by the attack of a cellular nucleophile to the electrophilic β-carbon (*) of a nitroalkene moiety. After the addition of the thiolate anion a protonation step takes place to form a nitroalkylated protein. **(C)** Formation and known biological effects of nitro fatty acids (NO_2-FA). NO_2-FA can be endogenously generated during inflammation by a reaction of nitric dioxide (NO_2) with unsaturated fatty acids. Nitric dioxide can derive from different reactive nitrogen species (i.e nitric oxide, peroxynitrite) or precursor molecules like nitrate (NO_3^-) and nitrite (NO_2^-). NO_2-FA can also be directly supplemented as natural ingredients of olives, olive oil and plants NO_2-FA engage in cell signaling processes *via* the post-translational modification (PTM) of nucleophilic protein targets such as 5-LO, PPARγ, sEH, or NF-kB (proteins highlighted in green: activated/increased activity/expression; proteins highlighted in red: inhibited/decreased activity/expression). These PTMs induce profound changes in protein function and distribution and are therefore the leading cause for numerous biological effects. For a comprehensive overview on NFA targets and therapeutic effects see (Schopfer and Khoo, 2019). *5-LO: 5-lipoxygenase; NF-κB: nuclear factor-κB; NRF-2: nuclear factor erythroid 2-related factor 2; PPARγ: peroxisome proliferator–activated receptor γ; sEH; soluble epoxide hydrolase; I/R: ischemia/reperfusion.*

THERAPEUTIC EFFECTS OF NFA

The protective and beneficial effects of NFA could be demonstrated in a number of *in vivo* animal disease models. Thus, therapeutic effects were proposed for the following diseases:

Classical Inflammatory Diseases

Inflammation is part of the body's immune defense responses. However, inflammatory processes require complex regulation to warrant a local and temporal restriction of inflammation and avoid chronification potentially triggering some types of cancers, rheumatoid arthritis, periodontitis, asthma, and Crohn's disease. NFAs have been shown to modulate directly the activity of a number of pro-inflammatory enzymes or factors involved in the acute phase of inflammation, such as nuclear factor-κB (NF-κB, see section *NF-κB*), 5-Lipoxygenase (5-LO, see section *5-Lipoxygenase*), and Prostaglandin endoperoxide H synthases 2 (COX-2, see section *Prostaglandin endoperoxide H synthases 1 and 2*). However, NFA mediated effects not only might contribute to symptom relief but also to active enhanced resolution of inflammation. Resolution of inflammation includes abrogation of immune cell recruitment at sites of inflammation, removal of activated immune cells, and suppression of production of pro-inflammatory mediators. NFAs affect resolution by triggering activation of the resolution factor transcriptional factor peroxisome proliferator-activated receptor γ [PPARγ, see section *PPARγ* and for overview on the factors role in resolution see (Croasdell et al., 2015)]. The direct covalent binding of NFA to functionally important amino acid residues of inflammatory target proteins might facilitate strong and sustained pharmacological impacts. Furthermore, reactions with amino acids which are poorly conserved among closely related proteins and embedding

of the target amino acids into specific clefts can increase selectivity of binding of covalent drugs to an exclusive set of proteins (Singh et al., 2011) which might potentially also apply to NFA. This might allow targeting a unique set of regulatory key proteins in inflammation. The known target proteins mediating the anti-inflammatory effects of NFA are listed in section *Nucleophilic Targets Susceptible to Michael Addition by NFA to Explain Their Therapeutic Effects*.

Several *in vivo* models have shown a therapeutic effect of NFA in preclinical models of inflammation, *e.g.*, pretreating mice with NFA in a model of LPS-induced inflammation resulted in a reduced severity of multiorgan dysfunction compared to LPS alone. Expression of inflammatory mediators in the NFA-treated group was also reduced compared to the LPS group (Wang et al., 2010). In a model of inflammatory bowel disease, the addition of NFA resulted in attenuated colonic inflammation and improved the clinical symptoms of this disease. The activation of PPARγ played an important role in this protection (Borniquel et al., 2010). However, the route of NFA administration seems to be important as demonstrated by Mather et al. They showed in a model of allergic contact dermatitis (ACD) that the administration of NFA subcutanously induced an immunosuppressive responses, including an increased activity of regulatory T cells (Mathers et al., 2017). In contrast, a topical administration of NFA in the same mouse model exacerbated the inflammatory response, including the infiltration of neutrophils, inflammatory monocytes, and $\gamma\delta$ T cells (Mathers et al., 2018).

Cardiovascular Diseases

By 2030, it is expected that cardiovascular disease (CVD) will account for 25 million deaths worldwide. Even in underdeveloped countries, CVD surpasses infectious diseases, indicating a high medical need for new treatment options (Okwuosa et al., 2016). CVDs including hypertension, coronary heart disease, and atherosclerosis are potentially associated with an elevated generation of reactive oxygen species (ROS) and nitric oxide (NO) and compromised endogenous antioxidant defenses (Mann et al., 2009), suggesting that NFA could be important players in CVD. Indeed, a number of publications indicate that NFAs possess protective effects against CVD. Exemplified, NFA-induced endothelium-independent vasorelaxation, which possibly involves release of NO (Freeman et al., 2008). Furthermore, in animal models of atherosclerosis, NFAs have been shown to reduce infarct size, decrease neutrophil infiltration into the infarct zone to prevent myocyte apoptosis (Rudolph et al., 2010b), reduce lipid accumulation, and promote plaque stability (Rudolph et al., 2010a). Finally, antihypertensive effects of NFAs have been reported, *e.g.*, nitro-oleic acid inhibits angiotensin II-induced hypertension (Zhang et al., 2010; Charles et al., 2014; Klinke et al., 2014).

Cancer

Globally, cancer is the second leading cause of death. Inflammatory processes are crucially involved at all stages of tumor development, starting from tumor initiation, promotion, malignant transformation, tumor invasion, and tumor metastasis. Furthermore, some targets of NFA are well-recognized players in tumorigenesis, and oxidative stress modulates these different stages of inflammation-induced carcinogenesis. Thus, a role of NFA in tumorigenesis has been proposed. Recently, it has been demonstrated that NFAs suppress the growth of breast cancer by diminishing cancer cell viability along with tumor cell migration and invasion (Woodcock et al., 2018). Furthermore, NFAs have been shown to enhance the cytotoxic activity of DNA-damaging agents on growth of triple-negative breast cells and might therefore function as adjuvants in therapy of such types of cancers (Asan et al., 2019). Finally, NFAs suppress tumor growth by causing mitochondrial dysfunction and activation of the intrinsic pathway of apoptosis in colorectal cancer cells. Inhibition of the pro-inflammatory proteins, NF-κB and 5-lipoxygenase, which are involved in tumorigenesis, is considered a possible mode of action for NFAs, explaining their chemopreventive effects (Kühn et al., 2018).

Fibrosis

Fibrosis is characterized as the overgrowth and hardening of a connective tissue in response to an injury or damage. The precise pathophysiological mechanism of generation of fibrosis is rather complex and still unknown; however, there seems to be a connection between fibrotic events and chronic inflammation (Wynn, 2008). In 2014, Reddy et al. reported that nitro fatty acids abolished pulmonary fibrosis and reduced disease severity in a mouse model with a possible role of NFA-mediated activation of PPAR (Reddy et al., 2014). Recently, NFAs have been demonstrated to protect against steatosis and fibrosis during development of non-alcoholic fatty liver disease in mice fibrosis (Rom et al., 2019). Moreover, NFAs have been reported suppressing angiotensin II-mediated fibrotic remodeling and atrial fibrillation with mechanisms that still need further investigation (Rudolph et al., 2016). NFAs might therefore be a novel lipid-based therapeutic strategy against different types of fibrotic processes with molecular mechanisms that need to be addressed in future studies.

NUCLEOPHILIC TARGETS SUSCEPTIBLE TO MICHAEL ADDITION BY NFA TO EXPLAIN THEIR THERAPEUTIC EFFECTS

NFAs are potent electrophiles that alkylate susceptible thiols of multiple transcriptional regulatory proteins, affecting downstream gene expression and modulating metabolic as well as inflammatory signaling pathways (Trostchansky et al., 2013). In a study done by Khoo and Li et al., the effects of different NFA derivatives on the NF-kB and Nrf2 signaling pathways were investigated to better understand NFA structure–function relationships. This study demonstrated that NFA derivatives having varying carbon chain lengths and different positions of the nitroalkene group and show different potencies in affecting the above-mentioned signaling pathways (Khoo et al., 2018). Moreover, Gorczynski and Smitherman et al. demonstrated that

the potency of NFA to activate PPAR-γ may vary according to the position of the NO2 group, where the position of nitration plays an important role in optimal PPAR-γ activation (Gorczynski et al., 2009). A number of protein targets of NFA have already been identified, which might explain some of the therapeutic effects of NFA (**Figure 1C**).

PPAR γ

The transcriptional factor PPARγ is a nuclear receptor regulating lipid homeostasis, inflammatory signaling, and adipocyte differentiation. PPARγ activation in myeloid cells suppresses the expression of pro-inflammatory mediators, like interferon-γ (IFNγ) and nitric oxide synthase (iNOS or NOS2) (Tontonoz and Spiegelman, 2008). PPARγ has also been associated with neutrophil apoptosis along with clearance and resolution of inflammation (Konopleva et al., 2004). NFAs are partial agonists of PPARγ. For that reason, they can restore insulin sensitivity *in vivo*. Furthermore, unlike Rosiglitazone they cause no weight gain while reducing the insulin and glucose levels in $Lep^{ob/ob}$ mice. Therefore, this feature is considered as an advantage that highlights its beneficial actions and potentially reduces the adverse effects associated with full PPARγ agonists (Schopfer et al., 2010; Lamas Bervejillo et al., 2020). They are also weaker agonists of PPAR-α and β/δ (Baker et al., 2005; Schopfer et al., 2005). It has been shown that PPARγ agonists appear to have direct neuroprotective actions in several different animal models, like Alzheimer's disease (AD), stroke, multiple sclerosis (MS), Parkinson's disease (PD), and amyotrophic lateral sclerosis (Sundararajan et al., 2006). Therefore, activation of PPARγ could explain some of the anti-inflammatory and possible neuroprotective actions of NFAs. NFAs used as drugs might therefore be useful for the therapy of these diseases.

NF-κB

Nuclear factor-κB (NF-κB) plays a significant part during inflammatory responses and is involved in the initiation, development, metastasis, and resistance to the treatment of cancer. In unstimulated cells, NF-κB dimers are sequestered in the cytoplasm by the inhibitor of κB proteins, I-κB (canonical/classical NF-κB pathway). Upon activation, I-kB releases NF-κB, allowing it to translocate into the nucleus where it activates the transcription of pro-inflammatory cytokines and other inflammatory mediators. NF-κB is comprised of two subunits, *i.e.* p50 and p65 (Mitchell et al., 2016). NFAs can specifically nitroalkylate the p65 subunit of NF-κB and, to a lower extent, the p50 subunit. Alkylation inhibits the translocation and DNA-binding affinities of NF-κB and, in consequence, inhibits its pro-inflammatory activities (Cui et al., 2006; (Khoo et al., 2018). This causes the repression of NF-κB dependent target gene expression and cytokine production such as tumor necrosis factor α (TNFα), interleukin-6 (IL-6), monocyte chemoattractant protein 1 (MCP-1) and the vascular cell adhesion molecule 1 (VCAM-1) that plays an important role in monocyte rolling and adhesion which is essential for the inflammatory process (Cui et al., 2006; Villacorta et al., 2018). In line with these findings, NFAs were reported to suppress the growth of aggressive breast cancer cells by inhibiting NF-κB transcriptional activity, thereby suppressing downstream NF-κB target gene expression (Woodcock et al., 2018). Furthermore, NFAs are able to interfere with the initial toll-like receptor-4 (TLR4) signaling upstream of the NF-κB cascade by disrupting the recruitment of the receptor into lipid rafts and assembly of the adaptor protein TRAF6/IKKb/IkBa complex in vascular cells. However, the exact mechanism still remains to be defined (Villacorta et al., 2013). Inhibition of NF-κB could therefore play a certain role in NFA-induced anti-tumorigenic effects as well as in some of the NFA-induced anti-inflammatory effects.

Nrf2-Keap1

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of genes encoding for proteins that counteract oxidative stress triggered during cell and tissue injury and inflammation. Under basal conditions, the transcription factor Nrf2 is suppressed by cytosolic Keap1 (Kelch-like ECH-associated protein 1), which promotes rapid ubiquitination and proteasomal degradation of Nrf2 (Kobayashi and Yamamoto, 2005). NFA activate Nrf2-dependent antioxidant gene expression by nitroalkylation of the thiol residues of critical cysteines, such as Cys273 and 288, in the Nrf2 regulatory protein, Keap1, thus facilitating the translocation of Nrf2 into the nucleus (Kansanen et al., 2011). The expression of Nrf2-dependent genes, including heme oxygenase-1 (HO-1), glutathione peroxidase (GPx), glutathione reductase, glutathione S-transferase, or superoxide dismutase then promotes cell protection by attenuating the inflammatory response (Zhu et al., 2008; Dreger et al., 2010). Glutathione (GSH) itself is a tripeptide synthesized from glutamate, cysteine, and glycine. GSH is catalyzed by two cytosolic enzymes, which are γ-glutamylcysteine synthetase and GSH synthetase. GSH metabolism plays a crucial role in the defense against oxidative stress, nutrient metabolism, and regulation of cellular events essential for whole-body homeostasis (Wu et al., 2004). Regarding HO-1, nitrolinoleic acid was able to induce its activity in a cell culture model of pulmonary epithelial cells as well as in the lungs of rats (Iles et al., 2009). The therapeutic effects of activating Nrf2 through NFA could be useful for treatment of oxidative stress- and Nrf2-dependent diseases, such as cancer and several types of inflammatory and neurodegenerative diseases.

5-Lipoxygenase

Lipoxygenases (LO) catalyze the generation of reactive lipid mediators derived from arachidonic acid, such as leukotrienes and 5-HETEs. These oxidized products support inflammatory processes by acting as chemotactic and chemokinetic agents as well as bronchioconstrictive factors (Haeggström and Funk, 2011). Our own studies have demonstrated that nitro-oleic acid is a potent inhibitor of 5-LO *in vitro* and *in vivo*. This effect is because of a nitroalkylation of catalytically relevant cysteine residues, C416 and C418, resulting in a loss of enzyme activity (Hörnig et al., 2012; Awwad et al., 2014). Blocking 5-LO was a major mechanism responsible for the suppression of lipopolysaccharide-induced pulmonary inflammation in mice dosed with NFA (Awwad et al., 2014). The 5-LO-inhibitory

potency of NFA could be beneficial for the treatment of pulmonary diseases, such as bronchial asthma, but also for cardiovascular diseases and cancer with the well-documented pathophysiological role of this enzyme (Steinhilber et al., 2010). In this sense, treatment of pulmonary hypertension could be a promising target.

Soluble Epoxide Hydrolase

Another enzyme that possesses a reactive cysteine, which is essential for catalytic functioning, is soluble epoxide hydrolase (sEH). sEH catalyzes the hydration of epoxides which is crucial for the regulation of blood pressure by the modulation of epoxyeicosatrienoic acid (EET) levels and their influence on blood vessel relaxing tonus through the named endothelial hyperpolarization mechanism. The conserved cysteine residue, C521, which resides proximal to the catalytic center of sEH can be alkylated by electrophilic lipids, leading ultimately to the inhibition of the enzyme (Charles et al., 2011). In a C521S sEH redox-dead knock-in mouse model, it was shown that treatment with NFA protects mice from hypertension only with sEH wildtype C521. Mice with an sEH C521S mutation did not benefit from NFA treatment, suggesting an underlying Michael reaction of NFA with this cysteine (Charles et al., 2014).

Figure 1 summarizes the nucleophilic targets of NFA and their potential role in disease.

Microsomal Prostaglandin E2 Synthase-1

Microsomal prostaglandin E synthase 1 (mPGES1) is a terminal enzyme of the cyclooxygenase pathway which catalyzes the last step of the synthesis of the pro-inflammatory mediator prostaglandin E_2 (PGE_2). The isoprostane 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ2) is a naturally occurring degradation product of prostaglandin D_2 which is another bioactive product of the cyclooxygenase pathway. Notably, 15d-PGJ_2 is not a member of the class of nitro fatty acids as the Michael acceptor moiety consist of a cyclopentenone motif lacking a nitro group. Interestingly, Prage and Jakobsson et al. could demonstrate that 15d-PGJ_2 can inhibit mPGES1 by covalent modification of residue C59 and by noncovalent inhibition through binding at the substrate (PGH_2) binding site which can potentially explain some anti-inflammatory actions of 15d-PGJ_2 (Prage et al., 2012).

Prostaglandin Endoperoxide H Synthases 1 and 2

Prostaglandin endoperoxide H synthase is an important enzyme that catalyzes the conversion of arachidonic acid (AA) to prostaglandin G2 (PGG2) and its subsequent reduction to prostaglandin H2 (PGH2), which is expressed during inflammation. PGHS exists in two isoforms, PGHS-1 and -2, which are found in mammalian tissues. Trostchansky et al. demonstrated that nitration of the carbon chain of AA yields novel nitroarachidonic acid isomers with new biological properties and causes the diversion of arachidonic acid from its normal metabolizing pathways. Nitroarachidonic acid inhibited peroxidase activity in PGHS-1 and -2 (COX-1 and 2) as well as oxygenase activity in PGHS-1. In addition, both isoforms, PGHS-1 and -2, were unable to use nitroarachidonic as a substrate for oxygenase or peroxidase activity. These effects suggest their potential pharmacological relevance during inflammation (Trostchansky et al., 2011).

CD36

The membrane protein CD36 is the latest protein, identified as direct NFA target in macrophages (Vazquez et al., 2020). The CD36 protein is expressed on the surface of various cell types including immune cells and mediates long-chain fatty acid and cholesterol ester uptake among other functions. Binding of NFA to CD36 reduced mLDL (modified low density lipoprotein) uptake and both cholesterol and cholesteryl ester accumulation in macrophages potentially providing an explanation for the NFA-mediated athero-protective effects animal models.

FURTHER APPROVED OR CLINICALLY DEVELOPED THERAPEUTIC DRUGS CONTAINING MICHAEL ACCEPTORS

Examples of further synthetic and naturally occurring Michael acceptors that target noncatalytic cysteine thiols are described subsequently. Structures of drugs or structural scaffolds with the Michael acceptor moiety highlighted in red are shown in **Figure 2**.

Approved Drugs

Ibrutinib

Ibrutinib is an inhibitor of Bruton's tyrosine kinase (BTK) with well-recognized antineoplastic activity. For BTK inhibition, the drug uses a Michael acceptor moiety for irreversible binding to the target cysteine (Herman et al., 2011). Such inhibition induces a modest cancer cell apoptosis, abolishes proliferation, and prevents both B-cell activation and B-cell-mediated signaling. Ibrutinib was FDA-approved in 2013 for mantle cell lymphoma and later for chronic lymphocytic leukemia (CLL, 2014) and B-cell lymphoma-like Waldenström macroglobulinemia (Castillo et al., 2016). In 2017, ibrutinib was approved as a second-line chronic graft *versus* host disease (cGVHD) (Miklos et al., 2017). In addition, ibrutinib has also been shown to have potential effects against autoimmune arthritis (Akinleye et al., 2013). Some patients have had reported relapse during ibrutinib therapy, which was based on an acquired resistance to this drug, based in most cases of cytogenetic abnormalities (Byrd et al., 2020). Functional studies indicate that the C481S mutation in *BTK* is the reason for resistance to ibrutinib by preventing irreversible drug binding (Woyach et al., 2014).

Neratinib

Neratinib is an irreversible tyrosine-kinase inhibitor of epidermal growth factor receptor (EGFR)/human epidermal growth factor receptor 1 (HER1), HER2, and HER4. Neratinib comprises a quinolone core that reacts through a Michael addition with the same reactive substituents as afatinib (see the following) but with an affinity and pharmacological potency

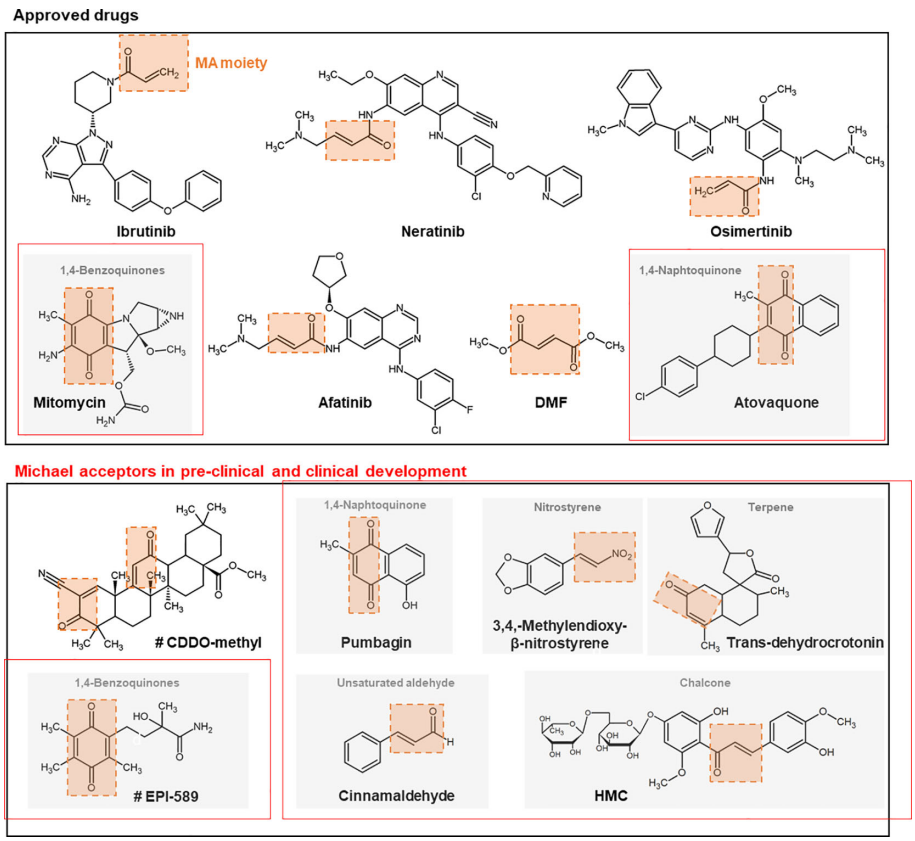


FIGURE 2 | Chemical structures + of different approved and (pre-) clinically developed therapeutic drugs containing Michael acceptors. The Michael acceptor moiety is highlighted in orange. Compounds marked with # are currently studied in clinical trials. *CDDO, 2-cyano-3,12-dioxo-oleana-1,9(11)-dien-28-oic acid; DMF, dimethyl fumarate; MA, Michael acceptor; HMC, hesperidin methyl chalcone.*

that is lower (Feldinger and Kong, 2015). The inhibition of tyrosine kinases lead to a G1-S phase arrest, which results in inhibition of tumor cell proliferation (Rabindran et al., 2004). It has been shown that neratinib is less potent in inhibiting proliferation of EGFR-positive cells compared to HER2-positive cells (Canonici et al., 2013). Furthermore, it has been demonstrated that neratinib can reverse membrane-bound ATP transporter-mediated multidrug resistance (Zhao et al., 2012). Neratinib was approved in 2017 as adjuvant treatment for patients with early-stage HER2-overexpressed/amplified breast cancer. Neratinib is further being evaluated in clinical trials for advanced/metastatic breast cancer and solid tumors, including HER2-mutated tumors (Feldinger and Kong, 2015).

Osimertinib

Osimertinib is a third-generation EGFR tyrosine kinase inhibitor that was marketed in 2017 to treat advanced or metastatic non-small-cell lung cancer (NSCLC) carrying a specific mutation. The drug targets cancer cells that contain the T790M mutation in the gene coding for EGFR but spares cancer cells with wildtype EGFR (Lategahn et al., 2019). However, within a time period of approximately 1 year, cancer cells can become resistant through various mechanisms, such as amplification of cMet and HER2. Nevertheless, the main mechanism of resistance to osimertinib is the mutation of the non-catalytic cysteine (C797S), representing the target amino acid of the drug for the Michael reaction (Patel et al., 2017). Therapeutic strategies to overcome osimertinib resistance are described elsewhere (Tang and Lu, 2018).

Afatinib

Afatinib is a protein kinase inhibitor that was approved in 2013 for the treatment of NSCLC. The chemical drug contains an electrophilic group capable of a Michael addition reaction to conserved cysteine residues inside the catalytic domains of EGFR, HER2, and HER4. This reaction inhibits irreversible enzymatic activity (Solca et al., 2012). Afatinib has also been investigated for breast cancer because of its additional activity against HER2 (Minkovsky and Berezov, 2008). However, a clinical phase II trial has indicated there is no benefit from afatinib alone or when combined with the microtubule assembly inhibitor vinorelbine (Jim Yeung, 2005) compared with treatment of the investigator's choice in women suffering from HER2-positive breast cancer with progressive brain metastases during or after therapy with trastuzumab, lapatinib, or both (Cortés et al., 2015).

Dimethyl Fumarate (DMF)

DMF is the ester of the unsaturated dicarboxylic fumaric acid. A number of *in vitro* and *in vivo* studies have shown a potent anti-inflammatory effect of DMF in a variety of diseases, *e.g.*, MS (Kappos et al., 2008), psoriasis (Nieboer et al., 1989; Reich et al., 2009), and asthma (Seidel et al., 2009). Currently, oral DMF is approved for MS (2013) and psoriasis (2017). The results of two large phase III trials testing DMF in remitting MS led to its rapid regulatory approval, first by the US Food and Drug Administration (FDA) in 2013 and then by the European Medicines Agency (EMA) in spring 2014 (Fox et al., 2012; Gold et al., 2015). In 2017, EMA approved an oral formulation of DMF for the treatment of adult patients with moderate-to-severe chronic plaque psoriasis (Mrowietz et al., 2018).

Similar to NFA, DMF can alkylate Keap1, which leads to a stabilization and translocation of Nrf2 (Seidel et al., 2009; Linker and Haghikia, 2016). Induction of Nrf2-mediated gene expression is considered the major mode of action responsible for suppression of neurodegenerative processes of MS. Additionally, Gillard et al. showed that DMF treatment led to significant inhibition of the nuclear translocation of p65 (canonical/classical NF-κB) and p52 (non-canonical NF-κB) signaling (Gillard et al., 2015) with a therapeutic relevance that requires further evaluation.

Michael Acceptors in Pre-Clinical and Clinical Development

Bardoxolone Methyl (Also Known as CDDO-Methyl Ester or RT-402)

CDDO-methyl ester (CDDO-Me), a semi-synthetic triterpenoid derived from oleanolic acid, is a promising chemotherapeutic and anti-inflammatory agent in clinical development (Couch et al., 2005; Celentano et al., 2019; Rossing et al., 2019; Tian et al., 2019). The structure of CDDO is comprised of two α, β-unsaturated carbonyl moieties, which are accessible for nucleophilic addition. An essential factor for potency is not the triterpenoid skeleton but the cyanoenone group whose absence greatly reduces the activity of CDDO-Me. This Michael acceptor structure can generate reversible adducts with cysteine residues in target proteins like Keap1 and IkB kinase, leading consequently to an activation of the NRF2/Keap1 pathway and inhibition of NF-κB signaling (Wang et al., 2014). Interestingly, the selective binding of CDDO-Me to cysteine residues of different proteins seems to be both context-dependent and dose-dependent. It has been shown that low concentrations of CDDO-Me protect cells against oxidative stress whereas higher concentrations are known to induce apoptosis (Wang et al., 2014). CDDO-Me and the related analog inhibit inflammatory responses and tumor growth *in vivo* and have also been considered for use in patients (Place et al., 2003). In particular, CDDO-Me underwent phase III clinical trials for chronic kidney disease (CKD) as well as phase I/II clinical trials for malignant diseases (Wang et al., 2014). However, it was discontinued for CKD owing to an increased risk of heart failure (de Zeeuw et al., 2013). Nevertheless, it is still being tested in clinical trials for treatment of obesity in adult men (NCT04018339, phase I), pulmonary hypertension (NCT03068130, phase III), chronic or diabetic kidney diseases (NCT03749447, phase III, and NCT03550443, phase III, respectively), Alport Syndrome, (NCT03019185, phase II/III) and autosomal dominant polycystic kidney disease (NCT03918447, phase III). Omaveloxolone (*N*-(2-Cyano-3,12-dioxo-28-noroleana-1,9(11)-dien-17-yl)-2,2-difluoropropanamide; CDDO- DFPA) is being further tested for the treatment of patients with Friedreich's Ataxia (NCT02255435, phase II).

Polyphenols

Polyphenols are major constituents of many herbal remedies exhibiting anti-inflammatory activities (González et al., 2011). They are characterized by the presence of multiple phenol structural units. Different types of polyphenols also contain a Michael acceptor unit within their structure. In addition, the oxidation of the parent polyphenol can lead to the formation of a reactive olefin, such as in the oxidation of a hydroquinone to a quinone. Over the last decade, there has been abundant attention to the possible health benefits of dietary plant polyphenols as antioxidants (Pandey and Rizvi, 2009). It has been reported that a number of polyphenolic extracts suppress tumor cell proliferation and reduce pro-inflammatory processes by inhibiting 5-LO (Leifert and Abeywardena, 2008), NF-κB, and mitogen-activated protein kinase signaling (Santangelo et al., 2007). The inactivation of NF-κB by polyphenols is thought to be mediated by their interaction with cysteine residues in either IκB kinase or the DNA-binding domain of NF-κB, particularly the Cy38 of the p65 subunit (Wang and Dubois, 2010). However, the beneficial effects of polyphenols have been mainly demonstrated by *in vitro* studies. Several factors, like low bioavailability, poor solubility, and high metabolism of some of the polyphenols, may account for the poor and difficult clinical translations of these compounds (Christensen, 2018). Chalcones, an example of polyphenols, are discussed subsequently.

Chalcones

Chalcones demonstrate a broad and versatile spectrum of pharmacological activities, including immunomodulation, anti-inflammatory, anticancer, antiviral, and antibiotic properties (Lee et al., 2015). Recently, a stable topical formulation has been tested containing the chalcone derivative hesperidin methyl chalcone (HMC) protecting the skin of mice towards UVB-induced oxidative stress and inflammation (Martinez et al., 2017). Other Chalcones have recently been identified as 5-LO inhibitors and urenyl chalcone derivatives exert a dual inhibition of cyclooxygenase-2 (COX-2)/5-LO activities (Lee et al., 2015). Further studies could show that chalcone derivatives inhibit secretory phospholipase A_2, COX enzymes, lipoxygenases, pro-inflammatory cytokine synthesis, neutrophil chemotaxis, immune cell phagocytosis, and production of ROS (Bukhari et al., 2014a; Bukhari et al., 2014b; Lee et al., 2015). Recently, a novel chalcone derivate (chalcone-*O*-alkylamine derivate) has been documented, demonstrating that it might be a multifunctional anti-AD agent (Bai et al., 2019). Besides all the promising potential of chalcone derivates, there are no approved drugs available to date.

Nitrostyrenes

3,4-Methylenedioxy-β-nitrostyrene has been identified as a NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome inhibitor with a Michael addition as the proposed mode of action. The activation of the NLRP inflammasome triggered caspase-1 activation and the release of the cytokine interleukin-1β, a pro-inflammatory mediator, which is involved in both acute as well as chronic inflammatory responses. Thus, NLRP3 has been implicated in the pathogenesis of several human diseases, such as gout, silicosis, type I/II diabetes, general endothelial dysfunction, erectile dysfunction, atherosclerosis, and AD (Baldwin et al., 2016; Pereira et al., 2019; Fais et al., 2019). Furthermore, the suppression of the inflammasome may efficiently reduce damaging processes, such as K+ efflux, lysosomal membrane destabilization, ROS generation, and ubiquitin/deubiquitination post-translational modifications (Baldwin et al., 2016). Therefore, the NLRP3 inflammasome is an attractive therapeutic target (Baldwin et al., 2016). Moreover, nitroalkene analog of α-tocopherol have been designed for the prevention and treatment of inflammation related diseases (Rodriguez-Duarte et al., 2018).

Quinones

1,4-benzoquinones are recognized for their anti-inflammatory, antioxidative, and anticancer activities (Schaible et al., 2014). A popular representative of this group is the active ingredient thymoquinone, isolated from *Nigella sativa* (Woo et al., 2012). Anti-inflammatory effects have been found to be associated with suppression of leukotriene formation (Werz, 2007) and 1,4-benzoquinone AA-861 is a well-recognized 5-LO inhibitor (Yoshimoto et al., 1982). EPI-589, a (R)-troloxamide quinone, is currently in clinical trials for PD. The estimated completion date was December 2019, but no results have been published as of yet (NCT02462603). Mitomycin, a benzoquinone, is used in the clinic for non-invasive or minimally invasive bladder cancers, and in combination with 5-fluorouracil (5-FU) as well as radiation during treatment of stage I-III anal cancer (Milla et al., 2014). Notably, mitomycin also contains a pharmacologically active aziridine group that leads to alkylations of target proteins.

Naphthoquinone

Naphthoquinone forms the structural basis of a number of natural compounds, most pre-eminently the K vitamins. Naphthoquinones are known for their antibiotic, antiviral, antifungal, antiphlogistic, and antipyretic properties (Hernández-Pérez et al., 1995; Kobayashi et al., 2011). The naphthoquinone plumbagin is a naturally compound in the medicinal herb *Plumbago zeylanica*. This herb has been safely used for centuries in Indian Ayurvedic and Oriental medicine for treating various ailments, including bacterial infections and allergic processes (Powolny and Singh, 2008). Furthermore, plumbagin has already been described to suppress NF-κB activation (Sandur et al., 2006). Plumbagin can also reduce the viability of human prostate cancer cells by triggering apoptosis. Adding N-acetylcysteine (NAC) significantly attenuated this effect (Powolny and Singh, 2008) indicating that the reaction of plumbagin with cellular proteins containing thiol groups might play an important role in the pharmacological activity of plumbagin. The naphthoquinone atovaquone is used to treat or prevent, *e.g.*, pneumocystis pneumonia (PCP) (only mild cases), toxoplasmosis, and malaria where it is one of the two component drugs along with malarone (National Institute of Diabetes and Digestive and Kidney Diseases, 2012).

Unsaturated Carboxylic Acids and Aldehydes

Unsaturated carboxylic acids and aldehydes are a structurally rather heterogeneous group. Examples are either NFA or cinnamaldehyde. NFA and their targets are described in detail above. Cinnamaldehyde is the main constituent of cinnamon. Cinnamaldehyde is a pleitropic bioactive compound that attracted lots of interest for its anticancer, anti-inflammatory, antidiabetic, and antifungal properties. It has also been reported to be beneficial against neurological diseases, *e.g.*, PD and AD (Rao and Gan, 2014). Cinnamaldehyde contains an α,β-unsaturated aldehyde and can act as a Michael acceptor. It is a potent activator of the transient receptor potential cation channel, subfamily A, member 1 (TRPA1) (Sandur et al., 2006), a Ca^{2+} channel that plays an important role in inflammatory and neuropathic pain, as well as the pathogenesis of AD (Lee et al., 2016). More detailed information about cinnamaldehyde and its potential as therapeutic agent is reviewed in Chen et al. (2017).

Terpenes

Some terpenes, which is the largest group of phytochemicals, contain a Michael acceptor unit (Butturini et al., 2011), *e.g.*, trans-dehydrocrotonin and crotonin. Both compounds originate from croton plants from the Amazonian region and have been associated with anti-inflammatory, anti-atherogenic, and anti-ulcerogenic properties (Hiruma-Lima et al., 2002). Furthermore, other diseases that affect the cardiovascular system, such as diabetes, have been shown to have positive effects from aqueous extracts of the stem barks of Croton cuneatus Klotz, which significantly reduced blood glucose levels in diabetic rats (Torrico et al., 2007).

A vast number of many other terpenes (Vasas and Hohmann, 2014) and phenolic (Kris-Etherton et al., 2002) compounds have been shown to possess protective effects regarding the cardiovascular system, including relaxation in conductance vessels, antithrombotic properties, lowering low-density lipoproteins (main cholesterol transporter for atheroma formation), or reduction of coronary heart disease and cardiovascular risk factors, as well as reversal of endothelial dysfunction.

These findings could indicate that the Michael acceptor moiety and reactivity of the drugs with thiols of target proteins are relevant to the therapeutic effects triggered by terpenes.

Therapeutic Effects of the Endogenous Michael Donor, GSH, and GSH Inhibitors

GSH is an abundant natural tripeptide found within almost all cells at concentrations of 0.5 to 10 mM (Lushchak, 2012). Oxidative stress can lead to chronic inflammation, which in turn could mediate most chronic diseases (Reuter et al., 2010). GSH is vital for protecting tissues against the degenerative effects of oxidative damage through the conjugation of chemically reactive electrophilic molecules from endogenous or exogenous agents and thus preventing unwanted reactions with important cell constituents (Reed, 1986; Lu, 1999). The Michael addition is one

of the mechanisms how GSH protects nucleic acids and proteins from these agents. Endogenous agents are described extensively by Wang and Ballatori (Wang and Ballatori, 1998). One example is the electrophilic eicosanoids, which contain α,β-unsaturated ketones and are biosynthesized during the oxidative metabolism of arachidonic acid. GSH adducts have been observed with molecules derived from lipoxygenases (Wang and Ballatori, 1998) and with electrophilic fatty acids (Batthyany et al., 2006).

GSH plays also an integral role in the clearance of drugs. The aforementioned drug, afatinib, undergoes extensive conjugation with GSH both in buffer and cytosol fractions deriving from liver and kidney tissues, whereas ibrutinib has exhibited much lower degree of GSH-dependent conjugation (Shibata and Chiba, 2015). The importance of GSH in drug clearance can be seen when patients accidentally take an overdose, *e.g.*, acetaminophen, a medication used to treat pain and fever, which is generally safe when used in the recommended dosage. However, when taken in overdose, it can cause a potentially fatal, hepatic centrilobular necrosis (James et al., 2003), which accounts for almost one-half of all patients with acute liver failure in the United States and Great Britain. At nontoxic doses, the metabolite of acetaminophen is efficiently detoxified by GSH, forming an acetaminophen-glutathione conjugate *via* Michael addition (Jollow et al., 1974). However, at toxic doses, the metabolite depleted hepatic GSH by as much as 80–90% (Mitchell et al., 1973; Jollow et al., 1974). Repletion of GSH using an antidote like N-acetylcysteine was able to prevent toxicity (Dargan and Jones, 2003).

Another important function of GSH is in the detoxification of small toxic molecules, thereby rendering them into less toxic derivatives. This activity accounts for one type of drug resistance, a key element in the failure of chemotherapy treatment. GSH can be combined with anticancer drugs to yield less toxic GSH conjugates with a higher water-solubility. The GSH conjugates of chemotherapeutics can penetrate out of the cells by the glutathione S-conjugate export (GS-X) pump or multidrug resistance-associated protein (MRP). Levels of GSH, glutathione-related enzymes, and the GS-X pump or MRP have been demonstrated to be elevated or overexpressed in a number of drug-resistant tumor cells (Zhang et al., 1998). A number of inhibitors to block or downregulate GSH to increase tumor responsiveness to chemotherapy are under investigation in several clinical trials (Trachootham et al., 2009; Singh et al., 2012). The most advanced drug is currently undergoing Phase III trials where the GSH inhibitor APR-246 and azacitidine or azacitidine alone is being compared in patients with TP53-mutated MDS (NCT03745716).

THERAPEUTIC OPTIONS FOR MICHAEL ACCEPTORS FOR NEURODEGENERATIVE/ NEUROINFLAMMATORY DISEASES

A link between Michael acceptors and neurodegenerative diseases has largely been established by a variety of sources. As Michael acceptors are present in the manufacturing, agricultural, and polymer industries, human exposure to these compounds is pervasive. Indeed, acrolein and methylvinyl ketone (MVK) are environmental pollutants while acrylamide (ACR) and methyl acrylate are dietary contaminants (reviewed in (Morgan et al., 2000; Friedman, 2003). Michael acceptors, because of their metastable and reactive properties, attack synaptic proteins and form complexes that accumulate at the nerve terminals. Consistently, elevated levels of acrolein and 4-hydroxy-nonenal (HNE) have been found in the degenerating neurons of the substantia nigra of PD patients (Yoritaka et al., 1996), where it has been hypothesized that they promote α-synuclein aggregation. Similarly, α,β-unsaturated aldehydes are generated endogenously as a break-down product of lipid peroxidation of ω-6 polyunsaturated fatty acids and have been thought to be responsible for synaptotoxicity and nerve terminal dysfunction in PD (Kehrer and Biswal, 2000; Friedman, 2003; LoPachin et al., 2008; Lee and Park, 2013; Fecchio et al., 2018). In AD, patients experienced increases in both α and β secretase levels, which has been linked to the presence of various lipid peroxidation products, including MDA, F2-isoprostanes, and HNE. Overall, elevated levels of Michael acceptor derivatives have also been detected in amyloid plaques (Markesbery and Carney, 1999). Evidence of lipid peroxidation was detected in Huntington's brain tissues, where, in particular, HNE was found to colocalize with Huntington inclusions (Lee et al., 2011).

In addition, ACR, acrolein, HNE, and other unsaturated carbonyl derivatives inhibit NO signaling at the nerve terminal, triggering neuroinflammatory processes, a common feature shared by most neurodegenerative diseases (Csala et al., 2015).

Interestingly, Michael acceptors have recently gained interest for their potential therapeutic properties with respect to neurodegenerative disease.

In this regard, recent studies have underlined the cytoprotective effects against oxidative stress that the synthetic triterpenoid (TP) derivatives of CDDO exert within either *in vivo* or *in vitro* animal model of neuronal deficits (Dinkova-Kostova et al., 2005; Tran et al., 2008; Dumont et al., 2009; Castellano et al., 2019). In particular, Dumont et al. found that three-month administration of CDDO-MA improved cognitive performance and reduced Aβ protein levels, which is the main component of senile plaques as well as plaque deposition in AD mouse models, by reducing inflammation, enhancing phagocytosis of the Aβ protein and plaques, and decreasing oxidative stress (Dumont et al., 2009).

Of note, additional studies pointed on the use of Bruton tyrosine kinase inhibitors in the treatment of AD as well as in MS (Montalban et al., 2019; Keaney et al., 2019). In addition, cysteine-targeting compounds such as ICE-like cysteine protease inhibitors (caspase I inhibitors) have been recently suggested as anti-apoptotic and anti-inflammatory agents to treat AD and PD patients, in which progressive neuronal death seems to be associated with caspase overactivation (LoPachin et al., 2008). The rationale of the use of Michael acceptors comes from the idea of developing compounds selectively targeting cysteine residues on caspase, thereby taking possible advantage

of the low occurrence of cysteine residues in the human proteome (2.3%) and thus potentially lessening off-target effects. However, as these compounds have multiple biological activities, the possibility to use them in the treatment of neurodegenerative diseases is far from clear, thus raising scepticism in the scientific arena (Parvez et al., 2018; Poganik and Aye, 2020).

FURTHER DISEASES WITH A POSSIBLE THERAPEUTIC EFFICACY OF MICHAEL ACCEPTORS

Beside the therapeutic effects described herein, Michael acceptors might play a potential role in a number of other diseases.

Recently, antidepressive effects of certain Michael acceptors have been reported. DHIPC, a 2′-hydroxy-4′,6′-diisoprenyloxychalcone derivate, exhibits antidepressant effects by increasing serotonin, noradrenaline, and 5 hydroxyindoleacetic acid levels in the hippocampus, hypothalamus, and brain cortex of DHIPC-treated mice (Zhao et al., 2018).

The plant-derived Michael acceptor curcumin possesses multiple modes of action. The drug is able to suppress liver fibrosis by modulation of a specific miRNA mediating the epigenetic regulation of liver fibrosis (Zheng et al., 2014). Furthermore, it is an effective treatment for idiopathic pulmonary fibrosis owing to inhibition of collagen secretion, fibroblast proliferation, and differentiation (Smith et al., 2010).

The Michael acceptor epalrestat is a reversible aldose reductase inhibitor preventing the conversion of glucose to sorbitol within the polyol pathway. In Japan, it is an approved drug for treatment of subjective and objective symptoms of diabetic neuropathy, the most common long-term complication in patients suffering from diabetes mellitus (Ramirez and Borja, 2008).

Antiparasitic properties are also benefits of Michael acceptor-containing drugs. K777 is an irreversible inhibitor of cruzan, a cysteine protease of *Trypanosoma cruzi*, which causes Chagas disease. Hybrid compounds comprising an electrophilic warhead and Michael acceptor-containing structure motif (*e.g.*, vinyl sulfone, pyrimidine nitrile group) are effective anti-malaria agents by targeting the parasitic food vacuole of *P. falciparum* within the low nanomolar range (Vale, 2016). Various plant-derived Michael acceptors also exhibit activity against leishmaniasis, such as the cyclopentenedione derivate, DCPC, isolated from *Piper carniconnectivum* roots or dihydrochalcones isolated from *P. elongate* (Paes-Gonçalves et al., 2012).

Furthermore, a number of drugs containing Michael acceptors exhibit anti-viral properties. Naturally occurring compounds like 15d-PGJ_2, celastrol, curcumin, and rosmarinic acid have well-documented anti-retroviral activity by targeting the Cys-rich domain of HIV-1 Tat, leading to inhibition of Tat-dependent transcription (Narayan et al., 2011). Rupintrivir (AG7088) was a promising Michael acceptor drug candidate that inhibits human rhino virus by targeting rhinoviral protease 3CP. Unfortunately, its development was discontinued during clinical phase II/III based on lack of efficiency in natural infection studies (Vale, 2016).

DISCUSSION: FUTURE DIRECTIONS AND CONCLUDING REMARKS

Over the past decades, the development of inhibitors covalently binding enzymes or other target proteins *via* Michael reaction was deprioritized by the pharmaceutical industry. This was mainly because of safety concerns about indiscriminate and unselective reactivity of the covalent-modification drugs with potentially off-target proteins, thereby causing unpredictable toxicity. However, recently, several efficient and safe covalently binding inhibitors of protein kinases have been successfully approved for cancer treatment, changing the perspective on this class of drugs. Binding of noncatalytic cysteine residues with acrylamides and other α, β-unsaturated carbonyl compounds is currently the preferred strategy used for the development of Michael acceptor-containing drugs. There is rising agreement that covalent binding of target proteins using Michael acceptor moieties can improve pharmacodynamic properties, such as efficacy, potency, selectivity, and duration of pharmacological effects. However, to avoid toxicity, the scaffold encompassing the electrophilic warhead needs rather careful, prolonged, and sophisticated drug design, including computational and molecular modelling methods applied. As such, designed covalent inhibitors might possess significant advantages over non-covalent inhibitors such that covalent warheads can target unique residues of selected target proteins with a higher pharmacodynamic efficacy and less susceptibility to the phenomenon of drug resistance. Along with biomedical drugs, covalently binding inhibitors on the basis of Michael acceptor moieties might therefore play a pivotal role in the drug market of the 21st century.

AUTHOR CONTRIBUTIONS

MP, JR, and TM contributed to the design and conception of the review. MP wrote the first draft of the manuscript. BK, JF, NH, CB, CM, SC, GM, IM, OA, UH, KZ, DS, and TM wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by the Else Kröner-Fresenius-Foundation (EKFS) as well as the Graduate School TRIP (Translational Research Innovation-Pharma), the German Research Foundation (DFG project MA-5825/1-2), the DFG Sonderforschungsbereich SFB-1039, and the Aarhus University Research Foundation (AUFF). TM was recipient of a Heisenberg fellowship from the German Research Foundation (DFG-MA-5825/2-1).

REFERENCES

Akinleye, A., Chen, Y., Mukhi, N., Song, Y., and Liu, D. (2013). : Ibrutinib and novel BTK inhibitors in clinical development. *J. Hematol. Oncol.* 6, 59. doi: 10.1186/1756-8722-6-59

Asan, A., Skoko, J. J., Woodcock, C.-S. C., Wingert, B. M., Woodcock, S. R., Normolle, D., et al. (2019). Electrophilic fatty acids impair RAD51 function and potentiate the effects of DNA-damaging agents on growth of triple-negative breast cells. *J. Biol. Chem.* 294 (2), 397–404. doi: 10.1074/jbc.AC118.005899

Awwad, K., Steinbrink, S. D., Frömel, T., Lill, N., Isaak, J., Häfner, A.-K., et al. (2014). Electrophilic fatty acid species inhibit 5-lipoxygenase and attenuate sepsis-induced pulmonary inflammation. *Antioxidants Redox Signaling* 20 (17), 2667–2680. doi: 10.1089/ars.2013.5473

Bai, P., Wang, K., Zhang, P., Shi, J., Cheng, X., Zhang, Q., et al. (2019). Development of chalcone-O-alkylamine derivatives as multifunctional agents against Alzheimer's disease. *Eur. J. Medicinal Chem.* 183, 111737. doi: 10.1016/j.ejmech.2019.111737

Baker, P. R.S., Lin, Y., Schopfer, F. J., Woodcock, S. R., Groeger, A. L., Batthyany, C., et al. (2005). Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands. *J. Biol. Chem.* 280 (51), 42464–42475. doi: 10.1074/jbc.M504212200

Baker, L. M.S., Baker, P. R.S., Golin-Bisello, F., Schopfer, F. J., Fink, M., Woodcock, S. R., et al. (2007). Nitro-fatty acid reaction with glutathione and cysteine. Kinetic analysis of thiol alkylation by a Michael addition reaction. *J. Biol. Chem.* 282 (42), 31085–31093. doi: 10.1074/jbc.M704085200

Baldwin, A. G., Brough, D., and Freeman, S. (2016). Inhibiting the Inflammasome: A Chemical Perspective. *J. Med. Chem.* 59 (5), 1691–1710. doi: 10.1021/acs.jmedchem.5b01091

Batthyany, C., Schopfer, F. J., Baker, P. R.S., Durán, R., Baker, L. M.S., Huang, Y., et al. (2006). Reversible post-translational modification of proteins by nitrated fatty acids in vivo. *J. Biol. Chem.* 281 (29), 20450–20463. doi: 10.1074/jbc.M602814200

Bauer, R. A. (2015). Covalent inhibitors in drug discovery: from accidental discoveries to avoided liabilities and designed therapies. *Drug Discov. Today* 20 (9), 1061–1073. doi: 10.1016/j.drudis.2015.05.005

Borniquel, S., Jansson, E. A., Cole, M. P., Freeman, B. A., and Lundberg, J. O. (2010). Nitrated oleic acid up-regulates PPARgamma and attenuates experimental inflammatory bowel disease. *Free Radical Biol. Med.* 48 (4), 499–505. doi: 10.1016/j.freeradbiomed.2009.11.014

Bukhari, S. N.A., Tajuddin, Y., Benedict, V. J., Lam, K. W., Jantan, I., Jalil, J., et al. (2014a). Synthesis and evaluation of chalcone derivatives as inhibitors of neutrophils' chemotaxis, phagocytosis and production of reactive oxygen species. *Chem. Biol. Drug design* 83 (2), 198–206. doi: 10.1111/cbdd.12226

Bukhari, S. N. A., Lauro, G., Jantan, I., Bifulco, G., and Amjad, M. W. (2014b). Pharmacological evaluation and docking studies of α,β-unsaturated carbonyl based synthetic compounds as inhibitors of secretory phospholipase A_2, cyclooxygenases, lipoxygenase and proinflammatory cytokines. *Bioorg. Med. Chem.* 22 (15), 4151–4161. doi: 10.1016/j.bmc.2014.05.052

Bürkle, A. (2002). "Posttranslational Modification," in *Encyclopedia of genetics*. Eds. S. Brenner, J. H. Miller and W. J. Broughton (San Diego: Academic Press), 1533.

Butturini, E., Cavalieri, E., de Prati, A. C., Darra, E., Rigo, A., Shoji, K., et al. (2011). Two naturally occurring terpenes, dehydrocostuslactone and costunolide, decrease intracellular GSH content and inhibit STAT3 activation. *PLoS One* 6 (5), e20174. doi: 10.1371/journal.pone.0020174

Byrd, J. C., Furman, R. R., Coutre, S. E., Flinn, I. W., Burger, J. A., Blum, K., et al. (2020). Ibrutinib Treatment for First-Line and Relapsed/Refractory Chronic Lymphocytic Leukemia: Final Analysis of the Pivotal Phase Ib/II PCYC-1102 Study. *Clin. Cancer Res.* 26 (15), 3918–3927. doi: 10.1158/1078-0432.CCR-19-2856

Canonici, A., Gijsen, M., Mullooly, M., Bennett, R., Bouguern, N., Pedersen, K., et al. (2013). Neratinib overcomes trastuzumab resistance in HER2 amplified breast cancer. *Oncotarget* 4 (10), 1592–1605. doi: 10.18632/oncotarget.1148

Castellano, J. M., Garcia-Rodriguez, S., Espinosa, J. M., Millan-Linares, M. C., Rada, M., and Perona, J. S. (2019). Oleanolic Acid Exerts a Neuroprotective Effect Against Microglial Cell Activation by Modulating Cytokine Release and Antioxidant Defense Systems. *Biomolecules* 9 (11), 683. doi: 10.3390/biom9110683

Castillo, J. J., Treon, S. P., and Davids, M. S. (2016). Inhibition of the Bruton Tyrosine Kinase Pathway in B-Cell Lymphoproliferative Disorders. *Cancer J. (Sudbury Mass.)* 22 (1), 34–39. doi: 10.1097/PPO.0000000000000170

Celentano, S., Capolongo, G., and Pollastro, R. M. (2019). Bardoxolone: a new potential therapeutic agent in the treatment of autosomal dominant polycystic kidney disease? *Giornale italiano di nefrologia organo ufficiale della Societa italiana di nefrologia* 36 (5).

Charles, R. L., Burgoyne, J. R., Mayr, M., Weldon, S. M., Hubner, N., Dong, H., et al. (2011). Redox regulation of soluble epoxide hydrolase by 15-deoxy-delta-prostaglandin J2 controls coronary hypoxic vasodilation. *Circ. Res.* 108 (3), 324–334. doi: 10.1161/CIRCRESAHA.110.235879

Charles, R. L., Rudyk, O., Prysyazhna, O., Kamynina, A., Yang, J., Morisseau, C., et al. (2014). Protection from hypertension in mice by the Mediterranean diet is mediated by nitro fatty acid inhibition of soluble epoxide hydrolase. *Proc. Natl. Acad. Sci. U. S. A.* 111 (22), 8167–8172. doi: 10.1073/pnas.1402965111

Chen, B.-J., Fu, C.-S., Li, G.-H., Wang, X.-N., Lou, H.-X., Ren, D.-M., et al. (2017). Cinnamaldehyde Analogues as Potential Therapeutic Agents. *Mini Rev. Med. Chem.* 17 (1), 33–43. doi: 10.2174/1389557516666160121120744

Christensen, L. P. (2018). "The Role of Direct and Indirect Polyphenolic Antioxidants in Protection Against Oxidative Stress," in *Polyphenols in human health and disease*, *2nd ed.*, vol. Volume 1 . Eds. R. R. Watson, S. Zibadi and V. R. Preedy (Amsterdam: Elsevier), 147–179.

Cortés, J., Dieras, V., Ro, J., Barriere, J., Bachelot, T., Hurvitz, S., et al. (2015). Afatinib alone or afatinib plus vinorelbine versus investigator's choice of treatment for HER2-positive breast cancer with progressive brain metastases after trastuzumab, lapatinib, or both (LUX-Breast 3): a randomised, open-label, multicentre, phase 2 trial. *Lancet Oncol.* 16 (16), 1700–1710. doi: 10.1016/S1470-2045(15)00373-3

Couch, R. D., Browning, R.G., Honda, T., Gribble, G. W., Wright, D. L., Sporn, M. B., et al. (2005). Studies on the reactivity of CDDO, a promising new chemopreventive and chemotherapeutic agent: implications for a molecular mechanism of action. *Bioorg. Med. Chem. Lett.* 15 (9), 2215–2219. doi: 10.1016/j.bmcl.2005.03.031

Croasdell, A., Duffney, P. F., Kim, N., Lacy, S. H., Sime, P. J., and Phipps, R. P. (2015). PPARγ and the Innate Immune System Mediate the Resolution of Inflammation. *PPAR Res.* 2015, 549691. doi: 10.1155/2015/549691

Csala, M., Kardon, T., Legeza, B., Lizák, B., Mandl, J., Margittai, É., et al. (2015). On the role of 4-hydroxynonenal in health and disease. *Biochim. Biophys. Acta* 1852 (5), 826–838. doi: 10.1016/j.bbadis.2015.01.015

Cui, T., Schopfer, F. J., Zhang, J., Chen, K., Ichikawa, T., Baker, P. R.S., et al. (2006). Nitrated fatty acids: Endogenous anti-inflammatory signaling mediators. *J. Biol. Chem.* 281 (47), 35686–35698. doi: 10.1074/jbc.M603357200

Dargan, P.II, and Jones, A. L. (2003). Management of paracetamol poisoning. *Trends Pharmacol. Sci.* 24 (4), 154–157. doi: 10.1016/S0165-6147(03)00053-1

Delmastro-Greenwood, M., Hughan, K. S., Vitturi, D. A., Salvatore, S. R., Grimes, G., Potti, G., et al. (2015). Nitrite and nitrate-dependent generation of anti-inflammatory fatty acid nitroalkenes. *Free Radic. Biol. Med.* 89, 333–341. doi: 10.1016/j.freeradbiomed.2015.07.149

Dinkova-Kostova, A. T., Liby, K. T., Stephenson, K. K., Holtzclaw, W.D., Gao, X., Suh, N., et al. (2005). Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. *Proc. Natl. Acad. Sci. U. S. A.* 102 (12), 4584–4589. doi: 10.1073/pnas.0500815102

Dreger, H., Westphal, K., Wilck, N., Baumann, G., Stangl, V., Stangl, K., et al. (2010). Protection of vascular cells from oxidative stress by proteasome inhibition depends on Nrf2. *Cardiovasc. Res.* 85 (2), 395–403. doi: 10.1093/cvr/cvp279

Dumont, M., Wille, E., Calingasan, N. Y., Tampellini, D., Williams, C., Gouras, G. K., et al. (2009). Triterpenoid CDDO-methylamide improves memory and decreases amyloid plaques in a transgenic mouse model of Alzheimer's disease. *J. Neurochemistry* 109 (2), 502–512. doi: 10.1111/j.1471-4159.2009.05970.x

Ehrentraut, S. F., and Colgan, S. P. (2012). Implications of protein post-translational modifications in IBD. *Inflamm. Bowel Dis.* 18 (7), 1378–1388. doi: 10.1002/ibd.22859

Fais, R. S., Rodrigues, F. L., Pereira, C. A., Mendes, A. C., Mestriner, F., Tostes, R. C., et al. (2019). The inflammasome NLRP3 plays a dual role on mouse

corpora cavernosa relaxation. *Sci. Rep.* 9 (1), 16224. doi: 10.1038/s41598-019-52831-0

Fazzari, M., Trostchansky, A., Schopfer, F. J., Salvatore, S. R., Sánchez-Calvo, B., Vitturi, D., et al. (2014). Olives and olive oil are sources of electrophilic fatty acid nitroalkenes. *PLoS One* 9 (1), e84884. doi: 10.1371/journal.pone.0084884

Fecchio, C., Palazzi, L., and de Laureto, P. P. (2018). α-Synuclein and Polyunsaturated Fatty Acids: Molecular Basis of the Interaction and Implication in Neurodegeneration. *Molecules (Basel Switzerland)* 23, (7). doi: 10.3390/molecules23071531

Feldinger, K., and Kong, A. (2015). Profile of neratinib and its potential in the treatment of breast cancer. *Breast Cancer (Dove Med. Press)* 7, 147–162. doi: 10.2147/BCTT.S54414

Fert-Bober, J., Murray, C.II, Parker, S. J., and van Eyk, J. E. (2018). Precision Profiling of the Cardiovascular Post-Translationally Modified Proteome: Where There Is a Will, There Is a Way. *Circ. Res.* 122 (9), 1221–1237. doi: 10.1161/CIRCRESAHA.118.310966

Fox, R. J., Miller, D. H., Phillips, J.T., Hutchinson, M., Havrdova, E., Kita, M., et al. (2012). Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. *New Engl. J. Med.* 367 (12), 1087–1097. doi: 10.1056/NEJMoa1206328

Freeman, B. A., Baker, P. R.S., Schopfer, F. J., Woodcock, S. R., Napolitano, A., and d'Ischia, M. (2008). Nitro-fatty acid formation and signaling. *J. Biol. Chem.* 283 (23), 15515–15519. doi: 10.1074/jbc.R800004200

Friedman, M. (2003). Chemistry, biochemistry, and safety of acrylamide. A review. *J. Agric. Food Chem.* 51 (16), 4504–4526. doi: 10.1021/jf030204

Ghosh, A. K., Samanta, I., Mondal, A., and Liu, W. R. (2019). Covalent Inhibition in Drug Discovery. *ChemMedChem* 14 (9), 889–906. doi: 10.1002/cmdc.201900107

Gillard, G. O., Collette, B., Anderson, J., Chao, J., Scannevin, R. H., Huss, D. J., et al. (2015). DMF, but not other fumarates, inhibits NF-κB activity in vitro in an Nrf2-independent manner. *J. Neuroimmunology* 283, 74–85. doi: 10.1016/j.jneuroim.2015.04.006

Gold, R., Phillips, J.T., Havrdova, E., Bar-Or, A., Kappos, L., Kim, N., et al. (2015). Delayed-Release Dimethyl Fumarate and Pregnancy: Preclinical Studies and Pregnancy Outcomes from Clinical Trials and Postmarketing Experience. *Neurol. Ther.* 4 (2), 93–104. doi: 10.1007/s40120-015-0033-1

González, R., Ballester, I., López-Posadas, R., Suárez, M. D., Zarzuelo, A., Martínez-Augustin, O., et al. (2011). Effects of flavonoids and other polyphenols on inflammation. *Crit. Rev. Food Sci. Nutr.* 51 (4), 331–362. doi: 10.1080/10408390903584094

Gorczynski, M. J., Smitherman, P. K., Akiyama, T. E., Wood, H. B., Berger, J. P., King, S.B., et al. (2009). Activation of peroxisome proliferator-activated receptor gamma (PPARgamma) by nitroalkene fatty acids: importance of nitration position and degree of unsaturation. *J. Med. Chem.* 52 (15), 4631–4639. doi: 10.1021/jm900326c

Grimble, R. F., and Tappia, P. S. (1998). Modulation of pro-inflammatory cytokine biology by unsaturated fatty acids. *Z. fur Ernahrungswissenschaft* 37(Suppl 1), 57–65.

Haeggström, J. Z., and Funk, C. D. (2011). Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease. *Chem. Rev.* 111 (10), 5866–5898. doi: 10.1021/cr200246d

Herman, S. E.M., Gordon, A. L., Hertlein, E., Ramanunni, A., Zhang, X., Jaglowski, S., et al. (2011). Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood* 117 (23), 6287–6296. doi: 10.1182/blood-2011-01-328484

Hernández-Pérez, M., Rabanal, R. M., de La Torre, M. C., and Rodríguez, B. (1995). Analgesic, anti-inflammatory, antipyretic and haematological effects of aethiopinone, an o-naphthoquinone diterpenoid from Salvia aethiopis roots and two hemisynthetic derivatives. *Planta Med.* 61 (6), 505–509. doi: 10.1055/s-2006-959358

Hiruma-Lima, C. A., Toma, W., de Gracioso, J. S., de Almeida, A. B. A., Batista, L. M., Magri, L., et al. (2002). Natural trans-crotonin: the antiulcerogenic effect of another diterpene isolated from the bark of Croton cajucara Benth. *Biol. Pharm. Bull.* 25 (4), 452–456. doi: 10.1248/bpb.25.452

Hörnig, M., Markoutsa, S., Häfner, A.-K., George, S., Wisniewska, J. M., Rödl, C. B., et al. (2012). Inhibition of 5-lipoxygenase by U73122 is due to covalent binding to cysteine 416. *Biochim. Biophys. Acta* 1821 (2), 279–286. doi: 10.1016/j.bbalip.2011.11.001

Iles, K. E., Wright, M. M., Cole, M. P., Welty, N. E., Ware, L. B., Matthay, M. A., et al. (2009). Fatty acid transduction of nitric oxide signaling: nitrolinoleic acid mediates protective effects through regulation of the ERK pathway. *Free Radic. Biol. Med.* 46 (7), 866–875. doi: 10.1016/j.freeradbiomed.2008.12.001

James, L. P., Mayeux, P. R., and Hinson, J. A. (2003). Acetaminophen-induced hepatotoxicity. *Drug Metab. Disposition: Biol. Fate chemicals* 31 (12), 1499–1506. doi: 10.1124/dmd.31.12.1499

Jim Yeung, S.-C. (2005). "Chapter 7 Preclinical studies of chemotherapy for undifferentiated thyroid carcinoma," in *Thyroid neoplasms, Bd. 4*. Ed. B. Haugen (Amsterdam, Boston: Elsevier (Advances in Molecular and Cellular Endocrinology, v. 4), 117–144.

Jollow, D. J., Thorgeirsson, S. S., Potter, W. Z., Hashimoto, M., and Mitchell, J. R. (1974). Acetaminophen-induced hepatic necrosis. VI. Metabolic disposition of toxic and nontoxic doses of acetaminophen. *Pharmacology* 12 (4–5), 251–271. doi: 10.1159/000136547

Kansanen, E., Bonacci, G., Schopfer, F. J., Kuosmanen, S. M., Tong, K.II, Leinonen, H., et al. (2011). Electrophilic nitro-fatty acids activate NRF2 by a KEAP1 cysteine 151-independent mechanism. *J. Biol. Chem.* 286 (16), 14019–14027. doi: 10.1074/jbc.M110.190710

Kappos, L., Gold, R., Miller, D. H., Macmanus, D. G., Havrdova, E., Limmroth, V., et al. (2008). Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. *Lancet (London England)* 372 (9648), 1463–1472. doi: 10.1016/S0140-6736(08)61619-0

Keaney, J., Gasser, J., Gillet, G., Scholz, D., and Kadiu, I. (2019). Inhibition of Bruton's Tyrosine Kinase Modulates Microglial Phagocytosis: Therapeutic Implications for Alzheimer's Disease. *J. Neuroimmune Pharmacol.* 14 (3), 448–461. doi: 10.1007/s11481-019-09839-0

Kehrer, J. P., and Biswal, S. S. (2000). The molecular effects of acrolein. *Toxicological Sci.* 57 (1), 6–15. doi: 10.1093/toxsci/57.1.6

Khoo, N. K.H., Li, L., Salvatore, S. R., Schopfer, F. J., and Freeman, B. A. (2018). Electrophilic fatty acid nitroalkenes regulate Nrf2 and NF-κB signaling:A medicinal chemistry investigation of structure-function relationships. *Sci. Rep.* 8 (1), 2295. doi: 10.1038/s41598-018-20460-8

Klinke, A., Möller, A., Pekarova, M., Ravekes, T., Friedrichs, K., Berlin, M., et al. (2014). Protective effects of 10-nitro-oleic acid in a hypoxia-induced murine model of pulmonary hypertension. *Am. J. Respir. Cell Mol. Biol.* 51 (1), 155–162. doi: 10.1165/rcmb.2013-0063OC

Kobayashi, M., and Yamamoto, M. (2005). Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxidants Redox Signaling* 7 (3–4), 385–394. doi: 10.1089/ars.2005.7.385

Kobayashi, K., Nishiumi, S., Nishida, M., Hirai, M., Azuma, T., Yoshida, H., et al. (2011). Effects of quinone derivatives, such as 1,4-naphthoquinone, on DNA polymerase inhibition and anti-inflammatory action. *Medicinal Chem. (Shariqah (United Arab Emirates)* 7 (1), 37–44. doi: 10.2174/157340611794072742

Konopleva, M., Elstner, E., McQueen, T. J., Tsao, T., Sudarikov, A., Hu, W., et al. (2004). Peroxisome proliferator-activated receptor gamma and retinoid X receptor ligands are potent inducers of differentiation and apoptosis in leukemias. *Mol. Cancer Ther.* 3 (10), 1249–1262.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., et al. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* 113 (9), 71–88. doi: 10.1016/S0002-9343(01)00995-0

Kühn, B., Brat, C., Fettel, J., Hellmuth, N., Maucher, I. V., Bulut, U., et al. (2018). Anti-inflammatory nitro-fatty acids suppress tumor growth by triggering mitochondrial dysfunction and activation of the intrinsic apoptotic pathway in colorectal cancer cells. *Biochem. Pharmacol.* 155, 48–60. doi: 10.1016/j.bcp.2018.06.014

Lamas Bervejillo, M., Bonanata, J., Franchini, G. R., Richeri, A., Marqués, J. M., Freeman, B. A., et al. (2020). A FABP4-PPARγ signaling axis regulates human monocyte responses to electrophilic fatty acid nitroalkenes. *Redox Biol.* 29, 101376. doi: 10.1016/j.redox.2019.101376

Lategahn, J., Keul, M., Klövekorn, P., Tumbrink, H. L., Niggenaber, J., Müller, M. P., et al. (2019). Inhibition of osimertinib-resistant epidermal growth factor receptor EGFR-T790M/C797S. *Chem. Sci.* 10 (46), 10789–10801. doi: 10.1039/c9sc03445e

Lee, S. E., and Park, Y. S. (2013). Role of lipid peroxidation-derived α, β-unsaturated aldehydes in vascular dysfunction. *Oxid. Med. Cell. Longev.* 2013, 629028. doi: 10.1155/2013/629028

Lee, J., Kosaras, B., Del Signore, S. J., Cormier, K., McKee, A., Ratan, R. R., et al. (2011). Modulation of lipid peroxidation and mitochondrial function improves neuropathology in Huntington's disease mice. *Acta Neuropathol.* 121 (4), 487–498. doi: 10.1007/s00401-010-0788-5

Lee, J. S., Bukhari, S. N. A., and Fauzi, N. M. (2015). Effects of chalcone derivatives on players of the immune system. *Drug Des. Dev. Ther.* 9, 4761–4778. doi: 10.2147/DDDT.S86242

Lee, K.-I., Lee, H.-T., Lin, H.-C., Tsay, H.-J., Tsai, F.-C., Shyue, S.-K., et al. (2016). Role of transient receptor potential ankyrin 1 channels in Alzheimer's disease. *J. Neuroinflamm.* 1392 (1), 92. doi: 10.1186/s12974-016-0557-z

Leifert, W. R., and Abeywardena, M. Y. (2008). Grape seed and red wine polyphenol extracts inhibit cellular cholesterol uptake, cell proliferation, and 5-lipoxygenase activity. *Nutr. Res. (New York N.Y.)* 28 (12), 842–850. doi: 10.1016/j.nutres.2008.09.001

Linker, R. A., and Haghikia, A. (2016). Dimethyl fumarate in multiple sclerosis: latest developments, evidence and place in therapy. *Ther. Adv. Chronic Dis.* 7 (4), 198–207. doi: 10.1177/2040622316653307

Liu, J., Qian, C., and Cao, X. (2016). Post-Translational Modification Control of Innate Immunity. *Immunity* 45 (1), 15–30. doi: 10.1016/j.immuni.2016.06.020

LoPachin, R. M., Barber, D. S., and Gavin, T. (2008). Molecular mechanisms of the conjugated alpha,beta-unsaturated carbonyl derivatives: relevance to neurotoxicity and neurodegenerative diseases. *Toxicological Sci.* 104 (2), 235–249. doi: 10.1093/toxsci/kfm301

Lu, S. C. (1999). Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J.* 13 (10), 1169–1183. doi: 10.1096/fasebj.13.10.1169

Lushchak, V.II (2012). Glutathione homeostasis and functions: potential targets for medical interventions. *J. Amino Acids* 2012, 736837. doi: 10.1155/2012/736837

Mann, G. E., Bonacasa, B., Ishii, T., and Siow, R. C. (2009). Targeting the redox sensitive Nrf2-Keap1 defense pathway in cardiovascular disease: protection afforded by dietary isoflavones. *Curr. Opin. Pharmacol.* 9 (2), 139–145. doi: 10.1016/j.coph.2008.12.012

Markesbery, W. R., and Carney, J. M. (1999). Oxidative alterations in Alzheimer's disease. *Brain Pathol. (Zurich Switzerland)* 9 (1), 133–146. doi: 10.1111/j.1750-3639.1999.tb00215.x

Martinez, R. M., Pinho-Ribeiro, F. A., Vale, D. L., Steffena, V. S., Vicentinic, F. T. M. C., Vignoli, J. A., et al. (2017). Trans-chalcone added in topical formulation inhibits skin inflammation and oxidative stress in a model of ultraviolet B radiation skin damage in hairless mice. *J. Photochem. Photobiol. B.* 171, 139–146. doi: 10.1016/j.jphotobiol.2017.05.002

Mathers, A. R., Carey, C. D., Killeen, M. E., Diaz-Perez, J. A., Salvatore, S. R., Schopfer, F. J., et al. (2017). Electrophilic nitro-fatty acids suppress allergic contact dermatitis in mice. *Allergy* 72 (4), 656–664. doi: 10.1111/all.13067

Mathers, A. R., Carey, C. D., Killeen, M. E., Salvatore, S. R., Ferris, L. K., Freeman, B. A., et al. (2018). Topical electrophilic nitro-fatty acids potentiate cutaneous inflammation. *Free Radical Biol. Med.* 115, 31–42. doi: 10.1016/j.freeradbiomed.2017.11.009

Miklos, D., Cutler, C. S., Arora, M., Waller, E. K., Jagasia, M., Pusic, I., et al. (2017). Ibrutinib for chronic graft-versus-host disease after failure of prior therapy. *Blood* 130 (21), 2243–2250. doi: 10.1182/blood-2017-07-793786

Milla, P., Fiorito, C., Soria, F., Arpicco, S., Cattel, L., and Gontero, P. (2014). Intravesical thermo-chemotherapy based on conductive heat: a first pharmacokinetic study with mitomycin C in superficial transitional cell carcinoma patients. *Cancer Chemother. Pharmacol.* 73 (3), 503–509. doi: 10.1007/s00280-014-2381-4

Minkovsky, N., and Berezov, A. (2008). BIBW-2992, a dual receptor tyrosine kinase inhibitor for the treatment of solid tumors. *Curr. Opin. Invest. Drugs (London Engl. 2000)* 9 (12), 1336–1346.

Mitchell, J. R., Jollow, D. J., Potter, W. Z., Davis, D. C., Gillette, J. R., and Brodie, B. B. (1973). Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *J. Pharmacol. Exp. Ther.* 187 (1), 185–194.

Mitchell, S., Vargas, J., and Hoffmann, A. (2016). Signaling via the NFκB system. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 8 (3), 227–241. doi: 10.1002/wsbm.1331

Montalban, X., Arnold, D. L., Weber, M. S., Staikov, I., Piasecka-Stryczynska, K., Willmer, J., et al. (2019). Placebo-Controlled Trial of an Oral BTK Inhibitor in Multiple Sclerosis. *New Engl. J. Med.* 380 (25), 2406–2417. doi: 10.1056/NEJMoa1901981

Morgan, D. L., Price, H. C., O'Connor, R. W., Seely, J. C., Ward, S. M., Wilson, R. E., et al. (2000). Upper respiratory tract toxicity of inhaled methylvinyl ketone in F344 rats and B6C3F1 mice. *Toxicological Sci.* 58 (1), 182–194. doi: 10.1093/toxsci/58.1.182

Mrowietz, U., Barker, J., Boehncke, W.-H., Iversen, L., Kirby, B., Naldi, L., et al. (2018). Clinical use of dimethyl fumarate in moderate-to-severe plaque-type psoriasis: a European expert consensus. *J. Eur. Acad. Dermatol. Venereology JEADV* 32 Suppl 3, 3–14. doi: 10.1111/jdv.15218

Narayan, V., Ravindra, K. C., Chiaro, C., Cary, D., Aggarwal, B. B., Henderson, A. J., et al. (2011). Celastrol inhibits Tat-mediated human immunodeficiency virus (HIV) transcription and replication. *J. Mol. Biol.* 410 (5), 972–983. doi: 10.1016/j.jmb.2011.04.013

National Institute of Diabetes and Digestive and Kidney Diseases (2012). *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury* (Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases).

Nieboer, C., de Hoop, D., van Loenen, A. C., Langendijk, P. N., and van Dijk, E. (1989). Systemic therapy with fumaric acid derivates: new possibilities in the treatment of psoriasis. *J. Am. Acad. Dermatol.* 20 (4), 601–608. doi: 10.1016/s0190-9622(89)70071-2

Okwuosa, I. S., Lewsey, S. C., Adesiyun, T., Blumenthal, R. S., and Yancy, C. W. (2016). Worldwide disparities in cardiovascular disease: Challenges and solutions. *Int. J. Cardiol.* 202, 433–440. doi: 10.1016/j.ijcard.2015.08.172

Paes-Gonçalves, H., Facundo, V. A., Santos, D. M. F., Silva, A. G. C., Ballico, L. J., Lima, D. K.S., et al. (2012). The leishmanicidal activity of a cyclopentenedione derivative isolated from the roots of a native Amazonian pepper (Piper carniconnectivum). *Rev. Bras. Farmacogn.* 22 (5), 1018–1023. doi: 10.1590/S0102-695X2012005000086

Pandey, K. B., and Rizvi, S.II (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* 2 (5), 270–278. doi: 10.4161/oxim.2.5.9498

Parvez, S., Long, M. J.C., Poganik, J. R., and Aye, Y. (2018). Redox Signaling by Reactive Electrophiles and Oxidants. *Chem. Rev.* 118 (18), 8798–8888. doi: 10.1021/acs.chemrev.7b00698

Patel, H., Pawara, R., Ansari, A., and Surana, S. (2017). Recent updates on third generation EGFR inhibitors and emergence of fourth generation EGFR inhibitors to combat C797S resistance. *Eur. J. Medicinal Chem.* 142, 32–47. doi: 10.1016/j.ejmech.2017.05.027

Pereira, C. A., Carlos, D., Ferreira, N. S., Silva, J. F., Zanotto, C. Z., Zamboni, D. S., et al. (2019). Mitochondrial DNA Promotes NLRP3 Inflammasome Activation and Contributes to Endothelial Dysfunction and Inflammation in Type 1 Diabetes. *Front. Physiol.* 10, 1557. doi: 10.3389/fphys.2019.01557

Place, A. E., Suh, N., Williams, C. R., Risingsong, R., Honda, T., Honda, Y., et al. (2003). The novel synthetic triterpenoid, CDDO-imidazolide, inhibits inflammatory response and tumor growth in vivo. *Clin. Cancer Res.* 9 (7), 2798–2806.

Poganik, J. R., and Aye, Y. (2020). Electrophile Signaling and Emerging Immuno- and Neuro-modulatory Electrophilic Pharmaceuticals. *Front. Aging Neurosci.* 12, 1. doi: 10.3389/fnagi.2020.00001

Powolny, A. A., and Singh, S. V. (2008). Plumbagin-induced apoptosis in human prostate cancer cells is associated with modulation of cellular redox status and generation of reactive oxygen species. *Pharm. Res.* 25 (9), 2171–2180. doi: 10.1007/s11095-008-9533-3

Prage, E. B., Morgenstern, R., Jakobsson, P.-J., Stec, D. F., Voehler, M. W., and Armstrong, R. N. (2012). Observation of two modes of inhibition of human microsomal prostaglandin E synthase 1 by the cyclopentenone 15-deoxy-Δ(12,14)-prostaglandin J(2). *Biochemistry* 51 (11), 2348–2356. doi: 10.1021/bi2019332

Rabindran, S. K., Discafani, C. M., Rosfjord, E. C., Baxter, M., Floyd, M.B., Golas, J., et al. (2004). Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res.* 64 (11), 3958–3965. doi: 10.1158/0008-5472.CAN-03-2868

Ramirez, M. A., and Borja, N. L. (2008). Epalrestat: an aldose reductase inhibitor for the treatment of diabetic neuropathy. *Pharmacotherapy* 28 (5), 646–655. doi: 10.1592/phco.28.5.646

Rao, P. V., and Gan, S. H. (2014). Cinnamon: a multifaceted medicinal plant. *Evid.-Based Complement. Altern. Med. eCAM* 2014:642942. doi: 10.1155/2014/642942

Reddy, A. T., Lakshmi, S. P., Zhang, Y., and Reddy, R. C. (2014). Nitrated fatty acids reverse pulmonary fibrosis by dedifferentiating myofibroblasts and promoting collagen uptake by alveolar macrophages. *FASEB J.* 28 (12), 5299–5310. doi: 10.1096/fj.14-256263

Reed, D. J. (1986). Regulation of reductive processes by glutathione. *Biochem. Pharmacol.* 35 (1), 7–13. doi: 10.1016/0006-2952(86)90545-9

Reich, K., Thaci, D., Mrowietz, U., Kamps, A., Neureither, M., and Luger, T. (2009). Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis â€ " A retrospective study (FUTURE). *J. der Deutschen Dermatologischen Gesellschaft* 7 (7), 603–610. doi: 10.1111/j.1610-0387.2009.07120.x

Reuter, S., Gupta, S. C., Chaturvedi, M. M., and Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Biol. Med.* 49 (11), 1603–1616. doi: 10.1016/j.freeradbiomed.2010.09.006

Rodriguez-Duarte, J., Dapueto, R., Galliussi, G., Turell, L., Kamaid, A., Khoo, N. K. H., et al. (2018). Electrophilic nitroalkene-tocopherol derivatives: synthesis, physicochemical characterization and evaluation of anti-inflammatory signaling responses. *Sci. Rep.* 8 (1), 12784. doi: 10.1038/s41598-018-31218-7

Rom, O., Xu, G., Guo, Y., Zhu, Y., Wang, H., Zhang, J., et al. (2019). Nitro-fatty acids protect against steatosis and fibrosis during development of nonalcoholic fatty liver disease in mice. *EBioMedicine* 41, 62–72. doi: 10.1016/j.ebiom.2019.02.019

Rossing, P., Block, G. A., Chin, M. P., Goldsberry, A., Heerspink, H. J.L., McCullough, P. A., et al. (2019). Effect of bardoxolone methyl on the urine albumin-to-creatinine ratio in patients with type 2 diabetes and stage 4 chronic kidney disease. *Kidney Int.* 96 (4), 1030–1036. doi: 10.1016/j.kint.2019.04.027

Rubbo, H. (2013). Nitro-fatty acids: novel anti-inflammatory lipid mediators. *Braz. J. Med. Biol. Res. = Rev. Bras. pesquisas medicas e biologicas* 46 (9), 728–734. doi: 10.1590/1414-431X20133202

Rudolph, T. K., Rudolph, V., Edreira, M. M., Cole, M. P., Bonacci, G., Schopfer, F. J., et al. (2010a). Nitro-fatty acids reduce atherosclerosis in apolipoprotein E-deficient mice. *Arteriosclerosis thrombosis Vasc. Biol.* 30 (5), 938–945. doi: 10.1161/ATVBAHA.109.201582

Rudolph, V., Rudolph, T. K., Schopfer, F. J., Bonacci, G., Woodcock, S. R., Cole, M. P., et al. (2010b). Endogenous generation and protective effects of nitro-fatty acids in a murine model of focal cardiac ischaemia and reperfusion. *Cardiovasc. Res.* 85 (1), 155–166. doi: 10.1093/cvr/cvp275

Rudolph, T. K., Ravekes, T., Klinke, A., Friedrichs, K., Mollenhauer, M., Pekarova, M., et al. (2016). Nitrated fatty acids suppress angiotensin II-mediated fibrotic remodelling and atrial fibrillation. *Cardiovasc. Res.* 109 (1), 174–184. doi: 10.1093/cvr/cvv254

Sandur, S. K., Ichikawa, H., Sethi, G., Ahn, K. S., and Aggarwal, B. B. (2006). Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) suppresses NF-kappaB activation and NF-kappaB-regulated gene products through modulation of p65 and IkappaBalpha kinase activation, leading to potentiation of apoptosis induced by cytokine and chemotherapeutic agents. *J. Biol. Chem.* 281 (25), 17023–17033. doi: 10.1074/jbc.M601595200

Santangelo, C., Varì, R., Scazzocchio, B., Di Benedetto, R., Filesi, C., and Masella, R. (2007). Polyphenols, intracellular signalling and inflammation. *Annali dell'Istituto superiore di Sanita* 43 (4), 394–405.

Schaible, A. M., Filosa, R., Temml, V., Krauth, V., Matteis, M., Peduto, A., et al. (2014). Elucidation of the molecular mechanism and the efficacy in vivo of a novel 1,4-benzoquinone that inhibits 5-lipoxygenase. *Br. J. Pharmacol.* 171 (9), 2399–2412. doi: 10.1111/bph.12592

Schopfer, F. J., and Khoo, N. K.H. (2019). Nitro-Fatty Acid Logistics: Formation, Biodistribution, Signaling, and Pharmacology. *Trends Endocrinol. metabolism: TEM* 30 (8), 505–519. doi: 10.1016/j.tem.2019.04.009

Schopfer, F. J., Lin, Y., Baker, P. R.S., Cui, T., Garcia-Barrio, M., Zhang, J., et al. (2005). Nitrolinoleic acid: an endogenous peroxisome proliferator-activated receptor gamma ligand. *Proc. Natl. Acad. Sci. U. S. A.* 102 (7), 2340–2345. doi: 10.1073/pnas.0408384102

Schopfer, F. J., Cole, M. P., Groeger, A. L., Chen, C.-S., Khoo, N. K.H., Woodcock, S. R., et al. (2010). Covalent peroxisome proliferator-activated receptor gamma adduction by nitro-fatty acids: selective ligand activity and anti-diabetic signaling actions. *J. Biol. Chem.* 285 (16), 12321–12333. doi: 10.1074/jbc.M109.091512

Seidel, P., Merfort, I., Hughes, J. M., Oliver, B. G. G., Tamm, M., and Roth, M. (2009). Dimethylfumarate inhibits NF-{kappa}B function at multiple levels to limit airway smooth muscle cell cytokine secretion. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297 (2), L326–L339. doi: 10.1152/ajplung.90624.2008

Shibata, Y., and Chiba, M. (2015). The role of extrahepatic metabolism in the pharmacokinetics of the targeted covalent inhibitors afatinib, ibrutinib, and neratinib. *Drug Metab. Disposition: Biol. Fate Chem.* 43 (3), 375–384. doi: 10.1124/dmd.114.061424

Singh, J., Petter, R. C., Baillie, T. A., and Whitty, A. (2011). The resurgence of covalent drugs. *Nat. Rev. Drug Discov.* 10 (4), 307–317. doi: 10.1038/nrd3410

Singh, S., Khan, A. R., and Gupta, A. K. (2012). Role of glutathione in cancer pathophysiology and therapeutic interventions. *J. Exp. Ther. Oncol.* 9 (4), 303–316.

Smith, M. R., Gangireddy, S. R., Narala, V. R., Hogaboam, C. M., Standiford, T. J., Christensen, P. J., et al. (2010). Curcumin inhibits fibrosis-related effects in IPF fibroblasts and in mice following bleomycin-induced lung injury. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 298 (5), L616–L625. doi: 10.1152/ajplung.00002.2009

Solca, F., Dahl, G., Zoephel, A., Bader, G., Sanderson, M., Klein, C., et al. (2012). Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker. *J. Pharmacol. Exp. Ther.* 343 (2), 342–350. doi: 10.1124/jpet.112.197756

Steinhilber, D., Fischer, A. S., Metzner, J., Steinbrink, S. D., Roos, J., Ruthardt, M., et al. (2010). 5-lipoxygenase: underappreciated role of a pro-inflammatory enzyme in tumorigenesis. *Front. Pharmacol.* 1, 143. doi: 10.3389/fphar.2010.00143

Sundararajan, S., Jiang, Q., Heneka, M., and Landreth, G. (2006). PPARgamma as a therapeutic target in central nervous system diseases. *Neurochemistry Int.* 49 (2), 136–144. doi: 10.1016/j.neuint.2006.03.020

Tang, Z.-H., and Lu, J.-J. (2018). Osimertinib resistance in non-small cell lung cancer: Mechanisms and therapeutic strategies. *Cancer Lett.* 420, 242–246. doi: 10.1016/j.canlet.2018.02.004

Tian, C., Gao, L., Zhang, A., Hackfort, B. T., and Zucker, I. H. (2019). Therapeutic Effects of Nrf2 Activation by Bardoxolone Methyl in Chronic Heart Failure. *J. Pharmacol. Exp. Ther.* 371 (3), 642–651. doi: 10.1124/jpet.119.261792

Tontonoz, P., and Spiegelman, B. M. (2008). Fat and beyond: the diverse biology of PPARgamma. *Annu. Rev. Biochem.* 77, 289–312. doi: 10.1146/annurev.biochem.77.061307.091829

Torrico, F., Cepeda, M., Guerrero, G., Melendez, F., Blanco, Z., Canelón, D. J., et al. (2007). Hypoglycaemic effect of Croton cuneatus in streptozotocin-induced diabetic rats. *Rev. bras. farmacogn.* 17 (2), 166–169. doi: 10.1590/S0102-695X2007000200005

Trachootham, D., Alexandre, J., and Huang, P. (2009). Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov.* 8 (7), 579–591. doi: 10.1038/nrd2803

Tran, T. A., McCoy, M. K., Sporn, M. B., and Tansey, M. G. (2008). The synthetic triterpenoid CDDO-methyl ester modulates microglial activities, inhibits TNF production, and provides dopaminergic neuroprotection. *J. Neuroinflamm.* 5, 14. doi: 10.1186/1742-2094-5-14

Trostchansky, A., Bonilla, L., Thomas, C. P., O'Donnell, V. B., Marnett, L. J., Radi, R., et al. (2011). Nitroarachidonic acid, a novel peroxidase inhibitor of prostaglandin endoperoxide H synthases 1 and 2. *J. Biol. Chem.* 286 (15), 12891–12900. doi: 10.1074/jbc.M110.154518

Trostchansky, A., Bonilla, L., González-Perilli, L., and Rubbo, H. (2013). Nitro-fatty acids: formation, redox signaling, and therapeutic potential. *Antioxid. Redox Signal.* 19 (11), 1257–1265. doi: 10.1089/ars.2012.5023

Vale, N. (2016). *Biomedical Chemistry. Current Trends and Developments. s.l.: De Gruyter*. doi: 10.1515/9783110468755

Vasas, A., and Hohmann, J. (2014). Euphorbia diterpenes: isolation, structure, biological activity, and synthesi-2012). *Chem. Rev.* 114 (17), 8579–8612. doi: 10.1021/cr400541j

Vazquez, M. M., Gutierrez, M. V., Salvatore, S. R., Puiatti, M., Dato, V. A., Chiabrando, G. A., et al. (2020). Nitro-oleic acid, a ligand of CD36, reduces cholesterol accumulation by modulating oxidized-LDL uptake and cholesterol efflux in RAW264.7 macrophages. *Redox Biol.* 36:101591. doi: 10.1016/j.redox.2020.101591

Villacorta, L., Chang, L., Salvatore, S. R., Ichikawa, T., Zhang, J., Petrovic-Djergovic, D., et al. (2013). Electrophilic nitro-fatty acids inhibit vascular inflammation by disrupting LPS-dependent TLR4 signalling in lipid rafts. *Cardiovasc. Res.* 98 (1), 116–124. doi: 10.1093/cvr/cvt002

Villacorta, L., Minarrieta, L., Salvatore, S. R., Khoo, N. K., Rom, O., Gao, Z., et al. (2018). In situ generation, metabolism and immunomodulatory signaling

actions of nitro-conjugated linoleic acid in a murine model of inflammation. *Redox Biol.* 15, 522–531. doi: 10.1016/j.redox.2018.01.005

Wang, W., and Ballatori, N. (1998). Endogenous glutathione conjugates: occurrence and biological functions. *Pharmacol. Rev.* 50 (3), 335–356.

Wang, D., and Dubois, R. N. (2010). Eicosanoids and cancer. *Nat. Rev. Cancer* 10 (3), 181–193. doi: 10.1038/nrc2809

Wang, H., Liu, H., Jia, Z., Jia, Z., Olsen, C., Litwin, S., et al. (2010). : Nitro-oleic acid protects against endotoxin-induced endotoxemia and multiorgan injury in mice. *Am. J. Physiol. Renal Physiol.* 298 (3), F754–F762. doi: 10.1152/ajprenal.00439.2009

Wang, Y.-Y., Yang, Y.-X., Zhe, H., He, Z.-X., and Zhou, S.-F. (2014). Bardoxolone methyl (CDDO-Me) as a therapeutic agent: an update on its pharmacokinetic and pharmacodynamic properties. *Drug Des. Dev. Ther.* 8, 2075–2088. doi: 10.2147/DDDT.S68872

Werz, O. (2007). Inhibition of 5-lipoxygenase product synthesis by natural compounds of plant origin. *Planta Med.* 73 (13), 1331–1357. doi: 10.1055/s-2007-990242

Woo, C. C., Kumar, A. P., Sethi, G., and Tan, K. H. B. (2012). Thymoquinone: potential cure for inflammatory disorders and cancer. *Biochem. Pharmacol.* 83 (4), 443–451. doi: 10.1016/j.bcp.2011.09.029

Woodcock, C.-S. C., Huang, Y., Woodcock, S. R., Salvatore, S. R., Singh, B., Golin-Bisello, F., et al. (2018). Nitro-fatty acid inhibition of triple-negative breast cancer cell viability, migration, invasion, and tumor growth. *J. Biol. Chem.* 293 (4), 1120–1137. doi: 10.1074/jbc.M117.814368

Woyach, J. A., Furman, R. R., Liu, T.-M., Ozer, H. G., Zapatka, M., Ruppert, A. S., et al. (2014). Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *New Engl. J. Med.* 370 (24), 2286–2294. doi: 10.1056/NEJMoa1400029

Wu, G., Fang, Y.-Z., Yang, S., Lupton, J. R., and Turner, N. D. (2004). Glutathione metabolism and its implications for health. *J. Nutr.* 134 (3), 489–492. doi: 10.1093/jn/134.3.489

Wynn, T. A. (2008). Cellular and molecular mechanisms of fibrosis. *J. Pathol.* 214 (2), 199–210. doi: 10.1002/path.2277

Yoritaka, A., Hattori, N., Uchida, K., Tanaka, M., Stadtman, E. R., and Mizuno, Y. (1996). Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. *Proc. Natl. Acad. Sci. U. S. A.* 93 (7), 2696–2701. doi: 10.1073/pnas.93.7.2696

Yoshimoto, T., Yokoyama, C., Ochi, K., Yamamoto, S., Maki, Y., Ashida, Y., et al. (1982). 2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow-reacting substance of anaphylaxis. *Biochim. Biophys. Acta* 713 (2), 470–473.

de Zeeuw, D., Akizawa, T., Audhya, P., Bakris, G. L., Chin, M., Christ-Schmidt, H., et al. (2013). Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. *New Engl. J. Med.* 369 (26), 2492–2503. doi: 10.1056/NEJMoa1306033

Zhang, K., Mack, P., and Wong, K. P. (1998). Glutathione-related mechanisms in cellular resistance to anticancer drugs. *Int. J. Oncol.* 12 (4), 871–882. doi: 10.3892/ijo.12.4.871

Zhang, J., Villacorta, L., Chang, L., Fan, Z., Hamblin, M., Zhu, T., et al. (2010). Nitro-oleic acid inhibits angiotensin II-induced hypertension. *Circ. Res.* 107 (4), 540–548. doi: 10.1161/CIRCRESAHA.110.218404

Zhao, X.-q., Xie, J.-d., Chen, X.-g., Sim, H. M., Zhang, X., Liang, Y.-j., et al. (2012). Neratinib reverses ATP-binding cassette B1-mediated chemotherapeutic drug resistance in vitro, in vivo, and ex vivo. *Mol. Pharmacol.* 82 (1), 47–58. doi: 10.1124/mol.111.076299

Zhao, D.-H., Wang, Y.-C., Zheng, L.-W., Liu, B.-Y., and Guan, L.-P. (2018). Antidepressant-like Effect of a Chalcone Compound, DHIPC and Its Possible Mechanism. *Iranian J. Pharm. Res. IJPR* 17 (1), 193–201.

Zheng, J., Wu, C., Lin, Z., Guo, Y., Shi, L., Dong, P., et al. (2014). Curcumin up-regulates phosphatase and tensin homologue deleted on chromosome 10 through microRNA-mediated control of DNA methylation–a novel mechanism suppressing liver fibrosis. *FEBS J.* 281 (1), 88–103. doi: 10.1111/febs.12574

Zhu, H., Jia, Z., Misra, B. R., Zhang, L., Cao, Z., Yamamoto, M., et al. (2008). Nuclear factor E2-related factor 2-dependent myocardiac cytoprotection against oxidative and electrophilic stress. *Cardiovasc. Toxicol.* 8 (2), 71–85. doi: 10.1007/s12012-008-9016-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 04 September 2020
doi: 10.3389/fphar.2020.529976

Dual Behavior of Long-Chain Fatty Acids and Their Cyclooxygenase/ Lipoxygenase Metabolites on Human Intestinal Caco-2 Cell Growth

Carolina E. Storniolo[1,2], *Marisol Cabral*[1,2], *Maria A. Busquets*[3,4], *Raquel Martín-Venegas*[2,5] *and Juan J. Moreno*[1,2,6]*

[1] Department of Nutrition, Food Sciences and Gastronomy, Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain, [2] Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona, Barcelona, Spain, [3] Department of Pharmacy, Pharmaceutical Technology and Physical-Chemistry, Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain, [4] Institute of Nanosciences and Nanotechnology, University of Barcelona, Barcelona, Spain, [5] Department of Biochemistry and Physiology, Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain, [6] CIBEROBN Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain

OPEN ACCESS

Edited by:
Emanuela Ricciotti, University of Pennsylvania, United States

Reviewed by:
Melania Dovizio, University of Studies G. d'Annunzio Chieti and Pescara, Italy
Jin Qian, Stanford University, United States

***Correspondence:**
Juan J. Moreno
jjmoreno@ub.edu

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *27 January 2020*
Accepted: *17 August 2020*
Published: *04 September 2020*

Citation:
Storniolo CE, Cabral M, Busquets MA, Martín-Venegas R and Moreno JJ (2020) Dual Behavior of Long-Chain Fatty Acids and Their Cyclooxygenase/ Lipoxygenase Metabolites on Human Intestinal Caco-2 Cell Growth. Front. Pharmacol. 11:529976. doi: 10.3389/fphar.2020.529976

Etiology of colorectal cancer (CRC) is related, at least in part, with nutritional profile and epidemiological data indicating a key role of dietary fat on CRC pathogenesis. Moreover, inflammation and eicosanoids produced from arachidonic acid might have a pivotal role in CRC development. However, the effect of specific fatty acids (FAs) on intestinal epithelial cell growth is not completely studied now. By this reason, the aim of this work is to unravel the effect of different saturated and unsaturated long-chain fatty acids (LCFA) and some LCFA metabolites on CRC cell line growth and their possible mechanisms of action. Our results demonstrated that oleic acid is a potent mitogenic factor to Caco-2 cells, at least in part, through 10-hydroxy-8-octadecenoic synthesized by lipoxigenase pathway, whereas polyunsaturated FAs such as eicosapentaenoic (EPA) acid has a dual behavior effect depending on its concentration. A high concentration, EPA induced apoptosis through intrinsic pathway, whereas at low concentration induced cell proliferation that could be related to the synthesis of eicosanoids such as prostaglandin E_3 and 12-hydroxyeicosapentaenoic acid and the subsequent induction of mitogenic cell signaling pathways (ERK 1/2, CREB, p38α). Thus, this study contributes to understand the complicated relationship between fat ingest and CRC.

Keywords: colorectal cancer, eicosapentaenoic acid, hydroxyeicosapentaenoic acids, oleic acid, prostaglandin

INTRODUCTION

Cancer causes around 7 million of deaths annually, becoming 12.5% in the entire world and colorectal cancer (CRC) is the third leading cause of cancer-related death in developed countries (Ullman and Itzkowitz, 2011). According to recent rates, the lifetime risk of developing CRC is 4.3% (NCI). Although a great effort has been made toward developing detection and surgical strategies, there has been little improvement in the outcome for patients with advanced disease.

CRC is linked with environmental factors, being lifestyle and nutritional profile the major but controllable implicated factors. In fact, around 90% of the CRC cases appear to be related to lifestyle, with the highest incidence in economically developed countries (Clinton et al., 2020). Refined carbohydrates, alcoholic beverages, red and processed meat, saturated fat, and a high energy intake, specially associated with abdominal body fatness, would favor CRC development. In contrast, high consumption of dietary fiber, fruits and vegetables, calcium, antioxidants, and vitamins would have the opposite effect (Meyerhardt et al., 2007; WCRF, 2011). Thus, westernized diet pattern is associated with an increased risk for CRC (Yusof et al., 2012).

According to the World Health Organization the average global intake of fat has increased over the last half-century (WHO). However, not all fats are the same and it is now well established that saturated, *trans* fats and unsaturated fats can act in opposite ways to influence human health, including promotion of cancer. Thus, the data indicated that not only the amount of dietary fat but also fat diet composition could be determinant in the pathogenesis of different neoplasms as CRC (Barone et al., 2014). Some experimental studies demonstrated that ω-3 PUFA protects against CRC, while ω-6 PUFA promotes this cancer development, proposing mechanisms like modulation of inflammation, cellular oxidative stress, membrane dynamics, and cellular receptors function (Larsson et al., 2004; Cockbain et al., 2012). However, in epidemiological studies there are many different results, and data seem to be inconclusive regarding the effect of these FAs on CRC. Some authors demonstrated an inversely relation between ω-3 or ω-6 PUFA consumption and CRC risk (Chao et al., 2005; Norat et al., 2005), others indicated a lack of association (Sasazuki et al., 2011; Song et al., 2014), while some shown a positive relationship (Daniel et al., 2009; Shen et al., 2012). In the other hand, studies including saturated fat consumption and CRC, demonstrated that there is a direct relation between these two factors (Giovannucci and Goldin, 1997; Rosignoli et al., 2008) but other authors have not found any significant relation (Williams et al., 2010. Oleic acid, ω-9 FA, is considered one of the healthier sources of fat in the diet. Also, oleic acid was attracted much attention as characteristic component of Mediterranean diet and has been linked to a protector effect against cancer (Escrich et al., 2007).

Eicosanoids produced from arachidonic acid (AA) might have a pivotal role in these events. AA biosynthesized from linoleic acid is the substrate of cyclooxygenase (COX) and lipoxygenase (LOX) for the production of eicosanoids such as 2-series prostaglandins (PGs), 4-series leukotrienes (LTs) and HETEs that may facilitate CRC progression by stimulating cell proliferation and survival, tumor cell invasiveness, and angiogenesis (Moreno, 2005; Moreno, 2009). PUFAs such as eicosapentaenoic acid (EPA) can also be released from cell membrane phospholipids and can act as substrate for COX and LOX pathways, giving rise to the 3-serie PGs, 4-serie LTs, and hydroxyeicosapentaenoic acids (HEPEs) (Smith, 2005) which effects on cell proliferation are poorly known.

Considering all together, the effect of FAs on intestinal epithelial cell growth is not completely understood now. By this reason, the aim of this study is to determine the effect of representative saturated and unsaturated long-chain FAs (LCFA) and some fatty acid metabolites on CRC cell line growth and their possible mechanisms of action.

Abbreviations: AA, arachidonic acid; CCCP, carbonyl cyanide 3-chlorophenylhydrazine; COX, cyclooxygenase; CRC, colorectal cancer; HEPE, hydroxyeicosapentaenoic acid; LCFA, long-chain fatty acids; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; SCFA, short-chain fatty acids; TMRE, tetramethylrhodamine ethyl ester.

MATERIAL AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), trypsin/EDTA, penicillin, and streptomycin were supplied by GIBCO (Paisley, Scotland, UK). Acridine orange, bovine serum albumin (BSA) de-fatty BSA, carbonyl cyanide 3-chlorophenylhydrazone (CCCP), Dulbecco's PBS, ethidium bromide, fetal bovine serum (FBS), nonessential amino acids, paraformaldehyde extra pure, propidium iodide, ribonuclease A from bovine pancreas, SC19220, tetramethylrhodamine ethyl ester perclorate (TMRE), and Triton X-100 were supplied by Sigma-Aldrich (St. Louis, MO, USA). 10-hydroxy-8-octadecenoic acid (10-HODE) was from Parchem (New Rochellem, NY, USA). Tissue culture supplies and sterile material were obtained from Corning, Nirco S.L., NORM-JECT and Biosigma S.R.L. (Italy). The BioRad Protein Assay was obtained from Bio-Rad Laboratories, Inc. Myristoleic, palmitoleic, stearic, oleic, elaidic, linoleic, α-linolenic, γ-linolenic, mead, arachidonic (AA), EPA, erucic and DHA acids, prostaglandin (PG) E_2, PGE_3, leukotriene (LT) B_4, LTB_5, 12-S-HETE, 12-S-HEPE, ketoprofen, baicalein, MK571, MK886 LY171883, zileuton, U75302, and the PPARγ ligand screening assay were supplied by Cayman Chemical Co. (Ann Arbor, MI, USA). Cell proliferation ELISA–BrdU (Colorimetric) Kit was from Roche (Basel, Switzerland). LY255283 was from Tocris Biosc. (Bristol, UK). AH23848 and ONO-329 were kindly provided by Glaxo-Wellcome (Stevenage, UK) and Ono Pharmaceutical Co. Ltd. (Osaka, Japan), respectively. The MebStain Apoptosis Kit was supplied by MBL International (Woburn, MD, USA) and the Multi Kinase Array (MKA) ElA by Symansis (Auckland, New Zealand).

Cell Culture

Caco-2 cells were derived from a moderately well-differentiated primary colon adenocarcinoma and were provided by American Type Culture Collection (HTB-37) (Manassas, VA, USA). The cells were routinely grown in 25 or 75 cm^2 plastic flasks at a density of 1×10^4 cells/cm^2 and cultured in DMEM with 4.5 g/L D-glucose and 2 mM L-glutamine, and supplemented with 1% (v/v) nonessential amino acids, 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were incubated at 37°C under a humidified atmosphere of 5% CO_2 in air. Cells grown to ~80% confluence were released by trypsinization and subcultured at a density of 1.5–2 $\times 10^4$ cells/cm^2 in 12 mm diameter plastic clusters and of 1×10^4 cells/cm^2 in 60 mm diameter plastic dishes. Growth

medium was replaced twice per week. Although cancerous in origin, Caco-2 cells undergo a gradual differentiation process that takes place spontaneously once confluence has been reached and that is completed after 21–25 days in culture (Martín-Venegas et al., 2006). Considering these, the experiments were performed in cells maintained for 3 days in culture to use undifferentiated Caco-2 cell cultures to perform all experiments. All experimentation products were diluted in DMSO (final concentration of DMSO was lesser than 0.1%). FAs stock solutions prepared in DMSO were further diluted in cell medium supplemented with FBS or cell medium containing 5% de-fatty BSA as a carrier to ensure FAs dissolution. Solutions containing equal amounts of de-fatty BSA were used as control.

Cell Growth and DNA Synthesis Assays

The effect of the treatments was assessed on Caco-2 cells clusters in 24-well plates (5–10 × 10^3 cells/cm^2). Cells were cultured for 96 h in DMEM supplemented with 10% FBS. Then, cells were incubated for 48 h in the presence of treatments. Finally, cells were washed, trypsinized, and counted under a microscope using ethidium bromide/acridine orange staining to count viable cells.

DNA synthesis in Caco-2 cells was assessed by a colorimetric immunoassay based on the measurement of BrdU incorporation during DNA synthesis (Cell proliferation ELISA, BrdU Kit, from Roche). Caco-2 cells were cultured at 1,000–1,500 cell/well in 96 well plates for 96 h in DMEM supplemented with 10% FBS. Then, cells were incubated for 48 h in the presence of compounds. Thereafter, cells were treated following the manufacturer instructions. Absorbance was measured at 450 nm in a plate reader (TECAN, Sunrise, Grödig, Austria).

TUNEL Assay

Degradation of chromosomal DNA was evaluated with TUNEL method using a MebStain apoptosis kit (MBL Int.). After 96 h in culture, Caco-2 cells were cultivated in media containing treatments for 48 h. Next, cells were fixed with 4% paraformaldehyde and permeabilized with 70% ethanol. Thereafter, 3′-OH DNA ends generated by DNA fragmentation were labeled with fluorescein-dUTP, mediated by terminal deoxynucleotidyl transferase, and were analyzed on an Epics XL flow cytometer (Coulter Corporation, San Francisco, CA).

PGE_2 and PGE_3 EIA Determination

PGE_2 and PGE_3 concentrations were determined in the Caco-2 culture medium. Cells were overnight with FBS or FBS plus EPA (10 μM). Two hundred fifty μl aliquots of the culture medium were acidified with 1 ml of formic acid and PGs were extracted in ethyl acetate. After discarding the aqueous phase, the organic phases were evaporated under a stream of N_2 and PGE_2 or PGE_3 were determined with a monoclonal enzyme immunoassay kit (MyBiosource, San Diego, CA) according to the manufacturer's protocol.

Measurement of Cell Signaling Pathways

Cells were seeded in 60 mm plastic clusters (10^4 cells/cm^2). After 4 days, the cultures were incubated with the treatments in absence of FBS. Maximal phosphorylation was observed after 5 min incubation for ERK1/2, Akt, and p38, and after 15 min for GSKβ and CREB. To measure the kinase activation with total cellular lysates, Caco-2 cells were lysed using a denaturing cell lysis buffer containing 6 M urea and protease (leupeptin 2 μg/ml, pepstatin 10 μM, aprotinin 3 μg/ml) and phosphatase (NaF 5 mM, $Na_4P_2O_7$ 2 mM, Na_3VO_4 1 mM) inhibitors. The resulting solutions containing 80–100 μg of proteins were then added to a kinase ELISA plate and the assay was performed following the manufacturer's recommendations (Symansis). Finally, optical density was measured at 450 nm. Thus, we studied the effect of PGE_2/PGE_3 on the phosphorylation of AKT1 (pS473), AKT2 (pS474), ERK1/2 (pT202/Y204; pT185/Y187), GSK3β (pS9), p38α (pT180/Y182), and CREB (pS133).

PPARγ Ligand Assay

FAs binding to PPARγ were studied with a fluorescence polarization-based single-step PPARγ ligand screening assay (Cayman Chem. Co., Ann Arbor, MI, USA). This assay is based on the competition of free ligand in the samples or standards for the affinity binding site of PPARγ occupied by a probe conjugated to fluorescein. Finally, the polarization was quantified as milli-polarization units (mP). This assay was adapted to be performed in a microcuvette with a luminescence spectrometer (AMINCO-Bowman Series 2, Spectronic Unicam, Leeds, UK).

Mitochondrial Membrane Potential Determination

Mitochondrial membrane depolarization (MMP) was measured through tetramethylrhodamine ethyl ester perclorate (TMRE) incorporation into active undifferentiated Caco-2 cell mitochondria. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was used as a positive control for MMP. Cell cultures were treated with experimental compounds for 20 min and, after tripsinization, cell suspension was labeled with TMRE for 30 min. Finally, samples were analyzed in FC500 flow cytometer (Coulter Corporation) to measure mitochondrial depolarization or hyperpolarization.

Data Analysis

The results are expressed as mean ± SEM. All data were compared by one-way ANOVA and the Student's t-test, using SPSS software (SPSS Inc., Chicago, IL). Significance was taken as $p < 0.05$.

RESULTS

Long-Chain Fatty Acids Affect Intestinal Epithelial Caco-2 Cell Growth

When undifferentiated Caco-2 cells were incubated with different LCFA in absence of FBS we observed that palmitoleic (10–100 μM), oleic (1–100 μM), myristoleic (100 μM), and elaidic (100 μM) acids as well as the lowest EPA and DHA concentration (10 μM) induced

cell proliferation (**Figure 1A**). In contrast saturated FAs such as stearic did not have this effect. These mitogenic effects of the above-mentioned monounsaturated FAs (MUFAs) and FUFAs were confirmed in DNA synthesis analysis except for palmitoleic (**Figure 1B**). Interestingly, it can be also seen that highest concentrations of some FAs like linoleic, α and γ-linolenic, AA, EPA, and DHA promote a decrease in cell number below control cell group suggesting a cytotoxic or apoptotic action of these PUFAs (**Figure 1A**). Then, cells were also incubated with FBS to assess the proliferative or antiproliferative capacity of these FAs in the presence of growth factors. **Figure 2** shows how oleic acid (1–10 μM) enhanced the mitogenic effect induced by FBS whereas linoleic, α- and γ-linolenic, and AA decreased cell growth. At 1–10 μM EPA and DHA did not modify cell growth induced by FBS, whereas at 100 μM the antiproliferative action of these PUFA was confirmed. Thus, saturated LCFAs did not have any effect on Caco-2 cell growth, oleic acid was the most mitogenic MUFA assayed; and EPA as a representative PUFA, presented a dual effect on Caco-2 cell growth depending on concentration and the presence of FBS.

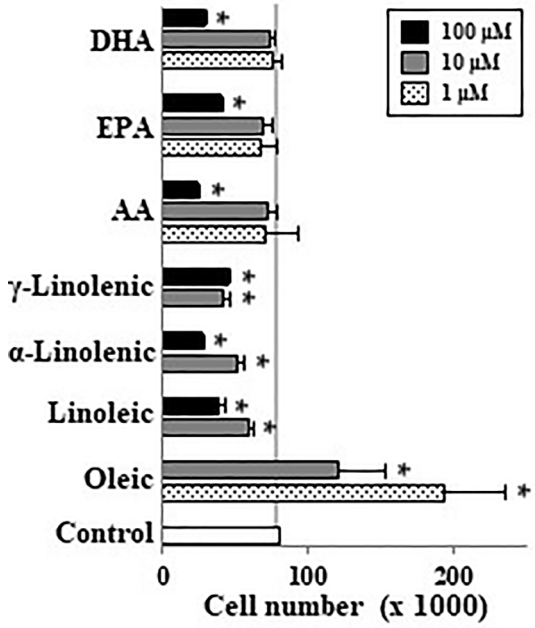


FIGURE 2 | Effect of different LCFA on Caco-2 cell growth in presence of 10% FBS. Oleic, linoleic, α-linolenic, γ-linolenic, AA, EPA, and DHA were incubated with Caco-2 cells at 1, 10, and 100 μM (dot, gray, and black bars, respectively) in presence of 10% FBS for 48 h and were then counted. Results are expressed like mean ± SEM (n = 4–12). *P < 0.05 *vs.* control group (white bar, cells cultured with 10% FBS).

Polyunsaturated Long-Chain Fatty Acids Induce Apoptosis in Caco-2 Cell Cultures

Considering that some LCFAs studied have antiproliferative effect at high concentrations, we analyzed their apoptotic activity by flow cytometry. Our results show that LCFAs such as α- and γ-linolenic, AA, EPA, and DHA induced apoptosis at high concentrations whereas oleic acid had no apoptotic activity (**Figure 3**). In addition, we measured the capacity of LCFAs to bind to PPARγ and to alter MMP (**Table 1**). We found that

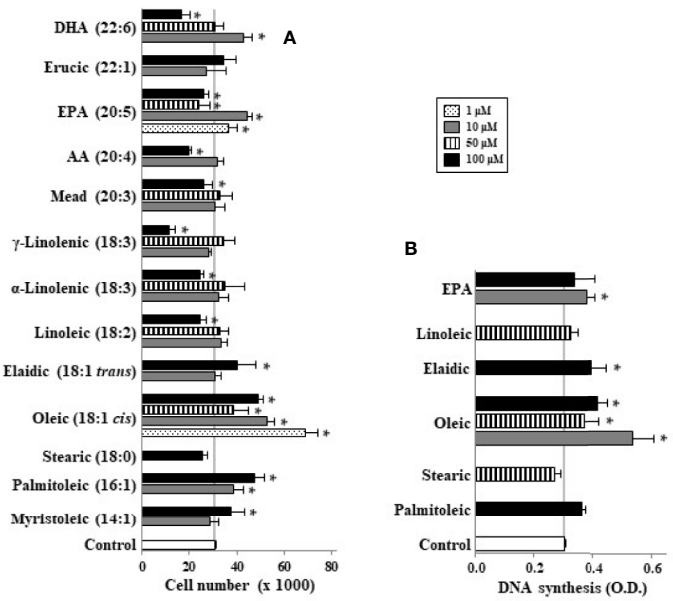


FIGURE 1 | Effect of different LCFA on Caco-2 cell growth **(A)** and DNA synthesis **(B)** in absence of growth factors. Myristoleic, palmitoleic, stearic, oleic, elaidic, linoleic, α-linolenic, γ-linolenic, mead, AA, EPA, erucic, and DHA were incubated with Caco-2 cells at 1, 10, 50, and 100 μM (dot, gray, line, and black bars, respectively) without FBS for 48 h and were then counted or DNA synthesis measured by cell BrdU incorporation. Results are expressed like mean ± SEM (n = 8–30). *P < 0.05 *versus* control group (white bar, cells cultured without FBS).

apoptotic LCFAs (α- and γ-linolenic, AA, EPA, and DHA) have affinity to PPARγ. Furthermore, AA, EPA, and DHA were capable to induce significant changes of MMP, both parameters implicated in the denominated intrinsic or mitochondrial pathway of apoptosis. Interestingly, low γ-linolenic, EPA, and DHA concentrations (10 μM) were not able to bind to PPARγ and to alter MMP (**Table 1**).

The Mitogenic Effect of Oleic Acid and EPA Can Be Related With Cyclooxygenase and Lipoxygenase Metabolite Release

Undifferentiated Caco-2 cells expresses COX-1 and COX-2 (Martin-Venegas et al., 2006), 5-, 12-, and 15-LOX (Martin-Venegas et al., 2014) as well as BLT receptors (Martin-Venegas et al., 2014) and EP receptors (Rodriguez-Lagunas et al., 2010). Here, we observed that cell proliferation induced by oleic acid or EPA at 10 μM was reverted by MK886 (5-LOX inhibitor), baicalein (12-LOX inhibitor), U75302 (BLT_1 antagonist), and LY255283 (BLT_1 and BLT_2 antagonist) (**Figure 4**). In addition, these experiments showed that ketoprofen (COX inhibitor), AH23838 (EP_4 antagonist), SC19220 (EP_1 antagonist) also reverted the mitogenic effect induced by EPA (**Figure 4**) whereas the mitogenic effect induced by oleic acid was similarly reverted by MK571 (cysteinyl leukotriene antagonist) and LY171883 (cysteinyl LT receptor antagonist) (**Figure 4**). All inhibitors and antagonists were used to optimal concentrations previously stablished (Cabral et al., 2013). The reversion of oleic/EPA mitogenic effects by AA cascade inhibitors and eicosanoid receptor antagonists suggests a role of oleic/EPA metabolites in the mitogenic effects induced by both FAs.

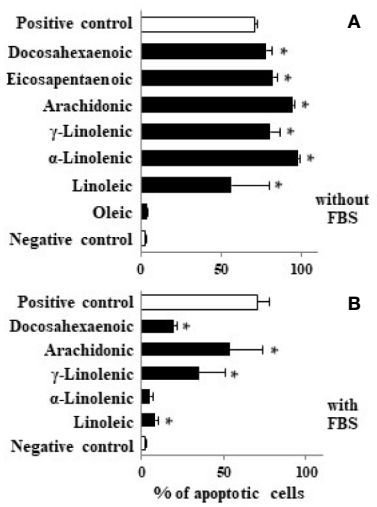


FIGURE 3 | Effect of different LCFA on Caco-2 cell apoptosis. Caco-2 cells were incubated with stearic, oleic, linoleic, α-linolenic, γ-linolenic, AA, EPA, and DHA at 100 μM (black bars) in absence **(A)** or presence of FBS **(B)** for 48 h and DNA fragmentation was measured. Values are mean ± SEM (n = 3–8). *P < 0.05 *versus* negative control group (cells cultured in absence of FBS, white bar). As positive control we used staurosporine (1 μM) in presence of 10% FBS (white bar).

Eicosanoids From EPA Such as PGE_3 and 12-S-HEPE Are Mitogenic Whereas LTB_5 Did Not Induce Caco-2 Cell Growth

EPA can be presented into cell membrane phospholipids (Lands et al., 1992), it is release by phospholipases (Nieves and Moreno, 2006), and it is metabolized by COX and LOX, giving rise to the

TABLE 1 | Effect of LCFAs on PPARγ ligand assay and mitochondrial membrane potential variation.

Treatment	PPARγ bind (mP)	ΔMMP (au)
Vehicle	108 ± 9	21 ± 1.9
Rosiglitazone (10 μM)	43 ± 2*	ND
CCCP (5 μM)	ND	6 ± 0.5*
Staurosporine (1 μM)	ND	70 ± 4*
Oleic (100 μM)	109 ± 12	21 ± 1.6
Linoleic (100 μM)	98 ± 10	22 ± 0.7
α-Linolenic (100 μM)	76 ± 8*	24 ± 0.2
γ-Linolenic (10 μM)	91 ± 8	22 ± 0.7
γ-Linolenic (100 μM)	71 ± 16*	22 ± 0.9
AA (100 μM)	70 ± 8*	28 ± 1.9*
EPA (10 μM)	89 ± 7	23 ± 1,3
EPA (100 μM)	63 ± 15*	34 ± 1.3*
DHA (10 μM)	82 ± 7	25 ± 0.9
DHA (100 μM)	56 ± 3*	38 ± 0.7*

*PPARγ binding was measured by fluorescence polarization and mitochondrial membrane potential variation (ΔMMP) was measured by flow cytometry as Material and Methods section described and was expressed as mP and arbitrary units (au), respectively. Results are expressed as mean ± SEM (n = 3–5). *P < 0.05 versus control group (vehicle). ND, non determined.*

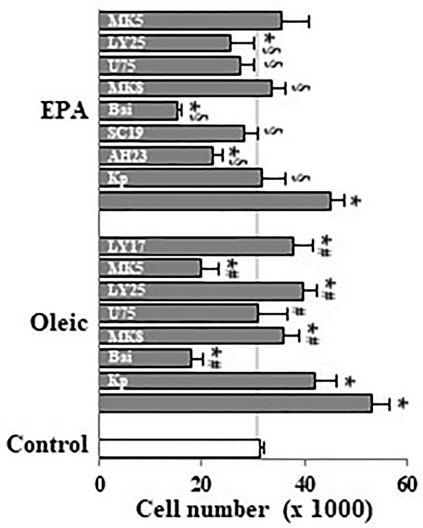


FIGURE 4 | Effect of pharmacological modulation of AA cascade on Caco-2 cell growth induced by oleic and EPA. Caco-2 cells were incubated without FBS for 48 h with oleic or EPA at 10 μM in presence or absence of ketoprofen (Kp, 5 μM), AH23848 (AH23, 20 nM), SC19220 (SC19, 60 nM), baicalein (Bai, 25 μM), MK886 (MK8, 10 μM), U75302 (U75, 5 μM), LY25283 (LY25, 25 μM), MK571 (MK5, 25 μM), or LY171883 (LY17, 25 μM) and cells were counted. Results are expressed as mean ± SEM (n = 8–16). *P < 0.05 *vs.* control group (white bar, cells cultured without FBS), #P < 0.05 *versus* oleic acid (10 μM), and §P < 0.05 *versus* EPA (10 μM).

3-series of prostanoids, 5-series leukotrienes, and HEPEs (Dommels et al., 2002). Our results show that PGE_2 concentrations in Caco-2 cell culture medium reach 1.52 ± 0.21 ng/ml (around 5 nM) whereas PGE_3 was not detected. Interestingly, EPA (10 μM) supplementation of Caco-2 cell culture medium increases PGE_3 synthesis (1.02 ± 0.13 ng/ml), whereas we observed the impairment of PGE2 (1.02 ± 0.09 ng/ml). In **Figures 5A, B** we observed that PGE_3 progressively increased Caco-2 cell growth and DNA synthesis up to 10 nM in a similar way to PGE_2. **Figures 5A, B** also show that Caco-2 cell growth and DNA synthesis induced by PGE_3 was totally inhibited by an EP_1 antagonist (SC19220) and by an EP_4 antagonist (AH23838). However, an EP_3 antagonist (ONO-AE3-240, 2nM) did not have any effect. Therefore, these results indicate that PGE_3 acts through EP_1 and EP_4 receptors, but not through the EP_3 receptor.

Our results also show that 12-S-HEPE (100–1000 nM) induced significant cell growth and DNA synthesis in Caco-2 cell cultures in the absence of growth factors, in a similar way to 12-S-HETE (100 nM). This mitogenic action of 12-S-HEPE was blocked by a COX inhibitor (ketoprofen), by a BLT_1 antagonist (U75302), and by a BLT_1 and BLT_2 antagonist (LY255283) (**Figures 5C, D**).

Previously we observed that LTB_4 has a mitogenic effect on Caco-2 cells (Cabral et al., 2013). However, our findings show that LTB_5 derived from EPA did not induce proliferation in the range of 1–100 nM (**Figure 5E**).

Finally, we studied the capacity of PGE_3 (10 nM) and 12-S-HEPE (100 nM) to phosphorylate pivotal elements in the cell signaling pathways implicated in the regulation of cell growth. Our findings show that PGE_3 presents a similar pattern to PGE_2. Thus, PGE_3 could increase the phosphorylation of ERK 1/2, CREB, GSKβ, and p38α (**Table 2**), cell signaling pathways involved in cell growth. Similar effects were induced by 12-S-HEPE (**Table 2**).

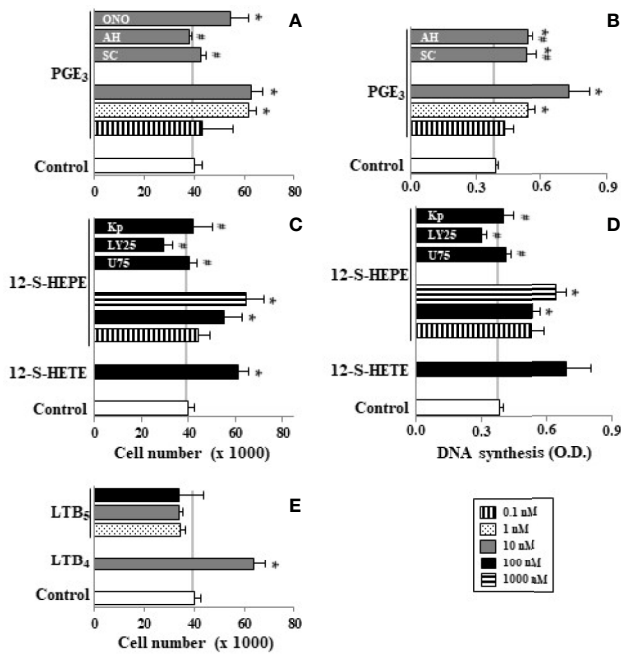


FIGURE 5 | Effect of PGE_3, 12-S-HEPE, and LTB_5 on Caco-2 cell growth. Caco-2 cells were incubated for 48 h with PGE_3 (0.1, 1, and 10 nM, line, dot, and gray bars, respectively) or PGE_3 (10 nM) plus SC19220 (SC, 60 nM) or AH 23848 (AH, 20 nM) or ONO-AE3-240 (ON, 2 nM) or with 12-S-HEPE (10, 100, and 1000 nM, line, gray, and dot bars, respectively) or with 12-S-HEPE (100 nM, gray bar) plus U 75302 (U, 5 μM) or LY 255283 (LY, 25 μM) or ketoprofen (Kp, 5 μM). Cell cultures were also incubated with LTB_5 (1, 10,and 100 nM, dot, gray, and black bars, respectively) or LTB_4 (10 nM, gray bar) in absence of FBS. Finally, cells were then counted **(A, C, E)** and DNA synthesis was measured **(B, D)**. Data are expressed as means ± SEM of three to four experiments performed in triplicate. *$P < 0.05$ *vs.* Caco-2 cell cultures in the absence of FBS, $^{\#}P < 0.05$ *vs.* cells incubated with 10 nM PGE_3 or 12-S-HEPE.

10-Hydroxy-8-Octadecenoic, LOX-Metabolite From Oleic Acid, Has Proliferative Effect in Caco-2 Cultures

Guerrero et al. (1997) and Clapp et al. (2006) reported the oxidation of oleic acid by bacterial and vegetal LOXs to produce 10-hydroxy-8-octadecenoic (10-HODE). Considering that undifferentiated Caco-2 cells have 5-, 12-, and 15-LOX activities (Cabral et al., 2013; Martin-Venegas et al., 2014), we hypothesized the implication of 10-HODE in the mitogenic effects of oleic acid. Our results show that 10-HODE acid induced significant cell growth and DNA synthesis in Caco-2 cell cultures in the absence of growth factors, in a similar way to oleic acid. This mitogenic action of 10-HODE was blocked by a BLT_1 antagonist (U75302), but not by BLT_1 and BLT_2 antagonist (LY255283) nor by MK571, a cysteinyl leukotriene antagonist (**Figure 6**). Furthermore, we observed that 10-HODE was able to induce some mitogenic cell signaling pathways such as ERK 1/2, CREB, or p38α (**Table 2**).

DISCUSSION

Undigested carbohydrates are fermented in the large intestine to form short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate. SCFAs and specially butyrate induces cellular differentiation and decrease proliferation of CRC-derived cell lines (Hass et al., 1997; Arantes et al., 2016). These effects may be related with the inhibition of histone deacetylase by DNA hypermethylation to promote cell differentiation (Boffa et al., 1978) and the inhibition of cell proliferation *via* p21, cyclin D1, and β-catenin pathway (Wang and Friedman, 1998). Thus, SCFAs have been reported to have important effects on intestinal epithelial cell growth/differentiation and consequently on CRC (Kles and Chang, 2006). However, there is a great debate about the role of saturated, monounsaturated, and polyunsaturated (ω-3 and ω-6) LCFAs in CRC, but few studies have been conducted on the potency of LCFAs and/or specific LCFA metabolites in modulating CRC cell line growth. Interestingly, our findings show that unsaturated LCFAs were mitogenic at physiological concentrations, whereas PUFAs such as EPA and DHA were mitogenic at low concentrations but at high concentrations might

TABLE 2 | Effect of eicosanoids (PGE_3 and 12-S-HEPE) and 10-HODE on cell signaling.

	O.D. (450 nm)				
Pathway	**Control**	**PGE_2**	**PGE_3**	**12-S-HEPE**	**10-HODE**
AKT1	0.18 ± 0.04	0.25 ± 0.04	0.23 ± 0.03	0.19 ± 0.02	0.21 ± 0.03
AKT2	0.17 ± 0.02	0.19 ± 0.02	0.18 ± 0.03	0.21 ± 0.01	0.19 ± 0.02
P38α	0.42 ± 0.04	0.92 ± 0.04*	0.99 ± 0.05*	0.85 ± 0.03*	0.83 ± 0.04*
GSKβ	0.19 ± 0.03	0.56 ± 0.08*	0.52 ± 0.07*	0.48 ± 0.04*	0.50 ± 0.06*
CREB	0.21 ± 0.02	0.82 ± 0.11*	0.85 ± 0.12*	0.76 ± 0.13*	0.69 ± 0.10*
ERK 1/2	0.37 ± 0.03	1.06 ± 0.12*	0.99 ± 0.11*	0.95 ± 0.14*	0.89 ± 0.13*

*Caco-2 cells were incubated with PGE_2 or PGE_3 (10 nM), 12-S-HEPE (100 nM), or 10-HODE (100 nM) for 5 or 15 min, cells were then collected and finally phosphorylated AKT1, AKT2, p38α, GSKβ, CREB, and ERK 1/2 were measured as described in the Material and Methods section and expressed as optical density (450 nm). Data are expressed as means ± SEM of two to four experiments performed in triplicate. *P < 0.05 vs. Caco-2 cell cultures in the absence of FBS.*

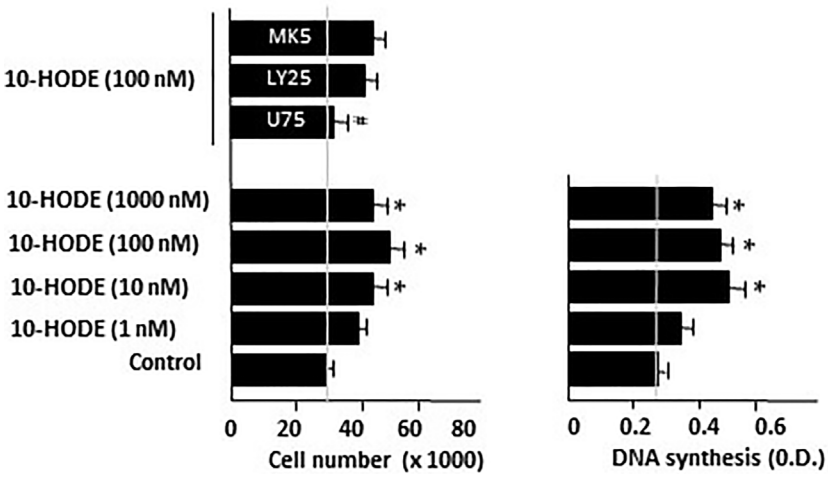


FIGURE 6 | Effect of 10-HODE on Caco-2 cell growth and DNA synthesis. 10-HODE was incubated with Caco-2 cells without FBS in presence of U75302 (U75, 5 μM), LY25283 (LY25, 25 μM), or MK571 (MK5, 25 μM) for 48 h and then cells were counted or DNA synthesis assayed by cell BrdU incorporation. Results are expressed as mean ± SEM (n = 6–9) *P < 0.05 *vs* control group (cells cultured without FBS), #P < 0.05 *vs* 10-HODE (100 nM), N.D., non determined.

have the opposite effects. In contrast, saturated LCFAs did not show any effects of Caco-2 cell growth.

Our findings show that monounsaturated LCFAs such as palmitoleic and oleic acids are mitogenic in undifferentiated Caco-2 cell cultures being oleic acid the most active. This effect was also observed by the $C_{18:1}$ *trans*, elaidic acid. These findings agree with Petrik et al. (2000), that reported that $Apc^{min/+}$ mice feed with oleic acid present a high number of intestinal tumors, and recently, this mitogenic effect of oleic acid has also been observed in prostate cancer cell growth (Liotti et al., 2018). Interestingly, stearoyl-CoA desaturases expression and activity and the subsequent conversion of saturated LCFA to monounsaturated LCFA, were linked to CRC pathogenesis (Vargas et al., 2015; Igal, 2016), and consequently an accumulation of monounsaturated LCFAs. It is important to note that oleic 10–100 μM is reached in human plasma (Kopf and Schmitz, 2013).

Cancer cells are characterized by higher rates of lipid biosynthesis in addition to increased glycolysis and lactate production than those of normal cells (Currie et al., 2013) as well as high fatty acid uptake that require the expression of CD36, over-expressed in the majority of tumor tissues (Kuemmerle et al., 2011). This suggests that catabolism of FAs may be the dominant bioenergetics source in cancer cells and, thus, be an important fuel source for cell proliferation. Interestingly, our study reports data that suggest an alternative mechanism to explain the mitogenic action of oleic acid. Thus, our findings prompt that the mitogenic effect of oleic acid appears dependent on LOX pathway metabolism and the subsequent production of oleic acid metabolites. Clapp et al. (2006) reported that lipoxygenation of oleic acid gives allylic hydroperoxides followed by conversion to enones. In this sense, Cabral et al. (2013 and 2015) reported that LTs from AA and hydroxyoctadecanoid acids from linoleic acid induced Caco-2 cell growth. Thus, we can consider the possible implication of a LOX pathway oleic-derived metabolite in these events. Guerrero et al. (1997) reported that 10-HODE is biosynthesized from oleic acid by microbial oxidation that Clapp

et al. (2006) attributed to LOX activity. The reversion of the mitogenic effects of oleic acid by LOX inhibitors suggest, by the first time in mammalian cells, that oleic acid is metabolized by LOXs and that 10-HODE or other oleic acid metabolite can be responsible, at least in part, of Caco-2 cell growth induced by this FA. Recently, we reported that LTs, HETEs, and hydroxyoctadecadienoic acids (Cabral et al., 2014; Cabral et al., 2015), with structural similarity to 10-HODE, have proliferative effects through BLT interaction and the subsequent COX-pathway activation. This study shows that 10-HODE from oleic acid was also able to induce proliferative cell signaling pathways and Caco-2 cell growth through common mechanisms with LTs and HETEs (Cabral et al., 2014; Cabral et al., 2015). Future studies should be designed to demonstrate 10-HODE synthesis by intestinal epithelial cells. Interestingly, Barone and co-workers (2014) observed a decrease in polyp number and polyp volume by olive oil diet. These apparent discrepancies put into consideration the fact that oleic acid and olive oil can exert different effect on CRC cell line growth considering that olive oil is a complex mix content bioactive compounds that can modulate oleic acid action. In this context, we recently reported that some minor compounds of extra virgin olive oil modulate the mitogenic effects of oleic acid on Caco-2 cells (Storniolo et al., 2019).

Dommels et al. (2002) reported that AA and EPA (10–100 μM) induced Caco-2 cell growth inhibition and cytotoxicity through peroxidation products generated during lipid peroxidation and COX activity. In agreement with these authors, we observed that other PUFAs such as α- and γ-linolenic and DHA were able to reduce cell number and that these findings were related with their capacity to induced apoptosis (mitochondrial membrane potential variation, DNA fragmentation) and binding to PPARγ, an event that Giros et al. (2009) did not consider involved in the apoptotic mechanism of ω-3 PUFAs on Caco-2 cells. These findings suggest a role of intrinsic pathway of apoptosis in the pro-apoptotic actions of these PUFAs. Interestingly, our findings indicate that although EPA as well as linoleic, α- and γ-linolenic, AA, and DHA are apoptotic at the highest concentration (around 100 μM) as was reported previously (Zhang et al., 2015; Storniolo, 2017), EPA and DHA have a mitogenic effect at 1–10 μM. In addition, we observed that this mitogenic effect of EPA is COX- and LOX-pathway dependent, suggesting that EPA metabolites could be involved in this event.

Experimental studies have shown that diets rich in fish oil significantly reduce the amount of AA present in membrane phospholipids (Mitjavila et al., 1996) and consequently the synthesis of AA metabolites such as PGE_2 (Moreno et al., 2001), but increase the release of EPA metabolites. Even though the theory of formation of the 3-series PGs by EPA has been studied for decades, we still do not fully understand the role of EPA metabolites such as PGE_3 in cancer cells (Yang et al., 2014). Here, we observed, for the first time, that PGE_3 increased cell growth and DNA synthesis in non-differentiated intestinal epithelial cells at concentrations reached in Caco-2 cell cultures supplemented with EPA. Thus, PGE_3 presents a proliferative action in a similar form to PGE_2 at nM concentrations that could be reached in colonic tissue as consequence of the immune cell eicosanoid biosynthesis (Le Faouder et al., 2013). These findings are, apparently, in disagreement with Fan et al. (2014) who reported that PGE_3 diminished the ability to support colonic stem cell expansion but using a non-physiological concentration (10 μM). Furthermore, we demonstrated that this PGE_3 proliferative effect was a consequence of interaction with the PGE_2 receptors EP_1 and EP_4, in agreement with their affinities (Moreno, 2017), and with a recent report showing similar effects of both PGs on the disruption of the intestinal epithelial barrier function (Rodriguez-Lagunas et al., 2013). Moreover, we observed that cell signaling pathways involved in the mitogenic action of PGE_3 are like those involved in PGE_2 action (Cabral et al., 2013), being p38α, CREB, and ERK 1/2 pathways involved in the mitogenic action of PGE_3.

HETEs have mitogenic effect on different types of cells and are also involved in the pathogenesis of cancer (Moreno, 2009). Recently, 12-S-HETE was reported to have a proliferative effect on Caco-2 cells (Cabral et al., 2013). To our knowledge, this is the first study to show that 12-S-HEPE from EPA has similar effect on intestinal epithelial cell growth with the implication of ERK 1/2, CREB, or P38α pathways. No specific cellular receptors for HETEs/HEPEs have been identified to date. However, it has been reported that the binding of 12-HETE to the BLT_2 receptor may be involved in its mitogenic action (Cabral et al., 2013). Here, we demonstrate that Caco-2 cell growth induced by 12-S-HEPE can be reverted by BLT_1 and BLT_2 antagonists and a COX inhibitor, which suggests that the 12-S-HEPE mitogenic action is, at least partly, due to PGs synthesis after 12-S-HEPE interaction with both BLT receptors; a mechanism previously described for LTB_4 and 12-HETE (Cabral et al., 2013) and 13-R-hydroxyoctadecadienoic acid (44), compounds structurally related with 12-S-HEPE.

EPA administered to patients with Crohn's disease (Ikehata et al., 1992) or ulcerative colitis (Salomon et al., 1990) increases the generation of LTB_5 and the LTB_5-LTB_4 ratio, which were related with an improvement in these patients. In our study, LTB_5 had no proliferative effect, while LTB_4 significantly induced Caco-2 cell growth, findings in agreement with Bortuzzo et al. (1996) who found a lower affinity of LTB_5 to the receptor of LTB_4. Moreover, since the treatment with 5-LOX inhibitor or cysteinyl LT receptor antagonist reduced the mitogenic effect of EPA, these results indirectly suggest that 5-series cysteinyl LTs could also be involved, at least in part, in the mitogenic EPA effects, in our experimental conditions. There is little literature about the affinity of EPA derived LTs and cysteinyl LT receptors but Wallace and McKnight (1990) reported that LTC_5 or LTD_5 have biological activity although less potent than LTs derived from AA. Furthermore, although LTB_5 did not have the mitogenic effect of its AA-derived partners, PGE_3 and 12-S-HEPE have considerable mitogenic effects on intestinal epithelial Caco-2 cells and may be involved in cell proliferation induced by EPA. The role of COX and/or LOX pathway on cancer cell line growth has been reported using cell lines such as HT-29, HCA7, or LoVo (Hawcroft et al., 2010; Ganesh et al., 2012;

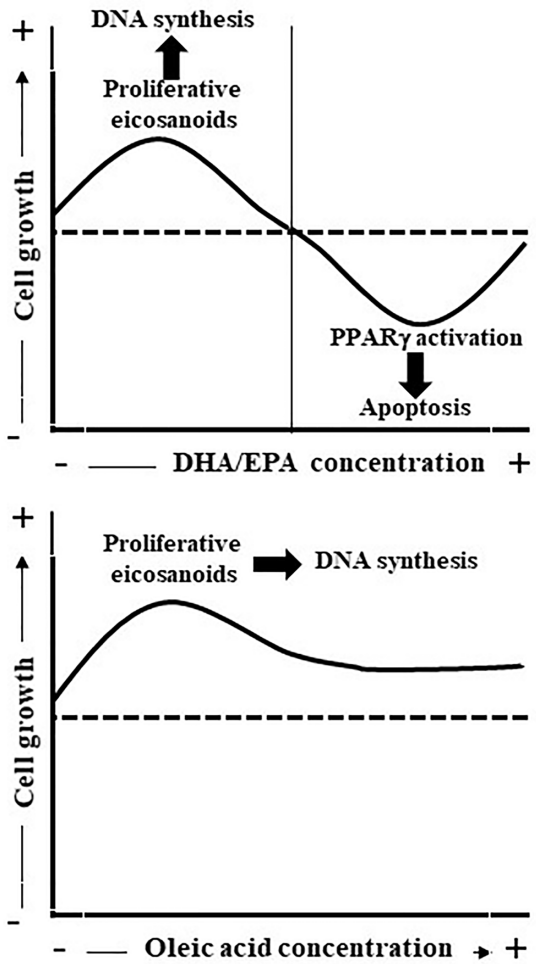


FIGURE 7 | Scheme represents the effect of oleic acid and EPA/DHA on Caco-2 cell growth and apoptosis. Oleic acid is mitogenic probably through LOX pathway metabolite synthesis whereas EPA/DHA present a dual effect on Caco-2 cell proliferation. Low EPA concentrations (up to 10 μM) induce Caco-2 cell growth as consequence, at least in part, of PGE_3 and 12-HEPE release whereas high EPA concentrations inhibit Caco-2 cell growth and induce apoptosis through binding to PPARγ.

Li et al., 2018). However, to our knowledge, it is the first time to report the effect of PGE_3, LTB_5, and 12-S-HEPE derived from EPA on epithelial cell growth. However, we believe that future research should analyze the role of EPA and EPA eicosanoids on non-transformed intestinal epithelial cell growth as well as intestinal epithelium development using *in vitro* and *in vivo* experimental models. Furthermore, it will be interesting to study the role of DHA metabolites to explain the proliferative effect of this PUFA at low concentrations.

In conclusion, the results obtained herein demonstrated that oleic acid is a potent mitogenic factor to undifferentiated Caco-2 cells probably through LOX pathway metabolite such as 10-HODE whereas PUFAs such as EPA or DHA have a dual behavior effect on Caco-2 cell growth depending on the FA concentration (**Figure 7**). A high concentration of EPA/DHA induced apoptosis, a process related with its binding to PPARγ. Meanwhile, low EPA concentration induced Caco-2 cell proliferation that could be related to the synthesis of mitogenic eicosanoids such as PGE_3 and 12-HEPE and the subsequent induction of mitogenic cell signaling pathways. Obviously, these conclusions do not exclude a direct effect of LCFA (oleic acid and EPA) or LCFA metabolites on Caco-2 cell growth through bind to GPR40 and the subsequent cell signaling activation involved in cell proliferation (Hass et al., 1997; Liu et al., 2013) or GPR120 that is abundantly expressed in intestine (Hirasawa et al., 2005; Wu et al., 2013), it is activated by LCFAs (Hirasawa et al., 2005) and is involved in the mitogenic effects of PUFAs (Hass et al., 1997). Thus, this study contributes to open new perspectives to understand the complicated relationship between fat ingest and CRC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

RM-V and JJM designed experiments and CES, MC, and MAB performed these experiments. CES, RM-V, and JJM carried out the data analysis and RM-V and JJM wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Ministerio de Ciencia e Innovación (BFU2007-61727/BFI, AGL2013-49083-C3-1-R and AGL2016-75329R), by Generalitat de Catalunya (2009SGR438) and by CIBEROBN (Instituto de Salud Carlos III).

ACKNOWLEDGMENTS

Cytometry analyses were performed at the Serveis Cientific-Tècnics of the University of Barcelona. Some of the results presented here appeared in CES doctoral thesis. We reward the comments and suggestions proposed by Dr. P.F. Marrero.

REFERENCES

Arantes, E. L., Dragano, N., Ramalho, A., Vitorino, D., de Souza, G. F., Lima, M. H., et al. (2016). Topical docosahexaenoic acid (DHA) accelerates skin wound healing in rats and activates GPR120. *Biol. Res. Nursery* 18, 411–419. doi: 10.1177/10998004155621617

Barone, M., Notarnicola, M., Caruso, M. G., Scavo, M. P., Viggiani, M. T., Tutino, V., et al. (2014). Olive oil and omega-3 polyunsaturated fatty acids suppress intestinal polyp growth by modulating the apoptotic process in ApcMin/+ mice. *Carcinogenesis* 35, 1613–1619. doi: 10.1093/carcin/bgu068

Boffa, L. C., Vidali, G., Mann, R. S., and Allfrey, V. G. (1978). Suppression of histone deacetylation in vivo and in vitro by sodium butyrate. *J. Biol. Chem.* 253, 3364–3366.

Bortuzzo, C., Hanif, R., Kashfi, K., Staiano-Coico, L., Shiff, S. J., and Rigas, B. (1996). The effect of leukotrienes B and selected HETEs on the proliferation of colon cancer cells. *Biochim. Biophys. Acta Lipids Lipid Metab.* 1300, 240–246. doi: 10.1016/0005-2760(96)00003-3

Cabral, M., Martín-Venegas, R., and Moreno, J. J. (2013). Role of arachidonic acid metabolites on the control of non-differentiated intestinal epithelial cell growth. *Int. J. Biochem. Cell. Biol.* 45, 1620–1628. doi: 10.1016/j.biocel.2013.05.009

Cabral, M., Martin-Venegas, R., and Moreno, J. J. (2014). Differential cell growth/apoptosis behavior of 13-hydroxyoctadecadienoic acid enantiomers in a colorectal cancer cell line. *Am. J. Physiol. Gastrointest. Liver Physiol.* 307, G664–G671. doi: 10.1152/ajpgi.00064.2014

Cabral, M., Martin-Venegas, R., and Moreno, J. J. (2015). Leukotriene D4-inducedCaco-2 cell proliferation is mediated by prostaglandin E2 synthesis. *Physiol. Rep.* 3, e12417. doi: 10.14814/phy2.12417

Chao, A., Thun, M. J., Connell, C. J., McCullough, M. L., Jacobs, E. J., Flanders, W. D., et al. (2005). Meat consumption and risk of colorectal cancer. *JAMA* 293, 172–182. doi: 10.1001/jama.293.2.172

Clapp, C. H., Strulson, M., Rodriguez, P. C., Lo, R., and Novak, M. J. (2006). Oxygenation of monounsaturated fatty acids by soybean lipoxygenase-1: Evidence for transient hydroperoxide formation. *Biochemistry* 45, 15884–15892. doi: 10.1021/bi0619425

Clinton, S. K., Giovannucci, E. L., and Hursting, S. D. (2020). The World Cancer Research Fund/American Institute for Cancer Research Third Expert Report on Diet, Nutrition, Physical Activity, and Cancer: Impact and Future Directions. *J. Nutr.* 150, 663–667. doi: 10.1093/jn/nxz268

Cockbain, A. J., Toogood, G. J., and Hull, M. A. (2012). Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut* 61, 135–149. doi: 10.1136/gut.2010.233718

Currie, E., Schulze, A., Zechner, R., Walther, T. C., and Farese, R. V. (2013). Cellular fatty acid metabolism and cancer. *Cell Metab.* 18, 153–161. doi: 10.1016/j.cmet.2013.05.017

Daniel, C. R., McCullough, M. L., Patel, R. C., Jacobs, E. J., Flanders, W. D., Thun, M. J., et al. (2009). Dietary intake of omega-6 and omega-3 fatty acids and risk of colorectal cancer in a prospective cohort of U.S. men and women. *Cancer Epidemiol. Biomarkers Prev.* 18, 516–525. doi: 10.1158/1055-9965.EPI-08-0750

Dommels, Y. E., Alink, G. M., Van Bladeren, P. J., and Van Ommen, B. (2002). Dietary n-6 and n-3 polyunsaturated fatty acids and colorectal carcinogenesis: Results from cultured colon cells, animal models and human studies. *Environ. Toxicol. Pharmacol.* 11, 297–308. doi: 10.1016/S1382-6689(02)00006-6

Escrich, E., Moral, R., Grau, L., Costa, I., and Solanas, M. (2007). Molecular mechanisms of the effects of olive oil and other dietary lipids on cancer. *Mol. Nutr. Food Res.* 51, 1279–1292. doi: 10.1002/mnfr.200700213

Fan, Y. Y., Davidson, L. A., Callaway, E. S., Goldsby, J. S., and Chapkin, R. S. (2014). Differential effects of 2-and 3-series E-prostaglandins on in vitro expansion of Lgr5+ colonic stem cells. *Carcinogenesis* 35, 606–612. doi: 10.1093/carcin/bgt412

Ganesh, R., Marks, D. J., Sales, K., Winslet, M. C., and Seifalian, A. M. (2012). Cyclooxygenase/lipoxygenase shunting lowers the anti-cancer effect of cyclooxygenase-2 inhibition in colorectal cancer cells. *World J. Surg. Oncol.* 10, 200. doi: 10.1186/1477-7819-10-200

Giovannucci, E., and Goldin, B. (1997). The role of fat, fatty acids, and total energy intake in the etiology of human colon cancer. *Am. J. Clin. Nutr.* 66, 1564S–1571S. doi: 10.1093/ajcn/66.6.1564S

Giros, A., Grzybowski, M., Sohn, V. R., Pons, E., Fernandez-Morales, J., Xicola, R. M., et al. (2009). Regulation of colorectal cancer cell apoptosis by the n-3 polyunsaturated fatty acids docosahexaenoic and eicosapentaenoic. *Cancer Prev. Res.* 1, 732–742. doi: 10.1158/1940-6207.CAPR-08-0197

Guerrero, A., Casals, I., Busquests, M., Leon, Y., and Manresa, A. (1997). Oxydation of oleic acid to €-10-hydroperoxy-8-octadecenoic and €-10-hydroxy-8-octadecenoic acids by Pseudomonas sp. *42A2 Biochim. Biophys. Acta* 1347, 75–81. doi: 10.1016/S0005-2760(97)00056-8

Hass, R., Busche, R., Luciano, L., Reale, E., and Engelhardt, W. V. (1997). Lack of butyrate is associated with induction of Bax and subsequent apoptosis in the proximal colon of guinea pig. *Gastroenterology* 112, 875–881. doi: 10.1053/gast.1997.v112.pm9041249

Hawcroft, G., Loadman, P. M., Belluzzi, A., and Hull, M. A. (2010). Effect of eicosapentaenoic acid on E-type prostaglandin synthesis and EP4 receptor signaling in human colorectal cancer cells. *Neoplasia* 12, 618–627. doi: 10.1593/neo.10388

Hirasawa, A., Tsumaya, K., Awaji, T., Katsumara, S., Adachi, T., Yamada, M., et al. (2005). Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.* 11, 90–94. doi: 10.1038/nm1168

Igal, R. A. (2016). Stearoyl CoA desaturase-1: New insights into a central regulator of cancer metabolism. *Biochim. Biophys. Acta* 1861, 1865–1880. doi: 10.1016/j.bbalip.2016.09.009

Ikehata, A., Hiwatashi, N., Kinouchi, Y., Yamazaki, H., Kumagai, Y., Ito, K., et al. (1992). Effect of intravenously infused eicosapentaenoic acid on the leukotriene generation in patients with active Crohn's disease. *Am. J. Clin. Nutr.* 56, 938–942. doi: 10.1093/ajcn/56.5.938

Kles, K. A., and Chang, E. B. (2006). Short-chain fatty acids impact on intestinal adaptation, inflammation, carcinoma, and failure. *Gastroenterology* 130, S100–S105. doi: 10.1053/j.gastro.2005.11.048

Kopf, T., and Schmitz, G. (2013). Analysis of non-esterified fatty acids in human samples by solid-phase-extraction and gas chromatography/mass spectrometry. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 938, 22–26. doi: 10.1016/j.jchromb.2013.08.016

Kuemmerle, N. B., Rysman, E., Lombardo, P. S., Flanagan, A. J., Lipe, B. C., Wells, W. A., et al. (2011). Lipoprotein lipase links dietary fat to solid tumor cell proliferation. *Mol. Cancer Ther.* 10, 427–436. doi: 10.1158/1535-7163.MCT-10-0802

Lands, W. B., Libelt, B., Morris, A., Kramer, N. C., Prewitt, T. E., Schmeisser, D., et al. (1992). Maintenance of lower proportions of (n-6) eicosanoid precursors in phospholipids of human plasma in response to added dietary (n-3) fatty acids. *Biochim. Biophys. Acta* 1180, 147–162. doi: 10.1016/0925-4439(92)90063-S

Larsson, S. C., Kumlin, M., Ingelman-Sundberg, M., and Wolk, A. (2004). Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *Am. J. Clin. Nutr.* 79, 935–945. doi: 10.1093/ajcn/79.6.935

Le Faouder, P., Baillif, V., Spreadbury, I., Motta, J. P., Rousset, P., Chene, G., et al. (2013). LC-MS/MS method for rapid and concomitant quantification of pro-inflammatory and pro-resolving polyunsaturated fatty acid metabolites. *J. Chromatogr. B.* 932, 123–133. doi: 10.1016/j.chromb.2013.06.014

Li, Y., Shi, J., Qi, S., Zhang, J., Peng, D., Chen, Z., et al. (2018). IL-33 facilitates proliferation of colorectal cancer dependent on COX2/PGE2. *J. Exp. Clin. Cancer Res.* 17, 196. doi: 10.1186/s13046-018-0839-7

Liotti, A., Cosimato, V., Mirra, P., Cali, G., Conza, D., Secondo, A., et al. (2018). Oleic acid promotes prostate cancer malignant phenotype via the G protein-coupled receptor FFA1/GPR40. *J. Cell. Physiol.* 233, 7367–7378. doi: 10.1002/jcp26572

Liu, Z., Xiao, Y., Yuan, Y., Zhang, X., Qin, C., Xie, J., et al. (2013). Effects of oleic acid on cell proliferation through an integrin-linked kinase signaling pathway in 786-O renal cell carcinoma cells. *Oncol. Lett.* 5, 1395–1399. doi: 10.3892/ol.2013.1160

Martín-Venegas, R., Roig-Pérez, S., Ferrer, R., and Moreno, J. J. (2006). Arachidonic acid cascade and epithelial barrier function during Caco-2 cell differentiation. *J. Lipid Res.* 47, 1416–1423. doi: 10.1194/jlr.M500564-JLR200

Martin-Venegas, R., Jáuregui, O., and Moreno, J. J. (2014). Liquid chromatography-tandem mass spectrometry analysis of eicosanoids and related compounds in cell models. *J. Chromatogr. B.* 964, 41–49. doi: 10.1016/j.jchromb.2014.05.024

Meyerhardt, J. A., Niedzwiecki, D., Hollis, D., Saltz, L. B., Hu, F. B., Mayer, R. J., et al. (2007). Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. *JAMA* 298, 754–764. doi: 10.1001/jama.298.7.754

Mitjavila, M. T., Rodríguez, M. C., Sáiz, M. P., Lloret, S., and Moreno, J. J. (1996). Effect of degree of unsaturation in dietary fatty acids on arachidonic acid mobilization by peritoneal macrophages. *Lipids* 31, 661–666. doi: 10.1007/BF02523839

Moreno, J. J., Carbonell, T., Sánchez, T., Miret, S., and Mitjavila, M. T. (2001). Olive oil decreases both oxidative stress and the production of arachidonic acid metabolites by the prostaglandin G/H synthase pathway in rat macrophages. *J. Nutr.* 131, 2145–2149. doi: 10.1093/jn/131.8.2145

Moreno, J. J. (2005). Arachidonic acid cascade enzyme inhibition and cancer. *Curr. Enzyme Inhibition* 2, 131–145. doi: 10.2174/1573408054022261

Moreno, J. J. (2009). New aspects of the role of hydroxyeicosatetraenoic acids in cell growth and cancer development. *Biochem. Pharmacol.* 77, 1–10. doi: 10.1016/j.bcp.2008.07.033

Moreno, J. J. (2017). Eicosanoid receptors: Targets for the treatment of disrupted intestinal epithelial homeostasis. *Eur. J. Pharmacol.* 796, 7–19. doi: 10.1016/j.ejphar.2016.12.004

National Cancer Institute *National cancer institute surveillance epidemiology and end results. Finding Statistics*. Available at: https://seer.cancer.gov/statfacts/html/colorect.html (Accessed May 22, 2017).

Nieves, D., and Moreno, J. J. (2006). Effect of arachidonic and eicosapentaenoic acid metabolism on RAW 264.7 macrophage proliferation. *J. Cell. Physiol.* 208, 428–434. doi: 10.1002/jcp.20678

Norat, T., Bingham, S., Ferrari, P., Slimani, N., Jenab, M., Mazuir, M., et al. (2005). Meat, fish, and colorectal cancer risk: The European prospective investigation into cancer and nutrition. *J. Nat. Cancer Inst.* 97, 906–916. doi: 10.1093/jnci/dji164

Petrik, M. B., McEntee, M. F., Johnson, B. T., Obukowicz, M. G., and Whelan, J. (2000). Highly unsaturated (n-3) fatty acids, but not α-linolenic, conjugated linoleic or γ-linolenic acids, reduce tumorigenesis in Apc(Min/+) mice. *J. Nutr.* 130, 2434–2443. doi: 10.1093/jn/130.10.2434

Rodriguez-Lagunas, M. J., Martín-Venegas, R., Moreno, J. J., and Ferrer, R. (2010). PGE_2 promotes Ca^{2+}-mediated epithelial barrier disruption through EP1 and EP4 receptors in Caco-2 cell monolayers. *Am. J. Physiol. Cell Physiol.* 299, 324–334. doi: 10.1152/ajpcell.00397.2009

Rodríguez-Lagunas, M. J., Storniolo, C. E., Ferrer, R., and Moreno, J. J. (2013). 5-Hydroxyeicosatetraenoic acid and leukotriene D4 increase intestinal epithelial paracellular permeability. *Int. J. Biochem. Cell. Biol.* 45, 1318–1326. doi: 10.1016/j.biocel.2013.04.005

Rosignoli, P., Fabiani, R., De Bartolomeo, A., Fuccelli, R., Pelli, M. A., and Morozzi, G. (2008). Genotoxic effect of bile acids on human normal and tumour colon cells and protection by dietary antioxidants and butyrate. *Eur. J. Nutr.* 47, 301–309. doi: 10.1007/s00394-008-0725-8

Salomon, P., Kornbluth, A. A., and Janowitz, H. D. (1990). Treatment of ulcerative colitis with fish oil n–3-omega-fatty acid: an open trial. *J. Clin. Gastroenterol.* 12, 157–161. doi: 10.1097/00004836-199004000-00009

Sasazuki, S., Inoue, M., Iwasaki, M., Sawada, N., Shimazu, T., Yamaji, T., et al. (2011). Intake of n-3 and n-6 polyunsaturated fatty acids and development of colorectal cancer by subsite: Japan Public Health Center-based prospective study. *Int. J. Cancer* 129, 1718–1729. doi: 10.1002/ijc.25802

Shen, X. J., Zhou, J. D., Dong, J. Y., Ding, W. Q., and Wu, J. C. (2012). Dietary intake of n-3 fatty acids and colorectal cancer risk: a meta-analysis of data from 489 000 individuals. *Br. J. Nutr.* 108, 1550–1556. doi: 10.1017/S0007114512003546

Smith, W. L. (2005). Cyclooxygenases, peroxide tone and the allure of fish oil. *Curr. Opin. Cell Biol.* 17, 174–182. doi: 10.1016/j.ceb.2005.02.005

Song, M., Chan, A. T., Fuchs, C. S., Ogino, S., Hu, F. B., Mozaffarian, D., et al. (2014). Dietary intake of fish, ω-3 and ω-6 fatty acids and risk of colorectal cancer: A prospective study in U.S. men and women. *Int. J. Cancer* 135, 2413–2423. doi: 10.1002/ijc.28878

Storniolo, C. E., Martínez-Hovelman, N., Martínez-Huélamo, M., Lamuela-Raventos, R. M., and Moreno, J. J. (2019). Extra Virgin Olive Oil Minor Compounds Modulate Mitogenic Action of Oleic Acid on Colon Cancer Cell Line. *J. Agric. Food Chem.* 67, 11420–11427. doi: 10.1021/acs.jafc.9b04816

Storniolo, C. E. (2017). *Efecto de componentes de la Dieta Mediterránea sobre la cascada del ácido araquidónico y la proliferación de células epiteliales intestinales* (University of Barcelona: Doctoral Thesis).

Ullman, T. A., and Itzkowitz, S. H. (2011). Intestinal inflammation and cancer. *Gastroenterology* 140, 1807–1816. doi: 10.1053/j.gastro.2011.01.057

Vargas, T., Moreno-Rubio, J., Herranz, J., Cejas, P., Molina, S., González-Vallinas, M., et al. (2015). ColoLipidGene: signature of lipid metabolism-related genes to predict prognosis in stage-II colon cancer patients. *Oncotarget* 6, 7348–7363. doi: 10.18632/oncotarget.3130

Wallace, J. L., and McKnight, G. W. (1990). Comparison of the damage-promoting effects of leukotrienes derived from eicosapentaenoic acid and arachidonic acid on the rat stomach. *J. Exp. Med.* 171, 1827–1832. doi: 10.1084/jem.171.5.1827

Wang, J., and Friedman, E. A. (1998). Short-chain fatty acids induce cell cycle inhibitors in colonocytes. *Gastroenterology* 114, 940–946. doi: 10.1016/S0016-5085(98)70313-0

Williams, C. D., Satia, J. A., Adair, L. S., Stevens, J., Galanko, J., Keku, T. O., et al. (2010). Associations of red meat, fat, and protein intake with distal colorectal cancer risk. *Nutr. Cancer* 62, 701–709. doi: 10.1080/01635581003605938

World Cancer Research Fund / American Institute for Cancer Research (2011).

World Health Organization. http://www.who.int/dietphysicalactivity/publications/trs916/kit/en/.

Wu, Q., Wang, H., Zhao, X., Shi, Y., Jin, M., Wan, B., et al. (2013). Identification of G-protein-coupled receptor 120 as a tumor-promoting receptor that induces angiogenesis and migration in human colorectal carcinoma. *Oncogene* 32, 5541–5550. doi: 10.1038/onc.2013.264

Yang, P., Jiang, Y., and Fischer, S. M. (2014). Prostaglandin E3 metabolism and cancer. *Cancer Lett.* 348, 1–11. doi: 10.1016/j.canlet.2014.03.010

Yusof, A. S., Isa, Z. M., and Shah, S. A. (2012). Dietary patterns and risk of colorectal cancer: a systematic review of cohort studies, (2000-2011). *Asian Pacific J. Cancer Prev.* 13, 4713–4717. doi: 10.7314/APJCP.2012.13.9.4713

Zhang, C., Yu, H., Shen, Y., Ni, X., Shen, S., and Das, U. N. (2015). Polyunsaturated fatty acids trigger apoptosis of colon cancer cells through a mitochondrial pathway. *Arch. Med. Sci.* 11, 1081–1094. doi: 10.5114/aoms.2015.54865

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constituted as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 29 October 2020
doi: 10.3389/fphar.2020.580897

Pleiotropic Functions of Cytochrome P450 Monooxygenase-Derived Eicosanoids in Cancer

Ying Luo[1] *and Jun-Yan Liu*[2]*†

[1]Department of Clinical Laboratory, Changning Maternity and Infant Health Hospital, East China Normal University, Shanghai, China, [2]Center for Novel Target & Therapeutic Intervention, Institute of Life Sciences, Chongqing Medical University, Chongqing, China

Eicosanoids are a class of functionally bioactive lipid mediators derived from the metabolism of long-chain polyunsaturated fatty acids (PUFAs) mediated by multiple enzymes of three main branches, including cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450s (CYPs). Recently, the role of eicosanoids derived by COXs and LOXs pathways in the control of physiological and pathological processes associated with cancer has been well documented. However, the role of CYPs-mediated eicosanoids, such as epoxyeicosatrienoic acids (EETs), epoxyoctadecenoic acids (EpOMEs), epoxyeicosatetraenoic acids (EpETEs), and epoxydocosapentaenoic acids (EDPs), as well as hydroxyeicosatetraenoic acids (HETEs), in tumorigenesis and cancer progression have not been fully elucidated yet. Here we summarized the association of polymorphisms of CYP monooxygenases with cancers and the pleiotropic functions of CYP monooxygenase-mediated eicosanoids (EETs, EpOMEs, EpETE, EDPs, and 20-HETE) in the tumorigenesis and metastasis of multiple cancers, including but not limited to colon, liver, kidney, breast and prostate cancers, which hopefully provides valuable insights into cancer therapeutics. We believe that manipulation of CYPs with or without supplement of ω-3 PUFAs to regulate eicosanoid profile is a promising strategy to prevent and/or treat cancers.

Keywords: CYP = cytochrome P450, eicosanoid, cancer, morphism, mechanism

OPEN ACCESS

Edited by:
Emer Smyth,
University of Pennsylvania,
United States

Reviewed by:
Milena Sokolowska,
University of Zurich, Switzerland
Jiiang-Huei Jeng,
National Taiwan University,
Taiwan

***Correspondence:**
Jun-Yan Liu
jyliu@cqmu.edu.cn

†ORCID:
Jun-Yan Liu
orcid.org/0000-0002-3018-0335

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *07 July 2020*
Accepted: *30 September 2020*
Published: *29 October 2020*

Citation:
Luo Y and Liu J-Y (2020) Pleiotropic Functions of Cytochrome P450 Monooxygenase-Derived Eicosanoids in Cancer. Front. Pharmacol. 11:580897. doi: 10.3389/fphar.2020.580897

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; ALL, acute lymphoblastic leukemia; AOM/DSS, azoxymethane/dextran sodium sulfate; CML, chronic myelogenous leukemia; COX, cyclooxygenase; CRC, colorectal cancer; CYP, cytochrome P450; DHA, docosahexaenoic acid; DHET, dihydroxyeicosatrienoic acid; DiHOME, dihydroxyoctadecenoic acid; EDP or EpDPE, epoxydocosapentaenoic acid; EEQ or EpETE, epoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; EGF, epidermal growth factor ; EMT, epithelial-mesenchymal transition; EPA, eicosatetraenoic acid; EpOME, epoxyoctadecenoic acid; FGF, fibroblast growth factor; GIST, gastrointestinal stromal tumors; GLA, γ-linolenic acid; HCC, hepatocellular carcinoma; HDoHE, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HNSCC, head and neck squamous cell carcinoma; LA, linoleic acid; LOX, lipoxygenase; mEH, microsomal epoxide hydrolase; NSCLC, non-small cell lung cancer; PDGF, platelet-derived growth factor; PLA_2, phospholipase A_2; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; RA, retinoic acid; sEH, soluble epoxide hydrolase; SREBP, sterol regulatory element binding protein; TNBC, triple-negative breast cancer; TNFα, tumor necrosis factor-α.

INTRODUCTION

Eicosanoids, a class of bioactive lipid mediators, are the metabolites of long-chain n-3 and n-6 polyunsaturated fatty acids (PUFAs) mediated by three primary enzymatic systems, cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450s (CYPs) enzymes. The common PUFAs include arachidonic acid (20:4 n = 6, AA), linoleic acid (18:2 n = 6, LA), γ-linolenic acid (18:3 n = 6, GLA), α-linolenic acid (18:3 n = 3, ALA), eicosatetraenoic acid (20:5 n = 3, EPA) and docosahexaenoic acid (22:6 n = 3, DHA). Eicosanoids are synthesized rapidly in response to multiple factors (e.g. allergy, infection, and injury) and act putatively through their cognate receptors in local cells. Although some eicosanoids exhibit immediate and short-lasting activity, they play an important role in many chronic diseases, including asthma, allergy, autoimmune diseases, and malignancies since they have pleiotropic functions, such as pro-inflammation, anti-inflammation, vasodilation, analgesia, and hyperalgesia (Dennis and Norris, 2015; Sokolowska et al., 2020). Interestingly, some eicosanoids were found to have dual actions, for example, lipoxins, resolvins, and protectins, which have been extensively reported to be anti-inflammatory and pro-resolving (Serhan et al., 2008). Emerging evidence showed the dominant roles of metabolites of PUFAs involved in the regulation of inflammation, pain, angiogenesis, and cancer (Zhang et al., 2014a). The COX- and LOX-mediated PUFAs metabolism have been well documented in tumorigenesis and cancer progression (Wang and Dubois, 2006; Cathcart et al., 2012; Knab et al., 2014; Tuncer and Banerjee, 2015). However, the roles of the CYP pathway-mediated metabolites of PUFAs in the pathogenesis of cancer has not been fully studied.

CYP enzymes catalyze a variety of oxidative and some reductive reactions involving thousands of substrates (Guengerich, 2007). The substrates of CYPs encompass xenobiotics, including substances that occur biologically but are exogenous to humans, such as antibiotics and synthetic organic chemicals (Porter and Coon, 1991), and endogenous compounds, such as cholesterol, testosterone, progesterone, prostaglandin H_2, corticosterone, retinoic acid, vitamin D_3, and some PUFAs, like AA and LA (Guengerich, 2017). CYP enzymes can mediate the metabolisms of the lipophilic endogenous and xenobiotic compounds into hydrophilic or polar compounds, which could be excreted from the body easily (Chang and Kam, 1999). Firstly, the substrate binds to the active sites of CYP enzymes. Then the reductive reaction of the heme iron from a ferric to a ferrous is occurred by an electron transferred from a reduced NADPH (Chang and Kam, 1999). After that, the oxygen molecule temporarily binds at the heme-containing active site (Zanger and Schwab, 2013). At last, the substrate molecule is inserted by an oxygen atom, and water is formed by other relevant atoms simultaneously. Therefore, CYP monooxygenases incorporate one atom of oxygen into their substrates (Ortiz de Montellano, 2010). Here we focus on the CYP-derived metabolites of PUFAs and their multiple functions in cancer.

The Cytochrome P450-Derived Eicosanoids of n-6 Polyunsaturated Fatty Acids

CYPs consisting of 57 functional genes in human are a superfamily of enzymes which mediate the metabolism of exogenous and endogenous compounds (Jamieson et al., 2017; Nelson et al., 2004). AA and LA are the most common substrates of the CYP enzyme system. CYP enzymes relevant to AA metabolism include two main branches: the ω-hydroxylase and epoxygenase pathways (Panigrahy et al., 2010). Epoxygenases (mainly CYP2C and CYP2J isoforms) convert AA to epoxyeicosatrienoic acids (EETs), including 5(6)-, 8(9)-, 11(12)-, and 14(15)-EET (Wu et al., 1997). ω-hydroxylases (mainly CYP4A and CYP4F isoforms) convert AA to 19-, and 20-hydroxyeicosatetraenoic acids (HETEs) (**Figure 1**) (Panigrahy et al., 2010). In addition, CYP4X1 and CYP2U1 can metabolize AA to 19- and 20-HETE, as well as 8(9)-, and 14(15)-EET (Chuang et al., 2004; Stark et al., 2008). LA is the primary exogenous precursor of essential fatty acids, obtained from many diets. Within the body, LA can be catalyzed to the formation of 9(10)- and 12(13)-epoxyoctadecenoic acids (EpOMEs) in the presence of CYP epoxygenases. Both EETs and EpOMEs are metabolically unstable and can be rapidly metabolized to corresponding fatty acid diols, dihydroxyeicosatrienoic acids (DHETs), and dihydroxyoctadecenoic acid (DiHOMEs), respectively, by soluble epoxide hydrolase (sEH) and microsomal EH (mEH) (**Figure 1**) (Zhang et al., 2014a). Recently, the CYP/sEH eicosanoid pathway related to inflammation and cancer gains many interests in academic researches.

The Cytochrome P450-Derived Eicosanoids of n-3 Polyunsaturated Fatty Acids

The n-3 PUFAs, mainly EPA and DHA, can be catalyzed by CYP isozymes into functional eicosanoids. EPA is metalized into ω/(ω-1)-hydroxyeicosapentaenoic acids (19- and 20-HEPE) by CYP ω-hydroxylases, and five regioisomeric epoxyeicosatetraenoic acids [5(6)-, 8(9)-, 11(12)-, 14(15)-, 17(18)-EEQ, or EpETE] by CYP epoxygenases (**Figure 1**) (Van Rollins et al., 1988). DHA can also be metalized into ω/(ω-1)-hydroxydocosahexaenoic acids (21- and 22-HDoHE) by CYP ω-hydroxylases, and six regioisomeric epoxydocosapentaenoic acids [4(5)-, 7(8)-, 10(11)-, 13(14)-, 16(17)-, 19(20)-EDP, or EpDPE), respectively, by CYP epoxygenases (**Figure 1**) (VanRollins et al., 1984). The epoxy metabolites EEQ and EDP can be further metabolized by sEH and mEH enzyme to the corresponding diols.

The Presence and Location of Cytochrome P450s in Organs

The CYP superfamily comprises 57 functional CYP genes and 58 pseudogenes in humans (Nelson et al., 2004). The CYPs have been reported to express in all human tissues investigated (Porter and Coon, 1991). They are expressed predominately in the endoplasmic reticulum membrane, cell surface, and mitochondria (Neve and Ingelman-Sundberg, 2010), with the greatest abundance in the liver (Porter and Coon, 1991), small intestine (Thelen and Dressman, 2009), and kidney (Renaud et al., 2011).

In humans, CYP2C and CYP2J are the predominant epoxygenases that metabolize PUFAs. CYP2C and CYP2J are widely distributed in the human body, including but not limited

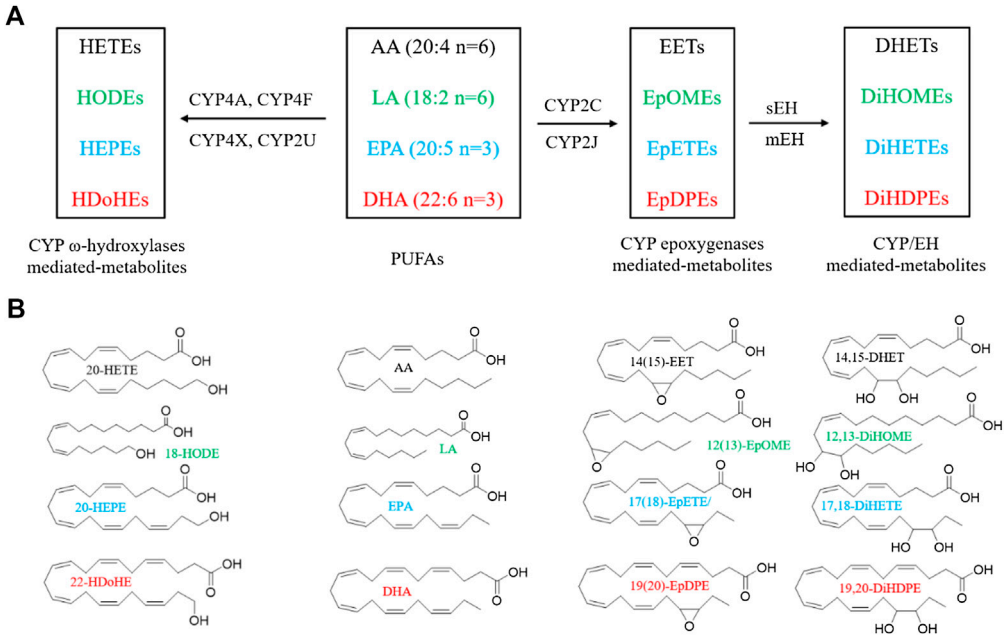


FIGURE 1 | Metabolism of PUFAs by CYP enzymes. **(A)** A simplified cascade of PUFAs discussed in this paper. AA, arachidonic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CYP, cytochrome P450; sEH, soluble epoxide hydrolase; DHET, dihydroxyeicosatrienoic acid; DiHOME, dihydroxy octadecamonoeneoic acid; DiHETE, dihydroxyeicosatetraenoic acid; DiHDTE, dihydroxydocosatetraenoic acid; EET, epoxyeicosatrienoic acid; EpOME, epoxyoctadecamonoeneoic acid; EEQ or EpETE, epoxyeicosatetreaenoic acid; EDP or EpDPE, epoxydocosapentaenoic acid; HDoHE, hydroxydocosahexaenoic acid; HEPE: hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; mEH, microsomal epoxide hydrolase; sEH, soluble epoxide hydrolase. The metabolites are from the fatty acids in the same color. **(B)** The chemical structures of PUFAs and the representative metabolites.

to the cardiovascular system, kidney, lung, brain, gastrointestinal tract, cerebral cortex, hippocampus, fetal nasal mucosa, and many other tissues (Nelson et al., 2004). The CYP2C family is located on chromosome 10 and consists of at least seven genes and/or pseudogenes. The CYP2C8, CYP2C9, CYP2C18, and CYP2C19 are most involved in the metabolism of PUFAs. They are also convinced to be involved in the progression of a malignant tumor (Xu et al., 2011). Besides the above-mentioned four CYP2C enzymes, CYP2J2 is another epoxygenase acting as a regulator that catalyzes the metabolism of PUFAs. In the human body, CYP2J2 is mainly distributed in cardiovascular tissues, such as cardiomyocytes, coronary endothelial cells, and aorta and vein of coronary smooth muscle cells. Liver enzymes account for only 1–2% of total CYP in the liver, jejunum, ileum, and colon. Limited by its content, CYP2J2 generally does not play a decisive role in exogenous metabolism in theory. However, CYP2J2 has been found to play a dominant role in the intestinal metabolism of certain drugs, such as antihistamines, terfenadine, and ebastine. In the kidney, CYP2J2 is expressed in proximal convoluted tubule and collecting tubule. In addition to the expression in tissues such as the heart, kidney, and liver, CYP2J2 is also highly expressed in the cerebral cortex, frontal lobe, and hippocampus. Dutheil et al. showed that a variety of CYPs are distributed in the human brain, and the expression level of CYP2J2 is about 20% of the total content of CYPs. Moreover, Ahmed E. et al. reported that CYP2J2 was expressed in the pancreatic islets, consistent with the finding that EETs, the metabolic products of AA, regulate the levels of insulin and glucagon (Zeldin et al., 1997). CYP2J2 and CYP2C9 enzymes are co-expressed in the pituitary gland, suggesting they can regulate the release of pituitary hormones including prolactin and growth hormone most likely via EETs (Snyder et al., 1989; Irusta et al., 2007).

In mammals, the CYP4 family primarily mediates the ω-hydroxylation of PUFAs, which includes 12 genes and 13 enzymes, such as, CYP4A, CYP4B, CYP4F, CYP4V, CYP4X, and CYP4Z. CYP4A, CYP4B, CYP4X, and CYP4Z are located on chromosome 1, while CYP4F and CYP4V on chromosome 19 and 4, respectively (Nelson et al., 2004). The CYP4 is the largest one in the human CYP family, only a few of which mediate the ω-hydroxylation of PUFAs (Simpson, 1997). In the CYP4 family, CYP4A11, CYP4F2, CYP4F3A, and CYP4F3B are the most studied. CYP4A11 has been reported to express primarily in the liver and kidney, which can be regulated by peroxisome proliferator-activated receptor-α (PPARα) and catalyze the metabolism of AA and lauric acid. CYP4F2 is also mainly expressed in the liver and kidney, and it is regulated by the sterol regulatory element-binding protein (SREBP). The P450 gene CYP4F3 is unusual, and CYP4F3A and CYP4F3B are two different spliceosomes. CYP4F3A is expressed in neutrophils and CYP4F3B is primarily expressed in the human liver and kidney. They are both the main ω-hydroxylases of long-chain PUFAs. In addition, CYP4F8, CYP4F22, and CYP4V2 have been found to express predominantly in extrahepatic tissues. Among these enzymes, only CYP4V2 exhibits fatty acid ω-hydroxylase activity. CYP4X1 and CYP4Z1 are both extrahepatic CYPs, the

former is highly expressed in the brain, skin, and airways, and is inducible by glucocorticoids and progesterone.

Other CYPs, such as CYP1A1, CYP 1A2, CYP1B1, CYP2D6, and CYP3A4, can detoxificate carcinogens regardless of whether they are expressed in the liver or kidney.

In addition to many studies investigating the expression of CYP epoxygenases in multiple organ tissues, a few studies summarized the expression of CYP epoxygenases in some specific cell types. Almost all the CYP epoxygenases were found in peripheral blood cells, vascular endothelial cells, and vascular smooth muscle cells (Xu et al., 2013; Sausville et al., 2019). In addition, CYP2J was reported to express in many cells, including but not limited to LS-174, ScaBER, SiHa, U251, A549, Tca-8113, Ncl-H446, HepG2, K562, HL-60, MOLT-4, Jurkat, Raji, autonomic ganglion nerves, and smooth muscle cells, pancreatic islet cells, Purkinje cells (DeLozier et al., 2007; Xu et al., 2013; Sausville et al., 2019).

The Association of Polymorphisms of Cytochrome P450s With Cancers

Recently, the expression of various CYP genes has been proved to be closely related to malignant tumors. Polymorphisms of CYPs have been suggested to influence susceptibility to cancers for many years. Genetic polymorphisms in CYPs have been reported to be associated with individuals variations in drug metabolism and disease susceptibility (Pikuleva and Waterman, 2013; Mittal et al., 2015). Here, we discuss some important polymorphisms of CYPs in cancers **(Table 1)**.

CYP1A1

CYP1A1 is a hepatic and extrahepatic enzyme that is regulated by the aryl hydrocarbon receptor signaling pathway. It has been always associated with the metabolism of pro-carcinogenic compounds to highly carcinogenic metabolites. For CYP1A1, four common variants (T3801C, A2455G, T3205C, and C2453A) were widely studied for the susceptibility to various cancers (Bozina et al., 2009). T3801C (rs4646903) and T3205C are situated in the 3′ noncoding region while A2455G (rs1048943) and C2453A are ascertained in exon 7, which results in the transition of isoleucine to valine on codon 462 and threonine to asparagine on codon 461, respectively (Li et al., 2004). The monomorphism of T3205C locus was reported in Indians (Singh et al., 2007), Americans (San Francisco) (Hirata et al., 2008), and Northeast Thai women (Wongpratate et al., 2020). Among several populations, the polymorphisms of T3801C and/or A2455G were reported to associate significantly with the increased risk in cervical cancer (Juarez-Cedillo et al., 2007; Li et al., 2016; Jain et al., 2017; Wang et al., 2017; Ding et al., 2018). An association of T3801C (CC) genotype with increased cervical cancer risk was reported among the Asians population by a meta-analysis study (Wu et al., 2013). However, there is a lack of significant association between T3801C and A2455G polymorphisms and cervical cancer risk in Chinese, Japanese, Israeli Jewish, Polish, Indian, and Thai populations (Sugawara et al., 2003; Gutman et al., 2009; Roszak et al., 2014; Tan et al., 2016; Wongpratate et al., 2020). The A allele of C2453A is associated with the risk of lung cancer (Gallegos-Arreola et al., 2008; Ezzeldin et al., 2017), laryngeal squamous cell carcinoma (Gajecka et al., 2005), thyroid cancer (Siraj et al., 2008), and cervical cancer (Wongpratate et al., 2020), but not associated with breast (Li et al., 2004; Singh et al., 2007; Amrani et al., 2016), colorectal (Little et al., 2006), or gastric cancer (Agudo et al., 2006).

CYP1A2

CYP1A2, which is similar to CYP1A1, can metabolize a broad range of foreign compounds and drugs. An SNP C>A (rs762551) was found in intron one of CYP1A2 (Womack et al., 2012), which influences the inducibility of CYP1A2 (Koonrungsesomboon et al., 2018). The highest CYP1A2 induction rate was reported in AA genotype (Sachse et al., 1999), and the high enzyme activity carriers were at high risk of lung cancer (Seow et al., 2001; Bu et al., 2014). On the other hand, the low activity or downregulation of CYP1A2 influenced by the SNP would result in the progression of hepatocellular carcinoma (HCC). As the substrate of CYP1A2, 17 β-estradiol can be metabolized to 2-hydroxyestradiol which is then converted to 2-methoxyestradiol that inhibits HCC cells proliferation by inducing apoptosis (Ren et al., 2016). More recently, the CYP1A2 SNP rs762551 was found to be significantly associated with the high risk in breast cancer in the Jordanian population (Al-Eitan et al., 2019).

CYP1B1

CYP1B1 not only mediates the metabolisms of xenobiotics, e.g. theophylline, ethoxyresorufin, and caffeine (Rochat et al., 2001; Zanger and Schwab, 2013) but also activates some procarcinogens, such as aromatic amines, heterocyclic amines, nitropolycyclic and polycyclic hydrocarbons (Chun and Kim, 2016). CYP1B1 mutations were a causative factor of diseases. For example, L432V and A119S (rs1056827) polymorphisms of the CYP1B1 gene were reported to increase the risk of developing endometrial cancer (Zhu et al., 2011) and laryngeal cancers (Yu et al., 2015).

CYP2A6

CYP2A6 is an essential hepatic enzyme involved in the metabolism of drugs, is responsible for a major metabolic pathway of nicotine. The first polymorphism identified of CYP2A6 was a nonsynonymous polymorphism (L160H) (rs1801272) which leads to no enzyme activity (Fernandez-Salguero et al., 1995). There are more than 30 non-synonymous polymorphisms in nine exons (Di et al., 2009). Some polymorphisms were found to be associated with smoking behavior, drug metabolism, and lung cancer risk (Di et al., 2009). The wild type CYP2A6*1A is with normal enzyme activity, and the CYP2A6*4, including CYP2A6*4A, CYP2A6*4B, and CYP2A6*4D, have no enzyme activity (Hukkanen et al., 2005). The CYP2A6*5 encoded an unstable enzyme activity since the substitution of Glycin-479 by valine occurred (Oscarson et al., 1999). CYP2A6 can activate procarcinogens, for instance, nitrosamines and aflatoxins. The absence of the CYP2A6 enzyme could reduce the risk of lung cancer because the

activation of procarcinogens would be decreased. Therefore, the phenotypes of CYP2A6*4 and CYP2A6*5 protect the carriers against lung cancer or other cancers (Raunio et al., 2001).

CYP2C9

CYP2C9 metabolizes about 15% of clinically administrated drugs. Two common non-synonymous polymorphisms of CYP2C9, R144C, rs1799853 (CYP2C9*2), and I359L, rs1057910 (CYP2C9*3), have been reported to be highly frequent in Caucasian populations (Sistonen et al., 2009; Van Booven et al., 2010). These two polymorphisms result in poor metabolic activity of CYP2C9 (Van Booven et al., 2010), and are positively associated with the risk of cancer. Individuals with CYP2C9*2 (R144C, rs1799853) polymorphism have a several-fold increased risk of head and neck squamous cell carcinoma (HNSSC) (Yadav et al., 2014). On the contrary, as the CYP2C9 (R144C, rs1799853, and I359L, rs1057910) variants metabolize AA less efficiently than CYP2C9 wild type, they were proved to retard the development of non-small cell lung cancer (NSCLC) due to the reduced ability to generate EETs (Sausville et al., 2018). Recently, the relationship of CYP2C9 polymorphism with colorectal cancer (CRC) susceptibility was investigated by a number of case-control studies. But the results were controversary. A meta-analysis of 13 studies involving a total of 20,879 subjects for CYP2C9 (R144C, rs1799853 and I359L, rs1057910) polymorphisms to evaluate the effect of CYP2C9 on genetic susceptibility for CRC suggest that the CYP2C9 (R144C, rs1799853 and I359L, rs1057910) polymorphisms are not associated with CRC susceptibility (Zhao et al., 2013). Also, the CYP2C9 (R144C, rs1799853, and I359L, rs1057910) polymorphisms are not associated with lung cancer risk among African-Americans and Caucasians in Los Angeles (Zhao et al., 2013) or in white Spanish (Garcia-Martin et al., 2002).

CYP2C19

Among the CYP2C subfamily, CYP2C19 is the most polymorphic (Lee, 2012). CYP2C19 polymorphism leads to differences in enzyme expression and metabolic activity between individuals. CYP2C19 polymorphisms classified the population to poor, extensive, and ultra-rapid metabolic activity (Reynald et al., 2012). The two primary point mutation sites of CYP2C19 are CYP2C19*2 and CYP2C19*3, which cause poor metabolizer phenotype of CYP2C19. CYP2C19 polymorphisms have been analyzed about the prostate, bladder, lung, liver, colorectal cancer, and other cancers. No association of the CYP2C19*2 allele and prostate cancer was identified in the Swedish and Danish population (Wadelius et al., 1999). But there is a weak association between the CYP2C19*2 allele and bladder cancer (Brockmoller et al., 1996). Yan et al. found that there was a significant interaction between CYP2C19*3 and smoking in increasing the risk of lung cancer in a Chinese population (Yan et al., 2014). Similar results were reported in the Japanese population (Tsuneoka et al., 1996). CYP2C19*3 was identified to be associated with breast cancer risk in women (Gan et al., 2011). In contrast, a decreased breast cancer risk for carriers of the CYP2C19*17 allele was observed in German women (Justenhoven et al., 2009), and a meta-analysis has found that CYP2C19*2 and CYP2C19*17 genotypes are associated with increased survival of breast cancer patients treated with tamoxifen (Bai et al., 2014). Zhou et al. found that CYP2C19*2 causes a poor metabolizer phenotype, while CYP2C19*3 is associated with the increased risk of digestive system cancer, especially in East Asians (Zhou et al., 2013). Moreover, poor metabolizer genotypes were found to be associated with the increased risks in many cancers, such as esophagus cancer, gastric cancer, lung cancer, head neck cancer, and hepatocellular carcinoma, suggesting the CYP2C19*2 and CYP2C19*3 most likely contributes to cancer susceptibility, particularly in the Asian populations (Wang et al., 2013).

CYP2D6

CYP2D6 is one of the most studied enzymes in the field of pharmacogenetics. It exhibits large interindividual variability on drug metabolism. The polymorphism of CYP2D6 causes different metabolizer genotypes, including poor, intermediate, efficient, or ultra-rapid ones (Ingelman-Sundberg, 2005). The number of CYP2D6 polymorphisms is over seventy-five, and the association between CYP2D6 polymorphisms and cancer risk has been studied for many years (Agundez, 2004). Over twenty years ago, London et al. reported that the presence of inactivating CYP2D6 alleles (CYP2D6*4, CYP2D6*3, CYP2D6*5, and CYP2D6*16) may decrease the risk of lung cancer among the African-Americans, suggesting the CYP2D6 genetic polymorphism is not the strong risk factor for lung cancer but may play a minor role (London et al., 1997). By a meta-analysis, a minor but statistically significant association of CYP2D6 polymorphism with lung cancer susceptibility was established (Rostami-Hodjegan et al., 1998). Recently, associations between childhood acute leukemia (CAL) and genetic polymorphism of CYP2D6*4 for homozygous alleles were reported, suggesting CYP2D6*4 polymorphism could play a vital role in the etiology of CAL (Ferri et al., 2018). The association of Liver cancer with CYP2D6 genotype was also established by Agundez et al. (Agundez et al., 1995) In the HCC patients, the CYP2D6*10 allelic frequency was significantly different from those of control subjects. CYP2D6*10 is also suggested to be a potential biomarker for hepatocarcinogenesis risk (Zhou et al., 2016). The role of CYP2D6 polymorphism in melanoma has been investigated by different research groups with consistent results, indicating individuals with defect genes are at increased risk (Wolf et al., 1992; Dolzan et al., 1995; Strange et al., 1999). In other cancers, CYP2D6 polymorphisms were demonstrated to be associated with prostate (Wadelius et al., 1999; Sobti et al., 2006), bladder (Abdel-Rahman et al., 1997; Ouerhani et al., 2008) and renal cancers (Ahmad et al., 2013).

CYP3A4

CYP3A4 is the best-studied gene polymorphism in the CYP3A family. A meta-analysis comprising 55 separate studies including 22,072 cancer cases and 25,433 controls found a significant association between CYP3A4*1B and cancer risk especially leukemia in the overall population (Zheng et al., 2018). In the Chinese Han population, a relationship between the TT genotype

of CYP3A4*1G (rs2242480) polymorphism and the risk of breast cancer was established (Liu et al., 2019). He et al. found that CYP3A4 A392G polymorphism, but not CYP3A5 Met235Thr, is associated with the increased risk in prostate cancer among Caucasians (He et al., 2014). Although there are many studies on CYP3A4 gene polymorphism and cancer susceptibility, the underlying mechanism is still unclear and needs to be further investigated.

THE FUNCTION OF CYTOCHROME P450-MEDIATED EICOSANOIDS IN CANCERS

The Roles of Epoxyeicosatrienoic Acids in Cancer

EETs were discovered in the early 1980s (Capdevila et al., 1981a; Capdevila et al., 1981b; Chacos et al., 1982). EETs have been found to function as the regulators of cardiac, vascular (Fleming, 2007; Fleming, 2008; Fleming, 2011; Fleming, 2016), and renal physiology (Imig, 2005; Capdevila and Wang, 2013; Capdevila et al., 2015), indicating many important roles on the homeostasis of healthy tissues. Nakagawa et al. reported that the loss of EETs is associated with hypertension (Nakagawa et al., 2006). More recently, EETs have also been found to be associated with tumorigenesis, cancer metastasis, and angiogenesis (Jiang et al., 2005; Jiang et al., 2007; Pozzi et al., 2007; Pozzi and Capdevila, 2008; Yang et al., 2009; Mitra et al., 2011; Panigrahy et al., 2012), recovery of cardiac tissue from ischemic insult (Seubert et al., 2007) and other pathophysiologic processes.

CYP-mediated biosynthesis of four EETs [5(6)-, 8(9)-, 11(12)-, and 14(15)-EET] has been implicated in tumor growth and angiogenesis, as well as suppression of inflammation in murine models of cancers (Panigrahy et al., 2011). Compared to adjacent normal tissue, human breast cancer tissue has a higher level of 14 (15)-EET, which was due to increased CYP2C8, CYP2C9, and CYP2J2 and decreased sEH (Wei et al., 2014). Overexpression of CYP2J2 was found to be overexpressed in human neoplastic tissue and human cell lines when compared with the adjacent normal tissue and normal cell lines, respectively (Jiang et al., 2005; Jiang et al., 2007). CYP2C9 was found to express in the vasculature of several human tumor samples and be the regulatory target of human peroxisomal proliferator-activated receptor-alpha (PPARα), which have anti-angiogenic and anti-tumorigenic properties (Pozzi et al., 2010). The elevated EET levels by CYP overexpression or directly provided by the pump showed the ability to promote cancer metastasis in a murine model of cancer (Panigrahy et al., 2012). 14 (15)-EET has been exhibited to promote the proliferation of vascular endothelial cells (Cheranov et al., 2008) and estrogen receptor-positive breast cancer epithelial cells (Mitra et al., 2011). The molecular mechanism underlying the function of EETs on cancer cell proliferation is partly through activation of the PI_3-kinase/AKT pathway and the $STAT_3$ pathway (Jiang et al., 2005). More recently, Guo et al. discovered the mechanisms by which cancer cell-intrinsic CYP monooxygenases promote tumor progression are associated with breast cancer mitochondria and EETs promoted the electron transport chain/respiration and inhibited AMPKα (Guo et al., 2017). In triple-negative breast cancer (TNBC), EETs are important metastasis drivers. EET concentrations are associated with the upregulation of CYP2C19 and CYP2J2 (Apaya et al., 2019). In addition, EETs promote epithelial-mesenchymal transition (EMT) and resistance via the STAT and AKT signaling pathways (Zhang et al., 2006; Jiang et al., 2007; Mitra et al., 2011; Wei et al., 2014; Luo et al., 2018).

EETs are also anti-inflammatory (Node et al., 1999), which makes the biological action of EETs on cancer more complicated. In the murine model, 11(12)-EET can decrease the adhesion of mononuclear to vascular endothelium induced by tumor necrosis factor-α (TNF-α) (Node et al., 1999). 14 (15)-EET can also inhibit the expression of TNF-α and IL-1β induced by LPS in mouse macrophages (Zhang et al., 2013b). These results supported that EETs are anti-inflammatory in human and mouse tissues.

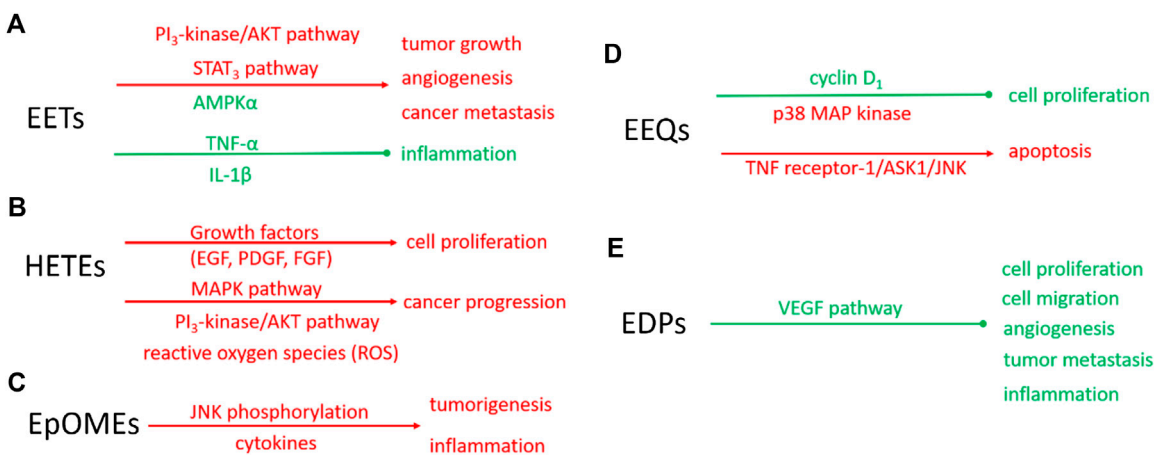


FIGURE 2 | Schematic diagram of molecular mechanisms of eicosanoids on the biological function of tumors. Red: indicating the enhanced signaling pathway or biological function; Green: indicating the suppressed signaling pathway or biological function solid line: proven pathways. The molecular mechanisms of EETs **(A)**, HETEs **(B)**, EpOMEs **(C)**, EEQs **(D)**, and EDPs **(E)**.

However, whether and how EETs play an anti-inflammatory role in cancer cells needs to be further investigated. The possible mechanisms underlying EETs regulate cancers are summarized in **Figure 2A**.

Recently, EETs were discovered to act the biological function in a receptor-dependent manner. However, the receptors of EETs have not been identified. Some research suggested that the actions of EETs are in part mediated via G protein-coupled receptor (GPCR) signaling. Five G protein-coupled receptors of the prostaglandin receptor family (PTGER2, PTGER4, PTGFR, PTGDR, and PTGER3IV) may be the EET receptors (Liu et al., 2017).

The Roles of Hydroxyeicosatetraenoic Acids in Cancer

The studies of the role of HETEs in cancer focus on 20-HETE. 20-HETE formed by CYP4 enzymes exhibits the proinflammatory function (Khanapure et al., 2007). In recent years, more attention has been paid to the promoting role of 20-HETE in cancer progression (Panigrahy et al., 2010; Alexanian and Sorokin, 2013). The endogenous 20-HETE formation has been implicated in cell proliferation by growth factors, including epidermal growth factor (EGF), platelet-derived growth factor

TABLE 1 | Examples of some cancers associated with cytochrome P450 enzymes.

CYPs	Polymorphism	Cancers	population	References
CYP1A1	T3801C (rs4646903) and/or A2455G (rs1048943)	Cervical cancer	Asians	Wu et al., 2013
			Caucasians and Asians	Ding et al., 2018
			Indians	Jain et al., 2017
			Mexican	Juarez-Cedillo et al., 2007
			Chinese	Li et al., 2016
	—		Caucasians	Wang et al., 2017
	C2453A	Lung cancer	Egyptian	Ezzeldin et al., 2017; Gallegos-Arreola et al., 2008
			Mexican	
	—	Laryngeal squamous cell carcinoma	Caucasian	Gajecka et al., 2005
		Thyroid cancer	Middle eastern	Siraj et al., 2008
		Cervical cancer	Northeast Thai	Wongpratate et al., 2020
	T3205C (rs1800031)	Breast cancer	Indians	Singh et al., 2007
		Endometrial cancer	Caucasian	Hirata et al., 2008
	—	Cervical cancer	Northeast Thai	Wongpratate et al., 2020
CYP1A2	rs762551	Lung cancer	Caucasians	Bu et al., 2014
		Breast cancer	Jordanian	Al-Eitan et al., 2019
CYP1B1	rs1056827	Endometrial cancer	Chinese	Zhu et al., 2011
		Laryngeal cancer	Chinese	Yu et al., 2015
CYP2A6	CYP2A6*4 and CYP2A6*5	Lung cancer	Japanese	Raunio et al., 2001
			Caucasian	
			—	
CYP2C9	rs1799853	Head and neck squamous cell carcinoma	Indian	Yadav et al., 2014
	rs1799853 and rs1057910	Non-small cell lung cancer	Caucasians	Sausville et al., 2018
CYP2C19	CYP2C19*2 (rs4244285)	Bladder cancer	German	Brockmoller et al., 1996
	CYP2C19*3	Lung cancer	Chinese	Yan et al., 2014
	—		Japanese	Tsuneoka et al., 1996
		Breast cancer	Chinese	Gan et al., 2011
		Digestive system cancer	East Asians	Zhou et al., 2013
	CYP2C19*17 (rs12248560)	Breast cancer	German	Justenhoven et al., 2009
CYP2D6	CYP2D6*1A	Bladder cancer	Egyptian	Abdel-Rahman et al., 1997
	CYP2D6*3 CYP2D6*4 CYP2D6*5 and CYP2D6*16	Lung cancer	African-Americans	London et al., 1997
	CYP2D6*4	Childhood acute leukemia	Italian	Ferri et al., 2018
		Malignant melanoma	British	Strange et al., 1999
	CYP2D6*3 and CYP2D6*4	Prostate cancer	Danish	Wadelius et al., 1999
	CYP2D6*10 (rs10655852)	Hepatocellular carcinoma	Chinese	Zhou et al., 2016
CYP3A4	CYP3A4*1B	Leukemia	Chinese	Zheng et al., 2018
	CYP3A4*1G (rs2242480)	Breast cancer	Chinese	Liu et al., 2019
	CYP3A4 A392G	Prostate cancer	Caucasians	He et al., 2014

(PDGF), and fibroblast growth factor (FGF). Guo et al. found CYP4/20-HETE pathway could influence the tumor volume. When implantation of glioma cells transfected with CYP4A1 into the rat, the tumor volume is a 10-fold increase compared with normal cells (Guo et al., 2008). In NSCLC cells, CYP4A/20-HETE increased the tumor growth rate and metastasis (Yu et al., 2011). The signaling mechanisms in CYP4/20-HETE induced cancer progression are mainly about the activation of MAPK pathway (Muthalif et al., 1998), PI_3K/Akt (Yu et al., 2011), and reactive oxygen species (ROS) (Guo et al., 2007), which was summarized as **Figure 2B**.

The Roles of Epoxyoctadecenoic Acids in Cancer

EpOMEs are the major epoxygenated fatty acids in human plasma produced from LA. EpOMEs , also called leukotoxins, have been shown to act as a responsible factor in circulatory shock, burn, pulmonary edema, and inflammation (Hanaki et al., 1991; Hayakawa et al., 1990; Kosaka et al., 1994; Ozawa et al., 1988; Totani et al., 2000). EpOMEs are pro-inflammatory in severe burn patients (Totani et al., 2000; Zheng et al., 2001). Recently, EpOMEs are found to have pro-cancer activity in a murine model of colorectal cancer (Wang et al., 2019). Evidence indicated that high LA diets increased the azoxymethane-induced colon tumorigenesis in rat models (Zheng et al., 2001; Wu et al., 2004; Fujise et al., 2007; Enos et al., 2016). Case-control studies showed opposing associations of serum n-3 and n-6 PUFAs with the risk of colorectal adenoma (Pot et al., 2008). Researchers found treatment with 12(13)-EpOME increased cytokine production and JNK phosphorylation *in vitro* and exacerbated azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced colon tumorigenesis *in vivo* (Wang et al., 2019). The underlying molecular mechanisms involved in the regulatory role of EpOMEs on cancer progression are not fully understood, partly because the specific receptors or direct cellular targets of EpOMEs are unknown. The effects of EpOMEs on cancers were summarized as **Figure 2C**. EpOMEs could be further metabolized to form DiHOMEs in the presence of sEH. Studies also showed DiHOMEs could induce chemotaxis, tissue injury, and cause mortality like EpOMEs in animal models (Moghaddam et al., 1997; Zheng et al., 2001).

The Roles of Epoxyeicosatetraenoic Acid in Cancer

Epidemiological studies revealed that dietary intake of EPA, the precursor of EEQs, decreases cancer risk (Bougnoux, 1999). Cui et al. have found that 17(18)-EEQ, but not 14(15)-, 11(12)-, 8(9)-, 5(6)-EEQ suppressed cell proliferation by down-regulating cyclin D_1 and activation of growth-suppressing p38 MAP kinase (Cui et al., 2011). Another research also revealed that a novel synthetic analog of 17(18)-EEQ activated TNF receptor-1/ASK1/JNK signaling to promote apoptosis in human breast cancer cells, manifesting the anticancer action of 17(18)-EEQ (Dyari et al., 2017). Up to now, the role of EEQ in cancer and its molecular mechanism has not been fully studied, which needs to be further studied in the future. The putative mechanism underlying EEQ in cancer was summarized as **Figure 2D**.

The Roles of Epoxydocosapentaenoic Acids in Cancer

EDPs are the metabolites of DHA mediated by CYP enzymes. They are suggested to be responsible for some of the beneficial effects of n-3 PUFAs and n-3 PUFA-rich diet (Arnold et al., 2010). Evidence has shown that the metabolites of n-3 PUFAs, mainly EDPs, mediate some effects in chronic disease conditions, such as hypertension, pain, and kidney diseases (Ulu et al., 2014; Deng et al., 2017; Hasegawa et al., 2017). More recently, some experimental studies showed that 16(17)- and 19(20)-EDPs are important mediators in suppressing inflammation and inhibiting angiogenesis, endothelial cell migration, endothelial cell proliferation, and tumor metastasis (Zhang et al., 2013a; Yanai et al., 2014; Hasegawa et al., 2017). EDPs are super unstable *in vivo* since they could be rapidly metabolized by sEH to form corresponding diols. Zhang et al. found 19,20-dihydroxydocosapentaenoic acid (19,20-DiHDPA), the metabolite of 19 (20)-EDP mediated by sEH, did not have any effect on tumor growth, indicating that the anticancer effect was from 19 (20)-EDP but not its diol metabolite (Zhang et al., 2013a). The putative mechanism of EDPs in cancer was summarized as **Figure 2E**.

CONCLUSION

Increasing studies supported the important role of CYP-derived eicosanoids in the progression of cancer and the resolution of inflammation. The action of EETs and EpOMEs has been investigated for decades while EEQs and EDPs are less-studied. Further studies are suggested to pay more attention to EEQs and EDPs since increasing the supplement of EPA and DHA with a focus on the biological activities of these eicosanoids and underlying mechanisms.

The additional complexity of the regulation of eicosanoids on inflammation, pain, angiogenesis, and cancer is the sEH enzyme, which can rapidly metabolize many CYPs-derived eicosanoids to corresponding fatty acid diols (Chacos et al., 1983; Zeldin et al., 1995; Zhang et al., 2014a). sEHs may modulate tumor angiogenesis by hydrolyzing pro-angiogenic EETs (Panigrahy et al., 2012). sEH also can hydrolyze anti-angiogenic epoxides of DHA (Zhang et al., 2013a). Co-inhibition of sEH resulted in a synergistic anti-inflammatory effect of the inhibitors of COXs and LOXs (Schmelzer et al., 2006; Liu et al., 2010; Hwang et al., 2011). Zhang et al. reported the anti-cancer effects of a dual inhibitor of sEH and COX (Zhang et al., 2014b). In short, the roles of CYPs/epoxides/sEH axis in cancer progression is substrate-dependent. Generally speaking, the CYP-mediated eicosanoids derived from ω-6 fatty acids (e.g. LA and AA) exacerbate cancer while the ones from ω-3 fatty acids (e.g.

EPA and DHA) are beneficial in the prevention and/or treatment of cancer. Manipulation of the CYPs/sEH pathway with or without supplement of ω-3 fatty acids to regulate target eicosanoids levels may be a promising strategy to prevent and/or treat cancers.

AUTHOR CONTRIBUTIONS

YL and JYL designed the paper frame; YL wrote the draft; JYL critically revised and finalized the paper. YL and JYL approved the final version.

REFERENCES

Abdel-Rahman, S. Z., Anwar, W. A., Abdel-Aal, W. E., Ghoneim, M. A., and Au, W. W. (1997). The CYP2D6 extensive metabolizer genotype is associated with increased risk for bladder cancer. *Cancer Lett.* 119, 115–122. doi:10.1016/s0304-3835(97)00265-6

Agudo, A., Sala, N., Pera, G., Capella, G., Berenguer, A., Garcia, N., et al. (2006). Polymorphisms in metabolic genes related to tobacco smoke and the risk of gastric cancer in the European prospective investigation into cancer and nutrition. *Cancer Epidemiol. Biomark. Prev.* 15, 2427–2434. doi:10.1158/1055-9965.epi-06-0072

Agundez, J. A. (2004) Cytochrome P450 gene polymorphism and cancer. *Curr. Drug Metabol.* 5, 211–224. doi:10.2174/1389200043335621

Agúndez, J. A. G., Leedesma, M. C., Benítez, J., Ladero, J. M., Rodríguez-Lescure, A., Díaz-Rubio, E., et al. (1995). CYP2D6 genes and risk of liver cancer. *Lancet* 345, 830–831. doi:10.1016/s0140-6736(95)92965-7

Ahmad, S. T., Arjumand, W., Seth, A., Nafees, S., Rashid, S., Ali, N., et al. (2013). Risk of renal cell carcinoma and polymorphism in phase I xenobiotic metabolizing CYP1A1 and CYP2D6 enzymes. *Urol. Oncol.: Semin. Orig. Invest.* 31, 1350–1357. doi:10.1016/j.urolonc.2011.12.009

Al-Eitan, L. N., Rababa'h, D. M., Alghamdi, M. A., and Khasawneh, R. H. (2019). Association of CYP gene polymorphisms with breast cancer risk and prognostic factors in the Jordanian population. *BMC Med. Genet.* 20, 148. doi:10.1186/s12881-019-0884-x

Alexanian, A. and Sorokin, A. (2013). Targeting 20-HETE producing enzymes in cancer—rationale, pharmacology, and clinical potential. *OncoTargets Ther.* 6, 243–255. doi:10.2147/OTT.S31586

Amrani, I., Bulatova, N., Awidi, A., Yousef, A.-M., Melhem, J. M., Al-Masri, M., et al. (2019). Lack of association between CYP1A1 M2 and M4 polymorphisms and breast carcinoma in Jordanian women: a case-control study. *Asian Pac. J. Cancer Prev. APJCP* 17, 387–393. doi:10.7314/apjcp.2016.17.1.387

Apaya, M. K., Shiau, J. Y., Liao, G. S., Liang, Y. J., Chen, C. W., Yang, H. C., et al. (2019). Integrated omics-based pathway analyses uncover CYP epoxygenase-associated networks as theranostic targets for metastatic triple negative breast cancer. *J. Exp. Clin. Canc. Res.* 38, 187. doi:10.1186/s13046-019-1187-y

Arnold, C., Markovic, M., Blossey, K., Wallukat, G., Fischer, R., Dechend, R., et al. (2010). Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of ω-3 fatty acids. *J. Biol. Chem.* 285, 32720–32733. doi:10.1074/jbc.m110.118406

Bai, L., He, J., He, G.-H., He, J.-C., Xu, F., and Xu, G.-L. (2014). Association of CYP2C19 polymorphisms with survival of breast cancer patients using tamoxifen: results of a meta-analysis. *Asian Pac. J. Cancer Prev. APJCP* 15, 8331–8335. doi:10.7314/apjcp.2014.15.19.8331

Bougnoux, P. (1999). n-3 polyunsaturated fatty acids and cancer. *Curr. Opin. Clin. Nutr. Metab. Care* 2, 121–126. doi:10.1097/00075197-199903000-00005

Bozina, N., Bradamante, V., and Lovrić, M. (2009). Genetic polymorphism of metabolic enzymes P450 (CYP) as a susceptibility factor for drug response, toxicity, and cancer risk. *Arh. Hig. Rada. Toksikol.* 60, 217–242. doi:10.2478/10004-1254-60-2009-1885

Brockmöller, J., Cascorbi, I., Kerb, R., and Roots, I. (1996). Combined analysis of inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferases M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. *Cancer Res* 56, 3915–3925

Bu, Z.-B., Ye, M., Cheng, Y., and Wu, W.-Z. (2014). Four polymorphisms in the cytochrome P450 1A2 (CYP1A2) gene and lung cancer risk: a meta-analysis. *Asian Pac. J. Cancer Prev. APJCP* 15, 5673–5679. doi:10.7314/apjcp.2014.15.14.5673

Capdevila, J., Chacos, N., Werringloer, J., Prough, R. A., and Estabrook, R. W. (1981a). Liver microsomal cytochrome P-450 and the oxidative metabolism of arachidonic acid. *Proc. Natl. Acad. Sci. U.S.A.* 78, 5362–5366. doi:10.1073/pnas.78.9.5362

Capdevila, J. H., Wang, W., and Falck, J. R. (2015). Arachidonic acid monooxygenase: genetic and biochemical approaches to physiological/pathophysiological relevance. *Prostag. Other Lipid Mediat.* 120, 40–49. doi:10.1016/j.prostaglandins.2015.05.004

Capdevila, J., Parkhill, L., Chacos, N., Okita, R., Masters, B. S. S., and Estabrook, R. W. (1981b). The oxidative metabolism of arachidonic acid by purified cytochromes P-450. *Biochem. Biophys. Res. Commun.* 101, 1357–1363. doi:10.1016/0006-291x(81)91597-7

Capdevila, J., and Wang, W. (2013). Role of cytochrome P450 epoxygenase in regulating renal membrane transport and hypertension. *Curr. Opin. Nephrol. Hypertens.* 22, 163–169. doi:10.1097/mnh.0b013e32835d911e

Cathcart, M.-C., O'Byrne, K. J., Reynolds, J. V., O'Sullivan, J., and Pidgeon, G. P. (2012). COX-derived prostanoid pathways in gastrointestinal cancer development and progression: novel targets for prevention and intervention. *Biochim. Biophys. Acta Rev. Cancer* 1825, 49–63. doi:10.1016/j.bbcan.2011.09.004

Chacos, N., Capdevila, J., Falck, J. R., Manna, S., Martin-Wixtrom, C., Gill, S. S., et al. (1983). The reaction of arachidonic acid epoxides (epoxyeicosatrienoic acids) with a cytosolic epoxide hydrolase. *Arch. Biochem. Biophys.* 223, 639–648. doi:10.1016/0003-9861(83)90628-8

Chacos, N., Falck, J. R., Wixtrom, C., and Capdevila, J. (1982). Novel epoxides formed during the liver cytochrome P-450 oxidation of arachidonic acid. *Biochem. Biophys. Res. Commun.* 104, 916–922. doi:10.1016/0006-291x(82)91336-5

Chang, G. W. M., and Kam, P. C. A. (1999). The physiological and pharmacological roles of cytochrome P450 isoenzymes. *Anaesthesia* 54, 42–50. doi:10.1046/j.1365-2044.1999.00602.x

Cheranov, S. Y., Karpurapu, M., Wang, D., Zhang, B., Venema, R. C., and Rao, G. N. (2012). An essential role for SRC-activated STAT-3 in 14,15-EET-induced VEGF expression and angiogenesis. *Blood* 111, 5581–5591. doi:10.1182/blood-2007-11-126680

Chuang, S. S., Helvig, C., Taimi, M., Ramshaw, H. A., Collop, A. H., Amad, M. A., et al. (2012). CYP2U1, a novel human thymus- and brain-specific cytochrome P450, catalyzes ω- and (ω-1)-Hydroxylation of fatty acids. *J. Biol. Chem.* 279, 6305–6314. doi:10.1074/jbc.m311830200

Chun, Y.-J., and Kim, D. (2016). Cancer activation and polymorphisms of human cytochrome P450 1B1. *Toxicol. Res.* 32, 89–93. doi:10.5487/tr.2016.32.2.089

Cui, P. H., Petrovic, N., and Murray, M. (2011). The ω-3 epoxide of eicosapentaenoic acid inhibits endothelial cell proliferation by p38 MAP kinase activation and cyclin D1/CDK4 down-regulation. *Br. J. Pharmacol.* 162, 1143–1155. doi:10.1111/j.1476-5381.2010.01113.x

DeLozier, T. C., Kissling, G. E., Coulter, S. J., Dai, D., Foley, J. F., Bradbury, J. A., et al. (2007). Detection of human CYP2C8, CYP2C9, and CYP2J2 in cardiovascular tissues. *Drug Metab. Dispos.* 35, 682–688. doi:10.1124/dmd.106.012823

Deng, B.-Q., Luo, Y., Kang, X., Li, C.-B., Morisseau, C., Yang, J., et al. (2017). Epoxide metabolites of arachidonate and docosahexaenoate function conversely in acute kidney injury involved in GSK3β signaling. *Proc. Natl. Acad. Sci. U.S.A.* 114, 12608–12613. doi:10.1073/pnas.1705615114

Dennis, E. A. and Norris, P. C. (2015). Eicosanoid storm in infection and inflammation. *Nat. Rev. Immunol.* 15, 511–523. doi:10.1038/nri3859

Deremer, D. L., Ustun, C., and Natarajan, K. (2008). Nilotinib: a second-generation tyrosine kinase inhibitor for the treatment of chronic myelogenous leukemia. *Clin. Therapeut.* 30, 1956–1975. doi:10.1016/j.clinthera.2008.11.014

Di, Y., Chow, V., Yang, L.-P., and Zhou, S.-F. (2012). Structure, function, regulation and polymorphism of human cytochrome P450 2A6. *Curr. Drug Metabol.* 10, 754–780. doi:10.2174/138920009789895507.

Ding, B., Sun, W., Han, S., Cai, Y., Ren, M., and Shen, Y. (2018). Cytochrome P450 1A1 gene polymorphisms and cervical cancer risk: a systematic review and meta-analysis. *Medicine (Baltimore)* 97, e0210. doi:10.1097/MD.0000000000010210

Dolzan, V., Rudolf, Z., and Breskvar, K. (1995). Human CYP2D6 gene polymorphism in Slovene cancer patients and healthy controls. *Carcinogenesis* 16, 2675–2678. doi:10.1093/carcin/16.11.2675

Dyari, H. R. E., Rawling, T., Chen, Y., Sudarmana, W., Bourget, K., Dwyer, J. M., et al. (2017). A novel synthetic analogue of ω-3 17,18-epoxyeicosatetraenoic acid activates TNF receptor-1/ASK1/JNK signaling to promote apoptosis in human breast cancer cells. *Faseb. J.* 31, 5246–5257. doi:10.1096/fj.201700033r

Enos, R. T., Velázquez, K. T., McClellan, J. L., Cranford, T. L., Nagarkatti, M., and Nagarkatti, P. S. (2016). High-fat diets rich in saturated fat protect against azoxymethane/dextran sulfate sodium-induced colon cancer. *Am. J. Physiol. Gastrointest. Liver Physiol.* 310, G906–G919. doi:10.1152/ajpgi.00345.2015

Ezzeldin, N., El-Lebedy, D., Darwish, A., El-Bastawisy, A., Hassan, M., Abd El-Aziz, S., et al. (2017). Genetic polymorphisms of human cytochrome P450 CYP1A1 in an Egyptian population and tobacco-induced lung cancer. *Gene Environ.* 39, 7. doi:10.1186/s41021-016-0066-4

Fernandez-Salguero, P., Hoffman, S. M., Cholerton, S., Mohrenweiser, H., Raunio, H., Rautio, A., et al. (1995). A genetic polymorphism in coumarin 7-hydroxylation: sequence of the human CYP2A genes and identification of variant CYP2A6 alleles. *Am. J. Hum. Genet.* 57, 651–660. Pubmed

Ferri, G. M., Guastadisegno, C. M., Intranuovo, G., Cavone, D., Birtolo, F., and Cecinati, V. (2018). Maternal exposure to pesticides, paternal occupation in the army/police force, and CYP2D6*4 polymorphism in the etiology of childhood acute leukemia. *J. Pediatr. Hematol. Oncol.* 40, e207–e214. doi:10.1097/mph.0000000000001105

Fleming, I. (2007). Epoxyeicosatrienoic acids, cell signaling and angiogenesis. *Prostag. Other Lipid Mediat.* 82, 60–67. doi:10.1016/j.prostaglandins.2006.05.003

Fleming, I. (2011). The cytochrome P450 pathway in angiogenesis and endothelial cell biology. *Canc. Metastasis Rev.* 30, 541–555. doi:10.1007/s10555-011-9302-3

Fleming, I. (2016). The factor in EDHF: cytochrome P450 derived lipid mediators and vascular signaling. *Vasc. Pharmacol.* 86: 31–40. doi:10.1016/j.vph.2016.03.001

Fleming, I. (2008). Vascular cytochrome p450 enzymes: physiology and pathophysiology. *Trends Cardiovasc. Med.* 18, 20–25. doi:10.1016/j.tcm.2007.11.002

Fujise, T., Iwakiri, R., Kakimoto, T., Shiraishi, R., Sakata, Y., Wu, B., et al. (2007). Long-term feeding of various fat diets modulates azoxymethane-induced colon carcinogenesis through Wnt/β-catenin signaling in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292, G1150–G1156. doi:10.1152/ajpgi.00269.2006

Gajecka, M., Rydzanicz, M., Jaskula-Sztul, R., Kujawski, M., Szyfter, W., and Szyfter, K. (2005). CYP1A1, CYP2D6, CYP2E1, NAT2, GSTM1 and GSTT1 polymorphisms or their combinations are associated with the increased risk of the laryngeal squamous cell carcinoma. *Mutat. Res. Fund Mol. Mech. Mutagen* 574, 112–123. doi:10.1016/j.mrfmmm.2005.01.027

Gallegos-Arreola, M. P., Figuera-Villanueva, L. E., Troyo-Sanroman, R., Morgn-Villela, G., Puebla-Prez, A. M., Flores-Marquez, M. R., et al. (2008). CYP1A1 *2B and *4 polymorphisms are associated with lung cancer susceptibility in Mexican patients. *Int. J. Biol. Markers* 23, 24–30. doi:10.1177/172460080802300104

Gan, C. Q., Wang, X. Y., Cao, Y. D., Ye, W. X., Liu, H., and Sun, Y. Y. (2011). Association of CYP2C19*3 gene polymorphism with breast cancer in Chinese women. *Genet. Mol. Res.* 10, 3514–3519. doi:10.4238/2011.december.5.3

García-Martín, E., Martínez, C., Ladero, J. M., Gamito, F. J., Rodriguez-Lescure, A., and Agúndez, J. A. (2002). Influence of cytochrome P450 CYP2C9 genotypes in lung cancer risk. *Canc. Lett.* 180, 41–46. doi:10.1016/s0304-3835(02)00015-0

Guengerich, F. P. (2017). Intersection of the roles of cytochrome P450 enzymes with xenobiotic and endogenous substrates: relevance to toxicity and drug interactions. *Chem. Res. Toxicol.* 30, 2–12. doi:10.1021/acs.chemrestox.6b00226.

Guengerich, F. P. (2007). Mechanisms of cytochrome P450 substrate oxidation: MiniReview. *J. Biochem. Mol. Toxicol.* 21, 163–168. doi:10.1002/jbt.20174

Guo, A. M., Arbab, A. S., Falck, J. R., Chen, P., Edwards, P. A., Roman, R. J., and Scicli, A. G. (2007). Activation of vascular endothelial growth factor through reactive oxygen species mediates 20-hydroxyeicosatetraenoic acid-induced endothelial cell proliferation. *J. Pharmacol. Exp. Therapeut.* 321, 18–27. doi:10.1124/jpet.106.115360

Guo, A. M., Sheng, J., Scicli, G. M., Arbab, A. S., Lehman, N. L., Edwards, P. A., et al. (2017). Expression of CYP4A1 in U251 human glioma cell induces hyperproliferative phenotype *in vitro* and rapidly growing tumors *in vivo*. *J. Pharmacol. Exp. Therapeut.* 327, 10–19. doi:10.1124/jpet.108.140889

Guo, Z., Sevrioukova, I. F., Denisov, I. G., Zhang, X., Chiu, T.-L., Thomas, D. G., et al. (2017). Heme binding biguanides target cytochrome P450-dependent cancer cell mitochondria. *Cell Chem. Biol* 24, 1314. doi:10.1016/j.chembiol.2017.09.012

Gutman, G., Morad, T., Peleg, B., Peretz, C., Bar-Am, A., Safra, T., and Grisaru, D. (2009). CYP1A1 and CYP2D6 gene polymorphisms in Israeli Jewish women with cervical cancer. *Int. J. Gynecol. Cancer* 19, 1300–1302. doi:10.1111/igc.0b013e3181b9fa5d

Hanaki, Y., Kamiya, H., Ohno, M., Hayakawa, M., Sugiyama, S., and Ozawa, T. (1991). Leukotoxin, 9,10-epoxy-12-octadecenoate: a possible responsible factor in circulatory shock and disseminated intravascular coagulation. *Jpn. J. Med.* 30, 224–228. doi:10.2169/internalmedicine1962.30.224

Hasegawa, E., Inafuku, S., Mulki, L., Okunuki, Y., Yanai, R., Smith, K. E., et al. (2017). Cytochrome P450 monooxygenase lipid metabolites are significant second messengers in the resolution of choroidal neovascularization. *Proc. Natl. Acad. Sci. U.S.A.* 114, E7545–E7553. doi:10.1073/pnas.1620898114

Hayakawa, M., Kosaka, K., Sugiyama, S., Yokoo, K., Aoyama, H., Izawa, Y., and Ozawa, T. (1990). Proposal of leukotoxin, 9,10-epoxy-12-octadecenoate, as a burn toxin. *Biochem. Int.* 21, 573–579

He, X.-F., Liu, Z.-Z., Xie, J.-J., Wang, W., Du, Y.-P., Chen, Y., and Wei, W. (2014). Association between the CYP3A4 and CYP3A5 polymorphisms and cancer risk: a meta-analysis and meta-regression. *Tumor Biol.* 35, 9859–9877. doi:10.1007/s13277-014-2241-1

Hirata, H., Hinoda, Y., Okayama, N., Suehiro, Y., Kawamoto, K., Kikuno, N., et al. (2008). CYP1A1, SULT1A1, andSULT1E1 polymorphisms are risk factors for endometrial cancer susceptibility. *Cancer* 112, 1964–1973. doi:10.1002/cncr.23392

Hukkanen, J., Jacob, P., 3rd, and Benowitz, N. L. (2005). Metabolism and disposition kinetics of nicotine. *Pharmacol. Rev.* 57, 79–115. doi:10.1124/pr.57.1.3

Hwang, S. H., Wagner, K. M., Morisseau, C., Liu, J.-Y., Dong, H., Wecksler, A. T., et al. (2011). Synthesis and Structure–Activity relationship studies of urea-containing pyrazoles as dual inhibitors of cyclooxygenase-2 and soluble epoxide hydrolase. *J. Med. Chem.* 54, 3037–3050. doi:10.1021/jm2001376

Imig, J. D. (2005). Epoxide hydrolase and epoxygenase metabolites as therapeutic targets for renal diseases. *Am. J. Physiol. Ren. Physiol.* 289, F496–F503. doi:10.1152/ajprenal.00350.2004

Ingelman-Sundberg, M. (2005). Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogen. J.* 5, 6–13. doi:10.1038/sj.tpj.6500285

Irusta, G., Murphy, M. J., Perez, W. D., and Hennebold, J. D. (2007). Dynamic expression of epoxyeicosatrienoic acid synthesizing and metabolizing enzymes in the primate corpus luteum. *Mol. Hum. Reprod.* 13, 541–548. doi:10.1093/molehr/gam044

Jain, V., Ratre, Y. K., Amle, D., Mishra, P. K., and Patra, P. K. (2017). Polymorphism of CYP1A1 gene variants rs4646903 and rs1048943 relation to the incidence of cervical cancer in Chhattisgarh. *Environ. Toxicol. Pharmacol.* 52, 188–192. doi:10.1016/j.etap.2017.04.009

Jamieson, K. L., Endo, T., Darwesh, A. M., Samokhvalov, V., and Seubert, J. M. (2017). Cytochrome P450-derived eicosanoids and heart function. *Pharmacol. Therap.* 179, 47–83. doi:10.1016/j.pharmthera.2017.05.005

Jiang, J.-G., Chen, C.-L., Card, J. W., Yang, S., Chen, J.-X., Fu, X.-N., et al. (2005). Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. *Cancer Res.* 65, 4707–4715. doi:10.1158/0008-5472.can-04-4173

Jiang, J.-G., Ning, Y.-G., Chen, C., Ma, D., Liu, Z.-J., Yang, S., et al. (2007). CytochromeP450 epoxygenase promotes human cancer metastasis. *Cancer Res.* 67, 6665–6674. doi:10.1158/0008-5472.can-06-3643

Juárez-Cedillo, T., Vallejo, M., Fragoso, J. M., Hernández-Hernández, D. M., Rodríguez-Pérez, J. M., Sánchez-García, S., et al. (2007). The risk of

developing cervical cancer in Mexican women is associated to CYP1A1 MspI polymorphism. *Eur. J. Cancer* 43, 1590–1595. doi:10.1016/j.ejca.2007.03.025

Justenhoven, C., Hamann, U., Pierl, C. B., Baisch, C., Harth, V., Rabstein, S., et al. (2009). CYP2C19*17 is associated with decreased breast cancer risk. *Breast Canc. Res. Treat.* 115, 391–396. doi:10.1007/s10549-008-0076-4

Khanapure, S., Garvey, D., Janero, D., and Gordon Letts, L. (2007). Eicosanoids in inflammation: biosynthesis, pharmacology, and therapeutic frontiers. *Curr. Top. Med. Chem.* 7, 311–340. doi:10.2174/156802607779941314

Knab, L. M., Grippo, P. J., and Bentrem, D. J. (2014). Involvement of eicosanoids in the pathogenesis of pancreatic cancer: the roles of cyclooxygenase-2 and 5-lipoxygenase. *World J. Gastroenterol.* 20, 10729–10739. doi:10.3748/wjg.v20.i31.10729

Koonrungsesomboon, N., Khatsri, R., Wongchompoo, P., and Teekachunhatean, S. (2018). The impact of genetic polymorphisms on CYP1A2 activity in humans: a systematic review and meta-analysis. *Pharmacogen. J.* 18, 760–768. doi:10.1038/s41397-017-0011-3

Kosaka, K., Suzuki, K., Hayakawa, M., Sugiyama, S., and Ozawa, T. (1994). Leukotoxin, a linoleate epoxide: its implication in the late death of patients with extensive burns. *Mol. Cell. Biochem.* 139, 141–148. doi:10.1007/bf01081737

Lee, S. J. (2012). Clinical application of CYP2C19 pharmacogenetics toward more personalized medicine. *Front. Genet.* 3, 318. doi:10.3389/fgene.2012.00318

Li, S., Li, G., Kong, F., Liu, Z., Li, N., Li, Y., and Guo, X. (2016). The association of CYP1A1 gene with cervical cancer and additional SNP-SNP interaction in Chinese women. *J. Clin. Lab. Anal.* 30, 1220–1225. doi:10.1002/jcla.22006

Li, Y., Millikan, R. C., Bell, D. A., Cui, L., Tse, C.-K. J., Newman, B., et al. (2004). Cigarette smoking, cytochrome P4501A1 polymorphisms, and breast cancer among African–American and white women. *Breast Cancer Res.* 6, R460–R473. doi:10.1186/bcr814

Little, J., Sharp, L., Masson, L. F., Brockton, N. T., Cotton, S. C., Haites, N. E., et al. (2006). Colorectal cancer and genetic polymorphisms ofCYP1A1,GSTM1andGSTT1: a case-control study in the Grampian region of Scotland. *Int. J. Canc.* 119, 2155–2164. doi:10.1002/ijc.22093

Liu, J.-Y., Yang, J., Inceoglu, B., Qiu, H., Ulu, A., Hwang, S.-H., Chiamvimonvat, N., and Hammock, B. D. (2010). Inhibition of soluble epoxide hydrolase enhances the anti-inflammatory effects of aspirin and 5-lipoxygenase activation protein inhibitor in a murine model. *Biochem. Pharmacol.* 79, 880–887. doi:10.1016/j.bcp.2009.10.025

Liu, X., Huang, X., Zhang, S., Niu, F., Ouyang, Y., Shou, Z., and Liu, J. (2019). Correlations between CYP3A4 polymorphism and susceptibility to breast cancer in Chinese Han population. *Int. J. Clin. Oncol.* 24, 179–188. doi:10.1007/s10147-018-1346-8

Liu, X., Qian, Z.-Y., Xie, F., Fan, W., Nelson, J. W., Xiao, X., et al. (2017). Functional screening for G protein-coupled receptor targets of 14,15-epoxyeicosatrienoic acid. *Prostag. Other Lipid Mediat.* 132, 31–40. doi:10.1016/j.prostaglandins.2016.09.002

London, S., Daly, A. K., Leathart, J. B., Navidi, W. C., Carpenter, C. C., and Idle, J. R. (1997). Genetic polymorphism of CYP2D6 and lung cancer risk in african-Americans and Caucasians in Los Angeles county. *Carcinogenesis* 18, 1203–1214. doi:10.1093/carcin/18.6.1203

Luo, J., Yao, J. F., Deng, X. F., Zheng, X. D., Jia, M., Wang, Y. Q., et al. (2018). 14, 15-EET induces breast cancer cell EMT and cisplatin resistance by up-regulating integrin alphavbeta3 and activating FAK/PI3K/AKT signaling. *J. Exp. Clin. Canc. Res.* 37, 23. doi:10.1186/s13046-018-0694-6

Mitra, R., Guo, Z., Milani, M., Mesaros, C., Rodriguez, M., Nguyen, J., et al. (2011). CYP3A4 mediates growth of estrogen receptor-positive breast cancer cells in part by inducing nuclear translocation of phospho-stat3 through biosynthesis of (±)-14,15-Epoxyeicosatrienoic acid (EET). *J. Biol. Chem.* 286, 17543–17559. doi:10.1074/jbc.m110.198515

Mittal, B., Tulsyan, S., Kumar, S., Mittal, R. D., and Agarwal, G. (2015). Cytochrome P450 in cancer susceptibility and treatment. *Adv. Clin. Chem.* 71, 77–139. doi:10.1016/bs.acc.2015.06.003

Moghaddam, M. F., Grant, D. F., Cheek, J. M., Greene, J. F., Williamson, K. C., and Hammock, B. D. (1997). Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat. Med.* 3, 562–566. doi:10.1038/nm0597-562

Muthalif, M. M., Benter, I. F., Karzoun, N., Fatima, S., Harper, J., Uddin, M. R., et al. (1998). 20-Hydroxyeicosatetraenoic acid mediates calcium/calmodulin-dependent protein kinase II-induced mitogen-activated protein kinase activation in vascular smooth muscle cells. *Proc. Natl. Acad. Sci. U.S.A.* 95, 12701–12706. doi:10.1073/pnas.95.21.12701

Nakagawa, K., Holla, V. R., Wei, Y., Wang, W. H., Gatica, A., Wei, S., et al. (2006). Salt-sensitive hypertension is associated with dysfunctional Cyp4a10 gene and kidney epithelial sodium channel. *J. Clin. Invest.* 116, 1696–1702. doi:10.1172/jci27546

Nelson, D. R., Zeldin, D. C., Hoffman, S. M., Maltais, L. J., Wain, H. M., and Nebert, D. W. (2004). Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics* 14, 1–18. doi:10.1097/00008571-200401000-00001

Neve, E. P., and Ingelman-Sundberg, M. (2010). Cytochrome P450 proteins: retention and distribution from the endoplasmic reticulum. *Curr. Opin. Drug Discov. Dev* 13, 78–85. doi:10.1016/j.cct.2009.10.001

Node, K., Huo, Y., Ruan, X., Yang, B., Spiecker, M., Ley, K., et al. (1999). Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 285, 1276–1279. doi:10.1126/science.285.5431.1276

Ortiz de Montellano, P. R. (2010). Hydrocarbon hydroxylation by cytochrome P450 enzymes. *Chem. Rev.* 110, 932–948. doi:10.1021/cr9002193

Oscarson, M., McLellan, R. A., Gullstén, H., Agúndez, J. A. G., Benítez, J., Rautio, A., et al. (1999). Identification and characterisation of novel polymorphisms in the CYP2A locus: implications for nicotine metabolism. *FEBS Lett.* 460, 321–327. doi:10.1016/s0014-5793(99)01364-2

Ouerhani, S., Marrakchi, R., Bouhaha, R., Ben Slama, M. R., Sfaxi, M., Ayed, M., et al. (2008). The role of CYP2D6*4 variant in bladder cancer susceptibility in Tunisian patients. *Bull. Cancer* 95, E1–E4. doi:10.1684/bdc.2008.0583

Ozawa, T., Nishikimi, M., Sugiyama, S., Taki, F., Hayakawa, M., and Shionoya, H. (1988). Cytotoxic activity of leukotoxin, a neutrophil-derived fatty acid epoxide, on cultured human cells. *Biochem. Int.* 16, 369–373

Panigrahy, D., Edin, M. L., Lee, C. R., Huang, S., Bielenberg, D. R., Butterfield, C. E., et al. (2012). Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. *J. Clin. Invest.* 122, 178–191. doi:10.1172/jci58128

Panigrahy, D., Greene, E. R., Pozzi, A., Wang, D. W., and Zeldin, D. C. (2011). EET signaling in cancer. *Canc. Metast. Rev.* 30, 525–540. doi:10.1007/s10555-011-9315-y

Panigrahy, D., Kaipainen, A., Greene, E. R., and Huang, S. (2010). Cytochrome P450-derived eicosanoids: the neglected pathway in cancer. *Canc. Metast. Rev.* 29, 723–735. doi:10.1007/s10555-010-9264-x

Pikuleva, I. A. and Waterman, M. R. (2013). Cytochromes p450: roles in diseases. *J. Biol. Chem.* 288, 17091–17098. doi:10.1074/jbc.r112.431916

Porter, T. D. and Coon, M. J. (1991). Cytochrome P-450. Multiplicity of isoforms, substrates, and catalytic and regulatory mechanisms. *J. Biol. Chem.* 266, 13469–13472

Pot, G. K., Geelen, A., van Heijningen, E.-M. B., Siezen, C. L. E., van Kranen, H. J., and Kampman, E. (2008). Opposing associations of serum n-3 and n-6 polyunsaturated fatty acids with colorectal adenoma risk: an endoscopy-based case-control study. *Int. J. Cancer* 123, 1974–1977. doi:10.1002/ijc.23729

Pozzi, A., and Capdevila, J. H. (2008). PPARalpha ligands as antitumorigenic and antiangiogenic agents. *PPAR Res.* 2008, 906542. doi:10.1155/2008/906542

Pozzi, A., Ibanez, M. R., Gatica, A. E., Yang, S., Wei, S., Mei, S., et al. (2007). Peroxisomal proliferator-activated receptor-α-dependent inhibition of endothelial cell proliferation and tumorigenesis. *J. Biol. Chem.* 282, 17685–17695. doi:10.1074/jbc.m701429200

Pozzi, A., Popescu, V., Yang, S., Mei, S., Shi, M., Puolitaival, S. M., et al. (2010). The anti-tumorigenic properties of peroxisomal proliferator-activated receptor α are arachidonic acid epoxygenase-mediated. *J. Biol. Chem.* 285, 12840–12850. doi:10.1074/jbc.m109.081554

Raunio, H., Rautio, A., Gullstén, H., and Pelkonen, O. (2001). Polymorphisms of CYP2A6 and its practical consequences. *Br. J. Clin. Pharmacol.* 52, 357–363. doi:10.1046/j.0306-5251.2001.01500.x

Ren, J., Chen, G. G., Liu, Y., Su, X., Hu, B., Leung, B. C. S., et al. (2016). Cytochrome P450 1A2 metabolizes 17β-estradiol to suppress hepatocellular carcinoma. *PLoS ONE* 11, e0153863. doi:10.1371/journal.pone.0153863

Renaud, H. J., Cui, J. Y., Khan, M., and Klaassen, C. D. (2011). Tissue distribution and gender-divergent expression of 78 cytochrome P450 mRNAs in mice. *Toxicol. Sci.* 124, 261–277. doi:10.1093/toxsci/kfr240

Reynald, R. L., Sansen, S., Stout, C. D., and Johnson, E. F. (2012). Structural characterization of human cytochrome P450 2C19. *J. Biol. Chem.* 287, 44581–44591. doi:10.1074/jbc.m112.424895

Rochat, B., Morsman, J. M., Murray, G. I., Figg, W. D., and McLeod, H. L. (2001). Human CYP1B1 and anticancer agent metabolism: mechanism for tumor-specific drug inactivation? *J. Pharmacol. Exp. Therapeut.* 296, 537–541.

Rostami-Hodjegan, A., Lennard, M. S., Woods, H. F., and Tucker, G. T. (1998). Meta-analysis of studies of the CYP2D6 polymorphism in relation to lung cancer and Parkinson's disease. *Pharmacogenetics* 8, 227–238. doi:10.1097/00008571-199806000-00005

Roszak, A., Lianeri, M., Sowińska, A., and Jagodziński, P. P (2014). CYP1A1 Ile462Val polymorphism as a risk factor in cervical cancer development in the Polish population. *Mol. Diagn. Ther.* 18, 445–450. doi:10.1007/s40291-014-0095-2

Sachse, C., Brockmöller, J., Bauer, S., and Roots, I. (1999). Functional significance of a C→A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. *Br. J. Clin. Pharmacol.* 47, 445–449. doi:10.1046/j.1365-2125.1999.00898.x

Sausville, L. N., Gangadhariah, M. H., Chiusa, M., Mei, S., Wei, S., Zent, R., et al. (2018). The cytochrome P450 slow metabolizers CYP2C9*2 and CYP2C9*3 directly regulate tumorigenesis via reduced epoxyeicosatrienoic acid production. *Cancer Res.* 78, 4865–4877. doi:10.1158/0008-5472.can-17-3977

Sausville, L. N., Williams, S. M., and Pozzi, A. (2019). Cytochrome P450 epoxygenases and cancer: a genetic and a molecular perspective. *Pharmacol. Ther.* 196, 183–194. doi:10.1016/j.pharmthera.2018.11.009

Schmelzer, K. R., Inceoglu, B., Kubala, L., Kim, I.-H., Jinks, S. L., Eiserich, J. P., et al. (2006). Enhancement of antinociception by coadministration of nonsteroidal anti-inflammatory drugs and soluble epoxide hydrolase inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13646–13651. doi:10.1073/pnas.0605908103

Seow, A., Zhao, B., Lee, E. J. D., Poh, W.-T., Teh, M., Eng, P., et al. (2001). Cytochrome P4501A2 (CYP1A2) activity and lung cancer risk: a preliminary study among Chinese women in Singapore. *Carcinogenesis* 22, 673–677. doi:10.1093/carcin/22.4.673

Serhan, C. N., Chiang, N., and Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.* 8, 349–361. doi:10.1038/nri2294

Seubert, J. M., Zeldin, D. C., Nithipatikom, K., and Gross, G. J. (2007). Role of epoxyeicosatrienoic acids in protecting the myocardium following ischemia/reperfusion injury. *Prostag. Other Lipid Mediat.* 82, 50–59. doi:10.1016/j.prostaglandins.2006.05.017

Simpson, A. E. (1997). The cytochrome P450 4 (CYP4) family. *Gen. Pharmacol.* 28, 351–359. doi:10.1016/S0306-3623(96)00246-7

Singh, V., Rastogi, N., Sinha, A., Kumar, A., Mathur, N., and Singh, M. P. (2007). A study on the association of cytochrome-P450 1A1 polymorphism and breast cancer risk in north Indian women. *Breast Canc. Res. Treat.* 101, 73–81. doi:10.1007/s10549-006-9264-2

Siraj, A. K., Ibrahim, M., Al-Rasheed, M., Abubaker, J., Bu, R., Siddiqui, S. U., et al. (2008). Polymorphisms of selected xenobiotic genes contribute to the development of papillary thyroid cancer susceptibility in Middle Eastern population. *BMC Med. Genet.* 9, 61. doi:10.1186/1471-2350-9-61

Sistonen, J., Fuselli, S., Palo, J. U., Chauhan, N., Padh, H., and Sajantila, A. (2009). Pharmacogenetic variation at CYP2C9, CYP2C19, and CYP2D6 at global and microgeographic scales. *Pharmacogen. Genom.* 19, 170–179. doi:10.1097/fpc.0b013e32831ebb30

Snyder, G. D., Yadagiri, P., and Falck, J. R. (1989). Effect of epoxyeicosatrienoic acids on growth hormone release from somatotrophs. *Am. J. Physiol.-Endocrinol. Metabol.* 256, E221–E226. doi:10.1152/ajpendo.1989.256.2.e221

Sobti, R. C., Onsory, K., Al-Badran, A. I., Kaur, P., Watanabe, M., Krishan, A., et al. (2006). CYP17, SRD5A2, CYP1B1, and CYP2D6 gene polymorphisms with prostate cancer risk in North Indian population. *DNA Cell Biol.* 25, 287–294. doi:10.1089/dna.2006.25.287

Sokolowska, M., Rovati, G. E., Diamant, Z., Untersmayr, E., Schwarze, J., Lukasik, Z., et al. (2020). Current perspective on eicosanoids in asthma and allergic diseases—EAACI Task Force consensus report, part I. *Allergy* doi:10.1111/all.14295

Stark, K., Dostalek, M., and Guengerich, F. P. (2008). Expression and purification of orphan cytochrome P450 4X1 and oxidation of anandamide. *FEBS J.* 275, 3706–3717. doi:10.1111/j.1742-4658.2008.06518.x

Strange, R. C., Ellison, T., Ichii-Jones, F., Bath, J., Hoban, P., Lear, J. T., et al. (1999). Cytochrome P450 CYP2D6 genotypes: association with hair colour, Breslow thickness and melanocyte stimulating hormone receptor alleles in patients with malignant melanoma. *Pharmacogenetics* 9, 269–276. doi:10.1097/00008571-199906000-00001

Sugawara, T., Nomura, E., Sagawa, T., Sakuragi, N., and Fujimoto, S. (2003). CYP1A1 polymorphism and risk of gynecological malignancy in Japan. *Int. J. Gynecol. Cancer* 13, 785–790. doi:10.1136/ijgc-00009577-200311000-00009

Tan, Y. H., Sidik, S. M., Husain, S. N. A. S., Lye, M. S., and Chong, P. P. (2016). CYP1A1 MspI polymorphism and cervical carcinoma risk in the multi-ethnic population of Malaysia: a case-control study. *Asian Pac. J. Cancer Prev. APJCP* 17, 57–64. doi:10.7314/apjcp.2016.17.1.57

Thelen, K., and Dressman, J. B. (2009). Cytochrome P450-mediated metabolism in the human gut wall. *J. Pharm. Pharmacol.* 61, 541–558. doi:10.1211/jpp.61.05.0002

Totani, Y., Saito, Y., Ishizaki, T., Sasaki, F., Ameshima, S., and Miyamori, I. (2000). Leukotoxin and its diol induce neutrophil chemotaxis through signal transduction different from that of fMLP. *Eur. Respir. J.* 15, 75–79. doi:10.1183/09031936.00.15107500

Tsuneoka, Y., Fukushima, K., Matsuo, Y., Ichikawa, Y., and Watanabe, Y. (1996). Genotype analysis of the CYP2C19 gene in the Japanese population. *Life Sci.* 59, 1711–1715. doi:10.1016/s0024-3205(96)00507-3

Tuncer, S., and Banerjee, S. (2015). Eicosanoid pathway in colorectal cancer: recent updates. *W. J. Gastroenterol.* 21, 11748–11766. doi:10.3748/wjg.v21.i41.11748

Ulu, A., Stephen Lee, K. S., Miyabe, C., Yang, J., Hammock, B. G., Dong, H., et al. (2014) An omega-3 epoxide of docosahexaenoic acid lowers blood pressure in angiotensin-II-dependent hypertension. *J. Cardiovasc. Pharmacol.* 64, 87–99. doi:10.1097/fjc.0000000000000094

Van Booven, D., Marsh, S., McLeod, H., Carrillo, M. W., Sangkuhl, K., Klein, T. E., and Altman, R. B. (2010). Cytochrome P450 2C9-CYP2C9. *Pharmacogen. Genom.* 20, 277–281. doi:10.1097/FPC.0b013e3283349e84

VanRollins, M., Baker, R. C., Sprecher, H. W., and Murphy, R. C. (1984). Oxidation of docosahexaenoic acid by rat liver microsomes. *J. Biol. Chem.* 259, 5776–5783.

VanRollins, M., Frade, P. D., and Carretero, O. A. (1988). Oxidation of 5,8,11,14,17-eicosapentaenoic acid by hepatic and renal microsomes. *Biochim. Biophys. Acta Gen. Subj.* 966, 133–149. doi:10.1016/0304-4165(88)90137-7

Wadelius, M., Autrup, J. L., Stubbins, M. J., Andersson, S.-O., Johansson, J.-E., Wadelius, C., et al. (1999). Polymorphisms in NAT2, CYP2D6, CYP2C19 and GSTP1 and their association with prostate cancer. *Pharmacogenetics* 9, 333–340. doi:10.1097/00008571-199906000-00008

Wang, D., and Dubois, R. N. (2006). Prostaglandins and cancer. *Gut* 55, 115–122. doi:10.1136/gut.2004.047100

Wang, H., Song, K., Chen, Z., and Yu, Y. (2013). Poor metabolizers at the cytochrome P450 2C19 loci is at increased risk of developing cancer in Asian populations. *PLoS ONE* 8, e73126. doi:10.1371/journal.pone.0073126

Wang, L. N., Wang, F., Liu, J., Jin, Y. H., Fang, C., and Ren, X. Q. (2017). CYP1A1 Ile462Val polymorphism is associated with cervical cancer risk in Caucasians not Asians: a meta-analysis. *Front. Physiol.* 8, 1081. doi:10.3389/fphys.2017.01081

Wang, W., Yang, J., Edin, M. L., Wang, Y., Luo, Y., Wan, D., et al. (2019). Targeted metabolomics identifies the cytochrome P450 monooxygenase eicosanoid pathway as a novel therapeutic target of colon tumorigenesis. *Cancer Res* 79, 1822–1830. doi:10.1158/0008-5472.can-18-3221

Wei, X., Zhang, D., Dou, X., Niu, N., Huang, W., Bai, J., et al. (2014). Elevated 14,15- epoxyeicosatrienoic acid by increasing of cytochrome P450 2C8, 2C9 and 2J2 and decreasing of soluble epoxide hydrolase associated with aggressiveness of human breast cancer. *BMC Cancer* 14, 841. doi:10.1186/1471-2407-14-841

Wolf, C. R., Smith, C. A. D., Gough, A. C., Moss, J. E., Vallis, K. A., Howard, G., et al. (1992). Relationship between the debrisoquine hydroxylase polymorphism and cancer susceptibility. *Carcinogenesis* 13, 1035–1038. doi:10.1093/carcin/13.6.1035

Womack, C. J., Saunders, M. J., Bechtel, M. K., Bolton, D. J., Martin, M., et al. (2012). The influence of a CYP1A2 polymorphism on the ergogenic effects of caffeine. *J. Int. Soc. Sports Nutr.* 9, 7. doi:10.1186/1550-2783-9-7

Wongpratate, M., Ishida, W., Phuthong, S., Natphopsuk, S., and Ishida, T. (2020). Genetic polymorphisms of the human cytochrome P450 1A1 (CYP1A1) and

cervical cancer susceptibility among Northeast Thai women. *Asian Pac. J. Cancer Prev. APJCP* 21, 243–248. doi:10.31557/apjcp.2020.21.1.243

Wu, B., Iwakiri, R., Ootani, A., Tsunada, S., Fujise, T., Sakata, Y., et al. (2004). Dietary corn oil promotes colon cancer by inhibiting mitochondria-dependent apoptosis in azoxymethane-treated rats. *Exp. Biol. Med.* 229, 1017–1025. doi:10.1177/153537020422901005

Wu, B., Liu, K., Huang, H., Yuan, J., Yuan, W., Wang, S., et al. (2013). MspI and Ile462Val polymorphisms in CYP1A1 and overall cancer risk: a meta-analysis. *PLoS ONE* 8, e85166. doi:10.1371/journal.pone.0085166

Wu, S., Chen, W., Murphy, E., Gabel, S., Tomer, K. B., Foley, J., et al. (1997). Molecular cloning, expression, and functional significance of a cytochrome P450 highly expressed in rat heart myocytes. *J. Biol. Chem.* 272, 12551–12559. doi:10.1074/jbc.272.19.12551

Xu, M., Ju, W., Hao, H., Wang, G., and Li, P. (2013). Cytochrome P450 2J2: distribution, function, regulation, genetic polymorphisms and clinical significance. *Drug Metabol. Rev.* 45, 311–352. doi:10.3109/03602532.2013.806537

Xu, X., Zhang, X. A., and Wang, D. W. (2011). The roles of CYP450 epoxygenases and metabolites, epoxyeicosatrienoic acids, in cardiovascular and malignant diseases. *Adv. Drug Deliv. Rev.* 63, 597–609. doi:10.1016/j.addr.2011.03.006

Yadav, S. S., Seth, S., Khan, A. J., Maurya, S. S., Dhawan, A., Pant, S., et al. (2014). Association of polymorphism in cytochrome P450 2C9 with susceptibility to head and neck cancer and treatment outcome. *Appl. Transl. Genom.* 3, 8–13. doi:10.1016/j.atg.2013.07.002

Yan, F., Xu, J.-F., Liu, X.-f., and Li, X.-H. (2014). Interaction between smoking and CYP2C19*3 polymorphism increased risk of lung cancer in a Chinese population. *Tumor Biol.* 35, 5295–5298. doi:10.1007/s13277-014-1689-3

Yanai, R., Mulki, L., Hasegawa, E., Takeuchi, K., Sweigard, H., Suzuki, J., et al. (2014). Cytochrome P450-generated metabolites derived from ω-3 fatty acids attenuate neovascularization. *Proc. Natl. Acad. Sci. U.S.A.* 111, 9603–9608. doi:10.1073/pnas.1401191111

Yang, S., Wei, S., Pozzi, A., and Capdevila, J. H. (2009). The arachidonic acid epoxygenase is a component of the signaling mechanisms responsible for VEGF-stimulated angiogenesis. *Arch. Biochem. Biophys.* 489, 82–91. doi:10.1016/j.abb.2009.05.006

Yu, P. J., Chen, W. G., Feng, Q. L., Chen, W., Jiang, M. J., and Li, Z. Q. (2015). Association between CYP1B1 gene polymorphisms and risk factors and susceptibility to laryngeal cancer. *Med. Sci. Mon. Int. Med. J. Exp. Clin. Res.* 21, 239–245. doi:10.12659/msm.893606

Yu, W., Chen, L., Yang, Y.-Q., Falck, J. R., Guo, A. M., Li, Y., et al. (2011). Cytochrome P450 ω-hydroxylase promotes angiogenesis and metastasis by upregulation of VEGF and MMP-9 in non-small cell lung cancer. *Cancer Chemother. Pharmacol.* 68, 619–629. doi:10.1007/s00280-010-1521-8

Zanger, U. M., and Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol. Ther.* 138, 103–141. doi:10.1016/j.pharmthera.2012.12.007

Zeldin, D. C., Foley, J., Boyle, J. E., Moomaw, C. R., Tomer, K. B., Parker, C., et al. (1997). Predominant expression of an arachidonate epoxygenase in islets of Langerhans cells in human and rat pancreas. *Endocrinology* 138, 1338–1346. doi:10.1210/endo.138.3.4970

Zeldin, D. C., Wei, S. Z., Falck, J. R., Hammock, B. D., Snapper, J. R., and Capdevila, J. H. (1995). Metabolism of epoxyeicosatrienoic acids by cytosolic epoxide hydrolase: substrate structural determinants of asymmetric catalysis. *Arch. Biochem. Biophys.* 316, 443–451. doi:10.1006/abbi.1995.1059

Zhang, B., Cao, H., and Rao, G. N. (2006). Fibroblast growth factor-2 is a downstream mediator of phosphatidylinositol 3-kinase-Akt signaling in 14,15-epoxyeicosatrienoic acid-induced angiogenesis. *J. Biol. Chem.* 281, 905–914. doi:10.1074/jbc.m503945200

Zhang, G., Kodani, S., and Hammock, B. D. (2014a). Stabilized epoxygenated fatty acids regulate inflammation, pain, angiogenesis and cancer. *Prog. Lipid Res.* 53, 108–123. doi:10.1016/j.plipres.2013.11.003

Zhang, G., Panigrahy, D., Hwang, S. H., Yang, J., Mahakian, L. M., Wettersten, H. I., et al. (2014b). Dual inhibition of cyclooxygenase-2 and soluble epoxide hydrolase synergistically suppresses primary tumor growth and metastasis. *Proc. Natl. Acad. Sci. U.S.A.* 111, 11127–11132. doi:10.1073/pnas.1410432111

Zhang, G., Panigrahy, D., Mahakian, L. M., Yang, J., Liu, J.-Y., Stephen Lee, K. S., et al. (2013a). Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6530–6535. doi:10.1073/pnas.1304321110

Zhang, W., Li, H., Dong, H., Liao, J., Hammock, B. D., and Yang, G. Y. (2013b). Soluble epoxide hydrolase deficiency inhibits dextran sulfate sodium-induced colitis and carcinogenesis in mice. *Anticancer Res.* 33, 5261–5271

Zhao, Y., Chen, Z.-X., Rewuti, A., Ma, Y.-S., Wang, X.-F., Xia, Q., et al. (2013). Quantitative assessment of the influence of cytochrome P450 1A2 gene polymorphism and colorectal cancer risk. *PLoS ONE* 8, e71481. doi:10.1371/journal.pone.0060607

Zheng, J., Plopper, C. G., Lakritz, J., Storms, D. H., and Hammock, B. D. (2001). Leukotoxin-diol. *Am. J. Respir. Cell Mol. Biol.* 25, 434–438. doi:10.1165/ajrcmb.25.4.4104

Zheng, Y., Xu, Y., Zhou, B. Y., Sun, L., Yu, P. B., Zhang, L., et al. (2018). CYP3A4*1B polymorphism and cancer risk: a meta-analysis based on 55 case-control studies. *Ann. Clin. Lab. Sci.* 48, 538–545

Zhou, J., Wen, Q., Li, S.-F., Zhang, Y.-F., Gao, N., Tian, X., et al. (2016). Significant change of cytochrome P450s activities in patients with hepatocellular carcinoma. *Oncotarget* 7, 50612–50623. doi:10.18632/oncotarget.9437

Zhou, X., Gu, Y., Wang, D.-N., Ni, S., Yan, J., and Zou, S. (2013). Eight functional polymorphisms in the estrogen receptor 1 gene and endometrial cancer risk: a meta-analysis. *PLoS ONE* 8, e60851. doi:10.1371/journal.pone.0066865

Zhu, Z. Y., Mu, Y. Q., Fu, X. M., Li, S. M., and Zhao, F. X. (2011). Association of CYP1B1 gene polymorphisms and the positive expression of estrogen alpha and estrogen beta with endometrial cancer risk. *Eur. J. Gynaecol. Oncol.* 32, 188–191. doi:10.1017/S0008423906459965

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 18 November 2020
doi: 10.3389/fphar.2020.595498

Eicosanoids in Cancer: New Roles in Immunoregulation

*Amber M. Johnson, Emily K. Kleczko and Raphael A. Nemenoff**

Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

Eicosanoids represent a family of active biolipids derived from arachidonic acid primarily through the action of cytosolic phospholipase A2-α. Three major downstream pathways have been defined: the cyclooxygenase (COX) pathway which produces prostaglandins and thromboxanes; the 5-lipoxygenase pathway (5-LO), which produces leukotrienes, lipoxins and hydroxyeicosatetraenoic acids, and the cytochrome P450 pathway which produces epoxygenated fatty acids. In general, these lipid mediators are released and act in an autocrine or paracrine fashion through binding to cell surface receptors. The pattern of eicosanoid production is cell specific, and is determined by cell-specific expression of downstream synthases. Increased eicosanoid production is associated with inflammation and a panel of specific inhibitors have been developed designated non-steroidal anti-inflammatory drugs. In cancer, eicosanoids are produced both by tumor cells as well as cells of the tumor microenvironment. Earlier studies demonstrated that prostaglandin E2, produced through the action of COX-2, promoted cancer cell proliferation and metastasis in multiple cancers. This resulted in the development of COX-2 inhibitors as potential therapeutic agents. However, cardiac toxicities associated with these agents limited their use as therapeutic agents. The advent of immunotherapy, especially the use of immune checkpoint inhibitors has revolutionized cancer treatment in multiple malignancies. However, the majority of patients do not respond to these agents as monotherapy, leading to intense investigation of other pathways mediating immunosuppression in order to develop rational combination therapies. Recent data have indicated that PGE2 has immunosuppressive activity, leading to renewed interest in targeting this pathway. However, little is known regarding the role of other eicosanoids in modulating the tumor microenvironment, and regulating anti-tumor immunity. This article reviews the role of eicosanoids in cancer, with a focus on their role in modulating the tumor microenvironment. While the role of PGE2 will be discussed, data implicating other eicosanoids, especially products produced through the lipoxygenase and cytochrome P450 pathway will be examined. The existence of small molecular inhibitors and activators of eicosanoid pathways such as specific receptor blockers make them attractive candidates for therapeutic trials, especially in combination with novel immunotherapies such as immune checkpoint inhibitors.

Keywords: cancer, eicosanoids, Tumor microenvironment, immunosuppression, T cells

OPEN ACCESS

Edited by:
Emanuela Ricciotti, University of Pennsylvania, United States

Reviewed by:
Dipak Panigrahy, Harvard Medical School, United States
Nune Markosyan, University of Pennsylvania, United States

****Correspondence:***
Raphael A. Nemenoff
Raphael.Nemenoff@cuanschutz.edu

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *16 August 2020*
Accepted: *07 October 2020*
Published: *18 November 2020*

Citation:
Johnson AM, Kleczko EK and Nemenoff RA (2020) Eicosanoids in Cancer: New Roles in Immunoregulation. Front. Pharmacol. 11:595498. doi: 10.3389/fphar.2020.595498

INTRODUCTION

Eicosanoids represent a family of lipid signaling molecules which are produced through the release of arachidonic acids from membrane phospholipids, and subsequent production through a series of oxygenases. Specifically, arachidonic acid is metabolized through three distinct pathways: cyclooxygenases (COX) to produce prostaglandins and thromboxanes, lipoxygenases (LOX) to produce hydroxyeicosatetraenoic acid (HETEs) and leukotrienes, and cytochrome P450 to produce epoxygenated fatty acids (EETs) (Smith, 1989). Since their discovery almost a century ago, this family of molecules have been implicated in almost every biological process including proliferation, differentiation, migration and invasion (Baker, 1990). In general, these molecules have short half-lives; they are produced in response to external stimuli such as growth factors or chemokines, and are released to act in either an autocrine or paracrine manner through binding to cell surface receptors. While the initial steps of eicosanoid production involve activation of intracellular phospholipases, production is mediated in a cell-specific fashion through a series of downstream synthases. Thus, the biological effects of eicosanoids in many diseases including cancer are context dependent, with distinct production by specific cell types signaling in turn to target cells.

In cancer, the role of eicosanoids has been examined for many years (Young, 1994; Wang and Dubois, 2010). Earlier studies focused largely on the role of prostaglandins, mainly prostaglandin E2 (PGE2) produced through the action of COX-1 or COX-2. Data from many investigators have shown that this eicosanoid is elevated in cancer. Studies demonstrated that PGE2 could act in an autocrine fashion to promote cell proliferation, migration and invasion (Sheng et al., 1998; Yip-Schneider et al., 2000; Raz, 2002). This resulted in the development of COX inhibitors, specifically COX-2 inhibitors as potential therapeutic agents in cancer (Jacoby et al., 2000; Csiki et al., 2005; Bertagnolli et al., 2006). While these agents had some efficacy, enthusiasm for targeting this pathway was diminished by the adverse side effects, specifically associated with risk for cardiovascular disease. Other eicosanoids were also considered pro-tumorigenic; however, their mechanism of action has not been well defined. Nevertheless, the consensus of these earlier studies was that eicosanoids in general promoted cancer progression, and these effects were mediated largely through direct effects on tumor cells.

The last 10 years has seen a significant "paradigm shift" from viewing cancer as a disease driven through activation of oncogenes and loss of tumor suppressor genes via somatic mutations, to a systemic disease involving an elaborate interplay between the transformed cancer cells and the surrounding microenvironment (Whiteside, 2008). These changes are reflected in the updated hallmarks of cancer defined by (Hanahan and Weinberg, 2011). In particular, one of these novel hallmarks is evasion of anti-tumor immunity. The focus on the ability of the immune system to inhibit, and potentially eliminate tumors has long been considered a potential therapeutic approach. However, the last 5 years has seen this come to fruition with the development of immunotherapy, specifically immune checkpoint inhibitors (Topalian et al., 2012; Topalian et al., 2015). These agents, which target pathways that cancer cells use to block anti-tumor effects of T cells have shown remarkable efficacy in a variety of malignancies, leading to FDA approval (Callahan and Wolchok, 2013; Gajewski et al., 2013). However, the overall response rate to this class of agents remains low (Camidge et al., 2019; Schoenfeld and Hellmann, 2020); thus a better understanding of mechanisms that control this response is required. Tumors are able to mobilize a variety of strategies to block anti-tumor T cells, and defining these pathways with a goal of developing rational combination therapies is being avidly pursued. In this context, it has become clear that specific eicosanoids have immunosuppressive activity. Thus, the role of these lipid mediators needs to be reexamined, beyond how they directly affect cancer cells, but also extending to an understanding of how they condition the tumor microenvironment. The prior development of both inhibitors and activators or eicosanoid pathways make these molecules attractive therapeutic targets (Narumiya et al., 1999). The goal of this review is to focus on the role of eicosanoids as modulators of the tumor microenvironment and specifically anti-tumor immunity. We will review published studies examining this, and also indicate where there are gaps in our knowledge regarding the role of this complex family in controlling immunity.

Pathways of Eicosanoid Production

Since there are numerous extensive reviews on pathways mediating eicosanoid production (Cathcart et al., 2011; Castelino, 2012; Dennis and Norris, 2015), we will only provide an overview of these pathways. In most cells the majority of arachidonic acid (AA) is esterified into membrane phospholipids (**Figure 1**). Release of AA is mediated through the action of the phospholipase A_2 (PLA_2) family of enzymes, and this usually represents the rate limiting step in eicosanoid production (Nakanishi and Rosenberg, 2006). The major isoform of PLA_2 implicated in regulated release of AA is Group IVA, designated $cPLA_2$ (Gronich et al., 1990; Balsinde and Dennis, 1997). This enzyme has specificity for arachidonic acid-containing phospholipids, is ubiquitously expressed and in resting cells is generally located in the cytoplasm (Clark et al., 1990; Bonventre, 1992; Leslie, 1997). Stimulation of cells resulting in increased levels of intracellular Ca^{2+} results in translocation of $cPLA_2$ to the membrane (Glover et al., 1995; Gronich et al., 1988). In most cells this translocation is to the nuclear envelope/endoplasmic reticulum (ER), which is the site of localization of many of the downstream effectors. In spite of extensive research over the past two decades, the factors that determine the site of localization are incompletely understood. However, the nuclear envelope/ER is the location of many of the downstream enzymes mediating AA release, including COX1/2 and 5-lipoxygenase (5-LO). While $cPLA_2$ is ubiquitously expressed, the downstream synthases show much greater degrees of cell-specific expression, resulting in differential expression by different cells in the tumor microenvironment.

Prostaglandin production is catalyzed through expression of either cyclooxygenase-1 or -2 (COX-1/2). In general COX-1 is

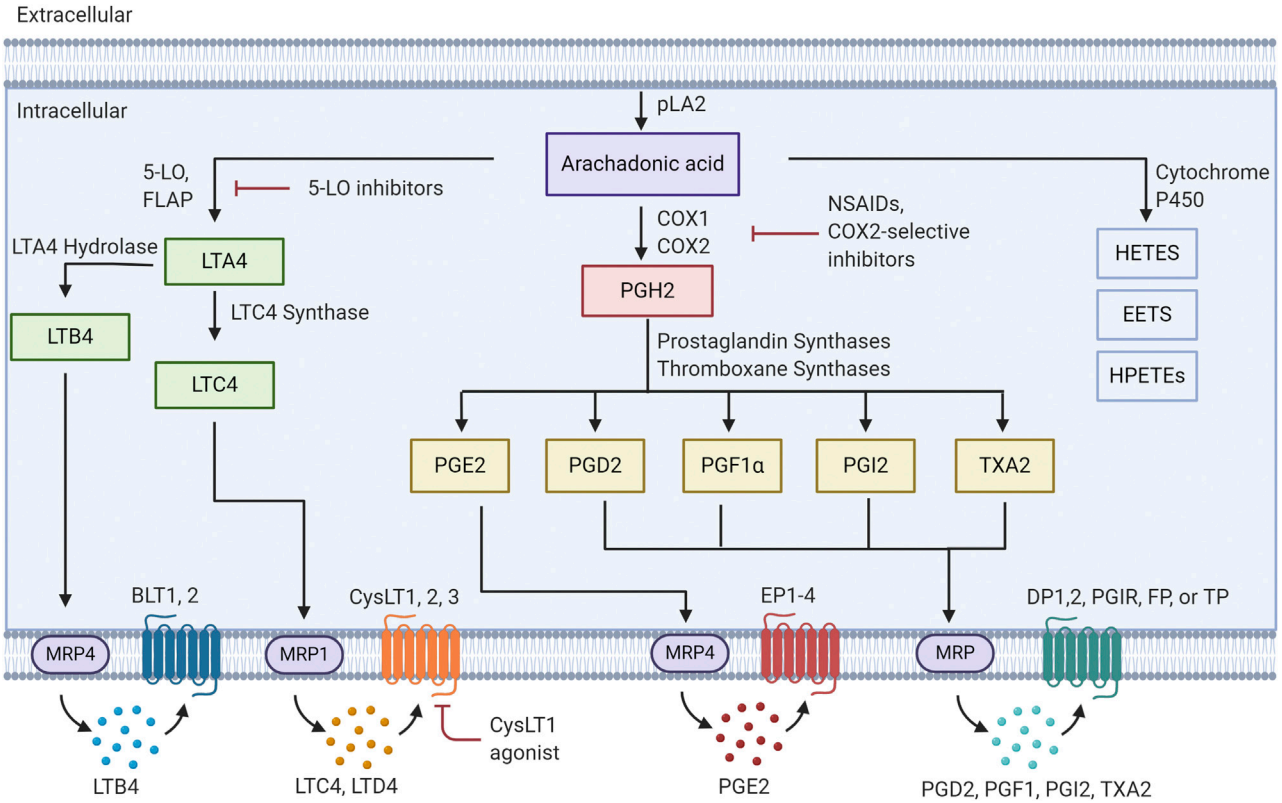


FIGURE 1 | Eicosanoid pathway Activation of PLA2 results in release of arachidonic acid from membrane phospholipids. This free arachidonic acid can be metabolized to a family of over 30 distinct bioactive lipids. These molecules are released from cells and act in an autocrine or paracrine fashion by binding to specific receptors.

expressed more widely in many cell types. However, COX-2 is expressed at low levels in most cell types, and is transcriptionally induced in response to inflammatory stimuli. Prostaglandin H2 (PGH2), which is produced through the metabolism of AA by COX-1/2 can then lead to multiple prostanoid products: prostaglandin E2, prostaglandin D2 (PGD2), prostacyclin (PGI2), prostaglandin F1α (PGF1α) and Thromboxane A2 (TXA2), via expression of downstream synthases: PGE2 synthases (mPGES-1,2), PGD synthases, PGI synthase (PGIS) and thromboxane synthases (Seo and Oh, 2017). These lipid mediators are then exported out of the cell, via multiple eicosanoid transporters, where they can bind in an autocrine or paracrine fashion to cell surface receptors. There are four PGE2 receptors (EP1,2,3,4), two PGD2 receptors (DP1,DP2), a PGI2 receptor (PGIR), a PGF1α receptor (FP) and a thromboxane receptor (TP) (Wang and DuBois, 2018). The majority of these receptors are members of the G-protein coupled receptor family and engage downstream signaling pathways. Finally, breakdown of these products is mediated through specific enzymes such as hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD) (Hughes et al., 2008; Tai, 2011).

Leukotrienes are produced through the action of 5-lipoxygenase, and 5-lipoxygenase activating protein (FLAP). FLAP serves to present AA to the 5-lipoxygenase enzyme, resulting in production of leukotriene A4 (LTA4) (Kanaoka and Austen, 2019). Subsequent metabolism of this product occurs through LTA4 hydrolase to produce leukotriene B4 (LTB4), or leukotriene C4 synthase (LTC4 synthase) to produce LTC4. LTC4 is subsequently exported and can be converted to leukotriene D4 (LTD4) through the actions of γ-glutamyl transpeptidases, and leukotriene E4 (LTE4) via membrane bound dipeptidases (Yokomizo et al., 2018). Specific G-protein coupled receptors for these products have been identified, with both a high and low affinity receptor for LTB4 (BLT1, BLT2) and two receptors for products of LTC4 synthase (CystLT1 and CystLT2) (Yokomizo et al., 2018). Antagonists for these receptors have been developed and the CystLT1 antagonists montelukast and zafirlukast are approved to treat bronchial asthma and allergic rhinitis (Capra et al., 2006). Recently a third receptor, designated as CystLT3 or GRP99 has been identified (Kanaoka et al., 2013). This receptor appears to be specific for LTE4.

Finally, the cytochrome P450 pathway results in the production of epoxyeicosatrienoic acids and the hydroxyeicosastetraeinoic (Chen and Wang, 2015). These molecules have been shown to have vasculo-protective effects through mediating vasodilation and angiogenesis (Evangelista et al., 2020). The role of these molecules in cancer has not been as well studied as the other classes of eicosanoids.

In addition to signaling through cell surface receptors, numerous eicosanoids can also signal by binding to and activating specific nuclear receptors of the peroxisome proliferator-activated receptor family (PPARs) specifically the peroxisome proliferator activated receptor family PPARγ or PPARδ (Berger and Moller, 2002; Nixon et al., 2003). In particular, the role of PPARγ has been extensively studied in multiple types of cancer (Berger and Moller, 2002; Grommes et al., 2004; Panigrahy et al., 2005; Nemenoff, 2007; Roman, 2008). While the role of these receptors will not be discussed in this review, it should be noted that the ability of eicosanoids to engage these receptors on different cell types adds to the complex biology of these mediators.

Regulators of Tumor Immunity

Pathways of immune evasion: The advent of immunotherapy has radically changed how lung cancer is treated, and altered the focus of preclinical research. While the ability of the immune system to recognize tumors has been appreciated for a long time, immune evasion was only recently defined as one of the hallmarks of cancer (Hanahan and Weinberg, 2011). A conceptual model to describe cancer-immune interactions is the immunoediting hypothesis. In this model, cancer cells develop a variety of strategies to avoid elimination by the host immune system (Schreiber et al., 2011). Initial recognition of cancer cells by the immune system is followed by adaptation of the cancer cells, leading to an equilibrium phase, and subsequently escape and cancer progression. Tumors have developed a variety of strategies to avoid T cell-mediated killing of cancer cells. These include loss of antigen presentation, through changes in MHC expression, or accessory pathways required for peptide binding (Pardoll, 2012; Gajewski et al., 2013), as well as changes in the relative populations of specific cell types comprising the tumor microenvironment, such as increases in immunosuppressive myeloid suppressor cells and tumor-associated macrophages (Mantovani et al., 2007; Whiteside, 2008; DeNardo et al., 2010). Based on these studies, it has been proposed for some time that therapeutic strategies targeting these pathways, and resulting in reestablishment of anti-tumor immunity would result in tumor elimination. Furthermore, these strategies could result in the formation of memory T cells, thus affording long term efficacy for these treatments. While initial attempts at immunotherapy were unsuccessful, recent studies have developed multiple strategies which have shown long-lasting effects in many cancers, resulting in a "immunotherapy revolution" in cancer (Topalian et al., 2015; Rothschilds and Wittrup, 2019).

Prominent among these pathways are immune checkpoints which are mechanisms designed to regulate the extent and the duration of the immune response critical for preventing autoimmune responses. Cancer cells have adopted these pathways to inactivate infiltrating T cells. The most well studied immune checkpoints are the CTLA4 and PD1/PD-L1 pathways (Pardoll, 2012; Gajewski et al., 2013). The PD-1/PD-L1 pathway involves upregulation of PD-L1 on cancer cells and other stromal cells, which binds to PD-1 expressed on activated T cells. This interaction inhibits T cell function resulting in what has been termed an "exhausted" phenotype. Similarly, CTLA4 expressed on T cells is bound by CD80 and CD86 expressed on cancer cells to inhibit T cell activation (Pardoll, 2012). Monoclonal antibodies targeting these interactions (anti-PD-1 or PD-L1, or anti-CTLA4) block these interactions, resulting in reactivation of T cells, and tumor elimination. These agents have shown surprising efficacy in a number of cancers including melanoma, non-small cell lung cancer (NSCLC), and head and neck squamous cell carcinoma, leading to current FDA approval for the treatment of 14 distinct malignancies, and the awarding of the Nobel Prize to the discoverers of this pathway. However, only approximately 20% of unselected cancer patients show a long-lasting response to this therapy (Doroshow et al., 2019; Havel et al., 2019; Tang et al., 2018). Thus, a major unmet need is to define the cellular and molecular mechanisms that determine the responsiveness to immune checkpoint inhibitors, and to develop rational combinations to increase patient responses.

The determinants mediating response to immunotherapy remain poorly understood. There clearly is an association of response to immunogenic burden and neoantigens (Rizvi et al., 2015). However, even in patients with high mutational burden there is a heterogeneity of response to checkpoint inhibitors. Immunosuppression can be mediated by a variety of mechanisms. In addition to immune checkpoints, recruitment of regulatory T cells (Treg) or myeloid derived suppressor cells (MDSC) can inhibit cytotoxic T cells independent of checkpoint pathways (Veglia et al., 2018; Togashi et al., 2019). Recently, Gajewski and coworkers have determined that in melanoma, patients that are resistant to checkpoint inhibitors have very few infiltrating T cells, designated an "uninflamed" tumor, compared to responders which have an "inflamed" phenotype comprising abundant infiltrating T cells (Gajewski et al., 2013). In their model, inherent properties of the cancer cells, distinct from mutational burden signal to the TME to limit T cell infiltration and interactions with cancer cells. Defining these pathways will be critical in allowing an increase in the response rate to immunotherapy. Other inherent properties of the cancer cell have also been associated with responsiveness. Ayers et al. have developed a gene signature involving responsiveness to interferon gamma (IFNγ) associate with response (Ayers et al., 2017). Our lab has demonstrated that at least in mouse models of lung cancer responsiveness to IFNγ is a determinant of response to anti-PD-1 therapy (Bullock et al., 2019).

Following the success with a single immune checkpoint inhibitor, a large number of clinical trials are currently examining combinations of immune checkpoints (e.g., anti-CTL4+antiPD-1) in multiple malignancies (Tang et al., 2018). While the response rate to these combinations is increased, there are still large numbers of patients that are resistant, and there is an increase in cytotoxicity in many of these trials. In addition to immune checkpoint inhibitors, many other immunosuppressive pathways have been identified which allow cancer cells to evade immune attack. These include increases in regulatory T cells (Treg) (Togashi et al., 2019), loss of antigen presentation (Johnson et al., 2018), (Rosenthal et al., 2019), or altered IFN signaling (Wang et al., 2016). Of particular interest is the role of

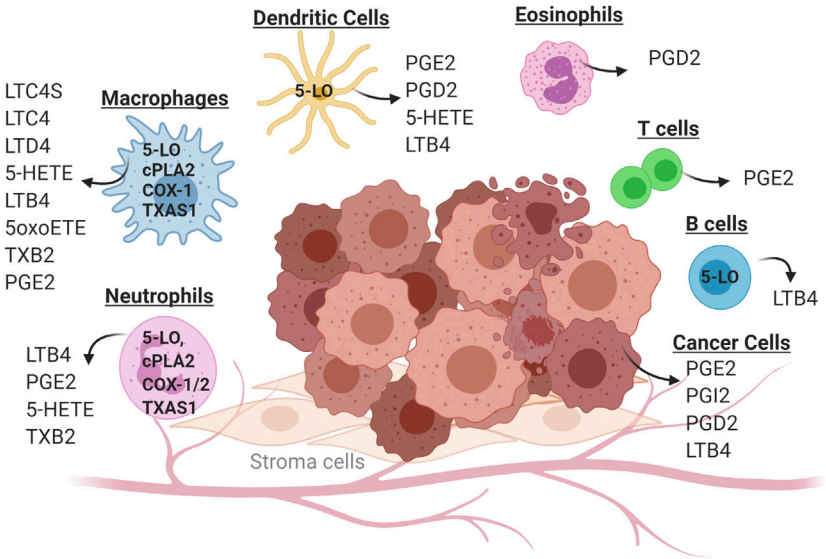


FIGURE 2 | Cell-specific production of eicosanoids in the tumor microenvironment. Solid tumors consist of cancer cells (transformed epithelial cells) in close contact with a non-transformed cells of the tumor microenvironment. These include inflammatory cells (neutrophils, macrophages, eosinophils and dendritic cells) as well as cells of the adaptive immune system (T cells and B cells). Individual cell types in the tumor microenvironment produce distinct panels of eicosanoid as a consequence of cell-specific expression of enzymes in this pathway. Some eicosanoids are produced by multiple cell types, such as PGE2 which can be produced by cancer cells, macrophages and neutrophils.

innate immunity in mediating immunosuppression. Innate immune cells comprise multiple subtypes, including neutrophils, monocytes/macrophages, myeloid derived suppressor cells and others. In addition to interacting with resident innate immune populations, cancer cells produce chemokines that attract specific populations of circulating myeloid cells. While many of these populations initially are mobilized to inhibit tumor growth, cross-talk between cancer cells results in phenotypic conversion of these populations. For example, monocyte/macrophages recruited to tumors have been shown to manifest a classic inflammatory phenotype, designated as M1, which can inhibit cancer progression (Mantovani et al., 2002; Murray et al., 2014). However, interactions with tumor cells can alter this phenotype and promote a phenotype associated with resolution of inflammation and wound healing, designated M2 (Sica et al., 2007).

Eicosanoids and Cancer

Studies dating back at least 40 years have shown that eicosanoids play a critical role in both cancer initiation and progression (Kisley et al., 2002; Edelman et al., 2008; Horn et al., 2009; Wang and Dubois, 2010; Cathcart et al., 2011). Production of eicosanoids has been shown to promote growth of cancer cells in an autocrine fashion by signaling through cell surface receptors (Tong et al., 2005; Nie, 2007; Moreno, 2009; Wang and Dubois, 2010; Cathcart et al., 2011; Schneider and Pozzi, 2011). Earlier studies have demonstrated that eicosanoids can control multiple biological processes in the setting of cancer, including cell proliferation, migration, and differentiation (Wang and Dubois, 2010). While these studies have focused on eicosanoids produced by cancer cells, our data and those of many others indicate that while tumor cells predominantly produce PGE2, the TME produces a broad spectrum of eicosanoids (Kudryavtsev et al., 2005; Greene et al., 2011; Panigrahy et al., 2011; Poczobutt et al., 2013; Moore and Pidgeon, 2017) (see **Figure 2**). These products are produced in large part by inflammatory cells, but act on other cell types, particularly cells of the adaptive immune system ($CD4^+$ and $CD8^+$ T cells). These observations increase the complexity of studying the role of individual eicosanoids, since it is necessary to consider the specific cell producing these agents, as well as the specific product produced. In fact, it is likely that the role of a particular product may differ depending on where and when it is produced during cancer progression. In the following sections, we will focus on eicosanoids that act on immunosuppressive pathways.

Role of Prostaglandins

The most studied prostaglandin is PGE2, produced mainly through the sequential action of COX-2 and microsomal PGE-synthase (mPGES-1). While earlier studies focused on the direct effects of PGE2 on cancer cells, there is emerging data indicating that this eicosanoid has important immunosuppressive pathways (Wang and DuBois, 2018). In many cases, PGE2 acts on innate immune cells to alter the tumor microenvironment. In pancreatic cancer, induction of COX-2, which is mediated through the ephrin A receptor two results in tumors in which T cells are largely excluded (Markosyan et al., 2019), and there are abundant increases in the numbers of myeloid derived suppressor cells. As such, these tumors are resistant to immune checkpoint inhibitors. Markosyan and co-workers demonstrated that genetic or pharmacologic inhibition of COX-2 alters the TME, resulting

in infiltration of T cells, and decreases in MDSC, suggesting that combinatorial therapy with COX-2 inhibitors such as celecoxib and anti-PD-1 may increase response (Markosyan et al., 2019). MDSCs act to suppress the anti-tumoral activity of T cells and other cell types (Veglia et al., 2018). In a lung cancer model in which Gprc5a is deleted, increased PGE2 synthesis was also associated with recruitment of MDSCs as well as promotion of alternatively activated M2 macrophages (Wang et al., 2020). PGE2 has been shown to signal through the EP2 receptor to induce an immunosuppressive phenotype in MDSC (Porta et al., 2020). These effects are mediated through nuclear accumulation of p50 NF-κB, and production of nitric oxide (NO). Recent data indicate that PGE2 can alter the phenotype of immune cells through regulation of epigenetic mechanisms. Specifically, PGE2 produced by cancer cells can act on myeloid cells to induce DNA methyltransferase 3A (DNMT3A), leading to alterations in DNA methylation and suppression of immunogenic genes (Rodríguez-Ubreva et al., 2017).

A number of additional mechanisms for the immunosuppressive effects of PGE2 have been proposed, and these appear to be cancer-type specific. Earlier studies showed that PGE2 collaborates with TGF-β to increase Tregs (Baratelli et al., 2005; Baratelli et al., 2010). More recently, PGE2 production has been implicated in directly mediating resistance to immune checkpoint inhibition (Zelenay et al., 2015). Induction of PD-L1 expression in tumor associated macrophages is mediated through PGE2 (Prima et al., 2017). PGE2 has been shown to induce expression of cell-surface ectonucleases CD39 and CD73 on $CD14^+$ monocytes (Al-Taei et al., 2017), resulting in production of adenosine is a breakdown product of ATP that mediates tumor progression and immunosuppression. PGE2 acting through the EP4 receptor has been shown to induce the immune checkpoint protein TIM3 on Jurkat T cells (Yun et al., 2019). Mesenchymal stem cells (MSC) from tumors selectively inhibited the function of NK cells in tumors, and this is mediated by PGE2 production by the MSC (Galland et al., 2017).

Dendritic cells are critical for engaging the adaptive immune system in inhibiting cancer progression. While distinct populations of dendritic cells have been defined, conventional dendritic cells (cDC) present tumor antigens to T cells, as well as produce cytokines to regulate T cell effector function (Wculek et al., 2020). While factors regulating recruitment of dendritic cells to tumors, data support a role for natural killer cells (NK) in cDC1 recruitment (Böttcher et al., 2018). PGE2 production by tumor cells inhibits this recruitment by impairing NK viability of cytokines production (Böttcher et al., 2018). The mechanisms responsible for inducing this population are not well understood. Recent data has demonstrated increased lipid accumulation in neutrophils mediated through fatty acid transporter 2 (Veglia et al., 2019) results in their promotion of immunosuppression. This is mediated at least in part through increased production of PGE2.

In colon cancer, PGE2 derived through COX-2 has been shown to lead to a feed forward mechanism, in which receptor-interacting protein kinase 3 (RIPK3) results in activation of myeloid suppressor cells and suppression of anti-tumor immunity (Yan et al., 2018). PGE2 leads to down-regulation of RIPK3, which in turn results in induction of COX-2 and increased production of PGE2 by the MDSCs, which in turn increases proliferation of cancer cells and inhibits the anti-tumor function of $CD8^+$ T cells.

The existence of four PGE2 receptors which signal through different pathways increases the complexity of developing effective therapeutic agents. Antagonists have been developed to target individual receptors. Antagonists against EP4, which signals through cAMP, have shown efficacy in multiple types of cancer (Majumder et al., 2018; Ching et al., 2020). EP3 antagonists have been shown to be effective in inhibiting growth of breast cancer cells (Hester et al., 2019), whereas EP1 antagonists are effective in models of hepatocellular carcinoma (Yang et al., 2019). The role of individual receptors in mediating immunosuppression has not been extensively examined, but these agents are likely to be more selective than broad inhibition of PGE2 production.

PGD2, in contrast has been shown to inhibit induction of expression of elevated levels of Indoleamine 2,3-dioxygenase (IDO) by macrophages (Bassal et al., 2016). Since IDO is associated with immunosuppression, these data would suggest that PGD2 would inhibit immunosuppression by blocking production of tryptophan metabolism (Bassal et al., 2016). However, PGD2 has also been shown to foster immunosuppression in acute promyelocytic leukemia through modifying Group 2 innate lymphoid cells and MDSCs (Trabanelli et al., 2017); these authors also show that strategies to decrease PGD2 relieve this immunosuppression.

In contrast to the general pro-tumorigenic effects of PGE2, prostacyclin produced through the action of prostacyclin synthase has been shown to inhibit both tumor initiation and progression (Keith et al., 2002; Keith et al., 2004; Li et al., 2018). Genetic mice with targeted overexpression of PGIS in the lung were protected against induction of lung tumors in response to either chemical carcinogenesis or exposure to cigarette smoke (Keith et al., 2002; Keith et al., 2004). While the mechanisms for these effects are not understood, immunostaining revealed alterations in macrophage staining in response to elevated prostacyclin. A clinical trial examining the effect of the prostacyclin analog iloprost in patients at risk for developing lung cancer demonstrated reduced progression of preneoplastic lesions in former smokers (Keith et al., 2011), underscoring the potential of prostacyclin analogs as chemopreventive agents. Elevated prostacyclin also inhibits tumor growth in mouse models of lung cancer (Li et al., 2018). This was mediated by increased numbers of $CD4^+$ T cells into the tumors. In other systems it has been shown that PGI2 regulates $CD4^+$ T cell populations, and can inhibit the development of Tregs (Aronoff et al., 2007; Li et al., 2018).

Role of Leukotrienes

Leukotrienes produced through the action of 5-lipoxygenase have been detected in both cancer cells and cells of the TME. In cancer cells, the majority of the published data support a pro-tumorigenic role for these lipid mediators, through direct effects on cell proliferation (Rioux and Castonguay, 1998; Steinhilber et al., 2010; Merchant et al., 2018). However, the

role of leukotrienes in the TME appears to be more complex. While some cancer cells can produce these lipids, leukotrienes are mainly produced by inflammatory cells, including neutrophils, macrophages, and eosinophils (Steinhilber et al., 2010; Wang and Dubois, 2010; Esser-von Bieren, 2017; Merchant et al., 2018). Studies in our lab using an immunocompetent model of lung cancer, demonstrated that resident macrophages are the highest expressers of 5-lipoxygenase (Poczobutt et al., 2016a).

Several studies have demonstrated that leukotriene production by tumor associated macrophages is critical for T cell recruitment. In particular, LTB4, produced through the actions of 5-lipoxygenase and LTA4 hydrolase, has been shown to be a potent chemotactic factor for the recruitment of T cells (Goodarzi et al., 2003; Jala et al., 2017a; Jala et al., 2017b; Tager et al., 2003). While other factors, specifically ligands which bind to CXCR3 on T cells, such as CXCL9 and CXCL10 are critical for T cell recruitment to tumors, deletion of BLT1 and CXCR3 result in equal impairments in T cell recruitment, and the double knockout mice do not show any additivity, suggesting that both of these pathways are required (Chheda et al., 2016). Consistent with these data, apoptotic cancer cells have been shown to reduce expression of 5-lipoxygenase in tumor associated macrophages, and this is associated with decreased T cell recruitment and tumor progression (Ringleb et al., 2018). Interestingly, the response of melanoma tumors to immune checkpoint inhibitors such as anti-PD-1 is abrogated if these tumors are growing in BLT1-deficient mice (Chheda et al., 2016). These data indicate that administration of analogs of LTB4 may augment the response to immune checkpoint blockade. This needs to be confirmed in relevant preclinical models. Furthermore, examination of LTB4 and its role in T cell recruitment needs to be examined in human tumors. A clinical trial using an LTB4 antagonist which was expected to inhibit lung cancer progression by acting on the tumor cells, actually resulted in worsening of disease (Jänne et al., 2014), suggesting that this agent is targeting the TME. Based on the preclinical data, it might be anticipated that this agent would decrease T cell recruitment to tumors, thus promoting progression.

Studies using the $APC^{min/+}$ mouse, a model for colorectal cancer demonstrate the complexity of this pathway. Initial studies using 5-lipoxygenase inhibitors, resulted in inhibition of tumor progression (Mohammed et al., 2011). However, more recent studies using mice deficient in expression of the LTB4 receptor BLT-1 ($APC^{min/+}/BLT^{-/-}$), resulted in increased tumor growth, suggesting an inhibitory role for this eicosanoid (Jala et al., 2017a; Jala et al., 2017b). Further, in $APC^{min/+}$ mice deficient in 5-lipoxygenase ($APC^{min/+}/5\text{-}LO^{-/-}$) tumor growth was inhibited. This is likely due to pro-tumorigenic effects of other 5-LO products, such as LTC4. Tumor-associated macrophages show a decreased expression for 5-lipoxygenase which appears to be mediated through direct cell-cell contact and MerTK signaling (Ringleb et al., 2018). These authors suggest that 5-LO may be marker for tumor associated macrophages (TAMs).

In fact, in colon cancer leukotrienes have been linked to a number of other hallmarks of cancer, including angiogenesis and altered metabolism (Burke et al., 2016). In ovarian cancer, 5-lipoxygenase products act to recruit tumor associated macrophages (Wen et al., 2015) and expression was associated with metastasis. These effects were likely mediated at least in part through regulation of MMPs. Since the role of eicosanoids are context dependent, additional studies are needed in other types of cancer (Wen et al., 2015).

In addition to binding to its cognate receptor, LTB4 can activate nuclear receptors, specifically peroxisome proliferator activated receptor-α (PPARα) (Devchand et al., 1996). While the role of this family of receptors in cancer is complex, and beyond the scope of this review, activation of PPARα by LTB4 in B cells promotes the generation of regulatory B cells (Breg) and promotes breast cancer metastasis (Wejksza et al., 2013). A role for leukotrienes has also been demonstrated in metastasis. Production of leukotrienes promotes formation of metastasis-initiating cells (MIC) in a breast cancer model (Wculek and Malanchi, 2015). These cells express leukotriene receptors, and in turn regulate the colonization and expansion of cancer cells in the premetastic niche.

These studies suggest potentially opposing effects of different 5-lipoxygenase products on cancer progression and immunity. Unfortunately, other than LTB4, there have not been extensive studies on the specific roles of the other leukotrienes (LTC4, D4 and E4). The existence of genetic mice which lack these products should be a valuable tool in examining the role of this pathway in greater detail.

Cytochrome P450 Metabolites

While there have been fewer studies examining these products, strong data indicate that EETs, and HETEs play an important role in tumor angiogenesis, and thus are likely to regulate anti-tumor immunity indirectly (Moreno, 2009; Panigrahy et al., 2011; Panigrahy et al., 2012; Chen and Wang, 2015). Cancer cells as well as cells of the TME have been shown to produce these products. Decreased production in lung cancer cells has been associated with slower disease progression (Sausville et al., 2018). There is very little known regarding the direct role of these products on either innate or adaptive immunity (Evangelista et al., 2020).

CONCLUSION AND FUTURE DIRECTIONS

The role of eicosanoids in cancer has been studied for many years, with publications as early as the 1960s (Hydovitz, 1968; Grimley et al., 1969). Initially the focus of these lipid mediators was on direct effects on cancer cells, focusing on proliferation and migration. This culminated in the development of selective COX-2 inhibitors as potential therapies for multiple types of cancer. However, the complications associated with these agents decreased enthusiasm for targeting eicosanoid pathways in cancer, and a decrease in the effort to define these pathways. More recently, with the explosion of interest in the tumor microenvironment, there has been a renewed interest in the role of eicosanoids in regulating the immune response and altering the composition of the TME. These studies have established a role of both products of the COX and 5-LO pathway in regulating anti-tumor immunity. Many of these

studies indicate an immunosuppressive role for these lipid mediators, distinct from their direct effects on cancer cells. Eicosanoids have been shown to modify the anti-tumor effects of cytotoxic T cells (DiMeo et al., 2008; Poczobutt et al., 2016b), alter the populations of innate immune cells to favor increases in immunosuppressive cells such as MDSC and tumor associated macrophages (Lone and Tasken, 2013; Esser-von Bieren, 2017), as well as modulating metabolic pathways such as IDO1 (Yang et al., 2001; Moore and Pidgeon, 2017). Thus, inhibitors of this pathway are attractive agents to use in combination with approved immunotherapies such as checkpoint inhibitors. Nevertheless, there are still important gaps in our understanding of eicosanoids in the TME.

From the experience with COX-2 inhibitors, it appears likely that systemic blockers of eicosanoid pathways will have undesirable side effects, limiting their utility as anti-cancer agents. Therefore, a potential strategy is to selectively target the action of specific eicosanoids on specific cell populations. This is well-illustrated in the case of leukotrienes. These mediators appear to promote cancer cell proliferation, and there have been numerous preclinical studies using inhibitors of 5-lipoxygenase demonstrating inhibitory effects on cancer progression (Rioux and Castonguay, 1998; Pace et al., 2004; Tong et al., 2005). However, since these molecules affect T cell recruitment into tumors, systemic blockade of this pathway may result in tumors with fewer infiltrating T cells, and this would be expected to block the action of immunotherapies such as checkpoint inhibitors (Rioux and Castonguay, 1998; Pace et al., 2004; Tong et al., 2005; Sharma et al., 2013; Satpathy et al., 2015; Chheda et al., 2016; Poczobutt et al., 2016). It is not known if these disparate effects are mediated through production of leukotrienes in different cell types, or whether the products produced by a specific cell type have opposing effects (Moore and Pidgeon, 2017). An additional complication is that targeting one arm of the eicosanoid pathway may result in promoting increased flux through another branch. For example, it has been shown that in some settings blocking 5-lipoxygenase activity will result in greater conversion of arachidonic acid through the COX pathway, resulting in increased prostaglandin production (Kudryavtsev et al., 2005). A more selective approach would involve blocking downstream targets of these enzymes targeting either the specific synthases or the receptors (see **Figure 2**). The majority of these receptors are G-protein coupled receptors, and these have been historically amenable to inhibition by small molecules. However, there are only a limited number of studies that have tested these compounds in relevant models of cancer, and their effects on the immune response to cancer is not well studied.

Finally, additional translational studies are required. Eicosanoids were originally thought to represent attractive targets for cancer therapy. However, the adverse effects associated with COX-2 inhibitors diminished the enthusiasm for targeting this pathway, which persists to some degree to this day. The advent of immunotherapy and the focus of targeting anti-tumor immunity has been a paradigm shift in cancer therapeutics and has provided new hope for many cancer patients. This has resulted in an explosion of clinical trials using combinations of immune checkpoint inhibitors and other therapies, often with little scientific rationale (Tang et al., 2018). Selectively targeting eicosanoid pathways has a strong basis in preclinical studies, and well-designed trials accompanied by analysis of tumor samples may lead to a renewed enthusiasm for these pathways. In addition, the availability of selective agonists and antagonists acting on specific eicosanoid receptors offer an opportunity to target specific biological roles of these molecules without the complications entailed in blocking entire pathways.

AUTHOR CONTRIBUTIONS

AJ prepared the figures and helped write the manuscript. EK helped write the manuscript. RN oversaw the manuscript and wrote the manuscript.

FUNDING

This work was supported by grants from the NIH (R01 CA162226 and P50 CA058187), LUNGevity Foundation and the Cancer League of Colorado. AJ was supported by the NIH NRSA T32 CA174648-01. Figures were created with BioRender.com.

REFERENCES

Al-Taei, S., Salimu, J., Spary, L. K., Clayton, A., Lester, J. F., and Tabi, Z. (2017). Prostaglandin E2-mediated adenosinergic effects on CD14+ cells: self-amplifying immunosuppression in cancer. *OncoImmunology* 6, e1268308. doi:10.1080/2162402x.2016.1268308

Aronoff, D. M., Peres, C. M., Serezani, C. H., Ballinger, M. N., Carstens, J. K., Coleman, N., et al. (2007). Synthetic prostacyclin analogs differentially regulate macrophage function via distinct analog-receptor binding specificities. *J. Immunol.* 178, 1628–1634. doi:10.4049/jimmunol.178.3.1628

Ayers, M., Lunceford, J., Nebozhyn, M., Murphy, E., Loboda, A., Kaufman, D. R., et al. (2017). IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J. Clin. Invest.* 127, 2930–2940. doi:10.1172/jci91190

Baker, R. R. (1990). The eicosanoids: a historical overview. *Clin. Biochem.* 23, 455–458. doi:10.1016/0009-9120(90)90255-s

Balsinde, J., and Dennis, E. A. (1997). Function and inhibition of intracellular calcium-independent phospholipase A2. *J. Biol. Chem.* 272, 16069–16072. doi:10.1074/jbc.272.26.16069

Baratelli, F., Lee, J. M., Hazra, S., Lin, Y., Walser, T. C., Schaue, D., et al. (2010). PGE(2) contributes to TGF-beta induced T regulatory cell function in human non-small cell lung cancer. *Am. J. Transl. Res.* 2, 356–367

Baratelli, F., Lin, Y., Zhu, L., Yang, S.-C., Heuzé-Vourc'h, N., Zeng, G., et al. (2005). Prostaglandin E2 Induces FOXP3 gene expression and T regulatory cell function in human CD4+T cells. *J. Immunol.* 175, 1483–1490. doi:10.4049/jimmunol.175.3.1483

Bassal, N. K., Hughes, B. P., and Costabile, M. (2016). Prostaglandin D2 is a novel repressor of IFNγ induced indoleamine-2,3-dioxygenase via the DP1 receptor and cAMP pathway. *Prostagl. Leukot. Essent. Fat. Acids* 110, 48–54. doi:10.1016/j.plefa.2016.01.006

Berger, J., and Moller, D. E. (2002). The mechanisms of action of PPARs. *Annu. Rev. Med.* 53, 409–435. doi:10.1146/annurev.med.53.082901.104018

Bertagnolli, M. M., Eagle, C. J., Zauber, A. G., Redston, M., Solomon, S. D., Kim, K., et al. (2006). Celecoxib for the prevention of sporadic colorectal adenomas. *N. Engl. J. Med.* 355, 873–884. doi:10.1056/nejmoa061355

Bonventre, J. V. (1992). Phospholipase A2 and signal transduction. *J. Am. Soc. Nephrol.* 3, 128–150

Böttcher, J. P., Bonavita, E., Chakravarty, P., Blees, H., Cabeza-Cabrerizo, M., Sammicheli, S., et al. (2018). NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell* 172, 1022–1037. doi:10.1016/j.cell.2018.01.004

Bullock, B. L., Kimball, A. K., Poczobutt, J. M., Li, H. Y., Kwak, J. W., Neuwelt, A. J., et al. (2019). Tumor-intrinsic response to IFNgamma shapes the tumor microenvironment and anti-PD-1 response in NSCLC. *Life Sci. Alliance* 2, e201900328. doi:10.26508/lsa.201900328

Burke, L., Butler, C. T., Murphy, A., Moran, B., Gallagher, W. M., O'Sullivan, J., et al. (2016). Evaluation of cysteinyl leukotriene signaling as a therapeutic target for colorectal cancer. *Front. Cell Dev. Biol.* 4, 103. doi:10.3389/fcell.2016.00103

Callahan, M. K., and Wolchok, J. D. (2013). At the bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. *J. Leukoc. Biol.* 94, 41–53. doi:10.1189/jlb.1212631

Camidge, D. R., Doebele, R. C., and Kerr, K. M. (2019). Comparing and contrasting predictive biomarkers for immunotherapy and targeted therapy of NSCLC. *Nat. Rev. Clin. Oncol.* 16 (6), 341–355. doi:10.1038/s41571-019-0173-9

Capra, V., Ambrosio, M., Riccioni, G., and Rovati, G. (2006). Cysteinyl-leukotriene receptor antagonists: present situation and future opportunities. *Curr. Med. Chem.* 13, 3213–3226. doi:10.2174/092986706778742963

Castelino, F. V. (2012). Lipids and eicosanoids in fibrosis. *Curr. Opin. Rheumatol.* 24, 649–655. doi:10.1097/bor.0b013e328356d9f6

Cathcart, M.-C., Lysaght, J., and Pidgeon, G. P. (2011). Eicosanoid signalling pathways in the development and progression of colorectal cancer: novel approaches for prevention/intervention. *Canc. Metastasis Rev.* 30, 363–385. doi:10.1007/s10555-011-9324-x

Chen, C., and Wang, D. W. (2015). Cytochrome P450-CYP2 family-epoxygenase role in inflammation and cancer. *Adv. Pharmacol.* 74, 193–221. doi:10.1016/bs.apha.2015.04.005

Chheda, Z. S., Sharma, R. K., Jala, V. R., Luster, A. D., and Haribabu, B. (2016). Chemoattractant receptors BLT1 and CXCR3 regulate antitumor immunity by facilitating CD8+ T cell migration into tumors. *J. Immunol.* 197, 2016–2026. doi:10.4049/jimmunol.1502376

Ching, M. M., Reader, J., and Fulton, A. M. (2020). Eicosanoids in cancer: prostaglandin E2 receptor 4 in cancer therapeutics and immunotherapy. *Front. Pharmacol.* 11, 819. doi:10.3389/fphar.2020.00819

Clark, J. D., Milona, N., and Knopf, J. L. (1990). Purification of a 110-kilodalton cytosolic phospholipase A2 from the human monocytic cell line U937. *Proc. Natl. Acad. Sci. U.S.A.* 87, 7708–7712. doi:10.1073/pnas.87.19.7708

Csiki, I., Morrow, J. D., Sandler, A., Shyr, Y., Oates, J., Williams, M. K., et al. (2005). Targeting cyclooxygenase-2 in recurrent non-small cell lung cancer: a phase II trial of celecoxib and docetaxel. *Clin. Canc. Res.* 11, 6634–6640. doi:10.1158/1078-0432.ccr-05-0436

DeNardo, D. G., Andreu, P., and Coussens, L. M. (2010). Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. *Canc. Metastasis Rev.* 29, 309–316. doi:10.1007/s10555-010-9223-6

Dennis, E. A., and Norris, P. C. (2015). Eicosanoid storm in infection and inflammation. *Nat. Rev. Immunol.* 15, 511–523. doi:10.1038/nri3859

Devchand, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Wahli, W. (1996). The PPARα-leukotriene B4 pathway to inflammation control. *Nature* 384, 39–43. doi:10.1038/384039a0

DiMeo, D., Tian, J., Zhang, J., Narushima, S., and Berg, D. J. (2008). Increased interleukin-10 production and Th2 skewing in the absence of 5-lipoxygenase. *Immunology* 123, 250–262. doi:10.1111/j.1365-2567.2007.02694.x

Doroshow, D. B., Sanmamed, M. F., Hastings, K., Politi, K., Rimm, D. L., Chen, L., et al. (2019). Immunotherapy in non-small cell lung cancer: facts and hopes. *Clin. Canc. Res.* 25, 4592–4602. doi:10.1158/1078-0432.ccr-18-1538

Edelman, M. J., Watson, D., Wang, X., Morrison, C., Kratzke, R. A., Jewell, S., et al. (2008). Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy-cancer and leukemia group B trial 30203. *J. Clin. Oncol.* 26, 848–855. doi:10.1200/jco.2007.13.8081

Esser-von Bieren, J. (2017). Immune-regulation and -functions of eicosanoid lipid mediators. *Biol. Chem.* 398, 1177–1191. doi:10.1515/hsz-2017-0146

Evangelista, E. A., Cho, C. W., Aliwarga, T., and Totah, R. A. (2020). Expression and function of eicosanoid-producing cytochrome P450 enzymes in solid tumors. *Front. Pharmacol.* 11, 828. doi:10.3389/fphar.2020.00828

Gajewski, T. F., Woo, S.-R., Zha, Y., Spaapen, R., Zheng, Y., Corrales, L., and Spranger, S. (2013). Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. *Curr. Opin. Immunol.* 25, 268–276. doi:10.1016/j.coi.2013.02.009

Galland, S., Vuille, J., Martin, P., Letovanec, I., Caignard, A., Fregni, G., and Stamenkovic, I. (2017). Tumor-derived mesenchymal stem cells use distinct mechanisms to block the activity of natural killer cell subsets. *Cell Rep.* 20, 2891–2905. doi:10.1016/j.celrep.2017.08.089

Glover, S., Bayburt, T., Jonas, M., Chi, E., and Gelb, M. H. (1995). Translocation of the 85-kDa phospholipase A2from cytosol to the nuclear envelope in rat basophilic leukemia cells stimulated with calcium ionophore or IgE/antigen. *J. Biol. Chem.* 270, 15359–15367. doi:10.1074/jbc.270.25.15359

Goodarzi, K., Goodarzi, M., Tager, A. M., Luster, A. D., and von Andrian, U. H. (2003). Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. *Nat. Immunol.* 4, 965–973. doi:10.1038/ni972

Greene, E. R., Huang, S., Serhan, C. N., and Panigrahy, D. (2011). Regulation of inflammation in cancer by eicosanoids. *Prostag. Other Lipid Mediat.* 96, 27–36. doi:10.1016/j.prostaglandins.2011.08.004

Grimley, P. M., Deftos, L. J., Weeks, J. R., and Rabson, A. S. (1969). Growth *in vitro* and ultrastructure of cells from a medullary carcinoma of the human thyroid gland: transformation by simian virus 40 and evidenc of thyrocalcitonin and prostaglandins. *J. Natl. Cancer Inst.* 42, 663–680. doi:10.1093/jnci/42.4.663

Grommes, C., Landreth, G. E., and Heneka, M. T. (2004). Antineoplastic effects of peroxisome proliferatoractivated receptor γ agonists. *Lancet Oncol.* 5, 419–429. doi:10.1016/s1470-2045(04)01509-8

Gronich, J. H., Bonventre, J. V., and Nemenoff, R. A. (1988). Identification and characterization of a hormonally regulated form of phospholipase A2 in rat renal mesangial cells. *J. Biol. Chem.* 263, 16645–16651

Gronich, J. H., Bonventre, J. V., and Nemenoff, R. A. (1990). Purification of a high-molecular-mass form of phospholipase A2 from rat kidney activated at physiological calcium concentrations. *Biochem. J.* 271, 37–43. doi:10.1042/bj2710037

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi:10.1016/j.cell.2011.02.013

Havel, J. J., Chowell, D., and Chan, T. A. (2019). The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat. Rev. Canc.* 19, 133–150. doi:10.1038/s41568-019-0116-x

Hester, A., Salzmann, B., Rahmeh, M., Kolben, T., Czogalla, B., Ditsch, N., et al. (2019). EP3 receptor antagonist L798,106 reduces proliferation and migration of SK-BR-3 breast cancer cells. *OncoTargets Ther.* 12, 6053–6068. doi:10.2147/ott.s204919

Horn, L., Backlund, M., and Johnson, D. H. (2009). Targeting the eicosanoid pathway in non-small-cell lung cancer. *Expert Opin. Ther. Targets* 13, 675–688. doi:10.1517/14728220902915567

Hughes, D., Otani, T., Yang, P., Newman, R. A., Yantiss, R. K., Altorki, N. K., et al. (2008). NAD+-Dependent 15-hydroxyprostaglandin dehydrogenase regulates levels of bioactive lipids in non-small cell lung cancer. *Canc. Prev. Res.* 1, 241–249. doi:10.1158/1940-6207.capr-08-0055

Hydovitz, J. D. (1968). Prostaglandins and diarrhea. *N. Engl. J. Med.* 278, 915. doi:10.1056/nejm196804182781626

Jacoby, R. F., Seibert, K., Cole, C. E., Kelloff, G., and Lubet, R. A. (2000). The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. *Cancer Res* 60, 5040–5044

Jala, V. R., Bodduluri, S. R., Satpathy, S. R., Chheda, Z., Sharma, R. K., and Haribabu, B. (2017a). The yin and yang of leukotriene B4 mediated inflammation in cancer. *Semin. Immunol.* 33, 58–64. doi:10.1016/j.smim.2017.09.005

Jala, V. R., Maturu, P., Bodduluri, S. R., Krishnan, E., Mathis, S., Subbarao, K., et al. (2017b). Leukotriene B4-receptor-1 mediated host response shapes gut microbiota and controls colon tumor progression. *OncoImmunology* 6, e1361593. doi:10.1080/2162402x.2017.1361593

Jänne, P. A., Paz-Ares, L., Oh, Y., Eschbach, C., Hirsh, V., Enas, N., et al. (2014). Randomized, double-blind, phase II trial comparing gemcitabine-cisplatin plus the LTB4 antagonist LY293111 versus gemcitabine-cisplatin plus placebo in first-line non-small-cell lung cancer. *J. Thorac. Oncol.* 9, 126–131. doi:10.1097/jto.0000000000000037

Johnson, D. B., Nixon, M. J., Wang, Y., Wang, D. Y., Castellanos, E., Estrada, M. V., et al. (2018). Tumor-specific MHC-II expression drives a unique pattern of resistance to immunotherapy via LAG-3/FCRL6 engagement. *JCI Insight* 3, e120360. doi:10.1172/jci.insight.120360

Kanaoka, Y., and Austen, K. F. (2019). Roles of cysteinyl leukotrienes and their receptors in immune cell-related functions. *Adv. Immunol.* 142, 65–84. doi:10.1016/bs.ai.2019.04.002

Kanaoka, Y., Maekawa, A., and Austen, K. F. (2013). Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4Ligand. *J. Biol. Chem.* 288, 10967–10972. doi:10.1074/jbc.c113.453704

Keith, R. L., Miller, Y. E., Hoshikawa, Y., Moore, M. D., Gesell, T. L., Gao, B., et al. (2002). Manipulation of pulmonary prostacyclin synthase expression prevents murine lung cancer. *Cancer Res.* 62, 734–740

Keith, R. L., Blatchford, P. J., Kittelson, J., Minna, J. D., Kelly, K., Massion, P. P., et al. (2011) Oral iloprost improves endobronchial dysplasia in former smokers. *Canc. Prev. Res.* 4, 793–802. doi:10.1158/1940-6207.capr-11-0057

Keith, R. L., Miller, Y. E., Hudish, T. M., Girod, C. E., Sotto-Santiago, S., Franklin, W. A., et al. (2004). Pulmonary prostacyclin synthase overexpression chemoprevents tobacco smoke lung carcinogenesis in mice. *Cancer Res* 64, 5897–5904. doi:10.1158/0008-5472.can-04-1070

Kisley, L. R., Barrett, B. S., Dwyer-Nield, L. D., Bauer, A. K., Thompson, D. C., and Malkinson, A. M. (2002). Celecoxib reduces pulmonary inflammation but not lung tumorigenesis in mice. *Carcinogenesis* 23, 1653–1660. doi:10.1093/carcin/23.10.1653

Kudryavtsev, I. A., Gudkova, M. V., Pavlova, O. M., Oreshkin, A. E., and Myasishcheva, N. V. (2005). Lipoxygenase pathway of arachidonic acid metabolism in growth control of tumor cells of different type. *Biochemistry (Mosc.)* 70, 1396–1403. doi:10.1007/s10541-005-0275-0

Leslie, C. C. (1997). Properties and regulation of cytosolic phospholipase A2. *J. Biol. Chem.* 272, 16709–16712. doi:10.1074/jbc.272.27.16709

Li, H. Y., McSharry, M., Walker, D., Johnson, A., Kwak, J., Bullock, B., et al. (2018). Targeted overexpression of prostacyclin synthase inhibits lung tumor progression by recruiting CD4+ T lymphocytes in tumors that express MHC class II. *OncoImmunology* 7, e1423182. doi:10.1080/2162402x.2017.1423182

Lone, A. M., and Tasken, K. (2013). Proinflammatory and immunoregulatory roles of eicosanoids in T cells. *Front. Immunol.* 4, 130. doi:10.3389/fimmu.2013.00130

Majumder, M., Nandi, P., Omar, A., Ugwuagbo, K. C., and Lala, P. K. (2018). EP4 as a therapeutic target for aggressive human breast cancer. *Int. J. Mol. Sci.* 19, 1019. doi:10.3390/ijms19041019

Mantovani, A., Marchesi, F., Porta, C., Sica, A., and Allavena, P. (2007). S10 Inflammation and breast cancer: hypothesis for targeted therapies. *Breast* 16 (Suppl. 2), S3–S33. doi:10.1016/s0960-9776(07)70033-4

Mantovani, A., Sozzani, S., Locati, M., Allavena, P., and Sica, A. (2002). Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 23, 549–555. doi:10.1016/s1471-4906(02)02302-5

Markosyan, N., Li, J., Sun, Y. H., Richman, L. P., Lin, J. H., Yan, F., et al. (2019). Tumor cell-intrinsic EPHA2 suppresses antitumor immunity by regulating PTGS2 (COX-2). *J. Clin. Invest.* 129, 3594–3609. doi:10.1172/jci127755

Merchant, N., Bhaskar, L. V. K. S., Momin, S., Sujatha, P., Reddy, A. B. M., and Nagaraju, G. P. (2018). 5-Lipoxygenase: its involvement in gastrointestinal malignancies. *Crit. Rev. Oncol.-Hematol.* 127, 50–55. doi:10.1016/j.critrevonc.2018.05.012

Mohammed, A., Janakiram, N. B., Li, Q., Choi, C.-I., Zhang, Y., Steele, V. E., et al. (2011). Chemoprevention of colon and small intestinal tumorigenesis in APCMin/+ mice by licofelone, a novel dual 5-LOX/COX inhibitor: potential implications for human colon cancer prevention. *Canc. Prev. Res.* 4, 2015–2026. doi:10.1158/1940-6207.capr-11-0233

Moore, G. Y., and Pidgeon, G. P. (2017). Cross-talk between cancer cells and the tumour microenvironment: the role of the 5-lipoxygenase pathway. *Int. J. Mol. Sci.* 18, 236. doi:10.3390/ijms18020236

Moreno, J. J. (2009). New aspects of the role of hydroxyeicosatetraenoic acids in cell growth and cancer development. *Biochem. Pharmacol.* 77, 1–10. doi:10.1016/j.bcp.2008.07.033

Murray, P. J., Allen, J. E., Biswas, S. K., Fisher, E. A., Gilroy, D. W., Goerdt, S., et al. (2014). Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 41, 14–20. doi:10.1016/j.immuni.2014.06.008

Nakanishi, M., and Rosenberg, D. W. (2006). Roles of cPLA2α and arachidonic acid in cancer. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1761, 1335–1343. doi:10.1016/j.bbalip.2006.09.005

Narumiya, S., Sugimoto, Y., and Ushikubi, F. (1999). Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* 79, 1193–1226. doi:10.1152/physrev.1999.79.4.1193

Nemenoff, R. A. (2007). Peroxisome proliferator-activated receptor-γ in lung cancer: defining specific versus "Off-Target— effectors. *J. Thorac. Oncol.* 2, 989–992. doi:10.1097/jto.0b013e318158cf0a

Nie, D. (2007). Cyclooxygenases and lipoxygenases in prostate and breast cancers. *Front. Biosci.* 12, 1574–1585. doi:10.2741/2170

Nixon, J. B., Kamitani, H., Baek, S. J., and Eling, T. E. (2003). Evaluation of eicosanoids and NSAIDs as PPARgamma ligands in colorectal carcinoma cells. *Prostaglandins Leukot. Essent. Fatty Acids* 68, 323–330. doi:10.1016/s0952-3278(03)00023-1

Pace, E., Profita, M., Melis, M., Bonanno, A., Paterno, A., Mody, C. H., et al. (2004). LTB4 is present in exudative pleural effusions and contributes actively to neutrophil recruitment in the inflamed pleural space. *Clin. Exp. Immunol.* 135, 519–527. doi:10.1111/j.1365-2249.2003.02387.x

Panigrahy, D., Edin, M. L., Lee, C. R., Huang, S., Bielenberg, D. R., Butterfield, C. E., et al. (2012). Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. *J. Clin. Invest.* 122, 178–191. doi:10.1172/JCI58128

Panigrahy, D., Huang, S., Kieran, M. W., and Kaipainen, A. (2005). PPARgamma as a therapeutic target for tumor angiogenesis and metastasis. *Canc. Biol. Ther.* 4, 687–693. doi:10.4161/cbt.4.7.2014

Panigrahy, D., Greene, E. R., Pozzi, A., Wang, D. W., and Zeldin, D. C. (2011). EET signaling in cancer. *Canc. Metastasis Rev.* 30, 525–540. doi:10.1007/s10555-011-9315-y

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Canc.* 12, 252–264. doi:10.1038/nrc3239

Poczobutt, J. M., De, S., Yadav, V. K., Nguyen, T. T., Li, H., Sippel, T. R., et al. (2016a). Expression profiling of macrophages reveals multiple populations with distinct biological roles in an immunocompetent orthotopic model of lung cancer. *J. Immunol.* 196, 2847–2859. doi:10.4049/jimmunol.1502364

Poczobutt, J. M., Nguyen, T. T., Hanson, D., Li, H., Sippel, T. R., Weiser-Evans, M. C. M., et al. (2016b). Deletion of 5-lipoxygenase in the tumor microenvironment promotes lung cancer progression and metastasis through regulating T cell recruitment. *J. Immunol.* 196, 891–901. doi:10.4049/jimmunol.1501648

Poczobutt, J. M., Gijon, M., Amin, J., Hanson, D., Li, H., Walker, D., et al. (2013). Eicosanoid profiling in an orthotopic model of lung cancer progression by mass spectrometry demonstrates selective production of leukotrienes by inflammatory cells of the microenvironment. *PloS One* 8, e79633. doi:10.1371/journal.pone.0079633

Porta, C., Consonni, F. M., Morlacchi, S., Sangaletti, S., Bleve, A., Totaro, M. G., et al. (2020). Tumor-derived prostaglandin E2 promotes p50 NF-κB-Dependent differentiation of monocytic MDSCs. *Cancer Res.* 80, 2874–2888. doi:10.1158/0008-5472.can-19-2843

Prima, V., Kaliberova, L. N., Kaliberov, S., Curiel, D. T., and Kusmartsev, S. (2017). COX2/mPGES1/PGE2pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. *Proc. Natl. Acad. Sci. U.S.A.* 114, 1117–1122. doi:10.1073/pnas.1612920114

Raz, A. (2002). Is inhibition of cyclooxygenase required for the anti-tumorigenic effects of nonsteroidal, anti-inflammatory drugs (NSAIDs)? *Biochem. Pharmacol.* 63, 343–347. doi:10.1016/s0006-2952(01)00857-7

Ringleb, J., Strack, E., Angioni, C., Geisslinger, G., Steinhilber, D., Weigert, A., et al. (2018). Apoptotic cancer cells suppress 5-lipoxygenase in tumor-associated macrophages. *J. Immunol.* 200, 857–868. doi:10.4049/jimmunol.1700609

Rioux, N., and Castonguay, A. (1998). Inhibitors of lipoxygenase: a new class of cancer chemopreventive agents. *Carcinogenesis* 19, 1393–1400. doi:10.1093/carcin/19.8.1393

Rizvi, N. A., Hellmann, M. D., Snyder, A., Kvistborg, P., Makarov, V., Havel, J. J., et al. (2015). Cancer immunology. Mutational landscape determines sensitivity

to PD-1 blockade in non-small cell lung cancer. *Science* 348, 124–128. doi:10.1126/science.aaa1348

Rodríguez-Ubreva, J., Català-Moll, F., Obermajer, N., Álvarez-Errico, D., Ramirez, R. N., Company, C., et al. (2017). Prostaglandin E2 leads to the acquisition of DNMT3A-dependent tolerogenic functions in human myeloid-derived suppressor cells. *Cell Rep.* 21, 154–167. doi:10.1016/j.celrep.2017.09.018

Roman, J. (2008). Peroxisome proliferator-activated receptor γ and lung cancer biology. *J. Invest. Med.* 56, 528–533. doi:10.2310/jim.0b013e3181659932

Rosenthal, R., Cadieux, E. L., Salgado, R., Bakir, M. A., Moore, D. A., Hiley, C. T., et al. (2019). Neoantigen-directed immune escape in lung cancer evolution. *Nature* 567, 479–485. doi:10.1038/s41586-019-1032-7

Rothschilds, A. M., and Wittrup, K. D. (2019). What, why, where, and when: bringing timing to immuno-oncology. *Trends Immunol.* 40, 12–21. doi:10.1016/j.it.2018.11.003

Satpathy, S. R., Jala, V. R., Bodduluri, S. R., Krishnan, E., Hegde, B., Hoyle, G. W., et al. (2015). Crystalline silica-induced leukotriene B4-dependent inflammation promotes lung tumour growth. *Nat. Commun.* 6, 7064. doi:10.1038/ncomms8064

Sausville, L. N., Gangadhariah, M. H., Chiusa, M., Mei, S., Wei, S., Zent, R., et al. (2018). The cytochrome P450 slow metabolizers CYP2C9*2 and CYP2C9*3 directly regulate tumorigenesis via reduced epoxyeicosatrienoic acid production. *Cancer Res.* 78, 4865–4877. doi:10.1158/0008-5472.can-17-3977

Schneider, C., and Pozzi, A. (2011). Cyclooxygenases and lipoxygenases in cancer. *Canc. Metastasis Rev.* 30, 277–294. doi:10.1007/s10555-011-9310-3

Schoenfeld, A. J., and Hellmann, M. D. (2020). Acquired resistance to immune checkpoint inhibitors. *Canc. Cell* 37, 443–455. doi:10.1016/j.ccell.2020.03.017

Schreiber, R. D., Old, L. J., and Smyth, M. J. (2011). Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 331, 1565–1570. doi:10.1126/science.1203486

Seo, M.-J., and Oh, D.-K. (2017). Prostaglandin synthases: molecular characterization and involvement in prostaglandin biosynthesis. *Prog. Lipid Res.* 66, 50–68. doi:10.1016/j.plipres.2017.04.003

Sharma, R. K., Chheda, Z., Jala, V. R., and Haribabu, B. (2013). Expression of leukotriene B4 receptor-1 on CD8+ T cells is required for their migration into tumors to elicit effective antitumor immunity. *J. Immunol.* 191, 3462–3470. doi:10.4049/jimmunol.1300967

Sheng, H., Shao, J., Morrow, J. D., Beauchamp, R. D., and DuBois, R. N. (1998). Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res.* 58, 362–366

Sica, A., Rubino, L., Mancino, A., Larghi, P., Porta, C., Rimoldi, M., et al. (2007). Targeting tumour-associated macrophages. *Expert Opin. Ther. Targets* 11, 1219–1229. doi:10.1517/14728222.11.9.1219

Smith, W. L. (1989). The eicosanoids and their biochemical mechanisms of action. *Biochem. J.* 259, 315–324. doi:10.1042/bj2590315

Steinhilber, D., Fischer, A. S., Metzner, J., Steinbrink, S. D., Roos, J., Ruthardt, M., et al. (2010). 5-lipoxygenase: underappreciated role of a pro-inflammatory enzyme in tumorigenesis. *Front. Pharmacol.* 1, 143. doi:10.3389/fphar.2010.00143

Tager, A. M., Bromley, S. K., Medoff, B. D., Islam, S. A., Bercury, S. D., Friedrich, E. B., et al. (2003). Leukotriene B4 receptor BLT1 mediates early effector T cell recruitment. *Nat. Immunol.* 4, 982–990. doi:10.1038/ni970

Tai, H.-H. (2011). Prostaglandin catabolic enzymes as tumor suppressors. *Canc. Metastasis Rev.* 30, 409–417. doi:10.1007/s10555-011-9314-z

Tang, J., Shalabi, A., and Hubbard-Lucey, V. M. (2018). Comprehensive analysis of the clinical immuno-oncology landscape. *Ann. Oncol.* 29, 84–91. doi:10.1093/annonc/mdx755

Togashi, Y., Shitara, K., and Nishikawa, H. (2019). Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. *Nat. Rev. Clin. Oncol.* 16, 356–371. doi:10.1038/s41571-019-0175-7

Tong, W.-G., Ding, X.-Z., Talamonti, M. S., Bell, R. H., and Adrian, T. E. (2005). LTB4 stimulates growth of human pancreatic cancer cells via MAPK and PI-3 kinase pathways. *Biochem. Biophys. Res. Commun.* 335, 949–956. doi:10.1016/j.bbrc.2005.07.166

Topalian, S. L., Hodi, F. S., Brahmer, J. R., Gettinger, S. N., Smith, D. C., McDermott, D. F., et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366, 2443–2454. doi:10.1056/NEJMoa1200690

Topalian, S. L., Drake, C. G., and Pardoll, D. M. (2015). Immune checkpoint blockade: a common denominator approach to cancer therapy. *Canc. Cell* 27, 450–461. doi:10.1016/j.ccell.2015.03.001

Trabanelli, S., Chevalier, M. F., Martinez-Usatorre, A., Gomez-Cadena, A., Salomé, B., Lecciso, M., et al. (2017). Tumour-derived PGD2 and NKp30-B7H6 engagement drives an immunosuppressive ILC2-MDSC axis. *Nat. Commun.* 8, 593. doi:10.1038/s41467-017-00678-2

Veglia, F., Perego, M., and Gabrilovich, D. (2018). Myeloid-derived suppressor cells coming of age. *Nat. Immunol.* 19, 108–119. doi:10.1038/s41590-017-0022-x

Veglia, F., Tyurin, V. A., Blasi, M., De Leo, A., Kossenkov, A. V., Donthireddy, L., et al. (2019) Fatty acid transport protein 2 reprograms neutrophils in cancer. *Nature* 569, 73–78. doi:10.1038/s41586-019-1118-2

Wang, D., and Dubois, R. N. (2010). Eicosanoids and cancer. *Nat. Rev. Canc.* 10, 181–193. doi:10.1038/nrc2809

Wang, D., and DuBois, R. N. (2018). Role of prostanoids in gastrointestinal cancer. *J. Clin. Invest.* 128, 2732–2742. doi:10.1172/jci97953

Wang, T., Jing, B., Xu, D., Liao, Y., Song, H., Sun, B., et al. (2020). PTGES/PGE2 signaling links immunosuppression and lung metastasis in Gprc5a-knockout mouse model. *Oncogene* 39, 3179–3194. doi:10.1038/s41388-020-1207-6

Wang, X., Schoenhals, J. E., Li, A., Valdecanas, D. R., Ye, H., Zang, F., et al. (2016). Suppression of type I IFN signaling in tumors mediates resistance to anti-PD-1 treatment that can Be overcome by radiotherapy. *Cancer Res.* 77 (4), 839–850. doi:10.1158/0008-5472.can-15-3142

Wculek, S. K., Cueto, F. J., Mujal, A. M., Melero, I., Krummel, M. F., and Sancho, D. (2020). Dendritic cells in cancer immunology and immunotherapy. *Nat. Rev. Immunol.* 20, 7–24. doi:10.1038/s41577-019-0210-z

Wculek, S. K., and Malanchi, I. (2015). Neutrophils support lung colonization of metastasis-initiating breast cancer cells. *Nature* 528, 413–417. doi:10.1038/nature16140

Wejksza, K., Lee-Chang, C., Bodogai, M., Bonzo, J., Gonzalez, F. J., Lehrmann, E., et al. (2013). Cancer-produced metabolites of 5-lipoxygenase induce tumor-evoked regulatory B cells via peroxisome proliferator-activated receptor α. *J. Immunol.* 190, 2575–2584. doi:10.4049/jimmunol.1201920

Wen, Z., Liu, H., Li, M., Li, B., Gao, W., Shao, Q., et al. (2015). Increased metabolites of 5-lipoxygenase from hypoxic ovarian cancer cells promote tumor-associated macrophage infiltration. *Oncogene* 34, 1241–1252. doi:10.1038/onc.2014.85

Whiteside, T. L. (2008).The tumor microenvironment and its role in promoting tumor growth. *Oncogene* 27, 5904–5912. doi:10.1038/onc.2008.271

Yan, G., Zhao, H., Zhang, Q., Zhou, Y., Wu, L., Lei, J., et al. (2018). A RIPK3-PGE2 circuit mediates myeloid-derived suppressor cell-potentiated colorectal carcinogenesis. *Cancer Res.* 78, 5586–5599. doi:10.1158/0008-5472.can-17-3962

Yang, H., Wang, M., Sun, H., Zhu, S., and Jin, J. (2019). Synergetic effect of EP1 receptor antagonist and (-)-Epigallocatechin-3-gallate in hepatocellular carcinoma. *Pharmacology* 104, 267–275. doi:10.1159/000502076

Yang, R., Chiang, N., Oh, S. F., and Serhan, C. N. (2001). "Metabolomics-lipidomics of eicosanoids and docosanoids generated by phagocytes," in *Current protocols in immunology*. Editor J. Coligan (Hoboken NJ: John Wiley).

Yip-Schneider, M. T., Barnard, D. S., Billings, S. D., Cheng, L., Heilman, D. K., Lin, A., et al. (2000). Cyclooxygenase-2 expression in human pancreatic adenocarcinomas. *Carcinogenesis* 21, 139–146. doi:10.1093/carcin/21.2.139

Yokomizo, T., Nakamura, M., and Shimizu, T. (2018). Leukotriene receptors as potential therapeutic targets. *J. Clin. Invest.* 128, 2691–2701. doi:10.1172/jci97946

Young, M. R. I. (1994). Eicosanoids and the immunology of cancer. *Canc. Metastasis Rev.* 13, 337–348. doi:10.1007/bf00666103

Yun, S. J., Lee, B., Komori, K., Lee, M. J., Lee, B. G., Kim, K., et al. (2019). Regulation of TIM-3 expression in a human T cell line by tumor-conditioned media and cyclic AMP-dependent signaling. *Mol. Immunol.* 105, 224–232. doi:10.1016/j.molimm.2018.12.006

Zelenay, S., van der Veen, A. G., Böttcher, J. P., Snelgrove, K. J., Rogers, N., Acton, S. E., et al. (2015). Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell* 162, 1257–1270. doi:10.1016/j.cell.2015.08.015

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 27 January 2021
doi: 10.3389/fphar.2020.617211

Extracellular ATP Mediates Cancer Cell Migration and Invasion Through Increased Expression of Cyclooxygenase 2

*Shilpa Sharma, Harshit Kalra and Ravi Shankar Akundi**

Neuroinflammation Research Lab, Faculty of Life Sciences and Biotechnology, South Asian University, New Delhi, India

The tumor microenvironment plays a major role in the ability of the tumor cells to undergo metastasis. A major player of tumors gaining metastatic property is the inflammatory protein, cyclooxygenase 2 (COX-2). Several tumors show upregulation of this protein, which has been implicated in mediating metastasis in various cancer types such as of colon, breast and lung. In this report, we show that the concentration of extracellular ATP (eATP) is increased in response to cell death mediated by chemotherapeutic agents such as doxorubicin. By using three different cell-lines—HeLa (cervical), IMR-32 (neuronal) and MCF-7 (breast)—we show that this eATP goes on to act on purinergic (P2) receptors. Among the various P2 receptors expressed in these cells we identified P2X7, in IMR-32 and MCF-7 cells, and P2Y12, in HeLa cells, as important in modulating cell migration and invasion. Downstream of the P2 receptor activation, both p42/44 mitogen-activated protein kinase (MAPK) and the p38 MAPK are activated in these cells. These result in an increase in the expression of COX-2 mRNA and protein. We also observe an increase in the activity of matrix metalloproteinase 2 (MMP-2) enzyme in these cells. Blocking the P2 receptors not only blocks migration and invasion, but also COX-2 synthesis and MMP-2 activity. Our results show the link between purinergic receptors and COX-2 expression. Increased levels of ATP in the tumor microenvironment, therefore, leads to increased COX-2 expression, which, in turn, affords migratory and invasive properties to the tumor. This provides P2 receptor-based anti-inflammatory drugs (PBAIDs) a potential opportunity to be explored as cancer therapeutics.

Keywords: cancer recurrence, COX-2, metastasis, P2 receptor, PBAIDs, prostaglandin E2, tumor microenvironment, residual ATP

OPEN ACCESS

Edited by:
Emanuela Ricciotti, University of Pennsylvania, United States

Reviewed by:
Sébastien Roger, Université de Tours, France
Elena Adinolfi, University of Ferrara, Italy

****Correspondence:***
Ravi Shankar Akundi
ravi.shankar@sau.ac.in

Specialty section:
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *14 October 2020*
Accepted: *23 December 2020*
Published: *27 January 2021*

Citation:
Sharma S, Kalra H and Akundi RS (2021) Extracellular ATP Mediates Cancer Cell Migration and Invasion Through Increased Expression of Cyclooxygenase 2. Front. Pharmacol. 11:617211. doi: 10.3389/fphar.2020.617211

Abbreviations: A740003, N-[1-[[(Cyanoamino)(5-quinolinylamino)methylene]amino]-2,2-dimethyl propyl]-3,4-dimethoxybenzeneacetamide; AR-C 69331, 2-(Propylthio)adenosine-5′-O-(β,γ-difluoromethylene)triphosphate tetrasodium salt; ADP, adenosine diphosphate; ATP, adenosine triphosphate; BzATP, 2′(3′)-O-(4-Benzoylbenzoyl)adenosine-5′-triphosphate tri(-triethyl ammonium) salt; COX, cyclooxygenase; eATP, extracellular ATP; MMP, matrix metalloproteinase; MRS2500, 2-iodo-N6-methyl-(N)-methanocarba-2′-deoxyadenosine 3′,5′-bisphosphate; NSAID, nonsteroidal anti-inflammatory drug; oATP, oxidized adenosine triphosphate (adenosine 5′-triphosphate-2′,3′-dialdehyde); PD 98059, 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; PGE2, prostaglandin E2; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; SB 202190, 4-(4-Fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)-1H-imidazole.

INTRODUCTION

Globally, the incidences of cancer detection are on the rise with more than 9.6 million deaths in 2018 alone (Bray et al., 2018). Despite advances in medical research, and an increase in the number of treatable cancers, it still remains one of the deadliest diseases worldwide with recurrence of cancer in treated patients being a major concern (Houssami et al., 2014; Holleczek et al., 2019). While the actual causes of tumor recurrence is not known, it is dependent on tumor size and its microenvironment (Avanzini and Antal, 2019). The tumor microenvironment has a range of inflammatory molecules that affect its progression and metastasis depending on the stage of the tumor and the immune phenotype (Wang and DuBois, 2015). One of these inflammatory modulators is the enzyme cyclooxygenase 2 (COX-2), responsible for the synthesis and release of prostaglandin E_2 (PGE_2) (Wang et al., 2007; Nakanishi and Rosenberg, 2013). The role of COX-2 in inflammation is well established (Akundi et al., 2005; Minghetti, 2007). In recent years, its role in cancer has also been observed, especially related to tumors acquiring metastatic potential and chemotherapy resistance (Ferrandina et al., 2002; Li et al., 2002). Many cancer types such as leukemia, breast cancer, pancreatic cancer, lung cancer and lymphomas show overexpression of COX-2 (Nakanishi et al., 2001; Ristimaki et al., 2002; Wun et al., 2004; Secchiero et al., 2005). This increase in COX-2 has been shown to enhance tumor progression through increased apoptosis resistance and increased metastatic properties (Choi et al., 2005; Singh et al., 2007; Karavitis and Zhang, 2013). Gonadotropins such as follicle-stimulating hormone or luteinizing hormone have been shown to increase migration and invasion in ovarian cancer cells via COX-2 (Feng et al., 2017). Although the nuclear factor κB (NF-κB) pathway has been implicated in the increased expression of COX-2 in cancer cells (Kim et al., 2014; Kuang et al., 2017), further upstream causes are not completely known.

On the other hand, several reports support the role of purinergic receptors in modulating tumor growth (Deli and Csernoch, 2008; Di Virgilio et al., 2018). Therapeutic interventions such as irradiation and chemotherapy have been shown to increase the levels of extracellular nucleotides in several organs (Schneider et al., 2015). Under normal physiological conditions, although there is only a few nmol/L concentration of ATP in the extracellular space, it has been shown to increase to several mmol/L levels under physiological stress such as hypoxia, inflammation and cancer (Khakh and North, 2006; Idzko et al., 2014). In addition to a non-regulated release of ATP from dying/damaged cells, active release of ATP also occurs through exocytic granules, microvesicles and various transporters and channels located on the tumor cells (Vultaggio-Poma et al., 2020). The role such huge amounts of extracellular ATP (eATP) may play in the tumor microenvironment has been debated with both pro- and anti-tumourogenic outcomes (Jiang et al., 2015; Avanzato et al., 2016; Pavlovic et al., 2020). This outcome largely depends on the type of receptor eATP acts on, of which there are ion-channel associated P2X receptors, G protein-coupled P2Y receptors, and adenosine-specific adenosine receptors (Fiebich et al., 2014). Expression of these purinergic receptors is found to be higher in tumor cells, thereby making them relevant to tumor progression (Di Virgilio et al., 2018). The type of P2 receptor expressed and the concentration of ATP in the microenvironment, which in turn is dependent on the activity of ectonucleotidases (Mandapathil et al., 2018; De Marchi et al., 2019; Soleimani et al., 2019), determine the progression of tumor. How the purinergic receptor activation mediates tumor progression is not completely known. ATP released by chemotherapy-sensitive cells can have an immunosuppressive function; thereby assisting tumor cells evade immune surveillance (Lecciso et al., 2017). However, mechanisms through which eATP enhances tumor progression remains to be addressed.

We have previously hypothesized that eATP released by dying cells acts as a second hit during inflammation leading to enhanced expression of COX-2 (Fiebich et al., 2014). In this study, we have extended this hypothesis to tumor cell progression. We propose that the high concentration of eATP in the tumor microenvironment is responsible for the increased COX-2 expression in tumor cells leading to their attaining metastatic properties. We show this through the use of different P2 receptor agonists and antagonists on tumor cell migration and invasion and expression of COX-2 in these cells. Our report, for the first time, provides evidence of the link between increased purinergic receptor expression in tumor cells and of increased COX-2 expression which is responsible for imparting metastasis.

MATERIALS AND METHODS

Cell Culture and Reagents

All reagents were purchased from Sigma Aldrich (Bengaluru, India), HiChem Life Sciences (Ghaziabad, India), or Fisher Thermo Scientific India (Mumbai, India). The human cervical cancer cell line, HeLa (kindly provided by Dr Yubaraj Pokharel, South Asian University, New Delhi, India), the breast cancer cell line, MCF-7 (kindly provided by Dr Seema Sehrawat, Shiv Nadar University, Dadri, India) and the human neuroblastoma cell-line, IMR-32 (from National Center for Cell Science, Pune, India), were cultured in Dulbecco's MEM supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 25 µg/ml amphotericin B, 1 mM sodium pyruvate, 2 mM L-glutamine and 10% heat-inactivated fetal calf serum (HiChem Life Sciences). Cells were cultured at a density of 5×10^4 cells/cm^2 in various formats (6-, 24- or 96-well plates) as per experimental requirement and were stimulated the following day when the cells reached ~70% confluency. Media was changed an hour prior to stimulation with the inhibitors added 30 min prior to the addition of ATP or P2 receptor agonists. ATP, apyrase and suramin were from Sigma Aldrich while all other P2 receptor agonists and antagonists, MAPK inhibitors, and COX inhibitors were obtained from Tocris Biosciences (Bio-Techne India, Pune, India). Doxorubicin was bought from HiMedia (HiChem Life Sciences).

Cell Death Analysis

HeLa, IMR-32, and MCF-7 cells were stimulated with 200 nM doxorubicin for 48 h. Cells were then washed, resuspended in ice-cold phosphate-buffered saline (PBS) and fixed in ethanol. Cells

were then stained with propidium iodide (PI, 20 μg/ml) in PBS for 10 min. PI-positive cells, indicating apoptosis, were counted on a flow cytometer and analyzed using BD FACS Suite software (BD Biosciences, San Jose, CA).

Quantitation of ATP Release

Extracellular ATP was measured in the culture supernatants of different cells treated with doxorubicin for 48 h. A bioluminescent ATP assay kit (Promega, New Delhi, India) was employed as per the manufacturer's instructions. Luminescence was recorded with a microplate reader (Synergy HT Biotek). To calculate the concentration of ATP released in the supernatant, an ATP standard curve of 0.78–500 nM range was employed.

Wound Healing Assay

Wound healing or scratch assay was performed as described elsewhere (Liang et al., 2007). Cells were plated in a 6-well plate and allowed to form a uniform monolayer. Scratch was made using a 200 μl tip in each well. The scratched wells were washed twice using media prior to treatment. The zero hour pictures of 6 random scratch areas were taken using a Nikon microscope. The plates were incubated in a humidified 5% CO_2 incubator kept at 37 °C and the same areas were imaged at different time intervals. For analysis percentage area healed was calculated using NIH ImageJ software (https://imagej.nih.gov/ij).

In vitro Migration Assay

Migration assay was performed as described elsewhere in a 24-well plate wherein Transwell inserts (Corning) of 8 μm pore size were placed (Liang et al., 2007). Cells were seeded at a density of 10,000 cells/insert on the upper chamber in serum-free media. Complete media containing the respective treatment was poured in the lower chamber of the transwell setup. At the end of the incubation point (27 h for HeLa, 18 h for IMR-32, or 12 h for MCF-7 cells), cells on the upper chamber were scrapped while the migrated cells from the lower side of the membrane were fixed in 70% ethanol and stained with 1 mg/ml Hoechst 44,432 for 5 min. The stained cells were imaged under a fluorescence microscope and counted using the NIH ImageJ software (https://imagej.nih.gov/ij).

Cell Invasion Assay

Transwell migration assay was modified using 0.1 mg/ml matrigel matrix (Corning) coating. 20,000 cells were plated above the matrigel coating in the transwell insert and allowed to invade along the treatment gradient. At the end of incubation (27 h for HeLa, 18 h for IMR-32, or 12 h for MCF-7 cells), cells on the upper chamber were scrapped while the invaded cells from the lower side of the membrane were fixed in 70% ethanol and stained with 1 mg/ml Hoechst 44,432 for 5 min. The stained cells were imaged and counted as described above.

Western Blot

Total cell lysates were prepared in a lysis buffer composed of 42 mM Tris-HCl, pH 6.8, 1.3% (w/v) sodium dodecylsulfate, 6.5% glycerol, 0.1 mM sodium orthovanadate, and protease inhibitor cocktail (from Sigma-Aldrich). Protein content was measured using the bicinchoninic acid method (Thermo Fisher Scientific) using bovine serum albumin (BSA) as standard. 2-Mercaptoethanol (final concentration 1%) and bromophenol blue (0.2 mg/ml) were added to the samples and heated at 95 °C for 5 min before electrophoresis. In total, 20–50 μg samples were loaded on a 7.5% (for COX-2 and MMP-2) or 12% (for p38 and p42/44 MAPK) polyacrylamide gel under reducing conditions. Separated proteins were transferred onto a polyvinylidene fluoride membrane (Merck LifeSciences, Mumbai, India) and blocked for 1 h with 5% BSA in Tris-buffered saline containing 0.1% Tween-20 (TBS-T) followed by primary antibody at 4 °C overnight. Primary antibodies used were rabbit anti-COX-2, rabbit anti-MMP-2, rabbit anti-phospho-p42/44 MAPK (detecting endogenous levels of p42/44 only when dually phosphorylated at Thr^{202} and Tyr^{204} of Erk1 and Thr^{185} and Tyr^{187} of Erk2), rabbit anti-phospho-p38 MAPK (detecting endogenous levels of p38 MAPK only when phosphorylated at Thr^{180} and/or Tyr^{182}), rabbit anti-p42/44 MAPK, and rabbit anti-p38 MAPK (all from Cell Signaling Tech, Danvers, MA, United States and used at 1:1,000 dilution in TBS-T containing 1% BSA). For the normalization of protein loaded, mouse anti-β-actin (Sigma-Aldrich) was used at 1:5,000 dilution. Secondary antibody was diluted 1:10,000 in 1% BSA in TBS-T for 1 h at RT and washed extensively. Proteins were detected using chemiluminescent solution made by mixing equal volumes of solution A (2.5 mM luminol, 0.396 mM p-coumaric acid and 0.1 M Tris-HCl, pH 8.5) and solution B (5.2 mM H_2O_2 and 0.1 M Tris-HCl pH 8.5).

TABLE 1 | List of primers used in the study.

Gene		Primer Sequence	Gene ID
P2X1	F	5′-TACGTGGTGCAAGAGTCAGG-3′	1519314411
	R	5′-CCAGGTCACAGTGCCAGTC-3′	
P2X4	F	5′-GCTTTCAACGGGTCTGTCA-3′	330448876
	R	5′-AGTGAAGTTTTCTGCAGCCTTT-3′	
P2X7	F	5′-GCGGTTGTGTCCCGAGTAT-3′	1854511
	R	5′-CCTTCCGGTCTGAATTCCTT-3′	
P2Y1	F	5′-CCCGAAACTGAGCTGCAC-3′	1519314089
	R	5′-TCAACTTAATTGGGGCATC-3′	
P2Y2	F	5′-CCTCAAGACCTGGAATGCGT-3′	20380402
	R	5′-GTAATAGACCAGCAGCGGCA-3′	
P2Y4	F	5′-TGGCAGTTTGGTTGGTCGTA-3′	109730011
	R	5′-TGGTCCCTTTGTTGCTGGTT-3′	
P2Y6	F	5′-CACCCACCACCTGTGTCTAC-3′	1407632
	R	5′-ACACAGATGTTCAGCGGCAG-3′	
P2Y12	F	5′-CTCTCTGTTGTCATCTGGGCA-3′	17389766
	R	5′-GCTGCCTGTTGGTCAGAATC-3′	
COX-2	F	5′-TGAGCATCTACGGTTTGCTG-3′	80,142
	R	5′-ATCATCAGACCAGGCACCA-3′	
β-Actin	F	5′-CCAACCGCGAGAAGATGA-3′	178023
	R	5′-CCAGAGGCGTACAGGGA-3′	

Gelatin Zymography

The proteolytic activity of matrix metalloproteinase (MMP-2) was analyzed by substrate-gel electrophoresis method as described elsewhere (Toth et al., 2012). Briefly, cells were plated in 6-well plates in complete media while treatment was

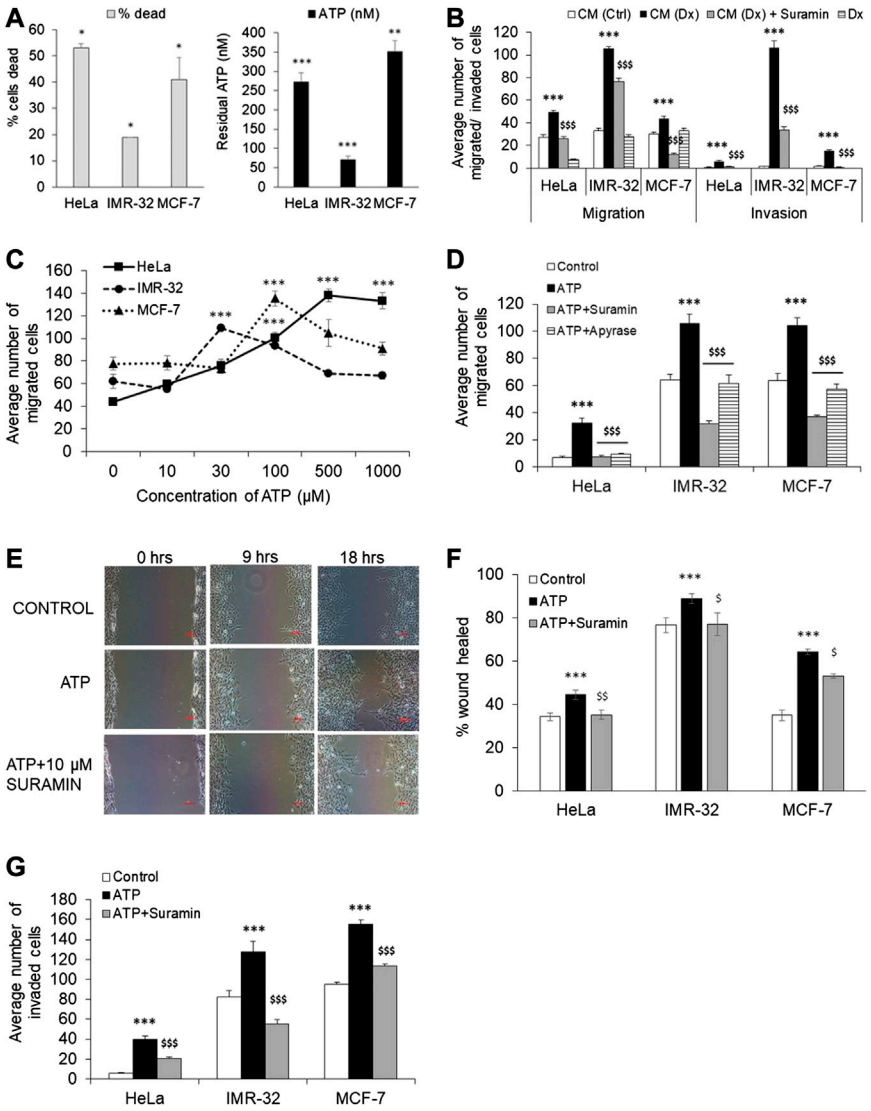


FIGURE 1 | Extracellular ATP enhances cancer cell migration and invasion. **(A)** HeLa, IMR-32 and MCF-7 cells were treated with 200 nM doxorubicin for 48 h following which the media was collected for measuring ATP levels (black bars) while the cells were stained with propidium iodide for cell death analysis through flow cytometry (gray bars). 10,000 cells (for HeLa and MCF-7) and 30,000 cells (for IMR-32) were run on a flow cytometer and the % cells dead depicted in gray bars. The amount of ATP released in the media was calculated based on a standard curve and represented here (n = 3 independent experiments). Data depicted mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Student's t-test. **(B)** Cell migration was assessed by culturing cells on top of a transwell insert in serum-free media while media containing serum was placed in the lower chamber. The same set up was used for invasion studies wherein the cells were instead plated on top of a matrigel in the transwell insert. Migration/invasion was analyzed 24 h after the addition in the lower chamber media from untreated HeLa cells (CM-Ctrl), from HeLa cells treated with 200 nM doxorubicin (CM-Dx), CM-Dx with addition of 10 μM suramin, or only doxorubicin (Dx). Cell counts from the lower chamber are depicted as mean ± SEM and are from three independent experiments. ***$p < 0.001$ with respect to cells treated with CM-Ctrl, $^{\$\$\$}p < 0.001$ with respect to cells treated with CM-Dx, for the same cell type, by Student's t-test. **(C)** Migration assay as described in **(B)** was performed in each cell-line stimulated with different concentration of ATP. The number of migrated cells to the bottom of the Transwell were counted and depicted as mean ± SEM. ***$p < 0.001$ with respect to unstimulated cells of the same type. **(D)** The migration assay as described in **(B)** was performed in each cell-line which were stimulated with ATP (1 mM for HeLa, 0.1 mM for MCF-7, and 30 μM for IMR-32) in the presence or absence of 10 μM suramin or 5 U/ml apyrase. Cell counts were done as in **(B)**. ***$p < 0.001$ with respect to unstimulated cells, $^{\$\$\$}p < 0.001$ with respect to ATP-treated cells of the same type. **(E)** Representative images of the wound healing assay in IMR-32 cells is depicted. Scale bar = 100 μ. **(F)** Confluent cultures of HeLa, IMR-32 or MCF-7 were subjected to a scratch followed by stimulation with ATP as mentioned in **(D)** above, alone or together with 10 μM suramin. The percentage of the wound healed after 27 h (for HeLa), 18 h (IMR-32) or 12 h (MCF-7) is depicted here. Paired Student's t-test shows significance of $p < 0.001$ with respect to control (***) or ATP-treated ($^{\$}p < 0.05$, $^{\$\$}p < 0.01$) cells for the respective cell-type ($n = 5$ independent experiments). **(G)** Cell invasion assay was conducted as mentioned in **(B)** with cells treated with ATP, alone or together with 10 μM suramin, as in **(F)**. Paired t-test shows significance of $p < 0.001$ with respect to control (***) or ATP-treated ($^{\$\$\$}$) cells.

given under serum-free conditions. The culture supernatant was collected, mixed with non-reducing dye and run on a SDS-PAGE gel containing 0.4% (m/v) gelatin. At the end of electrophoresis, the gels were washed in a buffer comprising of 2.5% Triton X-100, 5 mM calcium chloride, and 1 μM zinc chloride and incubated for 16–27 h in the activation buffer (1% Triton X-100, 0.2 M sodium chloride, 5 mM calcium chloride, and 1 μM zinc chloride). The gels were then stained with Coomassie blue wherein the presence of any clear white bands were indicative of the gelatinolytic activity of MMP-2.

RNA Isolation and Real Time PCR

Total cellular RNA was isolated from cells using Trizol reagent (Fisher ThermoScientific) as per the manufacturer's instructions. 2 μg total RNA was used for cDNA synthesis using the PrimeScript first strand cDNA Synthesis kit (DSS Takara, Advaita Biosciences). 1 μl of cDNA sample was used for real time PCR using SYBR Green Master Mix and 0.2 μM each of forward and reverse primers which were designed through Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and ordered through a commercial vendor (IDT). Sequences for the primers are tabulated in **Table 1**. The reactions were carried out in Applied Biosystem ViiA™ 7 system and mRNA expression analyzed using the $\delta\delta C_t$ method. Fold change was calculated as $2_t^{-\delta\delta C}$ where δC_t is the difference between the C_t values of COX-2/P2 receptor and β-actin for each cDNA sample and $\delta\delta C_t$ is the difference between the δC_t values of stimulated condition (ATP and its agonists along with P2 blockers) with that of the unstimulated one (control).

Statistical Analysis

All experiments were done in triplicates with each experiment made on cultures derived from at least three different thawed vials for each cell-line. Migration and invasion analyses were counted from at least 15 different fields per transwell from each passage. Normal distribution of data was assessed using the Shapiro-Wilk test. Data are presented as mean ± standard error of the mean (SEM) and were analyzed using Student's *t*-test, using Microsoft Excel software. Differences were considered significant when $p < 0.05$.

RESULTS

Doxorubicin Increases Levels of Extracellular ATP in Correlation to Cell Death

We had hypothesized that during chemotherapy drug-sensitive cells undergo cell death wherein their cellular contents, including ATP, are released into the microenvironment. To test this hypothesis, we checked the effect of the common chemotherapeutic agent, doxorubicin, on three different cell-lines—HeLa (cervical), IMR-32 (neuronal) and MCF-7 (breast). Flow cytometric data showed that at 200 nM dose, doxorubicin mediated 19% cell death in IMR-32 cells, 41% death in MCF-7 cells and 53% death in HeLa cells, 48 h post-stimulation (**Figure 1A**). Corresponding to this increase in cell death, there was also a concomitant increase in the levels of extracellular ATP (eATP) in the culture media in all 3 cases. In the case of HeLa cells, this corresponded to an increase to 272 ± 25 nM ATP detected in the media (3-fold over the levels measured in unstimulated cells, $p < 0.01$). The corresponding levels of eATP detected in the case of IMR-32 and MCF-7 cells were 71 ± 9 nM (15-fold, $p < 0.01$) and 351 ± 29 nM (5-fold, $p < 0.001$) after stimulation with doxorubicin. It has to be noted that these values represent residual eATP levels at the end of 48 h post-stimulation with doxorubicin. These results indicated that doxorubicin-mediated cell death resulted in a concomitant increase in the levels of eATP in the media which remain significantly high up to 48 h post-stimulation with doxorubicin.

Conditioned Media From Doxorubicin-Treated Cells Promotes Cancer Cell Migration and Invasion

In order to test whether the released ATP measured in the media obtained from dying cells promoted cancer cell metastasis, we used media obtained from HeLa cells treated with 200 nM doxorubicin (Dx) for 24 h as conditioned medium (CM-Dx) in migration and invasion assays. These assays were done in a Boyden chamber wherein cells were grown on top of a transwell insert in serum-free media while the conditioned media was added in the lower chamber. In the case of invasion assay, cells were grown on top of a thin matrigel layer on top of the insert. As control, we also used media obtained from unstimulated HeLa cells (CM-Ctrl). We found that conditioned media obtained from doxorubicin-treated cells (CM-Dx) significantly increased both migration and invasion in all three cell-lines (**Figure 1B**). CM-Dx-mediated 1.8-fold and 9-fold increase in migration and invasion, respectively, in HeLa cells ($p < 0.001$). Similarly, in MCF-7 cells, CM-Dx-mediated 1.5-fold and 10-fold increase in migration and invasion, respectively ($p < 0.001$). The highest migration (3-fold) and invasion (80-fold) was observed in the case of IMR-32 cells ($p < 0.001$). To confirm that the ATP present in the conditioned media was acting through P2 receptors, we co-stimulated the cells with suramin, a pan-P2 receptor antagonist. In all the three cell-lines, suramin significantly blocked both migration and invasion induced by CM-Dx ($p < 0.001$). We also confirmed that the presence of doxorubicin in CM-Dx did not influence cell migration by stimulating cells directly with doxorubicin. We found no significant change in the number of migrating cells when treated with doxorubicin alone (**Figure 1B**). A recent report shows that doxorubicin mediates MCF-7 cell migration through rhoA/myosin light chain 2 pathway (Liu et al., 2019). However, in that report cells were treated with doxorubicin for 3 h in a 6-well plate followed by their culture in the Transwell insert where migration was checked after 48 h. In our experiment, cells were directly stimulated on the Transwell insert and migration was checked after 12 h in the case of MCF-7 cells. Overall, our results suggest that media obtained from dying cells contains ATP whose effect on tumor cell migration and invasion could be blocked by a non-specific P2 receptor antagonist, suramin.

Exogenous ATP Mediates Migration in Different Cancer Cells

Since suramin significantly inhibited CM-Dx-mediated cell migration and invasion, we further confirmed the role of purinergic receptors in this process by directly adding ATP to the media exogenously. To test the concentration of ATP that promotes significant migration in cancer cells, we exogenously added ATP at different doses and measured cell migration using the Boyden chamber assay (**Figure 1C**). We found a dose-dependent increase in cell migration only in the case of HeLa cells which showed significant numbers of migrated cells from 10 μM onwards with the highest seen with 1 mM ATP. In the case of IMR-32 cells, maximal migration was observed with 30 μM ATP ($p < 0.001$) and was insignificant at 1 mM ATP. MCF-7 cells, on the other hand, showed significant migration at 100 μM ($p < 0.001$). Based on these preliminary observations, ATP was used at 1 mM for HeLa cells, 30 μM for IMR-32 cells and 100 μM for MCF-7 cells in all the subsequent experiments.

In order to further confirm that ATP is responsible for the migration of cells in the Boyden chamber assay, we used an ATP degrading enzyme, apyrase, which hydrolyzes ATP to AMP and inorganic phosphate. Exogenously added ATP increased migration in HeLa cells by 4.7-fold which was significantly inhibited in wells which were also treated with apyrase (**Figure 1D**). Similarly, the increase in eATP-mediated migration in IMR-32 and MCF-7 cells was also significantly inhibited by 45% in cells that were also co-treated with apyrase ($p < 0.001$ with respect to ATP-treated cells). These results suggest that migration is mediated through ATP, and that its hydrolysis by apyrase abolishes this property in all the three cell-lines.

Suramin Inhibits Cell Migration and Invasion Mediated by Exogenous ATP

The inhibition of CM-Dx-mediated cell migration by suramin and eATP-mediated migration by apyrase suggest that eATP acts through purinergic receptors. Accordingly, the effect of pan-P2 receptor antagonist, suramin, was tested in all three cell-lines. In the Boyden chamber assay, the number of migrated cells was significantly reduced by 77% in the case of HeLa cells which were treated with both ATP and suramin (**Figure 1D**). In the same way, we found 70 and 64% reduction in the number of migrated cells in the case of IMR-32 and MCF-7 cells which were co-treated with suramin ($p < 0.001$ with respect to ATP-treated cells). This suggested that eATP-mediated migration of cancer cells could be significantly inhibited through the use of a P2 receptor antagonist.

To further confirm that suramin prevented cancer cell progression, migration and proliferation of cells was measured through a wound healing assay wherein a wound/scratch was created in the center of the monolayer (**Figures 1E,F**). ATP significantly increased the closure of the wound in all three cell-lines ($p < 0.05$). In HeLa cells, the recovered area was 34 ± 2% at 27 h in control cells which was further increased to 44 ± 2% in cells treated with ATP ($p < 0.001$). In contrast, in cells that were co-stimulated with both ATP and suramin, the recovered wound area was the same as in control cells at 35 ± 2%, indicating that suramin blocked ATP-mediated wound closure ($p < 0.01$ with respect to ATP-treated cells). Similarly, in IMR-32 cells, 76 ± 3% wound was recovered in control cells at 18 h, while in ATP-treated cells the recovered wound was 89 ± 2% ($p < 0.001$). As in the case of HeLa cells, treatment with suramin failed the recovery of the wound and remained at 77 ± 5% ($p < 0.05$). In the case of more proliferative MCF-7 cells, the scratch was recovered by 35 ± 2% within 12 h in control cells which was greatly enhanced in cells treated with ATP (64 ± 1%, $p < 0.001$). In cells that were treated with both ATP and suramin, only 53 ± 1% area was recovered ($p < 0.05$ compared to ATP-treated cells). These results indicated that exogenous ATP mediated accelerated wound closure which was significantly inhibited through the use of a pan-P2 receptor antagonist.

Supporting this data, we also found that eATP mediated 7-fold increase in HeLa cells invasion through the matrigel ($p < 0.001$) which was also significantly inhibited by suramin (**Figure 1G**). In the case of IMR-32 cells, the invasion process was strongly inhibited with only 43% cells invaded compared to cells treated with eATP ($p < 0.001$). Similar result was observed with MCF-7 cells, wherein eATP-mediated invasion could be significantly inhibited by suramin. These results support the role of P2 receptors in cell migration and invasion.

TABLE 2 | Relative expression of P2 receptors in the three cell-lines.

	HeLa[a]	IMR-32	MCF-7
P2X1	21.6	17.9	18.7
P2X4	14.2	17.4	**9.7**
P2X7	17.7	**12.9**	15.2
P2Y1	22.4	n.d.	15.3
P2Y2	13.8	17.1	13.6
P2Y4	17.1	14.7	17.5
P2Y6	**7.9**	16.0	**11.8**
P2Y12	15.8	16.7	14.6

[a]*Total mRNA was isolated from unstimulated HeLa, IMR-32 and MCF-7 cells and cDNA synthesized.*

*Real time PCR analysis was done using specific primers given in **Table 1**. The mean δC_t values obtained from cDNA synthesized from at least three different RNA preparations is depicted here. The δC_t value was calculated from the C_t values of the receptor with that of the C_t value for β-actin. The mean C_t value for β-actin was 12.5 ± 0.2 for HeLa cells, 17.6 ± 0.5 for IMR-32 cells, and 13 ± 0.1 for MCF-7 cells. Entries in bold are receptors with highest expression based on dCt method. n.d.: not determined*

P2X7 Receptor Antagonists Inhibit eATP-Mediated Migration of IMR-32 and MCF-7 Cells

Since suramin could significantly inhibit eATP-mediated migration and invasion in all the three cell lines, we screened the expression of eight P2 receptors (3 P2X and 5 P2Y) through real time PCR. Among the ionotropic receptors, we found strong expression of P2X4 receptors in MCF-7 cells while P2X7 receptors were predominant in the IMR-32 cells (**Table 2**). Among the G protein coupled receptors, we found P2Y6 receptors to be strongly expressed in both HeLa and MCF-7 cells.

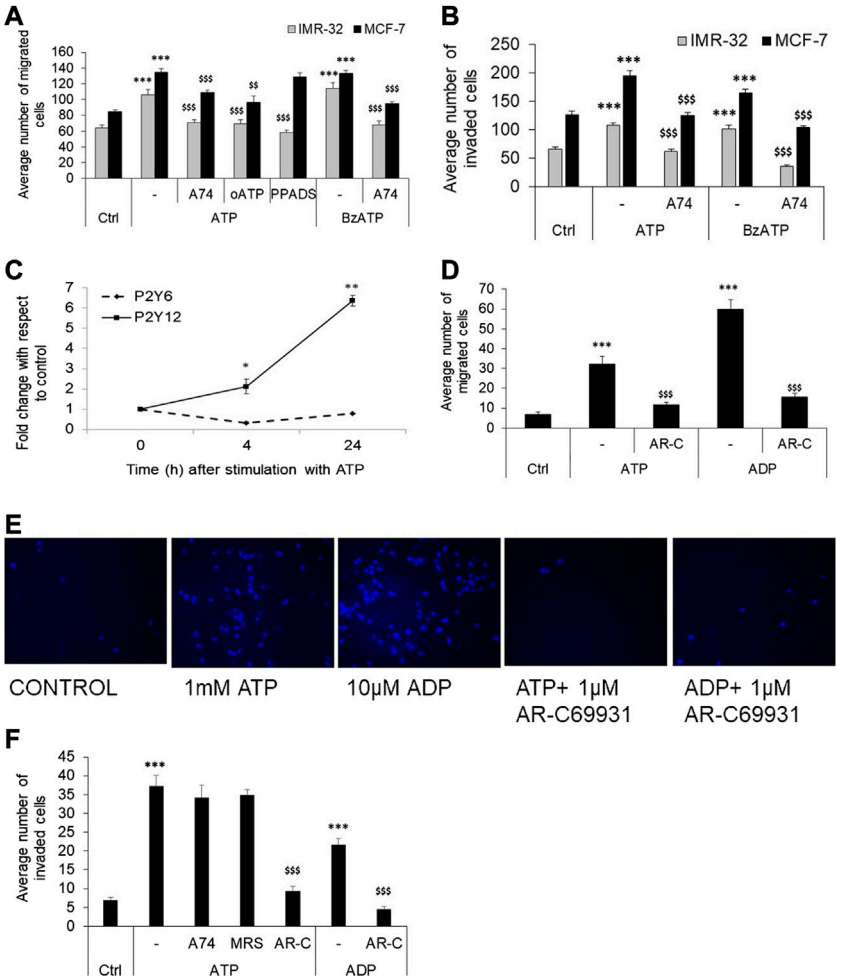


FIGURE 2 | P2X7 and P2Y12 receptor antagonists abolish cancer cell migration and invasion. **(A)** IMR-32 (gray bars) or MCF-7 (black bars) cells were plated on top of a transwell insert and treated with ATP (30 μM for IMR-32 or 100 μM for MCF-7), or 1 μM BzATP, in the presence or absence of 1 μM A740003 (A74), 1 μM oxidized ATP (oATP), or 1 μM PPADS. The number of migrated cells were stained, counted and depicted as mean ± SEM. Student's *t*-test shows significance of $p < 0.001$ with respect to unstimulated (***) or ATP/BzATP-treated ($^{\$\$\$}$) cells ($n = 5$ independent experiments). **(B)** Invasion assay as described in Methods was done in IMR-32 (gray bars) or MCF-7 (black bars) cells stimulated with ATP (30 μM for IMR-32 or 100 μM for MCF-7) or 1 μM BzATP, in the presence or absence of 1 μM A740003 (A74). The number of cells invading through the matrigel were counted and depicted as mean ± SEM. Student's *t*-test shows significance of $p < 0.001$ with respect to unstimulated (***) or ATP/BzATP-treated ($^{\$\$\$}$) cells. **(C)** HeLa cells were treated with 1 mM ATP, followed by RNA isolation at 4 and 24 h post-stimulation, for the analysis of P2Y6 and P2Y12 receptors. Fold change was calculated using $\delta\delta C_t$ method. Significances were calculated with respect to control cells (*$p < 0.05$, or **$p < 0.01$) using paired *t*-test. ($n = 5$ independent experiments) **(D)** HeLa cells were grown in the transwell chambers and stimulated with ATP (1 mM), or ADP (10 μM), with or without AR-C 69931 (1 μM, AR-C). The number of cells migrated to the lower chamber after 24 h was counted and represented as mean ± SEM. Student's *t*-test was used to calculate significance with respect to control cells (***$p < 0.001$), or with respect to ATP or ADP in the case of AR-C 69931 ($^{\$\$\$}p < 0.001$). **(E)** Representative images of HeLa cells, stained with Hoechst 44,432, post-migration in the transwell assay is depicted here. **(F)** HeLa cells were grown in the transwell chambers on top of a matrigel layer and stimulated with ATP (1 mM), or ADP (10 μM), with or without 1 μM AR-C 69931 (AR-C), 1 μM MRS 2500 (MRS), or 1 μM A740003 (A74). Data is depicted as given for **(D)**.

Further supporting these results, we found that exogenous stimulation of IMR-32 and MCF-7 cells with ATP enhanced P2X7 receptor expression by 2 ± 0.25-fold ($p < 0.01$), an effect also observed with the P2X7 receptor agonist, BzATP. Accordingly, we checked the effect of P2X7 receptor stimulation on cell migration and invasion. In both IMR-32 and MCF-7 cells, BzATP significantly increased cell migration, similar to the effect seen with eATP (**Figure 2A**). Furthermore, both eATP- and BzATP-mediated cell migration could be significantly inhibited by the P2X7 receptor-specific antagonist, A740003 ($p < 0.001$). A740003 has an IC_{50} of 40 nM for P2X7 receptors and >100 μM for all other P2X receptors (Illes et al., 2020), suggesting that eATP-mediated migration is dependent on P2X7 receptors. This was further confirmed through the use of another P2X7 receptor antagonist, oxidized ATP (oATP), which also showed significant inhibition by approximately 30%. Although BzATP has a higher specificity to P2X7 receptors (EC_{50} of 5 μM) compared to ATP itself (EC_{50} 100 μM), it also shows higher affinity to P2X1, P2X2, and P2X3 receptors (EC_{50} 2 nM for P2X1 receptors) (Illes et al., 2020).

Accordingly, we used a non-selective antagonist of P2X receptors, PPADS, which has an EC_{50} of 1 μM for P2X1, P2X2, and P2X3 receptors and 10–50 μM for P2X7 receptors (>500 μM for P2X4 receptors). PPADS (used at 1 μM here) showed 40% inhibition of eATP-mediated migration in the case of IMR-32 cells but had no effect on MCF-7 cells. This may likely suggest that other P2X receptors may be involved in eATP-mediated migration in IMR-32 cells; however, the significant inhibition by at least two specific antagonists of P2X7 receptors—A740003 and oATP—in both IMR-32 and MCF-7 cells points out to their role in metastasis.

Confirming these results, the invasion assays also showed that both ATP and BzATP mediated significant increase in the number of cells invading through the matrigel, which could be inhibited in wells where A740003 was used (**Figure 2B**). These results indicate that P2X7 receptors might play an important role in migration and invasion properties of IMR-32 and MCF-7 cells.

eATP-Mediated Migration in HeLa Cells Is P2Y12 Receptor-dependent

Although P2Y6 receptors were strongly expressed in HeLa cells, there was no change in their expression levels when stimulated with eATP (**Figure 2C**). In contrast, we found a potent increase in the expression of P2Y12 receptors by 2 ± 0.3-fold within 4 h of stimulation, and 6 ± 0.2-fold increase 24 h post-stimulation ($p < 0.001$). In order to identify the role of these receptors in HeLa cell migration and invasion, we used P2Y12 receptor agonist, ADP, and P2Y12 receptor antagonist, AR-C 69931, in the transwell assays. ADP has a specificity for P2Y1 (EC_{50} 5.09 μM), P2Y12 (EC_{50} 7.22 μM) and P2Y13 (EC_{50} 7.94 μM) receptors, while AR-C 69931 acts as a competitive antagonist at P2Y12 receptors (IC_{50} 9.4 μM) (Jacobson et al., 2020). A 4.7-fold increase in the migration of HeLa cells was observed with 1 mM ATP which was reduced by 65% in wells co-stimulated with AR-C 69931 ($p < 0.001$, **Figures 2D,E**). Furthermore, 10 μM ADP mediated a 9-fold increase in migration (60 ± 5 cells migrated in comparison to 32 ± 4 in ATP-stimulated condition and 7 ± 1 in case of control), which was also significantly inhibited by AR-C 69931 ($p < 0.001$).

In the invasion assay, 1 mM ATP mediated a 5-fold increase in invasion which was reduced by 75% when co-stimulated with AR-C 69931 ($p < 0.001$) (**Figure 2F**). To rule out the possible role of P2Y1 receptors, we used a very potent and selective antagonist of P2Y1 receptor, MRS2500 (EC_{50} 9.02 μM). There was no inhibition of ATP-mediated invasion in cells co-treated with MRS2500, suggesting that P2Y1 receptors may not be involved in this process, whose expression was also weak in HeLa cells (**Table 2**). Furthermore, since the dose of ATP used was very high, the likely involvement of P2X7 receptors was also ruled out through the use of the specific antagonist, A740003, which showed no inhibition of ATP-mediated invasion. Therefore, among the various inhibitors tested here, we found P2Y12 antagonist to be the most potent in inhibiting ATP-mediated invasion in HeLa cells. Accordingly, we found a 3-fold, but significant, increase in the number of invading cells with 10 μM ADP ($p < 0.001$), which was also reduced by 79% when co-stimulated with AR-C 69931. These results suggest that in HeLa cells P2Y12 receptors may play a major role in cell migration and invasion.

eATP-dependent Migration and Invasion of Tumor Cells Is Abolished by COX-2 Inhibitors

We had previously hypothesized that COX-2 was a downstream enzyme whose expression is modulated following the activation of the P2 receptor pathway (Fiebich et al., 2014). Therefore, we first wanted to identify whether COX-2 was required for P2 receptor-dependent migration and invasion of tumor cells. We used indomethacin, a nonselective COX inhibitor (IC_{50} 230 nM for COX-1 and IC_{50} 630 nM for COX-2), and celecoxib, a COX-2 specific inhibitor (IC_{50} 15 μM for COX-1 and 40 nM for COX-2). The inhibitors alone had no effect on cell migration in all the three cell-lines (**Figures 3A–C**). Addition of ATP led to 4-fold increase in migration and 2.5-fold increase in invasion in HeLa cells. However, co-stimulation with indomethacin significantly reduced both migration and invasion by 89% ($p < 0.001$) and 27% ($p < 0.05$). Celecoxib, on the other hand, reduced migration and invasion by 27% ($p < 0.001$) and 64% ($p < 0.01$).

Similar observations were made in the case of IMR-32 and MCF-7 cells too. In IMR-32 cells, eATP mediated a 2-fold increase in migration ($p < 0.001$, **Figure 3B**) which was reduced by 94% by indomethacin and 47% by celecoxib (both, $p < 0.001$). In the same manner, eATP-mediated invasion in IMR-32 cells was also reduced by 30% by indomethacin and by 58% by celecoxib ($p < 0.001$). In the case of MCF-7 cells, indomethacin inhibited eATP-mediated migration by 39% ($p < 0.001$) and invasion by 18% ($p < 0.05$) while celecoxib inhibited migration by 42% and invasion by 34% (both $p < 0.001$, **Figure 3C**). These results suggested that, across all the three cell-lines tested, exogenously added ATP mediated cancer cell migration or invasion required the activity of the enzyme COX-2.

eATP Mediates COX-2 Expression in Cancer Cell-Lines

Since inhibitors of COX-2 abolished P2 receptor-mediated migration and invasion in cancer cell-lines, we wanted to know whether exogenously added ATP affected COX-2 synthesis. In HeLa cells, stimulation with 1 mM ATP increased COX-2 mRNA expression by 2.6 ± 0.3-fold, which was significantly inhibited by suramin ($p < 0.05$, **Figure 3D**). A similar increase in COX-2 expression was also observed with 10 μM ADP, which was strongly inhibited by AR-C 69931. We then looked at the expression of COX-2 at the protein level for which we first tested the conditioned media obtained from dying cells. Conditioned media (CM) obtained from untreated cells (CM-Ctrl), did not show any COX-2 expression while cells that were exposed to CM obtained from doxorubicin-treated cells (CM-Dox) showed a strong increase in COX-2 expression (**Figure 3E**), suggesting that CM-Dox contains sufficient ATP coming from the dead cells to induce COX-2 expression in naïve cells.

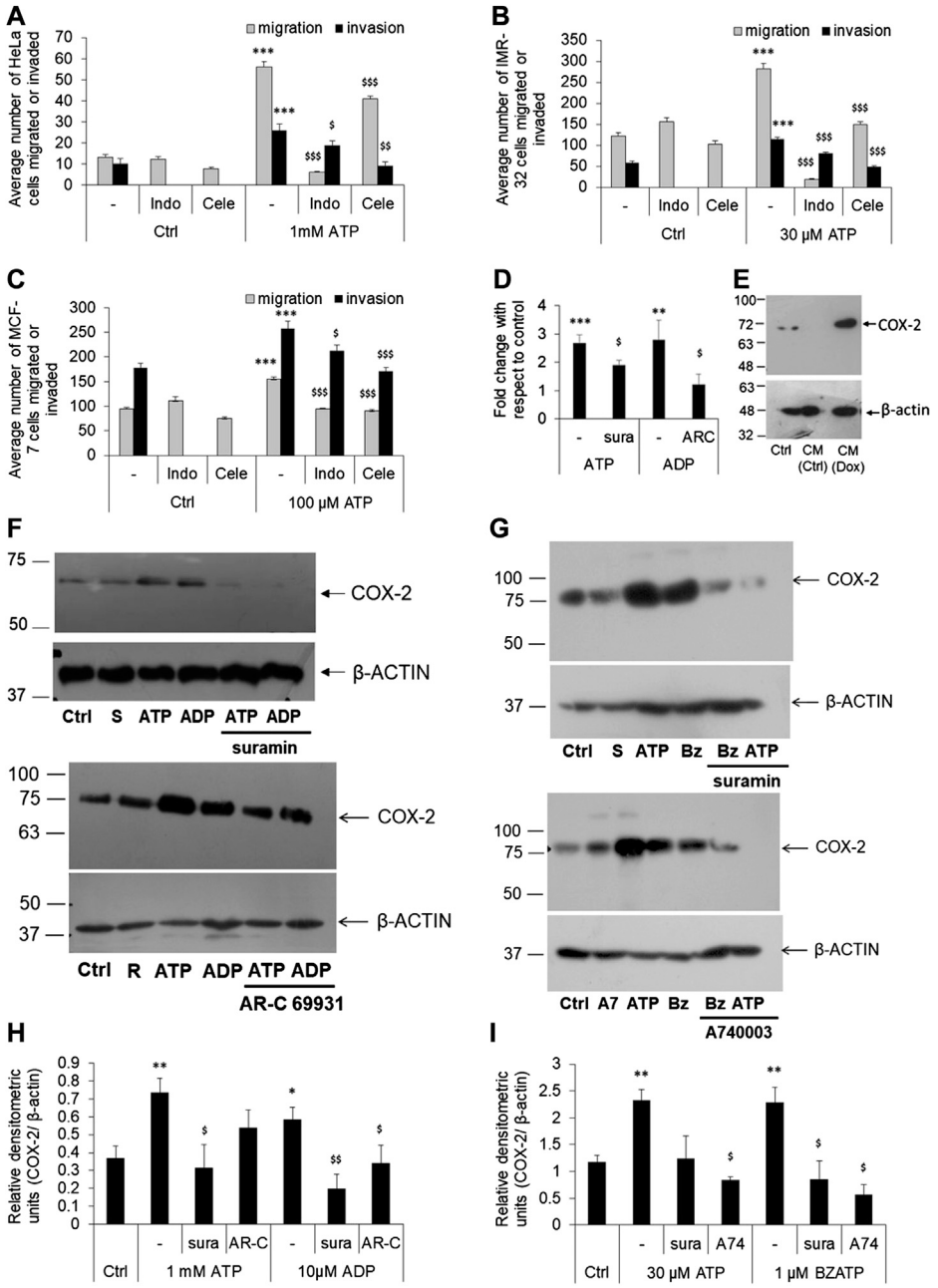


FIGURE 3 | P2 receptor-mediated cell migration or invasion is dependent on COX-2 expression. **(A–C)** HeLa **(A)**, IMR-32 **(B)** or MCF-7 **(C)** cells were plated on transwell inserts directly (for migration assay) or on top of a layer of matrigel (for invasion assays) and stimulated with ATP, with or without indomethacin (indo), or celecoxib (Cele)—both used at 100 nM. After 24 h, migrated/invaded cells from the bottom of the chamber were stained, counted, and depicted here as average number of cells ±SEM. Significances were calculated with respect to control cells (***$p < 0.001$) for ATP-treated cells, or with respect to ATP for the antagonists ($^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$) ($n = 5$ independent experiments). **(D)** HeLa cells were stimulated with ATP (1 mM), alone or with suramin (sura, 10 μM), or with ADP (10 μM), alone or with AR-C 69931 (AR-C, 1 μM). Total RNA was isolated for cDNA synthesis followed by real time PCR analysis for COX-2 expression. Fold change was calculated using $\delta\delta C_t$ method using β-actin as a housekeeping control. Significances were calculated with respect to control cells (**$p < 0.01$, or ***$p < 0.001$) for the agonists, or with respect to ATP or ADP in the case of antagonists ($^{\$}p < 0.05$) through paired *t*-test. **(E)** HeLa cells were treated with conditioned media (CM) obtained from doxorubicin-treated (CM-Dox) or normal (CM-Ctrl) cells as described in **Figure 1B**. 24 h after stimulation cell lysates were prepared for COX-2 expression. **(F,H)** HeLa cells were stimulated with 1 mM ATP or 10 μM ADP, with or without 10 μM suramin (sura) or 1 μM AR-C 69931 (AR-C) for 24 h. Cell lysates were then collected and run on SDS-PAGE for analysis of COX-2 expression. β-actin was used as a housekeeping control for relative densitometric analysis. A representative Western blot image is shown in F (S, suramin; R, AR-C 69931). Replicates of four Western blots from different passages of cells were used for densitometric analysis. **(G,I)** IMR-32 cells were stimulated with 30 μM ATP or 1 μM BzATP, with or without 10 μM suramin (sura) or 1 μM A740003 (A74) for 24 h. A representative image is shown in F (S, suramin; A7, A740003; Bz, BzATP) and densitometric analysis in **(I)**. Significances were calculated with respect to control cells (*$p < 0.05$, **$p < 0.01$) for the agonists, or with respect to ATP or ADP/BzATP in the case of antagonists ($^{\$}p < 0.05$, $^{\$\$}p < 0.01$).

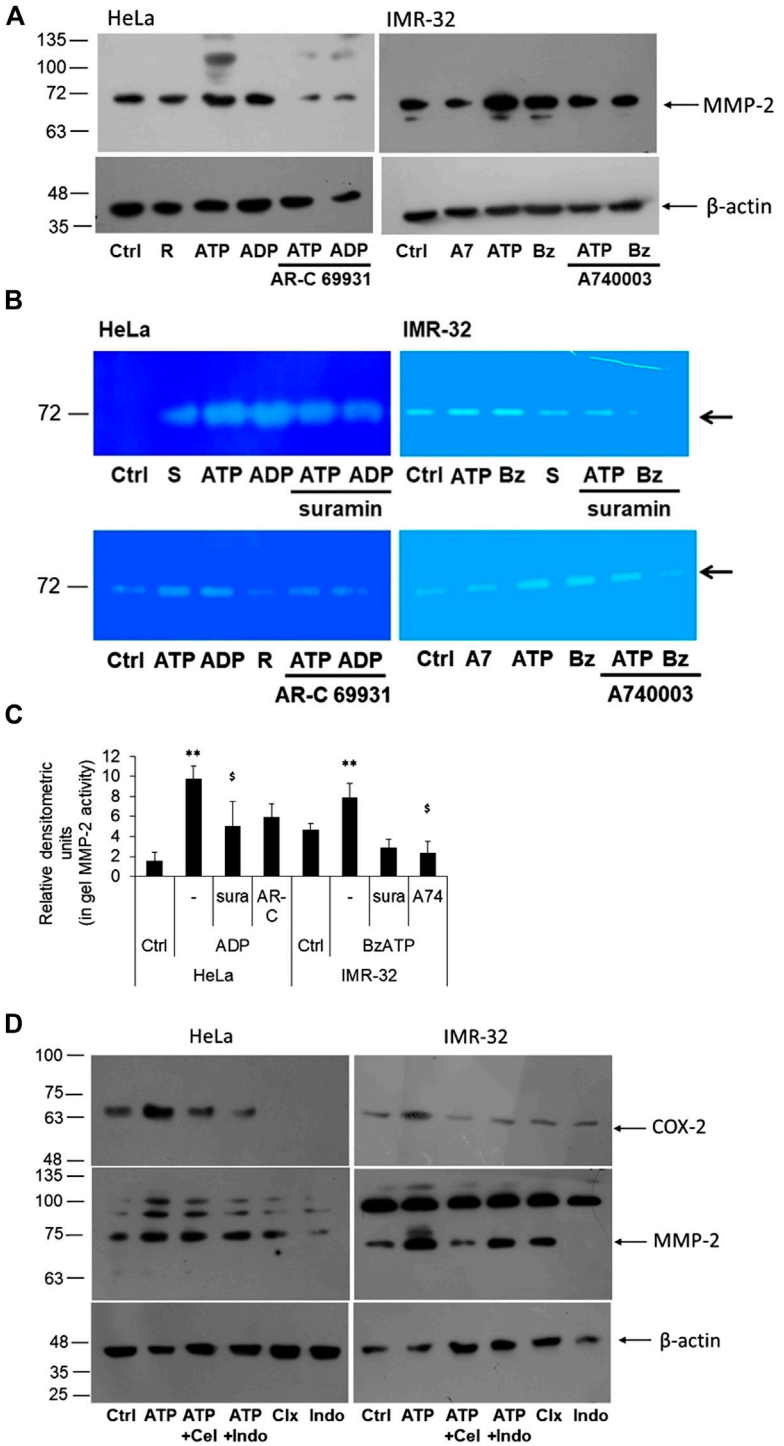


FIGURE 4 | P2 receptor antagonists block eATP-mediated MMP-2 activity. **(A)** HeLa and IMR-32 cells were stimulated with ATP (1 mM or 30 μM, respectively), 10 μM ADP or 1 μM BzATP (Bz), with or without the inhibitors AR-C 69931 (R, 1 μM) or A740003 (A7, 1 μM). 24 h post-stimulation cell lysates were prepared for the detection of MMP-2 through Western blotting as described in Materials and Methods. β-actin was used as a housekeeping control. **(B)** MMP-2 activity was observed through an in-gel assay as described in the Materials and Methods with cells stimulated in the same manner as described in **(A)** and also including the inhibitor suramin (S, 10 μM). Representative zymograph is depicted here. **(C)** Relative densitometry was made based on three independent gelatin zymography experiments. Student's *t*-test was done to calculate significance of agonist-treated cells with their unstimulated counterparts (**$p < 0.01$) and the antagonists with respect to their agonist counterparts ($^{\$}p < 0.05$). **(D)** HeLa and IMR-32 cells were stimulated with ATP (1 mM for HeLa and 30 μM for IMR-32 cells) for 24 h, with or without celecoxib (100 nM) or indomethacin (100 nM). Cell lysates were probed for the detection of COX-2 or MMP-2 levels with β-actin used as a housekeeping control.

Changes in the COX-2 mRNA were also reflected at the protein level. We found a significant increase in COX-2 expression in HeLa cells stimulated with either ATP (at 1 mM) or ADP (at 10 μM, **Figures 3F,H**). Furthermore, P2 receptor antagonists abolished this induced COX-2 expression. In the HeLa cells, the pan-P2 receptor antagonist suramin and the P2Y12 receptor-specific AR-C 69931 significantly inhibited COX-2 synthesis mediated by ATP or ADP. Similarly, in IMR-32 cells, there was a 2-fold increase in the levels of COX-2 in cells stimulated with 30 μM ATP or 1 μM BzATP ($p < 0.01$, **Figures 3G,I**). This increase in COX-2 expression was significantly reduced by the P2X7 receptor antagonist, A740003 ($p < 0.01$). These results indicated that eATP-mediated activation of P2Y12 receptors in HeLa cells or P2X7 receptors in IMR-32 cells show a common downstream target resulting in increased COX-2 expression in these cells.

P2 Receptor Activation Enhances MMP-2 Activity

An important enzyme required for the invasiveness of cancer cells is gelatinase or matrix metalloproteinases, of which matrix metalloproteinase 2 (MMP-2) has been widely reported in various cell culture studies (Hsu et al., 2015; Roomi et al., 2017). Therefore, we wanted to know whether exogenous addition of ATP also increased MMP-2 expression in these cells using Western blot and gelatin zymography. There was a moderate increase in the levels of intracellular MMP-2 in both HeLa and IMR-32 cells which could be inhibited through AR-C 69931 and A740003 in HeLa and IMR-32 cells, respectively (**Figure 4A**). Since MMP-2 is a secreted protein, we collected the media supernatant from stimulated cells and checked for its functional activity through in-gel gelatinase activity. In both HeLa and IMR-32 cells, eATP treatment showed marked increase in gelatinase activity which could be seen reduced in cells which were co-treated with suramin (**Figures 4B,C**). The P2Y12 receptor agonist ADP showed 6-fold increase in MMP-2 activity in HeLa cells ($p = 0.01$), which could be significantly reduced in cells which were also treated with suramin by 49% ($p < 0.05$). Similarly, in IMR-32 cells, the P2X7 receptor agonist BzATP (1 μM)-mediated increase in MMP-2 activity could be significantly reduced with its specific antagonist A740003 by 70% ($p < 0.05$). These results demonstrate that P2 receptor activation increases the activity of MMP-2, an important metastatic marker responsible for cell invasion.

In order to find out if the activity of MMP-2 was dependent on COX-2 expression, we stimulated the cells with celecoxib and indomethacin, 30 min prior to stimulation with ATP. We first confirmed the effect of the inhibitors on COX-2 levels. We found that both celecoxib and indomethacin reduced ATP-mediated increase in COX-2 levels in both HeLa and IMR-32 cells (**Figure 4D**). This suggested that these inhibitors not only inhibit the activity of the enzyme but also its expression within the cells. However, the effect of these inhibitors on MMP-2 expression was not remarkable, though in IMR-32 cells, celecoxib showed reduction in the active form of MMP-2.

P2 Receptor Activation Upregulates p42/44 and p38 MAPK Activity

We next investigated the signaling intermediates downstream of P2 receptor activation which were responsible for COX-2 expression. For this we looked at some of the kinases that were known to be involved in the induction of *COX-2* gene. The p42/44 and p38 mitogen-activated protein kinases (MAPK) are major modulators of COX-2 protein in various cells (Parvathenani et al., 2003; Yang et al., 2004; Akundi et al., 2005; Akter et al., 2021). To confirm their involvement, we used two well-known selective and cell-permeant antagonists of these kinases—SB202190, against p38 MAPK (K_d 38 nM), and PD98019, against mitogen-activated protein kinase kinase (MKK/MEK; IC_{50} 2–7 μM), which is upstream in the MAPK signaling pathway. We found that in both HeLa and IMR-32 cells, ATP-mediated increase in COX-2 levels could be reduced in cells which were pre-treated with SB202190 or PD98059 (**Figure 5A**). These results suggested that ATP-mediated increase in COX-2 levels in cancer cells involved the activation of MAPK signaling pathway.

Accordingly, we looked at the activity of MAPKs through phospho-specific antibodies wherein the phosphorylated forms indicate active MAPK form. ATP and the P2Y12 receptor agonist, ADP, significantly increased phosphorylated levels of p42/44 MAPK in HeLa cells by 2-fold (**Figures 5B,D**). However, the P2Y12 receptor antagonist, AR-C 69931 reduced the phosphorylated form of p42/44 MAPK by 45% in both ATP and ADP-stimulated cells. Similar observation was made in the IMR-32 cells where 30 μM ATP or 1 μM BzATP mediated a significant increase in the activation of p42/44 MAPK (*$p < 0.05$, **Figures 5C,E**) which could be abolished by 80% in cells which were pre-treated with the P2X7 receptor antagonist, A740003. These results implicate the role of p42/44 MAPK downstream of the P2 receptor pathway leading to COX-2 synthesis.

A similar effect was seen in the case of p38 MAPK activity. The addition of eATP (1 mM) showed a 3-fold increase in p38 MAPK activity which could be significantly abolished by 26% in cells pre-treated with AR-C 69931 (*$p < 0.05$, **Figures 5B,D**). In much the same way, ADP (10 μM) showed 3.7-fold increase in p38 MAPK activity (phosphorylation) which was reduced by 55% in cells treated with P2Y12 receptor antagonist, AR-C 69931. In IMR-32 cells, a 3-fold increase in the phosphorylated levels of p38 MAPK was seen with both ATP (30 μM) and the P2X7 receptor agonist, BzATP (1 μM)(*$p < 0.05$, **Figures 5C,E**). Irrespective of the stimulant used, the P2X7 receptor antagonist, A740003, strongly reduced the levels of phosphorylated p38 MAPK by over 60%. These results show that the p38 MAPK are activated downstream of P2X7 receptors in IMR-32 cells and P2Y12 receptors in HeLa cells.

DISCUSSION

We have hypothesized that eATP, which is found at very high concentrations in the tumor microenvironment, is responsible for

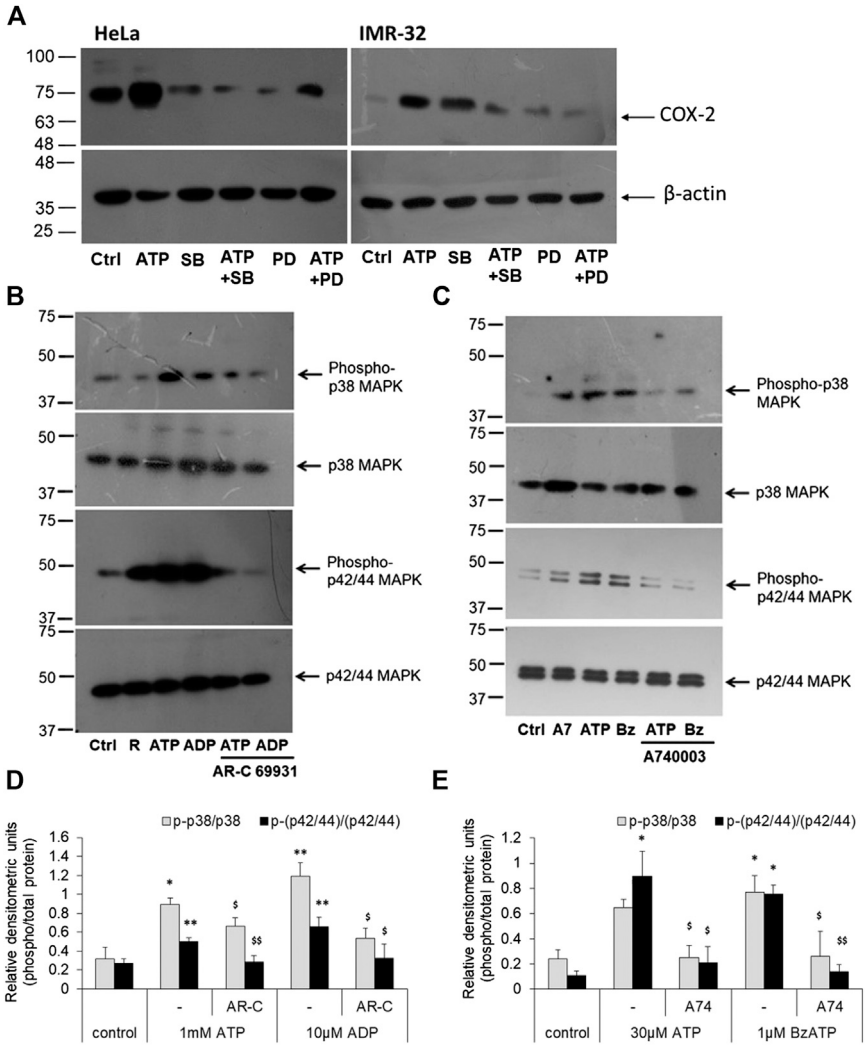


FIGURE 5 | P2 receptor stimulation increases p42/44 MAPK and p38 MAPK activity. **(A)** HeLa or IMR-32 cells were stimulated with ATP (1 mM for HeLa and 30 μM for IMR-32 cells) in the presence or absence of SB202190 (1 μM) or PD98059 (1 μM). The inhibitors were added 30 min prior to the addition of ATP and cells were harvested after 24 h for the detection of COX-2 protein. **(B)** HeLa cells were stimulated with 1 mM ATP or 10 μM ADP, with or without 1 μM AR-C 69931 (R) for 30 min. Cell lysates were then separated on SDS-PAGE and analyzed for phosphorylated or total forms of p42/44 MAPK or phosphorylated or total p38 MAPK. **(C)** IMR-32 cells were stimulated with 30 μM ATP or 1 μM BzATP (Bz), with or without 1 μM A740003 (A7), for 30 min. Cell lysates were then analyzed as described for HeLa cells. Representative images of Western blot are shown from a total of five independent experiments. **(D,E)** Densitometric analysis of phosphorylated p38 or p42/44 MAPK was done with respect to total p38 or p42/44 MAPK, respectively, for HeLa **(D)** or IMR-32 **(E)** cells. Significances were calculated with respect to control cells (*$p < 0.05$, **$p < 0.01$), or with corresponding agonists ($^{\$}p < 0.05$, $^{\$\$}p < 0.01$).

the increased expression of COX-2 which in turn affords the cells with migratory and invasive properties turning the tumor into a metastatic one. In order to test this hypothesis, we used three cell-lines of different tissue origins—HeLa (cervical), IMR-32 (neuronal) and MCF-7 (breast). While HeLa and MCF-7 are frequently used in cancer research, IMR-32 was also included in this study because of it being a neuroblastoma derived from a metastatic site in the abdomen and carrying two types of cells—a predominant smaller neuroblast-like cells and larger hyaline fibroblast cells (Tumilowicz et al., 1970). By using three different cell-lines, we wanted to check whether the mechanism of eATP-mediated tumor cell migration and invasion were common across cancer types. Although each of these cell-lines expressed different purinergic receptors, we found that the downstream effector was COX-2 in all cases which affords the cells with enhanced migration or invasion properties.

It has been well-established in literature that the levels of ATP increase in the tumor microenvironment (Idzko et al., 2014; Di Virgilio et al., 2018). This is especially true in the case of cancers that are therapeutically targeted wherein death of drug- or radiation-sensitive cells results in changing the contents of the microenvironment, of which ATP, among others, is a prominent molecule (Martins et al., 2009; Schneider et al., 2015; Kloss et al., 2019). We observed that the treatment with doxorubicin also showed an increase in the levels of eATP in the media 48 h post-stimulation. This "residual ATP" is the amount of ATP left in the

media after it had been degraded by ectonucleotidases and/or internalized through macropinocytosis besides the ATP that has bound to various P2 receptors. Treatment of acute myeloid leukemia cells with another chemotherapeutic agent, daunorubicin (200 nM), similarly showed the release of ATP in the range of 2–8 nM, 6 h post-stimulation (Pegoraro et al., 2020). The tumor microenvironment, therefore, remains enriched with ATP in the aftermath of chemotherapy. The amount of residual ATP depends on various factors such as the intracellular levels of ATP, which in turn depends on the metabolic activity of the cell, percentage of cells dying in response to the chemotherapeutic agent, and the expression levels and activity of membrane ectonucleotidases which are responsible for the degradation of eATP. This explains the differences we found in the levels of residual ATP between the three cell types. Among those we tested, HeLa cells were most sensitive to doxorubicin (53% cell death at 48 h) and therefore showed a high level of residual ATP. However, MCF-7 cells, despite slightly lower cell death at 41%, had the most measured ATP levels due to their higher proliferation rate. We have shown that this "residual ATP" has the ability to afford cancer cells with migration, invasion and increased expression of COX-2 through the use of conditioned media. Treatment of the cells with apyrase or suramin abolished eATP-mediated migration of cancer cells suggesting that the residual ATP is directly responsible for cell migration through P2 receptors. In light of such observations, ectonucleotidases such as CD73 and CD39 are increasingly gaining recognition as the new targets in cancer therapeutics (Moesta et al., 2020).

In this study, we screened eight P2 receptors which were commonly reported in cancer—the vast majority of these studies relate to P2X7, P2Y2 and P2Y12 receptors (Adinolfi et al., 2012; Li et al., 2013; Chadet et al., 2014; Jin et al., 2014). P2X7 receptors have been shown to express strongly in prostate cancer cells, in osteosarcoma, in lymphocyte-infiltrating gastric cancer, pancreatic ductal adenocarcinoma cell lines, and in patients with malignant pleural mesothelioma, and its antagonism has been shown to inhibit growth of tumors in various *in vivo* models (Qiu et al., 2014; Giannuzzo et al., 2015; Amoroso et al., 2016; Zhang et al., 2019; Calik et al., 2020). We found this true in the case of IMR-32 neuroblastoma and MCF-7 breast cancer cells. In both these cell types, BzATP significantly increased migration and invasion mediated by these cells, which could be inhibited through the use of P2X7 receptor-specific antagonist, A740003, and oATP. Antagonising P2X7 receptors has been shown to inhibit tumor growth in the case of human gliomas (Kan et al., 2020). In neuroblastoma cells, P2X7 inhibition not only reduced tumor progression but was also shown to influence metabolic activity via Akt pathway and angiogenesis through reduced vascular endothelial growth factor (VEGF) secretion (Amoroso et al., 2015). In the MDA-MB-231 cells, activation of P2X7 receptors induces calcium-activated SK3 potassium channels which assists in cell migration (Jelassi et al., 2011). P2X7 receptor has been shown to induce fast F-actin reorganization and formation of filopodia, thereby promoting invasion (Brisson et al., 2020). These roles of eATP-mediated morphological changes in early metastasis have also been shown to depend not only on P2X7 receptor stimulation but also on eATP which gets internalized through macropinocytosis (Cao et al., 2019). In addition to these roles, P2X7 receptors also modulate the tumor microenvironment wherein blockade of the receptors through systemic administration of A740003 showed an increase in $CD4^+$ cells but with diminished expression of ectonucleotidases, CD39 and CD73 (De Marchi et al., 2019). These observations and the current study support the various reports that have championed P2X7 receptors as a good therapeutic target. This is also reflected in the various preclinical studies which use P2X7 receptor antagonists as potential anti-tumour drugs (Di Virgilio et al., 2018; Lara et al., 2020).

We do not rule out the possible role of P2X4 receptors, which are the predominantly expressed receptors in MCF-7 cells, and of P2Y2 receptors in tumor progression. In the relatively more potent and metastatic breast cancer cell-line, MDA-MB-231, and in prostate cancer cells, P2Y2 receptors have been shown to be strongly expressed and whose knockdown prevented cell migration and invasion (Li et al., 2013; Eun et al., 2015). P2Y2 receptors further mediate inflammasome formation in radiotherapy-resistant breast cancer cells enhancing their invasiveness (Jin et al., 2018). MDA-MB-231 cells also strongly express P2Y6 receptors whose blockage with MRS 2578 or downregulation using siRNA inhibited migration and invasion (Ma et al., 2016). In P2Y6 knockout mice, lung tumor metastasis was prevented by reducing the recruitment of neutrophils (Qin et al., 2020). However, in our studies, we saw that there was no change in the levels of P2Y6 mRNA in cells stimulated with ATP unlike P2Y12 mRNA whose expression increased by 6-fold in 24 h. The expression of P2Y6 receptor has instead been shown to increase following hypoxia or epidermal growth factor treatment in MDA-MB-231 cells (Azimi et al., 2016). A specific antagonist of P2Y12 receptors, AR-C 69931, also significantly inhibited both ATP and ADP-mediated cell migration and invasion. P2Y12 receptor antagonists such as clopidogrel and ticagrelor are currently under clinical studies in breast, pancreatic and head and neck cancer (Ballerini et al., 2018; Elaskalani et al., 2020).

The fact that different cell types show upregulation of different P2 receptors suggests that it is essential to target each cancer type with the specific receptor expressed on its surface, in response to its microenvironment. For instance, in the case of hepatocellular carcinoma, P2Y11 receptors have been shown to play a major role in cell migration through ATP-induced calcium signaling (Khalid et al., 2017). P2Y11 receptors have also been implicated in pancreatic cancer cell migration through a p38 MAPK-dependent pathway (Shi et al., 2013). In the case of renal cancer cells, P2X6 receptors were found to increase cell migration and invasion involving calcium-mediated p42/44 MAPK signaling and MMP-9 activation (Gong et al., 2019). The diversity of P2 receptors, their promiscuity to different nucleotides, and the presence of ectonucleotidases, makes it difficult to target any one single receptor for a given cancer type (Fiebich et al., 2014; da Silva Ferreira et al., 2019). Moreover, mere expression of a receptor at high levels may not reflect its increased activity. This is especially true of P2X7 receptors whose opening would increase membrane permeability leading to cell death. However, in the case of tumors, it has been found that there is, instead, an increase in the expression of a non-pore forming

P2X7 receptor which is functionally redundant, thereby assisting tumor cell survival (Gilbert et al., 2019). The two splice variants of P2X7 receptors also show differential modulation in response to chemotherapy as seen in acute myeloid leukemia (Pegoraro et al., 2020). It has to also be noted that the physiological role of P2 receptors depends on the cell type they are expressed on—inhibiting P2Y12 receptors with ticagrelor in pancreatic ductal adenocarcinoma cells decreases their proliferative capacity through the Akt pathway (Elaskalani et al., 2020), inhibition of the same receptors on macrophages enhances their tumor cell phagocytic properties involving ER stress pathway (Pavlovic et al., 2020) and inhibits the formation of inflammasome (Huang et al., 2020), whose role in cancer progression continues to be sought (Hamarsheh and Zeiser, 2020). Therefore, targeting specific P2 receptors remains a challenge making therapeutic approach personalized depending on the nucleotide composition of the microenvironment and/or receptor expression. Our study, however, shows that, irrespective of the type of P2 receptor that is activated upstream, the downstream pathway leads to increased COX-2 expression, thereby providing a common pathway for therapeutic intervention. This has been true with both P2X7 and P2Y12 receptors *in vitro*.

COX-2 has been shown to be overexpressed in various cancers (Ristimaki et al., 2002; Yan et al., 2004). In the human lung cancer cells, COX-2 has been shown to afford cells with resistance to apoptosis, thereby making them withstand chemotherapy (Chen et al., 2010). Doxorubicin-resistant MCF-7 cells (MCF-7/DOX), which exhibit high invasiveness, strongly express COX-2 similar to that expressed in the highly metastatic breast cancer MDA-MB-231 cells (Kang et al., 2011). Increased COX-2 activity leads to increased PGE_2 synthesis, whose levels have been shown to be increased in the tumor microenvironment (Wan et al., 2013; Lala et al., 2018; Carter et al., 2019). Inhibition of COX-2 in MDA-MB-231 cells through upstream inhibition of the nuclear factor κB (NF-κB) signaling suppressed its invasiveness (Kim et al., 2014). The p42/44 MAPK signaling is upstream of the NF-κB-mediated COX-2 expression (Akundi et al., 2005), and has been shown to be activated following P2 receptor stimulation (Parvathenani et al., 2003; Yang et al., 2004; Lili et al., 2019). Similarly, p38 MAPK is essential for COX-2 synthesis, either directly through downstream activation of transcription factors (Fiebich et al., 2000; Akundi et al., 2005), or through stabilization of COX-2 mRNA post-transcription, thereby prolonging the effect of COX-2 or inflammation for a much longer period (Lasa et al., 2000; Harper and Tyson-Capper, 2008). Recently, we found that in macrophages, eATP enhanced LPS-mediated COX-2 synthesis by sustaining high levels of COX-2 mRNA through extended phosphorylation of cyclin-dependent kinase 9 and p38 MAPK (Akter et al., 2021). Our results here show that the presence of residual ATP within the tumor microenvironment provides a low-grade chronic inflammation. The resulting activation of P2 receptors leads to increased phosphorylation of p38 and p42/44 MAPK, thereby leading to

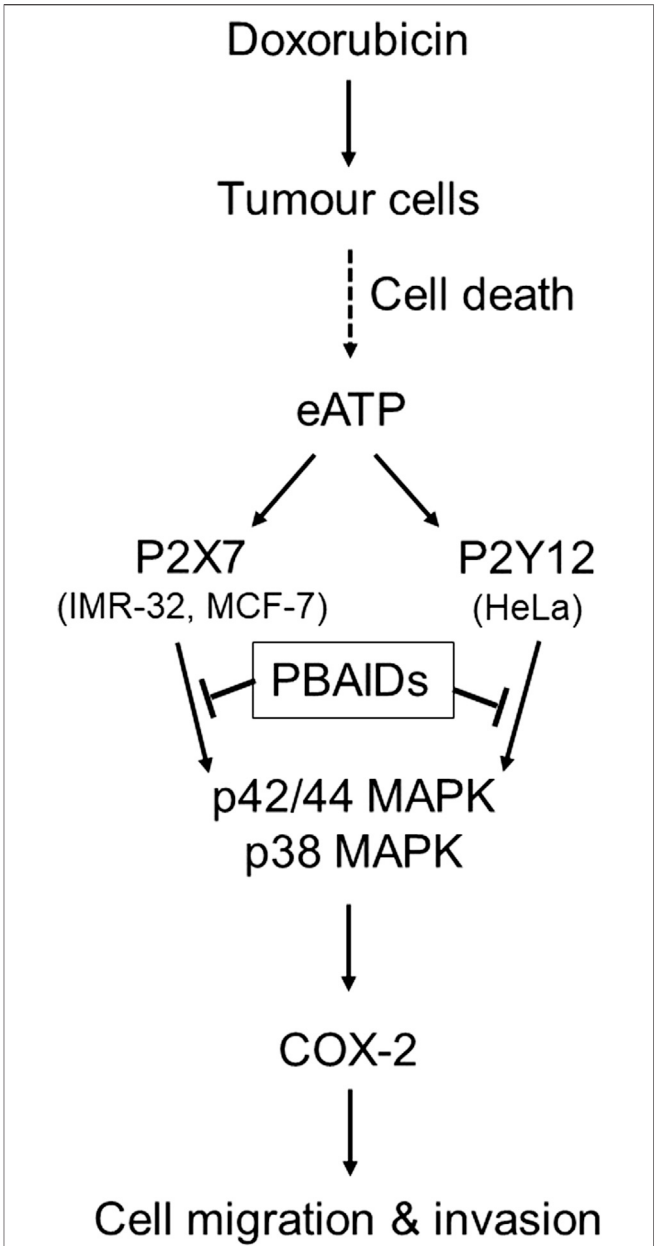


FIGURE 6 | Exogenous ATP mediates increased COX-2 expression leading to cancer cell metastasis. Treatment of tumors with chemotherapeutic agents such as doxorubicin leads to profound cell death and increased levels of exogenous ATP (eATP) in the extracellular milieu. This ATP acts on the P2 receptors of tumor cells in the vicinity through different P2 receptors depending on tumor type such as P2X7 receptors in case of IMR-32 and MCF-7 cells or P2Y12 receptors in case of HeLa cells. Activation of either of these P2 receptors leads to the activation of p38 and p42/44 MAPK which, in turn, induce the transcription of COX-2. Increased COX-2 synthesis has been shown to modulate tumor cell migration and invasiveness, thus contributing toward increased metastasis. P2 receptor-based anti-inflammatory drugs (PBAIDs), which are specific P2 receptor antagonists, have been shown to block the phosphorylation of MAPKs, synthesis of COX-2 and cell migration or invasion.

increased COX-2 synthesis. Antagonists of P2X7 or P2Y12 receptors, therefore, not only reduce the levels of phosphorylated p38 and p42/44 MAPK, but also show reduction in COX-2 levels and abolish migration/invasive properties of the cancer cells in response to eATP.

Inhibition of COX-2, especially through nonsteroidal anti-inflammatory drugs (NSAIDs), has been considered a therapeutic alternative for arresting tumor growth (Gupta and Dubois, 2001; Ulrich et al., 2006). However, chronic use of non-specific NSAIDs or COX-2 specific inhibitors (COXIBs) for other diseases had been counterproductive (in t' Veld et al., 2001; Juni et al., 2004). We had previously hypothesized that upstream inhibition of P2 receptors will result in reduced COX-2 expression without affecting its housekeeping role (Fiebich et al., 2014). These P2 receptor antagonists, dubbed as P2 receptor-based anti-inflammatory drugs (PBAIDs), would inhibit the downstream MAPK signaling, COX-2 synthesis and cancer cell migration/invasion. PBAIDs, therefore, provide a suitable alternative to NSAIDs for cancer treatment without the unpleasant gastrointestinal side-effects associated with long-term NSAID usage or the risks associated with COXIBs. PBAIDs such as suramin has been repurposed as an anti-cancer drug in other studies (Ahmed et al., 2016; Cheng et al., 2019; Wiedemar et al., 2020). We have also shown here that suramin inhibits eATP-mediated COX-2 and MMP-2 synthesis and prevents cancer cell migration and invasion. While the new generation specific P2 receptor antagonists have specificity of action on their side, suramin is an already approved drug by US FDA and could be repurposed as a PBAID.

In summary, we show that doxorubicin mediated cell death leads to an increase in the levels of eATP, which in turn leads to the activation of different P2 receptors depending on the cell type (**Figure 6**). P2 receptor antagonists are able to inhibit COX-2 expression and MMP-2 activity, thereby confirming that increased levels of eATP are responsible for the metastatic properties of these cells. Blocking of P2 receptors in tumor cells, *in vivo*, leads to reduction in tumor growth due to inhibition of COX-2. This has direct relevance to cancer recurrence where chemotherapeutic agents kill drug-sensitive cells leading to a build-up of eATP in the tumor microenvironment. This ATP may act on the P2 receptors of nearby drug-resistant cells thereby increasing their COX-2 expression. Such cells have the potential to turn metastatic leading to cancer recurrence over a period of time. Therefore, targeting the P2 receptor-COX-2 pathway is a promising strategy in the control of cancer resurgence. The fact that we found different P2 receptors for the different cell lines further suggest that each cancer type has its own specific P2 receptor target and, therefore, earlier reports on P2 receptor antagonists in cancer treatment have to be revisited.

DATA AVAILABILITY STATEMENT

All the datasets that have been used in the current study are available from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

Conceived and planned experiments, RA; experiments performed by SS and HK; manuscript writing and editing, SS and RA.

FUNDING

This work was supported in part by grants from the Council of Scientific and Industrial Research (CSIR) [37(1702)/17/EMR-II] and Science and Engineering Research Board (SERB) [EMR/2016/001442] to RA.

ACKNOWLEDGMENTS

The technical assistance of Manisha Singh and Nitin Sharma is sincerely acknowledged.

REFERENCES

Adinolfi, E., Raffaghello, L., Giuliani, A. L., Cavazzini, L., Capece, M., Chiozzi, P., et al. (2012). Expression of P2X7 receptor increases *in vivo* tumor growth. *Cancer Res.* 72 (12), 2957–2969. doi:10.1158/0008-5472.CAN-11-1947

Ahmed, K., Shaw, H. V., Koval, A., and Katanaev, V. L. (2016). A second WNT for old drugs: drug repositioning against WNT-dependent cancers. *Cancers* 8 (7), 66. doi:10.3390/cancers8070066

Akter, S., Sharma, R. K., Sharma, S., Rastogi, S., Fiebich, B. L., and Akundi, R. S. (2021). Exogenous ATP modulates prostaglandin E2 (PGE2) release in macrophages through sustained phosphorylation of CDK9 and p38 MAPK. *J. Leukoc. Biol.* doi:10.1002/JLB.3A1219-697RR

Akundi, R. S., Candelario-Jalil, E., Hess, S., Hüll, M., Lieb, K., Gebicke-Haerter, P. J., et al. (2005). Signal transduction pathways regulating cyclooxygenase-2 in lipopolysaccharide-activated primary rat microglia. *Glia* 51 (3), 199–208. doi:10.1002/glia.20198

Amoroso, F., Capece, M., Rotondo, A., Cangelosi, D., Ferracin, M., Franceschini, A., et al. (2015). The P2X7 receptor is a key modulator of the PI3K/GSK3β/VEGF signaling network: evidence in experimental neuroblastoma. *Oncogene* 34 (41), 5240–5251. doi:10.1038/onc.2014.444

Amoroso, F., Salaro, E., Falzoni, S., Chiozzi, P., Giuliani, A. L., Cavallesco, G., et al. (2016). P2X7 targeting inhibits growth of human mesothelioma. *Oncotarget* 7 (31), 49664–49676. doi:10.18632/oncotarget.10430

Avanzato, D., Genova, T., Fiorio Pla, A., Bernardini, M., Bianco, S., Bussolati, B., et al. (2016). Activation of P2X7 and P2Y11 purinergic receptors inhibits migration and normalizes tumor-derived endothelial cells via cAMP signaling. *Sci. Rep.* 6, 32602. doi:10.1038/srep32602

Avanzini, S., and Antal, T. (2019). Cancer recurrence times from a branching process model. *PLoS Comput. Biol.* 15 (11), e1007423. doi:10.1371/journal.pcbi.1007423

Azimi, I., Beilby, H., Davis, F. M., Marcial, D. L., Kenny, P. A., Thompson, E. W., et al. (2016). Altered purinergic receptor-Ca^{2+} signaling associated with hypoxia-induced epithelial-mesenchymal transition in breast cancer cells. *Mol. Oncol.* 10 (1), 166–178. doi:10.1016/j.molonc.2015.09.006

Ballerini, P., Dovizio, M., Bruno, A., Tacconelli, S., and Patrignani, P. (2018). P2Y12 receptors in tumorigenesis and metastasis. *Front. Pharmacol.* 9, 66. doi:10.3389/fphar.2018.00066

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and

mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J. Clin.* 68 (6), 394–424. doi:10.3322/caac.21492

Brisson, L., Chadet, S., Lopez-Charcas, O., Jelassi, B., Ternant, D., Chamouton, J., et al. (2020). P2X7 receptor promotes mouse mammary cancer cell invasiveness and tumour progression, and is a target for anticancer treatment. *Cancers* 12 (9), 2342. doi:10.3390/cancers12092342

Calik, I., Calik, M., Sarikaya, B., Ozercan, I. H., Arslan, R., Artas, G., et al. (2020). P2X7 receptor as an independent prognostic indicator in gastric cancer. *Bosn. J. Basic Med. Sci.* 20 (2), 188–196. doi:10.17305/bjbms.2020.4620

Cao, Y., Wang, X., Li, Y., Evers, M., Zhang, H., and Chen, X. (2019). Extracellular and macropinocytosis internalized ATP work together to induce epithelial-mesenchymal transition and other early metastatic activities in lung cancer. *Cancer Cell Int.* 19, 254. doi:10.1186/s12935-019-0973-0

Carter, B. Z., Mak, P. Y., Wang, X., Tao, W., Ruvolo, V., Mak, D., et al. (2019). An ARC-regulated IL1β/Cox-2/PGE2/β-catenin/ARC Circuit controls leukemia-microenvironment interactions and Confers drug resistance in AML. *Cancer Res.* 79 (6), 1165–1177. doi:10.1158/0008-5472.CAN-18-0921

Chadet, S., Jelassi, B., Wannous, R., Angoulvant, D., Chevalier, S., Besson, P., et al. (2014). The activation of P2Y2 receptors increases MCF-7 breast cancer cells migration through the MEK-ERK1/2 signalling pathway. *Carcinogenesis* 35 (6), 1238–1247. doi:10.1093/carcin/bgt493

Chen, W., Bai, L., Wang, X., Xu, S., Belinsky, S. A., and Lin, Y. (2010). Acquired activation of the Akt/cyclooxygenase-2/Mcl-1 pathway renders lung cancer cells resistant to apoptosis. *Mol. Pharmacol.* 77 (3), 416–423. doi:10.1124/mol.109.061226

Cheng, B., Gao, F., Maissy, E., and Xu, P. (2019). Repurposing suramin for the treatment of breast cancer lung metastasis with glycol chitosan-based nanoparticles. *Acta Biomater.* 84, 378–390. doi:10.1016/j.actbio.2018.12.010

Choi, E. M., Heo, J. I., Oh, J. Y., Kim, Y. M., Ha, K. S., Kim, J. I., et al. (2005). COX-2 regulates p53 activity and inhibits DNA damage-induced apoptosis. *Biochem. Biophys. Res. Commun.* 328 (4), 1107–1112. doi:10.1016/j.bbrc.2005.01.072

da Silva Ferreira, N. C., Alves, L. A., and Soares-Bezerra, R. J. (2019). Potential therapeutic applications of P2 receptor antagonists: from bench to clinical trials. *Curr. Drug Targets* 20 (9), 919–937. doi:10.2174/1389450120666190213095923

De Marchi, E., Orioli, E., Pegoraro, A., Sangaletti, S., Portararo, P., Curti, A., et al. (2019). The P2X7 receptor modulates immune cells infiltration, ectonucleotidases expression and extracellular ATP levels in the tumor microenvironment. *Oncogene* 38 (19), 3636–3650. doi:10.1038/s41388-019-0684-y

Deli, T., and Csernoch, L. (2008). Extracellular ATP and cancer: an overview with special reference to P2 purinergic receptors. *Pathol. Oncol. Res.* 14 (3), 219–231. doi:10.1007/s12253-008-9071-7

Di Virgilio, F., Sarti, A. C., Falzoni, S., De Marchi, E., and Adinolfi, E. (2018). Extracellular ATP and P2 purinergic signalling in the tumour microenvironment. *Nat. Rev. Cancer* 18 (10), 601–618. doi:10.1038/s41568-018-0037-0

Elaskalani, O., Domenichini, A., Abdol Razak, N. B., E Dye, D., Falasca, M., and Metharom, P. (2020). Antiplatelet drug ticagrelor enhances chemotherapeutic efficacy by targeting the novel P2Y12-AKT pathway in pancreatic cancer cells. *Cancers* 12 (1), 250. doi:10.3390/cancers12010250

Eun, S. Y., Ko, Y. S., Park, S. W., Chang, K. C., and Kim, H. J. (2015). P2Y2 nucleotide receptor-mediated extracellular signal-regulated kinases and protein kinase C activation induces the invasion of highly metastatic breast cancer cells. *Oncol. Rep.* 34 (1), 195–202. doi:10.3892/or.2015.3972

Feng, D., Zhao, T., Yan, K., Liang, H., Liang, J., Zhou, Y., et al. (2017). Gonadotropins promote human ovarian cancer cell migration and invasion via a cyclooxygenase 2-dependent pathway. *Oncol. Rep.* 38 (2), 1091–1098. doi:10.3892/or.2017.5784

Ferrandina, G., Lauriola, L., Zannoni, G. F., Fagotti, A., Fanfani, F., Legge, F., et al. (2002). Increased cyclooxygenase-2 (COX-2) expression is associated with chemotherapy resistance and outcome in ovarian cancer patients. *Ann. Oncol.* 13 (8), 1205–1211. doi:10.1093/annonc/mdf207

Fiebich, B. L., Akter, S., and Akundi, R. S. (2014). The two-hit hypothesis for neuroinflammation: role of exogenous ATP in modulating inflammation in the brain. *Front. Cell. Neurosci.* 8, 260. doi:10.3389/fncel.2014.00260

Fiebich, B. L., Mueksch, B., Boehringer, M., and Hüll, M. (2000). Interleukin-1beta induces cyclooxygenase-2 and prostaglandin E(2) synthesis in human neuroblastoma cells: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB. *J. Neurochem.* 75 (5), 2020–2028. doi:10.1046/j.1471-4159.2000.0752020.x

Giannuzzo, A., Pedersen, S. F., and Novak, I. (2015). The P2X7 receptor regulates cell survival, migration and invasion of pancreatic ductal adenocarcinoma cells. *Mol. Cancer* 14, 203. doi:10.1186/s12943-015-0472-4

Gilbert, S. M., Oliphant, C. J., Hassan, S., Peille, A. L., Bronsert, P., Falzoni, S., et al. (2019). ATP in the tumour microenvironment drives expression of nfP2X7, a key mediator of cancer cell survival. *Oncogene* 38 (2), 194–208. doi:10.1038/s41388-018-0426-6

Gong, D., Zhang, J., Chen, Y., Xu, Y., Ma, J., Hu, G., et al. (2019). The m6A-suppressed P2RX6 activation promotes renal cancer cells migration and invasion through ATP-induced Ca^{2+} influx modulating ERK1/2 phosphorylation and MMP9 signaling pathway. *J. Exp. Clin. Cancer Res.* 38 (1), 233. doi:10.1186/s13046-019-1223-y

Gupta, R. A., and Dubois, R. N. (2001). Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. *Nat. Rev. Cancer* 1 (1), 11–21. doi:10.1038/35094017

Hamarsheh, S., and Zeiser, R. (2020). NLRP3 inflammasome activation in cancer: a double-edged sword. *Front. Immunol.* 11, 1444. doi:10.3389/fimmu.2020.01444

Harper, K. A., and Tyson-Capper, A. J. (2008). Complexity of COX-2 gene regulation. *Biochem. Soc. Trans.* 36 (Pt 3), 543–545. doi:10.1042/BST0360543

Holleczek, B., Stegmaier, C., Radosa, J. C., Solomayer, E. F., and Brenner, H. (2019). Risk of loco-regional recurrence and distant metastases of patients with invasive breast cancer up to ten years after diagnosis—results from a registry-based study from Germany. *BMC Cancer* 19 (1), 520. doi:10.1186/s12885-019-5710-5

Houssami, N., Macaskill, P., Marinovich, M. L., and Morrow, M. (2014). The association of surgical margins and local recurrence in women with early-stage invasive breast cancer treated with breast-conserving therapy: a meta-analysis. *Ann. Surg Oncol.* 21 (3), 717–730. doi:10.1245/s10434-014-3480-5

Hsu, J. Y., Chang, K. Y., Chen, S. H., Lee, C. T., Chang, S. T., Cheng, H. C., et al. (2015). Epidermal growth factor-induced cyclooxygenase-2 enhances head and neck squamous cell carcinoma metastasis through fibronectin up-regulation. *Oncotarget* 6 (3), 1723–1739. doi:10.18632/oncotarget.2783

Huang, B., Qian, Y., Xie, S., Ye, X., Chen, H., Chen, Z., et al. (2020). Ticagrelor inhibits the NLRP3 inflammasome to protect against inflammatory disease independent of the P2Y12 signaling pathway. *Cell. Mol. Immunol.* doi:10.1038/s41423-020-0444-5

Idzko, M., Ferrari, D., and Eltzschig, H. K. (2014). Nucleotide signalling during inflammation. *Nature* 509 (7500), 310–317. doi:10.1038/nature13085

Illes, P., Muller, C. E., Jacobson, K. A., Grutter, T., Nicke, A., Fountain, S. J., et al. (2020). Update of P2X receptor properties and their pharmacology: IUPHAR Review 30. *Br. J. Pharmacol.*: doi:10.1111/bph.15299

in t' Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., et al. (2001). Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease, *N. Engl. J. Med.* 345 (21), 1515–1521. doi:10.1056/NEJMoa010178

Jacobson, K. A., Delicado, E. G., Gachet, C., Kennedy, C., von Kügelgen, I., Li, B., et al. (2020). Update of P2Y receptor pharmacology: IUPHAR review 27. *Br. J. Pharmacol.* 177 (11), 2413–2433. doi:10.1111/bph.15005

Jelassi, B., Chantôme, A., Alcaraz-Pérez, F., Baroja-Mazo, A., Cayuela, M. L., Pelegrin, P., et al. (2011). P2X(7) receptor activation enhances SK3 channels-and cystein cathepsin-dependent cancer cells invasiveness. *Oncogene* 30 (18), 2108–2122. doi:10.1038/onc.2010.593

Jiang, J. X., Riquelme, M. A., and Zhou, J. Z. (2015). ATP, a double-edged sword in cancer. *Oncoscience* 2 (8), 673–674. doi:10.18632/oncoscience.230

Jin, H., Eun, S. Y., Lee, J. S., Park, S. W., Lee, J. H., Chang, K. C., et al. (2014). P2Y2 receptor activation by nucleotides released from highly metastatic breast cancer cells increases tumor growth and invasion via crosstalk with endothelial cells. *Breast Cancer Res.* 16 (5), R77. doi:10.1186/bcr3694

Jin, H., Ko, Y. S., and Kim, H. J. (2018). P2Y2R-mediated inflammasome activation is involved in tumor progression in breast cancer cells and in radiotherapy-resistant breast cancer. *Int. J. Oncol.* 53 (5), 1953–1966. doi:10.3892/ijo.2018.4552

Jüni, P., Nartey, L., Reichenbach, S., Sterchi, R., Dieppe, P. A., and Egger, M. (2004). Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. *Lancet* 364 (9450), 2021–2029. doi:10.1016/S0140-6736(04)17514-4

Kan, L. K., Seneviratne, S., Drummond, K. J., Williams, D. A., O'Brien, T. J., and Monif, M. (2020). P2X7 receptor antagonism inhibits tumour growth in human

high-grade gliomas. *Purinergic Signal.* 16 (3), 327–336. doi:10.1007/s11302-020-09705-2

Kang, J. H., Song, K. H., Jeong, K. C., Kim, S., Choi, C., Lee, C. H., et al. (2011). Involvement of Cox-2 in the metastatic potential of chemotherapy-resistant breast cancer cells. *BMC Cancer* 11, 334. doi:10.1186/1471-2407-11-334

Karavitis, J., and Zhang, M. (2013). COX2 regulation of breast cancer bone metastasis. *Oncoimmunology* 2 (3), e23129. doi:10.4161/onci.23129

Khakh, B. S., and North, R. A. (2006). P2X receptors as cell-surface ATP sensors in health and disease. *Nature* 442 (7102), 527–532. doi:10.1038/nature04886

Khalid, M., Brisson, L., Tariq, M., Hao, Y., Guibon, R., Fromont, G., et al. (2017). Carcinoma-specific expression of P2Y11 receptor and its contribution in ATP-induced purinergic signalling and cell migration in human hepatocellular carcinoma cells. *Oncotarget* 8 (23), 37278–37290. doi:10.18632/oncotarget.16191

Kim, M. J., Kim, H. S., Lee, S. H., Yang, Y., Lee, M. S., and Lim, J. S. (2014). NDRG2 controls COX-2/PGE_2-mediated breast cancer cell migration and invasion. *Mol. Cell* 37 (10), 759–765. doi:10.14348/molcells.2014.0232

Kloss, L., Dollt, C., Schledzewski, K., Krewer, A., Melchers, S., Manta, C., et al. (2019). ADP secreted by dying melanoma cells mediates chemotaxis and chemokine secretion of macrophages via the purinergic receptor P2Y12. *Cell Death Dis.* 10 (10), 760. doi:10.1038/s41419-019-2010-6

Kuang, W., Deng, Q., Deng, C., Li, W., Shu, S., and Zhou, M. (2017). Hepatocyte growth factor induces breast cancer cell invasion via the PI3K/Akt and p38 MAPK signaling pathways to up-regulate the expression of COX2. *Am. J. Transl. Res.* 9 (8), 3816–3826.

Lala, P. K., Nandi, P., and Majumder, M. (2018). Roles of prostaglandins in tumor-associated lymphangiogenesis with special reference to breast cancer. *Cancer Metastasis Rev.* 37 (2-3), 369–384. doi:10.1007/s10555-018-9734-0

Lara, R., Adinolfi, E., Harwood, C. A., Philpott, M., Barden, J. A., Di Virgilio, F., et al. (2020). P2X7 in cancer: from molecular mechanisms to therapeutics. *Front. Pharmacol.* 11, 793. doi:10.3389/fphar.2020.00793

Lasa, M., Mahtani, K. R., Finch, A., Brewer, G., Saklatvala, J., and Clark, A. R. (2000). Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. *Mol. Cell Biol.* 20 (12), 4265–4274. doi:10.1128/mcb.20.12.4265-4274.2000

Lecciso, M., Ocadlikova, D., Sangaletti, S., Trabanelli, S., De Marchi, E., Orioli, E., et al. (2017). ATP release from chemotherapy-treated dying leukemia cells elicits an immune suppressive effect by increasing regulatory T cells and tolerogenic dendritic cells. *Front. Immunol.* 8, 1918. doi:10.3389/fimmu.2017.01918

Li, G., Yang, T., and Yan, J. (2002). Cyclooxygenase-2 increased the angiogenic and metastatic potential of tumor cells. *Biochem. Biophys. Res. Commun.* 299 (5), 886–890. doi:10.1016/s0006-291x(02)02707-9

Li, W. H., Qiu, Y., Zhang, H. Q., Liu, Y., You, J. F., Tian, X. X., et al. (2013). P2Y2 receptor promotes cell invasion and metastasis in prostate cancer cells. *Br. J. Cancer* 109 (6), 1666–1675. doi:10.1038/bjc.2013.484

Liang, C. C., Park, A. Y., and Guan, J. L. (2007). *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration *in vitro*. *Nat. Protoc.* 2 (2), 329–333. doi:10.1038/nprot.2007.30

Lili, W., Yun, L., Tingran, W., Xia, W., and Yanlei, S. (2019). P2RX7 functions as a putative biomarker of gastric cancer and contributes to worse prognosis. *Exp. Biol. Med.* 244 (9), 734–742. doi:10.1177/1535370219846492

Liu, C. L., Chen, M. J., Lin, J. C., Lin, C. H., Huang, W. C., Cheng, S. P., et al. (2019). Doxorubicin promotes migration and invasion of breast cancer cells through the upregulation of the RhoA/MLC pathway. *J. Breast Cancer* 22 (2), 185–195. doi:10.4048/jbc.2019.22.e22

Ma, X., Pan, X., Wei, Y., Tan, B., Yang, L., Ren, H., et al. (2016). Chemotherapy-induced uridine diphosphate release promotes breast cancer metastasis through P2Y6 activation. *Oncotarget* 7 (20), 29036–29050. doi:10.18632/oncotarget.8664

Mandapathil, M., Boduc, M., Roessler, M., Güldner, C., Walliczek-Dworschak, U., and Mandic, R. (2018). Ectonucleotidase CD39 expression in regional metastases in head and neck cancer. *Acta Otolaryngol.* 138 (4), 428–432. doi:10.1080/00016489.2017.1405278

Martins, I., Tesniere, A., Kepp, O., Michaud, M., Schlemmer, F., Senovilla, L., et al. (2009). Chemotherapy induces ATP release from tumor cells. *Cell Cycle* 8 (22), 3723–3728. doi:10.4161/cc.8.22.10026

Minghetti, L. (2007). Role of COX-2 in inflammatory and degenerative brain diseases. *Subcell. Biochem.* 42, 127–141. doi:10.1007/1-4020-5688-5_5

Moesta, A. K., Li, X. Y., and Smyth, M. J. (2020). Targeting CD39 in cancer. *Nat. Rev. Immunol.* 20 (12), 739–755. doi:10.1038/s41577-020-0376-4

Nakanishi, M., and Rosenberg, D. W. (2013). Multifaceted roles of PGE2 in inflammation and cancer. *Semin. Immunopathol.* 35 (2), 123–137. doi:10.1007/s00281-012-0342-8

Nakanishi, Y., Kamijo, R., Takizawa, K., Hatori, M., and Nagumo, M. (2001). Inhibitors of cyclooxygenase-2 (COX-2) suppressed the proliferation and differentiation of human leukaemia cell lines. *Eur. J. Cancer* 37 (12), 1570–1578. doi:10.1016/s0959-8049(01)00160-5

Parvathenani, L. K., Tertyshnikova, S., Greco, C. R., Roberts, S. B., Robertson, B., and Posmantur, R. (2003). P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. *J. Biol. Chem.* 278 (15), 13309–13317. doi:10.1074/jbc.M209478200

Pavlović, N., Kopsida, M., Gerwins, P., and Heindryckx, F. (2020). Inhibiting P2Y12 in macrophages induces endoplasmic reticulum stress and promotes an anti-tumoral phenotype. *Int. J. Mol. Sci.* 21 (21), 8177. doi:10.3390/ijms21218177

Pegoraro, A., Orioli, E., De Marchi, E., Salvestrini, V., Milani, A., Di Virgilio, F., et al. (2020). Differential sensitivity of acute myeloid leukemia cells to daunorubicin depends on P2X7A versus P2X7B receptor expression. *Cell Death Dis.* 11 (10), 876. doi:10.1038/s41419-020-03058-9

Qin, J., Zhang, Z., Fu, Z., Ren, H., Liu, M., Qian, M., et al. (2020). The UDP/P2y6 axis promotes lung metastasis of melanoma by remodeling the premetastatic niche. *Cell. Mol. Immunol.* 17 (12), 1269–1271. doi:10.1038/s41423-020-0392-0

Qiu, Y., Li, W. H., Zhang, H. Q., Liu, Y., Tian, X. X., and Fang, W. G. (2014). P2X7 mediates ATP-driven invasiveness in prostate cancer cells. *PLoS One* 9 (12), e114371. doi:10.1371/journal.pone.0114371

Ristimäki, A., Sivula, A., Lundin, J., Lundin, M., Salminen, T., Haglund, C., et al. (2002). Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Res.* 62 (3), 632–635.

Roomi, M. W., Kalinovsky, T., Rath, M., and Niedzwiecki, A. (2017). Modulation of MMP-2 and MMP-9 secretion by cytokines, inducers and inhibitors in human glioblastoma T-98G cells. *Oncol. Rep.* 37 (3), 1907–1913. doi:10.3892/or.2017.5391

Schneider, G., Glaser, T., Lameu, C., Abdelbaset-Ismail, A., Sellers, Z. P., Moniuszko, M., et al. (2015). Extracellular nucleotides as novel, underappreciated pro-metastatic factors that stimulate purinergic signaling in human lung cancer cells. *Mol. Cancer* 14, 201. doi:10.1186/s12943-015-0469-z

Secchiero, P., Barbarotto, E., Gonelli, A., Tiribelli, M., Zerbinati, C., Celeghini, C., et al. (2005). Potential pathogenetic implications of cyclooxygenase-2 overexpression in B chronic lymphoid leukemia cells. *Am. J. Pathol.* 167 (6), 1599–1607. doi:10.1016/S0002-9440(10)61244-8

Shi, K., Queiroz, K. C., Stap, J., Richel, D. J., and Spek, C. A. (2013). Protease-activated receptor-2 induces migration of pancreatic cancer cells in an extracellular ATP-dependent manner. *J. Thromb. Haemostasis* 11 (10), 1892–1902. doi:10.1111/jth.12361

Singh, B., Berry, J. A., Shoher, A., Ayers, G. D., Wei, C., and Lucci, A. (2007). COX-2 involvement in breast cancer metastasis to bone. *Oncogene* 26 (26), 3789–3796. doi:10.1038/sj.onc.1210154

Soleimani, A., Taghizadeh, E., Shahsavari, S., Amini, Y., Rashidpour, H., Azadian, E., et al. (2019). CD73; a key ectonucleotidase in the development of breast cancer: recent advances and perspectives. *J. Cell. Physiol.* 234 (9), 14622–14632. doi:10.1002/jcp.28187

Toth, M., Sohail, A., and Fridman, R. (2012). Assessment of gelatinases (MMP-2 and MMP-9) by gelatin zymography. *Methods Mol. Biol.* 878, 121–135. doi:10.1007/978-1-61779-854-2_8

Tumilowicz, J. J., Nichols, W. W., Cholon, J. J., and Greene, A. E. (1970). Definition of a continuous human cell line derived from neuroblastoma. *Cancer Res.* 30 (8), 2110–2118.

Ulrich, C. M., Bigler, J., and Potter, J. D. (2006). Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. *Nat. Rev. Cancer* 6 (2), 130–140. doi:10.1038/nrc1801

Vultaggio-Poma, V., Sarti, A. C., and Di Virgilio, F. (2020). Extracellular ATP: a feasible target for cancer therapy. *Cells* 9 (11). doi:10.3390/cells9112496

Wan, L., Pantel, K., and Kang, Y. (2013). Tumor metastasis: moving new biological insights into the clinic. *Nat. Med.* 19 (11), 1450–1464. doi:10.1038/nm.3391

Wang, D., and DuBois, R. N. (2015). Immunosuppression associated with chronic inflammation in the tumor microenvironment. *Carcinogenesis* 36 (10), 1085–1093. doi:10.1093/carcin/bgv123

Wang, M. T., Honn, K. V., and Nie, D. (2007). Cyclooxygenases, prostanoids, and tumor progression. *Cancer Metastasis Rev.* 26 (3-4), 525–534. doi:10.1007/s10555-007-9096-5

Wiedemar, N., Hauser, D. A., and Mäser, P. (2020). 100 Years of suramin. *Antimicrob. Agents Chemother.* 64 (3). doi:10.1128/AAC.01168-19

Wun, T., McKnight, H., and Tuscano, J. M. (2004). Increased cyclooxygenase-2 (COX-2): a potential role in the pathogenesis of lymphoma. *Leuk. Res.* 28 (2), 179–190. doi:10.1016/s0145-2126(03)00183-8

Yan, M., Rerko, R. M., Platzer, P., Dawson, D., Willis, J., Tong, M., et al. (2004). 15-Hydroxyprostaglandin dehydrogenase, a COX-2 oncogene antagonist, is a TGF-beta-induced suppressor of human gastrointestinal cancers. *Proc. Natl. Acad. Sci. U.S.A.* 101 (50), 17468–17473. doi:10.1073/pnas.0406142101

Yang, L., Cranson, D., and Trinkaus-Randall, V. (2004). Cellular injury induces activation of MAPK via P2Y receptors. *J. Cell. Biochem.* 91 (5), 938–950. doi:10.1002/jcb.10774

Zhang, Y., Cheng, H., Li, W., Wu, H., and Yang, Y. (2019). Highly-expressed P2X7 receptor promotes growth and metastasis of human HOS/MNNG osteosarcoma cells via PI3K/Akt/GSK3beta/beta-catenin and mTOR/HIF1alpha/VEGF signaling. *Int. J. Cancer* 145 (4), 1068–1082. doi:10.1002/ijc.32207

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership