CHOLESTEROL AND OXYSTEROLS AS SIGNAL MOLECULES IN HUMAN PATHOPHYSIOLOGY AND CANCER: IMPLICATIONS FOR NEW THERAPEUTIC STRATEGIES

EDITED BY: Vincenzo Pezzi, Rosa Sirianni and Michihisa Umetani
PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-278-7
DOI 10.3389/978-2-88963-278-7

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: researchtopics@frontiersin.org

CHOLESTEROL AND OXYSTEROLS AS SIGNAL MOLECULES IN HUMAN PATHOPHYSIOLOGY AND CANCER: IMPLICATIONS FOR NEW THERAPEUTIC STRATEGIES

Topic Editors:
Vincenzo Pezzi, University of Calabria, Italy
Rosa Sirianni, University of Calabria, Italy
Michihisa Umetani, University of Houston, United States

Citation: Pezzi, V., Sirianni, R., Umetani, M., eds. (2019). Cholesterol and Oxysterols as Signal Molecules in Human Pathophysiology and Cancer: Implications for New Therapeutic Strategies. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88963-278-7

Table of Contents

frontiers in Endocrinology

EDITORIAL
published: 23 October 2019
doi: 10.3389/fendo.2019.00732

Editorial: Cholesterol and Oxysterols as Signal Molecules in Human Pathophysiology and Cancer: Implications for New Therapeutic Strategies

Rosa Sirianni[1], *Michihisa Umetani*[2,3] *and Vincenzo Pezzi*[1]*

[1] Department of Pharmacy and Health and Nutritional Science, University of Calabria, Cosenza, Italy, [2] Center for Nuclear Receptors and Cell Signaling, University of Houston, Houston, TX, United States, [3] Department of Biology and Biochemistry, College of Natural Sciences and Mathematics, University of Houston, Houston, TX, United States

Keywords: cholesterol (chol), oxysterol, cancer, signal molecules, nuclear receptor (NR)

Editorial on the Research Topic

Cholesterol and Oxysterols as Signal Molecules in Human Pathophysiology and Cancer: Implications for New Therapeutic Strategies

Cholesterol (Chol) is a lipid essential for membrane biogenesis and functionality, cell proliferation, and cell differentiation (1), however its "popularity" comes from hypercholesterolemia, a main risk factor for cardiovascular disease, neurodegeneration, inflammatory bowel disease, and cancer (2, 3). Chol can be the precursor of steroid hormones, bile acids, and other sterol metabolites responsible for a number of specific effects on human physiology (4). Among Chol metabolites, increasing attention is drawn by the family of oxidation products termed oxysterols that have been recently recognized as ligands for nuclear receptors involved in the regulation of cell viability and metabolism (5). Effects promoted by these Chol oxidation products appear to be dependent on their concentration, cell specific, dynamic condition of the cellular and tissue environment (6). For these reasons, the knowledge of molecular mechanisms that regulate the balance of Chol and oxysterol metabolism in different tissues is of crucial importance for understanding the occurrence of several diseases including cancer.

This collection is focused on the role of Chol and/or oxysterols as signaling molecules whose alteration can determine the onset or progression of pathologies. Consequently, modulation of sterol homeostasis represent a promising therapeutic strategy. Yamauchi and Rogers in this collection reviewed the connection between sterol metabolism and atherosclerosis as well as cancers. Their paper focuses on several drugs including those currently used in clinic (i.e., statins to block Chol synthesis and enhance LDL uptake) and those targeting sterol metabolism in preclinical and clinical studies (i.e., drugs targeting SREBPs and SREBP regulators, LXR, ABC transporters, ACAT, and sterol hydroxylases). Chol-free reconstituted or synthetic HDL (sHDL) represent a new reasonable strategy for delivering chemotherapeutic drugs to cancer cells. HDL receptor (Scavenger receptor type B-I; SR-BI), is highly expressed in endocrine cancers, notably due to the high demand for Chol by cancer cells. Binding sHDL loaded with a chemotherapic will deplete Chol while assuring specificity for cancer cells. This attractive approach is reviewed by Morin et al. suggesting the application of sHDLs as endocrine cancer therapeutics.

OPEN ACCESS

Edited and reviewed by:
Claire Perks,
University of Bristol, United Kingdom

***Correspondence:**
Vincenzo Pezzi
v.pezzi@unical.it

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *17 September 2019*
Accepted: *10 October 2019*
Published: *23 October 2019*

Citation:
Sirianni R, Umetani M and Pezzi V (2019) Editorial: Cholesterol and Oxysterols as Signal Molecules in Human Pathophysiology and Cancer: Implications for New Therapeutic Strategies. Front. Endocrinol. 10:732. doi: 10.3389/fendo.2019.00732

Patients suffering from hyperlipidemia or metabolic syndrome clearly evidence a link between male reproductive function and Chol homeostasis. Sèdes et al. in this collection highlighted molecular mechanisms involving nuclear receptors LXRs and FXRα and their sterol ligands important for testicular function. Drugs targeting these nuclear receptors could be used in the clinic either to treat male infertility or as new approach for male contraception.

Another interesting aspect is evidenced by Oguro reviewing in this collection recent advances in our understanding of the roles of Chol and its metabolites as signaling molecules in the regulation of hematopoiesis and hematologic malignancies. In particular, Chol metabolism to steroids and oxysterols promotes hematologic cancers, and statins that inhibit *de novo* Chol synthesis have cytotoxic effects in malignant hematopoietic cells.

The therapeutic potential of statins for the treatment of solid cancer has been reviewed in this collection by Chimento et al. Statins prevent cancer growth and metastasis by interfering with Chol activities which include signal molecules on membrane rafts, substrate for steroids, oxysterols, and Vitamin D3 synthesis, ligand for estrogen-related receptor alpha (ERRα). This last aspect that proposes Chol as an endogenous ERRα agonist has been reviewed in depth by Casaburi et al. In several cancer cells, the expression and the activity of ERRα, together with its cofactors (PGC-1 α/β), is further influenced by oncogenic signals and can thus be re-directed to induce metabolic programs favoring tumor growth and progression (7). Based on these considerations, the use of therapeutic strategies aimed to reduce Chol levels, such as statins or drugs targeting the SREBP metabolic pathways, could be a promising option to counteract metabolic rewiring in cancer cells where ERRα plays a pivotal role. Another relevant signal molecule binding nuclear receptors and involved in cancer progression is the oxysterol 27-hydroxycholesterol (27HC). 27HC is an abundant Chol metabolite in the human circulation and promotes breast cancer cell proliferation (8, 9). Hiramitsu et al. in this collection clearly describe how this oxysterol promotes also lung cancer cell proliferation through Estrogen Receptor β/PI3K-Akt signaling. Thus, lowering 27HC levels may lead to a novel approach for the treatment of lung cancer. Oxysterols are also involved in the pathology of three major gastrointestinal cancer (hepatocellular carcinoma, pancreatic, and colon cancer). Kovac et al. in this collection suggest an interesting theory on the circadian clock effects on gastrointestinal carcinogenesis by regulating lipid metabolism and beyond. With this in mind, classical therapies (statins) to modulate Chol in gastrointestinal cancers, must be administered at a proper time and for a proper duration (chronotherapy) for successful therapies.

In conclusion, from this collection it emerges that regulation of sterol homeostasis is a complex network of transcription factors, protein modifiers, sterol transporters/carriers, sterol sensors disregulated in various pathological settings such as atherosclerosis, cancers, and neurodegenerative diseases. In this context Chol and oxysterol as signal molecules are an appealing target to counteract these pathologies. New strategies aimed to modulate their levels and actions in different tissues are worth to be developed.

AUTHOR CONTRIBUTIONS

RS, MU, and VP contributed conception and design of the study. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

MU was supported by National Institutes of Health grant HL127037. VP and RS were supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC) project no. IG20122 and IG15230.

REFERENCES

1. Simons K, Ikonen E. How cells handle cholesterol. *Science.* (2000) 290:1721–6. doi: 10.1126/science. 290. 5497.1721
2. Maxfield F, Tabas I. Role of cholesterol and lipid organization in disease. *Nature.* (2005) 438:612–21. doi: 10.1038/nature04399
3. Kuzu OF, Noory MA, Robertson GP. The role of cholesterol in cancer. *Cancer Res.* (2016) 76:2063–70. doi: 10.1158/0008-5472.CAN-15-2613
4. Griffiths WJ, Abdel-Khalik J, Yutuc E, Morgan AH, Gilmore I, Hearn T, et al. Cholesteromics: an update. *Anal Biochem.* (2017) 524:56–67. doi: 10.1016/j.ab.2017.01.009
5. Umetani M. Re-adopting classical nuclear receptors by cholesterol metabolites. *J Steroid Biochem Mol Biol.* (2016) 157:20–6. doi: 10.1016/j.jsbmb.2015.11.002
6. Brown AJ, Jessup W. Oxysterols: sources, cellular storage and metabolism, and new insights into their roles in cholesterol homeostasis. *Mol Aspects Med.* (2009) 30:111–22. doi: 10.1016/j.mam.2009.02.005
7. Deblois G, St-Pierre J, Giguere V. The PGC-1/ERR signaling axis in cancer. *Oncogene.* (2013) 32:3483–90. doi: 10.1038/onc.2012.529
8. DuSell CD, Umetani M, Shaul PW, Mangelsdorf DJ, McDonnell DP. 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator. *Mol Endocrinol.* (2008) 22:65–77. doi: 10.1210/me.2007-0383
9. Wu Q, Ishikawa T, Sirianni R, Tang H, McDonald JG, Yuhanna IS, et al. 27-hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Rep.* (2013) 5:637–45. doi: 10.1016/j.celrep.2013.10.006

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 Endocrinology

MINI REVIEW
published: 19 July 2018
doi: 10.3389/fendo.2018.00369

Cholesterol: A Gatekeeper of Male Fertility?

Lauriane Sèdes, Laura Thirouard, Salwan Maqdasy, Manon Garcia, Françoise Caira, Jean-Marc A. Lobaccaro, Claude Beaudoin and David H. Volle**

Université Clermont Auvergne, INSERM U 1103, CNRS, UMR 6293, Génétique Reproduction et Développement, Clermont-Ferrand, France

Cholesterol is essential for mammalian cell functions and integrity. It is an important structural component maintaining the permeability and fluidity of the cell membrane. The balance between synthesis and catabolism of cholesterol should be tightly regulated to ensure normal cellular processes. Male reproductive function has been demonstrated to be dependent on cholesterol homeostasis. Here we review data highlighting the impacts of cholesterol homeostasis on male fertility and the molecular mechanisms implicated through the signaling pathways of some nuclear receptors.

Keywords: cholesterol, fertility, testis, spermatozoa, nuclear receptors

OPEN ACCESS

Edited by:
Vincenzo Pezzi,
University of Calabria, Italy

Reviewed by:
Riccardo Pierantoni,
Università degli Studi della Campania "Luigi Vanvitelli" Caserta, Italy
Michaela Luconi,
Università degli Studi di Firenze, Italy

****Correspondence:***
Claude Beaudoin
claude.beaudoin@uca.fr
David H. Volle
david.volle@inserm.fr

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *16 March 2018*
Accepted: *19 June 2018*
Published: *19 July 2018*

Citation:
Sèdes L, Thirouard L, Maqdasy S, Garcia M, Caira F, Lobaccaro J-MA, Beaudoin C and Volle DH (2018) Cholesterol: A Gatekeeper of Male Fertility? Front. Endocrinol. 9:369. doi: 10.3389/fendo.2018.00369

Infertility is a major public health issue defined by the World Health Organization as the inability of a couple to conceive a child after 1 year of unprotected regular sex. About 15% of couples worldwide are reported to be infertile (1); and male disorders are diagnosed in 30–50% of cases (2). Azoospermia is one of the most severe case of infertility; which is defined by the total absence of spermatozoa in the ejaculate and affects more than 20% of infertile men (3).

Male infertility can be divided into two categories: abnormalities of excretory origin, which define a defect of sperm delivery in the genital tract and abnormalities of secretory origin, which correspond an alteration of the production of the spermatozoa by the testis. The aetiologies of testicular lesions involve multiple genetic, environmental and/or behavioral factors. However, in 30% of cases, male fertility disorders remain unexplained (4). It is therefore necessary to better understand the physiology of the testis in order to identify the causes of infertility that are still idiopathic.

Male reproductive function has been demonstrated to be highly dependent on cholesterol homeostasis. Indeed, cholesterol is the precursor of steroid synthesis which is crucial for normal sperm production (5–7). Moreover, many experimental and clinical data have highlighted the importance of lipid metabolism in the control of testicular physiology and male fertility (8). As example, the depletion of cholesterol in plasma and tissues in a mouse model deficient for the gene encoding 24-dehydrocholesterol reductase (*Dhcr24*) leads to mouse infertility (9).

CHOLESTEROL HOMEOSTASIS

Cholesterol is essential for mammalian cell functions and integrity. It is an important structural component maintaining the permeability and fluidity of the cell membrane. Within the cells, the cholesterol homeostasis is tightly regulated to ensure normal cellular processes. Cholesterol homeostasis is strictly regulated at the cellular level.

Cholesterol is also a precursor for steroid hormones synthesis such as bile acids and vitamin D. Oxidized derivatives of cholesterol, known as oxysterols, are also implicated in numerous biological processes.

The main source of cholesterol is food. Dietary cholesterol is first routed from the small intestine to the liver, and then redistributed to the requesting organs. Cholesterol is uptake from lipoproteins; the Scavenger receptor B type 1 (SR-B1) is the cell surface high-density lipoprotein (HDL) receptor that mediates HDL-cholesterol ester (HDL-CE) uptake. Next to this, less than half of the cholesterol originates from *de novo* synthesis. Cholesterol synthesis is a multi-step enzymatic process. Synthesis of cholesterol begins from the acetyl-CoA, which is transported from the mitochondria to the cytosol. A series of reactions give rise to Hydroxy-methylglutaryl-CoA (HMG-CoA), which is then converted to mevalonate by HMG-CoA reductase. Finally, multi-steps will then give rise to cholesterol.

The cellular efflux of cholesterol is essential to maintain the homeostasis, as cells in peripheral organs do not express genes involved in the catabolism of cholesterol. The efflux of cholesterol requires acceptors such as HDL. There is either a simple diffusion (aqueous diffusion pathway) or a facilitated one through SR-BI pathway. There could also be active process *via* members of the ATP-binding cassette (ABC) family transporters such as ABCA1 and ABCG1.

Within the body, the liver plays an important role in maintaining cholesterol homeostasis by regulating absorption and synthesis to prevent over accumulation in the plasma and tissues (10, 11) (**Figure 1**). The small intestine is also an important actor of *de novo* synthesis of cholesterol, bile synthesis, and absorption and fecal excretion. Molecular mechanisms involved have been quite well defined in the organs such as liver, adipose tissue, and intestine (12). The cross talks between liver and intestine illustrate the complex mechanisms involved in the regulation of cholesterol homeostasis through the involvement of several factors such as SREBP-2 (sterol regulatory element-binding protein), LXR (Liver X receptor, NR1H2, NR1H3), and FXRα (Farnesoid X receptor, NR1H4). Many studies suggest that SREBP and LXRs work in a coordinated manner to maintain cholesterol homeostasis (11, 13). SREBP2 up-regulates the expression of genes involved in the biosynthesis and uptake of cholesterol when cholesterol level is low.

LXRα and LXRβ are ligand-activated nuclear receptors; which are critical for the elimination of excess cholesterol. LXRα is primarily expressed in macrophages, intestine, liver, adipose, and kidney, whereas LXRβ is more ubiquitous (14). The major endogenous LXRs agonists that have been identified are oxysterols [20(S)-, 22(R)-, 24(S)-, 25-, 27-hydroxycholesterol; 24(S), 25-epoxycholesterol] and cholesterol biosynthetic intermediates [desmosterol, follicular fluid meiosis-activating sterol (FF-MAS), or testis meiosis-activating sterol (T-MAS)] (15, 16). Two synthetic LXRα/β agonists were developed and have been used widely such as T0901317 and GW3965 (17). The physiologically most important oxysterols are generated in cells by mitochondrial or endoplasmic reticulum cholesterol hydroxylases belonging to the cytochrome P450 family (CYP46A1, CH25H, CYP27A1 CYP3A4, and CYP7A1). Several natural compounds derived from plants have also been shown to activate (phytosterols, diterpenes…) or inhibit (naringenin) LXRs activity (15). Studies from LXR-knockout mice have demonstrated that LXRs are important in the control of cholesterol homeostasis. Activation of LXRs protects cells from cholesterol accumulation by suppressing cholesterol biosynthesis, activating the conversion of cholesterol to bile acids in the liver, and decreasing intestinal cholesterol absorption (18, 19). Indeed, LXRα-knockout mice fed with a high-cholesterol diet show accumulation of cholesteryl-esters in the liver (20). LXRα is the prevalent isoform acting as hepatic sensor of cholesterol. While *Lxrα* knock-out mice showed an impaired hepatic function when fed high fat diet, mice lacking *Lxrβ* are able to resist to the deleterious impact of diet enriched in cholesterol (20, 21).

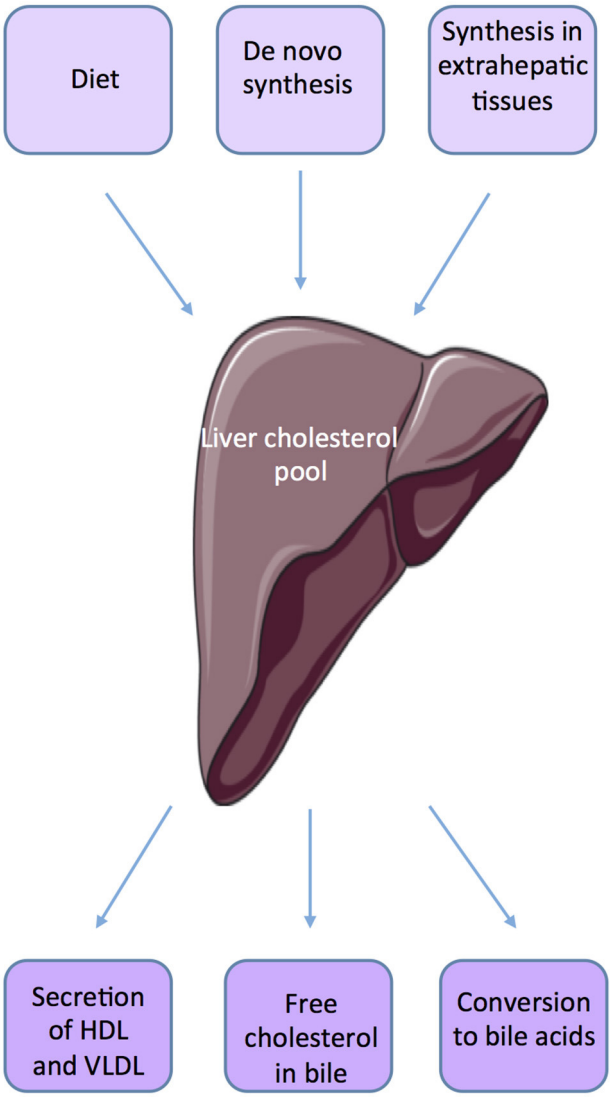

FIGURE 1 | Source of cholesterol.

Cholesterol is the precursor for bile acids (BAs) synthesis within the liver. These molecules are the main constituents of bile and ensure solubilisation and emulsification of fat to help digestion (22). Their synthesis and excretion are major mechanisms of cholesterol catabolism in mammals.

Enzymatic modifications of cholesterol result in the production of primary BAs: cholic acid (CA) and chenodeoxycholic (CDCA) (23). There are two main BA synthesis pathways (24). The ≪classical≫ pathway involves CYP7A1 and CYP8B1 enzymes while the ≪alternative≫ pathway involves the cholesterol 27-hydroxylase CYP27A1 and 25-hydroxycholesterol 7-alpha-hydroxylase CYP7B1. Before their excretion, BAs are, in part, conjugated with glycine or taurine in the liver. This leads to the production of tauro-, or glyco-conjugates BAs. Stored in the gallbladder, primary BAs and conjugates are discharged during the meal into the duodenum to facilitate fat digestion. In the ileum, BAs are in part deconjugated and modified to give rise to secondary BAs (25). Deoxycholic acid (DCA) and lithocholic acid (LCA) are derived respectively from CA and CDCA. BAs have been described as signaling molecules that signal through two main receptors: the nuclear Farnesoid X receptor alpha (FXRα; NR1H4) (26–28) and the membrane G protein-coupled bile acid receptor TGR5 (GPBAR1) (24, 29).

In addition, the nuclear receptor FXRα plays important role on cholesterol homeostasis through the regulation of bile acid metabolism in the liver and in the intestine. In the liver, FXR reduces the expression of the genes encoding key enzymes of bile acids biosynthesis pathways: the cholesterol 7α-hydroxylase (Cytochrome P450 7a1; CYP7A1) and the sterol 12α-hydroxylase (CYP8B1). In the intestine, FXRα activates the expression of the Fibroblast Growth Factor 19 (FGF19 in human) or FGF15 (mouse) which acts in an endocrine manner on hepatocytes *via* the Fibroblast Growth Factor Receptor 4 (FGFR4) to repress *Cyp7a1* expression (30, 31).

THE TESTIS, CHOLESTEROL HOMEOSTASIS

The testis produces male gametes, namely spermatozoa. It is composed of a network of seminiferous tubules, separated from each other by the interstitial space. These two compartments define the two major functions of the testis with the production of gametes (exocrine function) and sexual hormone (endocrine function). Leydig cells within the interstitial space provide part of the endocrine function, and the exocrine function takes places within the seminiferous tubules. Their efficiencies are based on the specific actions of the different cell types and their interactions.

The purpose of this special issue is to collect recent acquisition on the role of cholesterol and/or derivatives as signaling molecules, whose alteration can determine the onset or progression of different pathologies. Here, we will highlight the roles of pathways activated by cholesterol and derivatives through nuclear receptors on male fertility disorders.

The Testicular Endocrine Function

In human and rodents, testosterone is the main type of circulating androgens, which is mainly synthesized by the Leydig cells. Testis also produces small amounts of other hormones such as estrogen and progesterone.

Maintenance of an adequate concentration of intra-testicular testosterone is essential for testicular function, especially for spermatogenesis (32). The hypothalamo-pituitary axis exerts a major control on testicular steroidogenesis. Gonadotropin Releasing Hormone (GnRH) secreted by the hypothalamus, stimulates the synthesis and release of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) by the gonadotropic cells of the anterior pituitary (33). The LH acts directly on the Leydig cells to control their steroidogenic activity *via* the activation of the luteinizing hormone/choriogonadotropin receptor, LHCGR. In return, sex steroids exert a negative feedback on their own synthesis. LH is involved in the regulation of steroidogenesis at different levels. LH pathway promotes the activation of cholesterol ester hydroxylase increasing the free cholesterol pool (34) and also promotes the transfer of cholesterol from the outer membrane to the inner membrane of the mitochondria. LH signaling pathway leads to the transcriptional activation of LH target genes such as *StAR* (35), *Cyp11a1* (36), and *3βHsd* (37).

The major role of cholesterol in the endocrine function has been well established, as cholesterol is the precursor of steroids. There are multiple sources of cholesterol that contribute to steroidogenesis such as *de novo* synthesized, (b) stored cholesteryl esters, exogenous lipoprotein-supplied cholesterol as well as plasma membrane-derived cholesterol. The plasma lipoproteins are the main source of steroid synthesis. The homeostasis of the interstitial tissue, mainly in Leydig cells, involved factors of cholesterol homeostasis such as HMG-CoA reductase, HSL, and ACAT (38, 39). Among others, the links between cholesterol and steroid synthesis are highlighted by the control of the SCAP/SREBP pathway, a regulator of cholesterol homeostasis by cAMP the secondary messenger of the LH pathways.

Sex steroid hormones share a common biosynthesis pathway, with cholesterol as unique precursor. Leydig cells are able to *de novo* synthesize cholesterol or can also use stored cholesterol ester to ensure steroidogenesis function (40). A recent study shows that the plasma membrane is one of the richest source of free cholesterol available for acute steroidogenesis (41). The first enzymatic step of steroidogenesis involves the conversion of cholesterol to pregnenolone, which occurs within mitochondria. It requires the transport of cholesterol from the outer mitochondrial membrane to the inner membrane. This limiting step is mainly provided by the Steroidogenic Acute Regulatory protein (STAR) (42). This crucial point has been highlighted by the use of transgenic mouse models. Mice lacking *Star* present gonadal insufficiencies due to steroidogenesis defects associated with lipid accumulation in the interstitial compartment (43). Recently, using a mitochondrial reconstitution assay system, it has been shown that SNARE proteins (Soluble N-ethylmaleimide-sensitive-factor Attachment *protein* REceptor) are vital components contributing to cholesterol transport within the mitochondria (44). Then, the biosynthesis involves the oxidative cleavage of the side-chain of cholesterol by cholesterol side-chain cleavage enzyme (cytochrome P450scc, CYP11A1), a mitochondrial cytochrome P450 oxidase, to give pregnenolone (45). Pregnenolone may be converted to testosterone through two pathways called

Delta 4 (Δ4) and Delta 5 (Δ5) (46). The Delta 4 (Δ4) pathway involves conversion of pregnenolone to progesterone by the action of the enzyme 3β-Hydroxysteroid Dehydrogenase (3βHSD). Progesterone is then metabolized by cytochrome P450 17α-hydroxylase/17,20 lyase (CYP17A1) in androstenedione, substrate of the 17β-hydroxysteroid dehydrogenase enzyme (17βHSD) for testosterone synthesis. The Delta 5 (Δ5) pathway is initiated by the CYP17A1 enzyme, that allows the conversion of pregnenolone to dehydroepiandrosterone (DHEA). Then, 17βHSD and 3βHSD enzymes ensure its conversion into androstenediol or androstenedione, and testosterone. The 3βHSD enzyme allows switching from Δ5 to Δ4 pathway. In primates including humans, the Δ5 pathway predominates because CYP17A1 enzyme has a low activity 17, 20 lyase for the conversion of 17α-hydroxyprogesterone to androstenedione (47, 48). A recent proteomic analysis of murine testis lipid droplets (LDs) revealed that these LDs contained a large number of enzymes involved in metabolism and steroidogenesis. Testicular LDs could be an active organelle functionally involved in steroidogenesis (49).

Androgens exert autocrine action on Leydig cells via the androgen receptor (AR) to control their own synthesis. The AR knockout mice in Leydig cells have decreased plasma testosterone concentrations, despite high rates of LH. This hypo-androgenicity is explained by a defect in the expression of key genes encoding steroidogenic enzymes *Cyp17a1*, *3βhsd,* and *17βhsd*. This was shown to be associated with an arrest of spermatogenesis at the stage of round spermatid causing infertility (50, 51). In humans, mutations on the gene encoding the receptor of adrogen AR (*NR3C4*) result in androgen resistance syndrome (52). Estrogen action is also important in regulating testicular function. Estrogen mainly mediated their signal through the activation of two specific estrogen receptors (ER), ERα and ERβ (53, 54). Estrogen effects on steroidogenesis have been elucidated using mouse model. It has been shown that ERα regulates fetal and neonatal steroidogenesis and ERβ is implicated in the control of neonatal gametogenesis. *Erα* is express in Leydig cells during fetal and neonatal life and its deletion increases testosterone production during fetal life from 13.5 dpc (55). In human, estrogens also play a crucial role in the control of reproductive functions. Studies in patients with inactivating mutations of *ERα* or *CYP19* (aromatase cytochrome P450) suggest a role of estrogens signaling in human male fertility (56). Next to the steroid receptors, it has been shown that numerous nuclear receptors are implicated in the regulation of steroids synthesis. This was recently well reviewed (57, 58). Nuclear receptor family has been described to locally control the expression and activity of enzymes involved in steroidogenesis. Among these, the Steroidogenic Factor 1 (SF-1) and the Liver Receptor Homolog 1 (LRH-1) are positive regulators of hormone steroids synthesis. They stimulate the expression of common target genes such as *Star*, *Cyp11a1*, *Cyp17a1,* and *3βhsd* encoding enzymes of steroidogenesis (59–62). SF-1 and LRH-1 are also involved in the regulation of cholesterol biosynthesis by regulating the expression of *Sr-b1* (scavenger receptor class B type I) gene which provides plasma cholesterol to steroidogenic tissues (63, 64), and the enzyme Hydroxymethylglutaryl-CoA synthase and reductase (HMG CoA) involved in the biosynthesis of cholesterol (65, 66).

In addition, it has been shown that LXRα is expressed within Leydig cells (8, 67, 68). It was demonstrated that in 2.5-month-old LRXα-deficient mice, testosterone production is significantly lower than in wild type control mice. This was associated with a decrease of the expression of *3βhsd* enzyme (67). Interestingly, LXR agonist T0901317 increased testosterone concentration in wild-type mice in association with an accumulation of StAR at both mRNA and protein levels (67).

Next to this, a negative regulation of steroid synthesis involving receptors of bile acids has been defined as an important regulator of testicular physiology. BAs receptors, FXRα and TGR5, are expressed within the testis (69, 70) and have been recently shown to be implicated in testis dysfunction (71). Detectable levels of bile acids have been measured in the testis of mice in normal physiological conditions (72) and recently, it was shown that testis expresses key enzymes of BAs synthesis and is able to produce BAs (73).

The bile acids nuclear receptor FXRα is implicated in steroidogenesis regulation. In 2007, the presence of FXRα within mouse Leydig cells was demonstrated for the first time (69). While *Fxrα*$^{-/-}$ male presented the same plasma testosterone level than control mice, induction of FXRα with a synthetic agonist in wild type mice results in a decrease of androgen production independently of the hypothalamo-pituitary axis. The activation of the FXRα receptor in Leydig cells induces transcription of the gene encoding the orphan nuclear receptors SHP (Small Heterodimer Partner, *Nr0b2*) and DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1, *Nr0b1*), two orphan nuclear receptors, which are negative regulators of steroidogenic Leydig cells activity. Indeed, SHP AND DAX-1 inhibit the expression of *Lrh1* and *Sf1*. SHP also interacts with LRH1 thereby inhibiting its trans-activating activity. This decreases expressions of genes encoding key enzymes of steroidogenesis and thus leads to lower testosterone levels (69). In that line, through the regulation of the endocrine function, it has been demonstrated that BA-FXRα pathways also regulate male sexual maturation (74). Mice fed a diet supplemented with BAs during pubertal age show increased incidence of infertility. This is associated with altered differentiation and increase apoptosis of germ cells due to lower testosterone levels. This is sustained by the fact that supplementation with testosterone abolished the effect of CA-diet on germ cell apoptosis.

These overall data clearly suggest a complex regulation of testicular steroidogenesis by nuclear receptors whose activities are regulated by cholesterol derivatives such as oxysterols and bile acids (**Figure 2**).

The Testicular Exocrine Function

Germ cell differentiation is closely related to somatic Sertoli cells functions, which provide structural and nutritional supports (**Figure 3**).

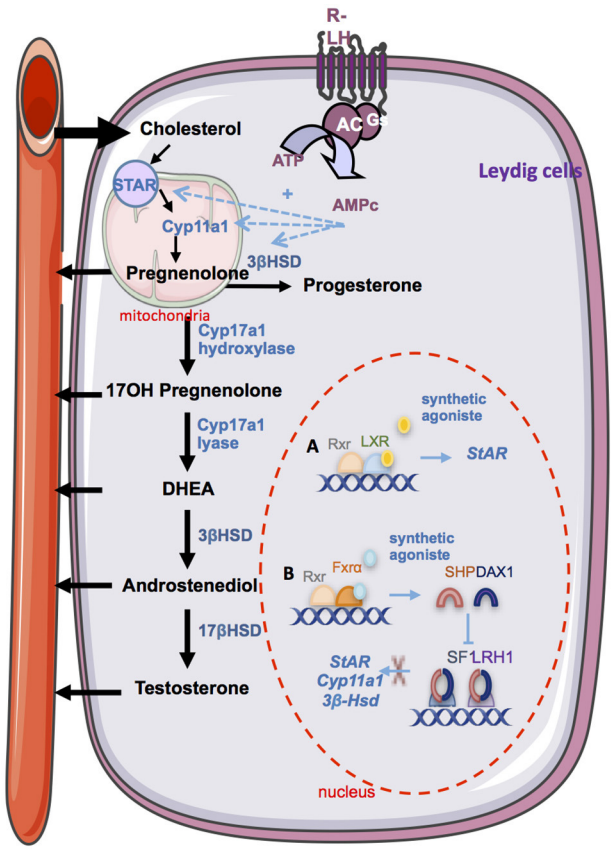

FIGURE 2 | The endocrine function. Steroidogesesis occurs in Leydig cells from cholesterol. The first step involves the STAR protein which allow the transport of cholesterol within the mitochondria. Then, cholesterol is converted in pregnenolone by the CYP11A1 enzyme. Several enzymatic transformation steps will convert pregnenolone into testosterone. The differents enzymes are marked in blue. LH via it receptor (R-LH) induces the increase expression of some key enzymes of spermatogenesis. Some nuclear receptors have been described as regulators of spermatogenesis. **(A)** The activation of LXRs by a synthetic agonist induces the upregulation of Star. **(B)** The activation of FXRα by a synthetic agonist induce the increase *Shp* of *Dax1* which in turn inhibits the trans activating activity of SF1 and LRH1 on the promotor of steroidogenic enzyme such as *Star, Cyp11a1,* and *3bhsd*. STAR, steroidogenic acute regulatory protein; Cyp11a1, cytochrome p450scc cholesterol side chain clivage; Cyp17a1, cytochrome P450 17α-hydroxylase/17,20 lyase; 3βHsd, 3β-hydroxysteroid dehydrogenase; 17βHsd, 17β-hydroxysteroid dehydrogenase; DHEA, dehydroepiandrosterone; RXR, retinoid acid receptor; LXR, liver X receptor; FXR, farnesoid X receptor; Shp, small heterodimer partner; Dax1, dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1; SF1, steroidogenic factor 1; LRH1, liver receptor homolog 1.

The Sertoli Cells

One primary function of Sertoli cells is to set up the blood testis barrier (BTB) at puberty under the action of androgens, retinoids and thyroid hormones (75–77). The BTB is composed of specialized junctions established between adjacent Sertoli cells near the basement membrane of seminiferous tubules. That defines Sertoli cells polarity giving them different structural and functional poles. The BTB divides the seminiferous tubules in basal and apical compartments. Self-renewal and differentiation of spermatogonia as well as progression to the stage pre-leptotene spermatocyte I occur in the basal compartment. The two meiotic divisions and post-meiotic steps take place in the apical compartment. The BTB regulates and restricts the passage of nutritional substances, vital molecules, and toxic compounds in the apical compartment of the seminiferous tubules. BTB also defines an immune privileged environment. It isolates many antigens present on the surface of post-meiotic germ cells from the systemic circulation thus preventing the emergence of the corresponding antibodies and autoimmune diseases leading to infertility (78). Sertoli cells also participate to the secretion of components of the extracellular matrix necessary to maintain the integrity of the seminiferous epithelium and intercellular junctions (79). In addition, they synthesize proteases and protease inhibitors contributing to the dynamic of junction complexes allowing the migration of germ cells (80). Sertoli cells provide a set of essential nutrients for germ cells survival and differentiation, including amino acids, carbohydrates, lipids and vitamins (80). Sertoli cells are able to metabolize glucose into pyruvate and lactate (81) which is the preferential energy substrate for post-meiotic germ cells to support their metabolic activity. Sertoli cells secrete numerous growth factors modulating the proliferation and/or differentiation of germ cells (82). In addition Sertoli cells secrete severals transport proteins that will ensure the passage of metal ions (Transferrin) (83) or hormone (androgen-binding protein, retinoid binding protein) (84) through the BTB for the metabolic requirement of the post-meiotic germ cells.

Sertoli cells provide large amounts of lipids used for spermatogenesis. *In vitro*, it has been demonstrated that Sertoli cells are able to synthesize cholesterol from acetate (85). However, this *de novo* synthesis is not a sufficient source of cholesterol to ensure spermatogenesis *in vivo*. Therefore, it is necessary to import cholesterol from the blood circulation. In rodent, it has been demonstrated that high density lipoprotein (HDL) is the primary source of cholesterol used by Sertoli cells (86). The high-density lipoprotein transporter SR-B1 is essential to import cholesterol from blood. SR-B1 is an integral membrane glycoprotein expressed in Leydig and Sertoli cells (87, 88). This transporter facilitates the uptake of HDL. It has been demonstrated that the overexpression of SR-B1 is associated with an increase of esterified cholesterol in Sertoli cells (89). SR-B1 is also implicated in the phagocytosis of apoptotic germ cells. This may also increase cholesterol levels in Sertoli cells (90). Another important point is the elimination of cholesterol overflow. Cholesterol is esterified and stored in lipid droplets to limit toxicity. Sertoli cells maintain cholesterol homeostasis through reverse cholesterol transport. This reverse transport primarily relies on the cholesterol transporter ATP-binding cassette A1 (ABCA1) (91). Genetic mutations in *ABCA1* lead to the absence of HDL in the plasma and the accumulation of esterified cholesterol in tissues, increasing the incidence of atherosclerosis (92). ABCA1 is highly expressed in the testis and it was observed that within the seminiferous tubules, ABCA1 is more expressed in Sertoli cells (91). Authors demonstrated that TM4 Sertoli cells lacking *Abca1* or primary Sertoli cells cultured from *Abca1*$^{-/-}$ mice fail to efflux cholesterol. *In vivo*, *Abca1*$^{-/-}$

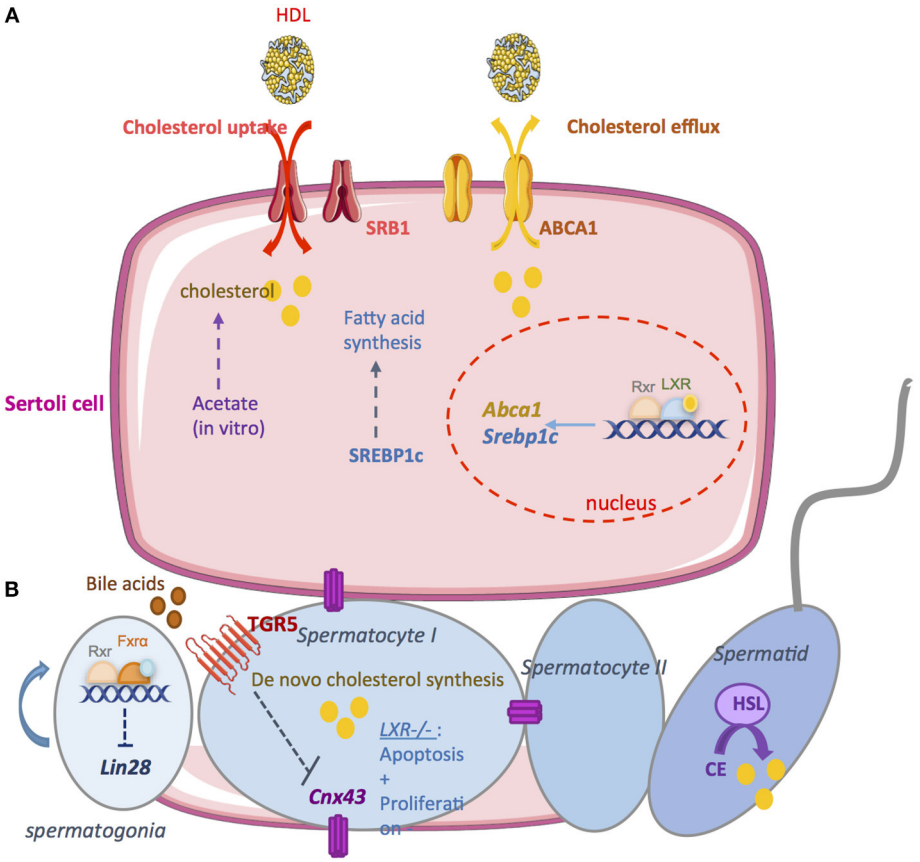

FIGURE 3 | The exocrine function. **(A)** A large amount of lipids used for spermatogenesis are provided by Sertoli cells. *In vitro*, it has been demonstrated that Sertoli cells are able to synthesize cholesterol from acetate. Therefore, it is necessary to import cholesterol from the blood circulation. high density lipoprotein (HDL) is the primary source of cholesterol used by Sertoli cells. SRB1 facilitates the uptake of HDL within mitochondria. Sertoli cells maintain cholesterol homeostasis through reverse cholesterol transport which relies on the cholesterol transporter ATP-binding cassette A1 (ABCA1). LXR/RXR heterodimers increase the cellular cholesterol content by enhancing the levels of SREBP-1c and decrease cholesterol levels by increasing ABCAI expression. **(B)** Germ cells are able to produce cholesterol *de novo*. Cholesterol *de novo* synthesis was increased during the development of pachytene, leptotene, and zygotene stages of spermatocyte I. During the latest steps, the hormone sensitive lipase (HSL) mediates the hydrolysis of cholesterol esters. The bile acids nuclear receptor FXRα regulate germ cell physiology. FXRα inhibits the expression of the pluripotency marker Lin28. $FXR^{-/-}$ mice present an increase number of undifferenciated spermatogonia that induce an increase number of spermatozoa. The G coupled bile acids receptor TGR5 also regulates germ cell physiology. Activation of TGR5 by Bile acids in spermatocytes decrease Cnx43 expression inducing the rupture of blood testis barrier. Nuclear receptor for oxysterols LXRs are also important regulators of germ cells physiology. In $LXR^{-/-}$ mice, germ cells apoptosis increase while there is a decrease in the proliferation rate. This could explain the complete loss of germ cells in these mice leading to infertility.

mice present lipid accumulation in Sertoli. At 6 months, intra-testicular testosterone levels and sperm counts are significantly reduced in *Abca1*$^{-/-}$ mice compared with controls. Thereby, the fertility of *Abca1*$^{-/-}$ mice is reduced across their reproductive lifespans. These results indicate that ABCA1 play important role to Sertoli cells function and male fertility.

Regarding the cellular signaling, it has been demonstrated that LXRβ is expressed in Sertoli cells. The *Lxrβ*$^{-/-}$ knockout mice are fertile with few observed defects (57, 67). However, these mice present cholesteryl ester accumulation in the Sertoli cells. This is associated with a decrease of germ cell proliferation rate. Interestingly, it was shown that men with azoospermia present a significant decrease of *Lxrβ* expression within the testis associated with fewer proliferating germ cells (93). Mice deficient for both LXRs isoforms present progressive testicular degeneration associated with germ cell depletion. *Lxrα*$^{-/-}$;*Lxrβ*$^{-/-}$ are reported to be infertile by 7–9 months of age (67, 68). As in *Lxrβ*$^{-/-}$ mice, lipid accumulation in Sertoli cells appears to be the earliest phenotype of *Lxrα*$^{-/-}$;*Lxrβ*$^{-/-}$ mice. A recent study further investigate the roles of LXRβ in Sertoli cells by generating a mouse model that re-expresses *Lxrβ* only in Sertoli cells in the *Lxrα*$^{-/-}$;*Lxrβ*$^{-/-}$ mice (94). Authors showed that this *Lxrβ* re-expression is fundamental for Sertoli cells to maintain functional BTB and to sustain the germ cell pool. As expected, *Lxrβ* re-expression restored lipid homeostasis in Sertoli cells and regulates the endocrine function of Leydig cells. However, these mice are infertile with spermatogenesis defects. Furthermore, it was also demonstrated that wild-type animals exposed to the synthetic LXR agonist T0901317, within diet, exhibited increased levels of the LXR target genes sterol

regulatory element-binding protein-1c (SREBP-1c) and ABCA1 (14, 95). Another study comforts the previous results in MSC-1 cells treated with T0901317 where ABCA1 levels were also increased (68).

These data clearly demonstrate the main roles of LXRs in the regulation lipid homeostasis of Sertoli cell and thus male fertility.

The Germ Cell Lineage

Spermatogenesis is a highly coordinated process leading to the production of male haploid gametes differentiated from diploid germ stem cells. Spermatogenesis begins with a proliferative phase with the differentiation of type A spermatogonia located in contact with the basement membrane of the seminiferous tubules. A_s (A single) spermatogonia possess the ability to renew the population of undifferentiated germ cells. Spermatogonia can also enter in the differentiation process. Then spermatogonia engaged in meiosis, the diploid spermatocyte gives rise to haploid cells called secondary spermatocyte or spermatocyte II and then round spermatids. Meiosis step is followed by spermiogenesis; which is the terminal process of spermatogenesis differentiation. The spermatids nucleus will be reorganized leading to chromatin compaction (96). Firstly the majority of histones is replaced by small basic transition proteins (TNP1 and TNP2). Secondly, transition proteins will be replaced by specific testis nuclear protein, protamines (PRM1 and PRM2) (97) to protect the genome from physical and chemical attack.

There is an intimate association between cholesterol metabolism and fertility during spermatogenesis. Cholesterol is required for the mass production of germ cells during spermatogenesis. A study determined that cholesterol *de novo* synthesis was increased during the development of pachytene, leptotene, and zygotene stages (98), which was associated with increased diameter and surface area of germ cells. In later stages, the rate of cholesterol synthesis tends to decrease and remains low throughout the following phases of spermatogenesis. During the last steps of spermatogenesis the Hormone-sensitive lipase (HSL) is known to mediate the hydrolysis of cholesterol esters. It has been shown that the HSL is localized in elongating spermatids and spermatozoa. The most striking phenotype of $Hsl^{-/-}$ mice is male sterility caused by oligospermia (99). $Hsl^{-/-}$ mice were found to accumulate diacylglycerols and cholesterol esters in the testis. A recent study further investigate $Hsl^{-/-}$ mice testis. Authors showed that spermatogenesis is arrested just before spermiogenesis elongation step (100). An important pathway of lipid metabolism must be lost in $Hsl^{-/-}$ testes causing an arrest of cell differentiation.

Like any animal cell, spermatozoa have a lipid bilayer plasma membrane. The proportion of the different constituents gives to the spermatic plasma membrane unique properties. The spermatic membrane has been shown to be rich in polyunsaturated fatty acids (PUFAs) important for ensuring the viability and mobility of the spermatozoon (101–103). The high proportion of PUFA also seems to play an important role in the process of membrane fusion between spermatozoon and oocyte. Indeed, PUFAs contribute to flexibility and membrane fluidity (104, 105). The high proportion of PUFA, especially docosahexaenoic acid (DHA) in humans, is important for fertility. Indeed, one study showed a decrease in PUFAs and an increase in saturated fatty acids (SFA) in spermatozoa of asthenozoospermic men compared to sperm of normozoospermic men (106). Another study also found a negative correlation between body mass index and DHA and a positive correlation between DHA and normal sperm parameters (107). Sterols are the second major components of the plasma membrane. Cholesterol is the most abundant sterol found in the spermatic membrane of many species (102). Spermatozoa are enriched in cholesterol within the seminiferous tubules. Indeed, in the spermatocyte stage, the germ cells are able to synthesize *de novo* cholesterol to allow the increase of their membrane surface (98). Sertoli cells also participate in this process by providing to sperm cells *de novo* synthesized cholesterol or derived from the bloodstream (85, 89). More recently, the presence of oxidized cholesterol derivatives has been described in several species including mice (108). These oxysterols could influence the membrane fluidity (109). The spermatic membrane is also composed of complex lipids such as phospholipids (PL) and sphingolipids (77, 85). The lipid composition of spermatozoa is of great importance during capacitation in the female genital tract and also in membrane fusion events during fertilization. After spermiation, the lipid composition of the spermatic membrane is not definitive and will undergo many modifications during the epididymal transit and the capacitation process. Lipids largely influence post-testicular maturation of spermatozoa. During the epididymal transit, the lipid composition of the gamete is strongly reshuffled (110–112). These modifications influence the membrane dynamics in order to confer greater membrane fluidity. These lipid rearrangements are finely regulated. The epididymis produces lipid vesicles allowing protein transfer to spermatozoa. Finally, capacitation is a stage of post-testicular maturation partly regulated by the lipid composition of the spermatic membrane (113). Therefore, regulation of the lipid profile of gametes is essential for maintaining male fertility. It has recently been shown that the lipid composition of spermatozoa varies according to the dietary intake of lipids (114), demonstrating a direct correlation between diet and gamete composition.

Mammalian spermatozoa are specialized and polarized cells, with one specific function which is the fertilization of the female oocyte. This fertilizing capacity is acquired during a multistep process after spermiation called post testicular maturation. Spermatozoa maturation starts during its epididymal transit and ends in the female genital tract. The maturation of spermatozoa remains the major function of the epididymis. Epididymal maturation is essential for the acquisition of fertilizing power but is also essential to ensure normal embryonic development (115). Epididymal maturation consists of morphological, functional, and biochemical changes.

Regarding the signaling pathways involved, the LXR signaling pathways control implicated in the regulation of spermatogenesis (67). Spermatogenesis results from a balance between cell

proliferation, cell differentiation, and cell death. Proliferation and apoptosis are respectively altered in $Lxr\beta^{-/-}$ and $Lxr\alpha^{-/-}$ mice, whereas these mice did not show any fertility troubles. In the $Lxr\beta^{-/-}$ mice the lower proliferation rate was compensated by a decreased apoptosis rate and in $Lxr\alpha^{-/-}$ mice the higher apoptosis level was associated with an increased proliferation rate. The lack of both LXRs led to a dramatic decline in proliferation and increase in apoptosis. This could explain the complete loss of germ cells in these mice and thus infertility. Recently, the implication of LXRs in the physiopathology of defective spermatogenesis in non-obstructive azoospermia (NOA) patients was investigated (93). Indeed, abnormalities observed in NOA patients look like those observed in $Lxr^{-/-}$ mice. It has been shown that *Lxr* mRNA levels were decreased in NOA specimens and positively correlated with germ cell number. As expected, authors showed a decreased level of *Idol* (Inducible Degrader of the LDL receptor) and *Srebp1c* (Sterol regulatory element-binding protein 1), which are LXR target genes.

Next to this, a recent study has shown that the G coupled bile acids receptor TGR5 was expressed within the testis (70). It has been shown that BA-TGR5 signaling pathways alter testicular epithelium integrity leading to adult male fertility disorders (72). Indeed, after 4 months of dietary cholic acid (CA) supplementation, 25% of male mice were sterile and in fertile ones a 20% decrease of pups per litter was reported. Male exposed to BAs presented lower spermatozoa in the tail of the epididymis explaining the fertility defect. Interestingly, authors showed that $Tgr5^{-/-}$ mice were preserved of the CA deleterious effects on the testis. At the molecular level, BAs exposure induced a repression of cell-cell interactions network through the down-regulation of N-cadherin as well as connexin 43 (CX43) expression. This leads to germ cell sloughing, rupture of the BTB and then spermatids apoptosis. In humans, a reduction in the expression of CX43 was shown to be associated with the degree of spermatogenesis defect (116–118).

Recently, it has been shown that bile acids nuclear receptor FXRα regulates germ cell physiology (73). Authors showed that FXRα plays a role in the establishment and maintenance of the undifferentiated germ cell pool that in turn influences male fertility. $Fxr\alpha^{-/-}$ males present an extended fertility with aging. In $Fxr\alpha^{-/-}$ males, the maintenance of fertility capacities results from a higher number of undifferentiated germ cells during aging associated with high spermatozoa production. FXRα regulates the expression of several pluripotency factors within the germ cell lineage. *In vitro* approaches show that FXRα controls the expression of the pluripotency marker Lin28 in the germ cells.

Obesity and Male Fertility

In the last decades infertility has become a global public health issue affecting 15% of all reproductive couples. It has been estimated that 70 million couples worldwide experience subfertility or infertility (119). Male factors are responsible for ∼25% of cases of infertility (2). Currently, the etiology of low semen quality is poorly understood. Many physiological, environmental and genetic factors have been implicated (120). Metabolic syndrome (MetS) is a group of risk factors such as high blood pressure, high blood sugar, unhealthy cholesterol levels, and abdominal fat. The association between MetS and male hypogonadism is well established and reviewed (121). Cross-sectional studies have found that between 20 and 64% of obese men have low total testosterone levels (122). A number of studies also show that low testosterone is associated with insulin resistance and an increased risk for diabetes mellitus and MetS in men. Testosterone supplementation seems to be beneficial on inflammation, muscle mass, lipid profile, sexual function, and improves comorbidities of obesity, MetS, or cardiovascular disease in males (121).

Evidence from human studies indicates that male obesity and components of the diet could play an important role in the deregulation of spermatogenesis, sperm maturation, or fertilizing ability. Obese or overweight men could present a decrease sperm quantity, quality, and motility (123, 124). A study showed differences between obese and lean patients regarding the acrosome reaction (125). Sperm acrosome reaction is impaired in obese men associated with altered circulating levels of estradiol and sperm cholesterol content. Obese men can also present an increase sperm DNA damage or lower embryo implantation rate (122, 126). The majority of studies in humans focuses on the impact of obesity on male fertility and do not take into account the plasma lipid profile. It is reported that 65% of infertile men show hypercholesterolemia and/or triglyceridemia (127). The impact of dyslipidemia on male fertility remains controversial. Even if cholesterol and lipid homeostasis is essential for male fecundity (57, 128), a recent study reported that hypercholesterolemia is not associated with sperm concentration or motility in men (129). It has also been shown that there is no correlation between sperm concentration and serum total cholesterol or triglyceride in human (130).

Some animal studies revealed that high cholesterol diet can impair fertility and lower sperm quality. Rabbit fed a High fat diet (HFD) is an experimental model of metabolic syndrome (MetS) that closely similar to the human syndrome (131–133). This model has been characterized by hyperglycaemia, hypercholesterolemia, hypertension, hypogonadotropic hypogonadism, penile alterations (131, 132, 134) or nonalcoholic steatohepatitis. This model is very useful to study the deleterious impact of MetS symptoms such as hypercholesterolemia on male fertility. It has been demonstrated that supplementation with testosterone (131, 132) with the FXRα agonist the obeticholic acid (133, 134) normalize several MetS symptoms including HFD-induced penile alterations. These animals presented abnormal sperm morphology, decreased sperm number and declined percentage of motile sperm or acrosome reaction (134–136, 138, 139). It has been shown that administration of tamoxifen, used in the treatment of idiopathic male infertility to HFD rabbits partially restored sperm motility, but further decreased morphology and increased spontaneous acrosome reaction (136). It has also been shown that hypercholesterolemic rabbits present lower testicular efficiency related to both a decrease in spermatogonial cells and an increase in germ cell apoptosis (137). On the other hand,

spermiogenesis, which is the last step of spermatogenesis, was also affected in these animals. In post-meiotic germ cells from hyper-cholesterolemic rabbits, abnormal development of acrosome, nucleus, and inaccurate tail implantation were associated with actin–alpha-tubulin–GM1 sphingolipid altered distribution. It was recently shown in rabbits that a cholesterol-enriched diet increased lipid deposition in the seminiferous tubules and disrupted the BTB (138). Total protein levels of the tight-junction protein 1 (ZO-1) and occludin and their distribution patterns were markedly affected, impairing fertility.

A similar study has demonstrated a disruption of BTB in mice exposed to High Fat Diet (HFD) (139). It has been reported that male rodents exposed to HFD present a decreased number of motile spermatozoa associated with a decline of fertilization rates (140–142). Intracellular reactive oxygen species (ROS) and sperm DNA damage were elevated in the HFD group compared with controls that could explain the decrease sperm motility (141). It has been demonstrated that HFD induces a decrease in testosterone production associated with a decrease level of steroidogenic enzyme (143, 144). A recent study suggests that the increase of autophagy process could be a cause of fertility defects induced by HFD (145). Authors demonstrate that inhibiting autophagy with chloroquine improves the decreased fertility of HFD male mice. Furthermore, the excessive activation of autophagy was also observed in sperm samples from obese, sub-fertile male patients.

In addition, some studies were interested in the impact of paternal or maternal exposure to HFD on male offspring (146, 147). It has been shown that preconception exposure of fathers to HFD impairs the motility of spermatozoa of male offspring, despite their control diet consumption (146). Feeding females with a HFD through pregnancy and lactation induces a decrease on LH and testosterone levels in male offspring (147). If translatable to human health, these studies suggest that reproductive defects may be amplified throughout generations because of calorie dense diet.

Interestingly, in the last decade, it has been proposed that derivatives of bile acids (BAs) could be interesting molecules for the treatment of metabolic diseases such as diabetes or obesity. Regarding the links between BA signaling pathways and male testicular physiology (72–74), a study has investigated the consequences of a long term exposure to molecules that activate BA signaling pathways to treat obesity. For that purpose, mice were fed HFD to induce obesity and concomitantly treat with BAs (148). Even if BAs improves abnormalities induced by the HFD such as body weight, glycemia, or lipidic profiles, co-exposure of HFD and BAs leads to a higher level of infertility. This was associated with altered germ cell proliferation, default of endocrine function and abnormalities in cell-cell interactions within the seminiferous tubule.

CONCLUSIONS

Male fertility disorders represent serious health problem and in 30% of the cases the cause remains unknown. Understanding the various pathologic mechanisms causing male infertility represents an active area of research. As reviewed here, cholesterol homeostasis is crucial for testicular functions such as steroidogenesis, Sertoli cells function, or germ cell differentiation. Thus, altered concentrations of plasma cholesterol can affect the reproductive function leading to male infertility. This link between lipid homeostasis and male fertility disorders is clearly evident in patients suffering from hyperlipidemia or metabolic syndrome. Lipid-lowering drugs could probably ameliorate some reproductive symptoms. However, epidemiological data are currently scarce and controversial.

All these data could help in proposing new molecular markers to diagnose cases of infertility or to define whether the results obtained on the incriminated nuclear receptors LXRs and FXRα which can be modulated by ligands can lead to concepts transferable to the clinic either to develop new strategies to treat infertile men or new approached for male contraception. Such perspectives will require the development of testicular-specific LXR or FXR modulators to treat testicular disorders and therefore male disorders without causing their impacts that may lead to metabolic disorders.

In the opposite way, modulating some of these receptors through the use of agonist or antagonist molecules would improve the symptoms of metabolic syndrome diseases. However, it has been shown in mice that modulating nuclear receptors signaling induce deleterious effects on testicular physiology leading to infertility. In that line, as molecules modulating the BA receptor signaling pathways have been proposed to treat metabolic diseases, it will be important to define the molecular mechanisms involved in the deleterious impact of BAs on testis physiology. Such studies will be essential to define how to pharmacologically or genetically modulate bile acid receptors to treat metabolic disorders while minimizing impacts on male reproductive functions. In addition, the identification of the crosstalk between increased BA levels and metabolic diseases on fertility capacities of male mice should be a mechanism explaining rare clinical situations of infertility following bariatric chirurgical approach in some obese men.

It will be interesting in the future to identify at the clinical levels, using biopsies of patients suffering from liver disorders (cholestasis, hepatitis…), the potential involvement of bile acid signaling pathways in the human regulation of male fertility. According to previous published data, it will be necessary to analyse both BA levels as well as the BA pool composition. Indeed, it appears that secondary BA, namely DCA, is the one whose levels are inversely correlated with fertility. Such analyses on human cohort will be useful to define if BA levels or pool composition might be a useful biomarker linking liver disorders and male infertility; or even if BA could be markers of idiopathic infertility.

In the recent decades, assisted medical procreation (AMP) has been importantly developed to overpass the fertility disorders. Next to *In vitro* fecundation, important efforts have been done in order to develop approach of *in vitro* production of germ cells from spermatogonial stem cells. This strategy is important to propose AMP to azoospermic men. The recent data regarding the role of FXRalpha in the establishment of the

pool of undifferentiated spermatogonia, suggest that FXRalpha modulators might be useful in such approaches to increase the rate and efficiency to produce male games.

AUTHOR CONTRIBUTIONS

LS, LT, SM, MG, FC, JML, CB and DV have participated to the writing of this review.

FUNDING

This work was funded by Inserm, CNRS, Clermont Université, Ligue contre le Cancer (Comité Puy de Dôme to DV), Nouveau Chercheur Auvergne (#R12087CC to DV), and Plan Cancer—Cancer-Environnement InCa/Inserm (C14012CS to DV). DV's team received support by the French government IDEX-ISITE initiative 16-IDEX-0001 (CAP 20-25).

REFERENCES

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol.* (2015) 13:37. doi: 10.1186/s12958-015-0032-1
2. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. *Fertil Steril.* (2002) 77:873–82.
3. Jarvi K, Lo K, Grober E, Mak V, Fischer A, Grantmyre J, et al. The workup and management of azoospermic males. *Can Urol Assoc J.* (2015) 9:229–35. doi: 10.5489/cuaj.3209
4. Cavallini G. Male idiopathic oligoasthenoteratozoospermia. *Asian J Androl.* (2006) 8:143–57. doi: 10.1111/j.1745-7262.2006.00123.x
5. Parton RG, Hancock JF. Lipid rafts and plasma membrane microorganization: insights from Ras. *Trends Cell Biol.* (2004) 14:141–7. doi: 10.1016/j.tcb.2004.02.001
6. Simons K, Ikonen E. How cells handle cholesterol. *Science* (2000) 290:1721–6.
7. Yokoyama S. Release of cellular cholesterol: molecular mechanism for cholesterol homeostasis in cells and in the body. *Biochim Biophys Acta.* (2000) 1529:231–44. doi: 10.1016/S1388-1981(00)00152-9
8. El-Hajjaji FZ, Oumeddour A, Pommier AJC, Ouvrier A, Viennois E, Dufour J, et al. Liver X receptors, lipids and their reproductive secrets in the male. *Biochim Biophys Acta* (2011) 1812:974–81. doi: 10.1016/j.bbadis.2011.02.004
9. Wechsler A, Brafman A, Shafir M, Heverin M, Gottlieb H, Damari G, et al. Generation of viable cholesterol-free mice. *Science* (2003) 302:2087. doi: 10.1126/science.1090776
10. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* (1990) 343:425–30. doi: 10.1038/343425a0
11. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell* (2006) 124:35–46. doi: 10.1016/j.cell.2005.12.022
12. Goedeke L, Fernández-Hernando C. Regulation of cholesterol homeostasis. *Cell Mol Life Sci.* (2012) 69:915–30. doi: 10.1007/s00018-011-0857-5
13. Calkin AC, Tontonoz P. Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. *Nat Rev Mol Cell Biol.* (2012) 13:213–24. doi: 10.1038/nrm3312
14. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, et al. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev.* (2000) 14:2819–30.
15. Komati R, Spadoni D, Zheng S, Sridhar J, Riley KE, Wang G. Ligands of therapeutic utility for the liver X receptors. *Molecules* (2017) 22:e88. doi: 10.3390/molecules22010088
16. Mutemberezi V, Guillemot-Legris O, Muccioli GG. Oxysterols: from cholesterol metabolites to key mediators. *Prog Lipid Res.* (2016) 64:152–69. doi: 10.1016/j.plipres.2016.09.002
17. Ratni H, Wright MB. Recent progress in liver X receptor-selective modulators. *Curr Opin Drug Discov Devel.* (2010) 13:403–13.
18. Wang Y, Rogers PM, Su C, Varga G, Stayrook KR, Burris TP. Regulation of cholesterologenesis by the oxysterol receptor, LXRalpha. *J Biol Chem.* (2008) 283:26332–9. doi: 10.1074/jbc.M804808200
19. Baranowski M. Biological role of liver X receptors. *J Physiol Pharmacol.* (2008) 59(Suppl. 7):31–55.
20. Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, et al. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell* (1998) 93:693–704.
21. Alberti S, Schuster G, Parini P, Feltkamp D, Diczfalusy U, Rudling M, et al. Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice. *J Clin Invest.* (2001) 107:565–73. doi: 10.1172/JCI9794
22. Poupon R, Chignard N, Rosmorduc O, Barbu V, Housset C. [Biliary function and its regulation]. *Med Sci.* (2004) 20:1096–9. doi: 10.1051/medsci/200420121096
23. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem.* (2003) 72:137–74. doi: 10.1146/annurev.biochem.72.121801.161712
24. Martinot E, Sèdes L, Baptissart M, Lobaccaro J-M, Caira F, Beaudoin C, et al. Bile acids and their receptors. *Mol Aspects Med.* (2017) 56:2–9. doi: 10.1016/j.mam.2017.01.006
25. Ridlon JM, Kang D-J, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res.* (2006) 47:241–59. doi: 10.1194/jlr.R500013-JLR200
26. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. *Science* (1999) 284:1362–5.
27. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile acids: natural ligands for an orphan nuclear receptor. *Science* (1999) 284:1365–8.
28. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell* (1999) 3:543–53.
29. Maruyama T, Tanaka K, Suzuki J, Miyoshi H, Harada N, Nakamura T, et al. Targeted disruption of G protein-coupled bile acid receptor 1 (Gpbar1/M-Bar) in mice. *J Endocrinol.* (2006) 191:197–205. doi: 10.1677/joe.1.06546
30. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab.* (2005) 2:217–25. doi: 10.1016/j.cmet.2005.09.001
31. Holt JA, Luo G, Billin AN, Bisi J, McNeill YY, Kozarsky KF, et al. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev.* (2003) 17:1581–91. doi: 10.1101/gad.1083503
32. Woolveridge I, Bryden AA, Taylor MF, George NJ, Wu FC, Morris ID. Apoptosis and expression of apoptotic regulators in the human testis following short- and long-term anti-androgen treatment. *Mol Hum Reprod.* (1998) 4:701–7.
33. Shacham S, Harris D, Ben-Shlomo H, Cohen I, Bonfil D, Przedecki F, et al. Mechanism of GnRH receptor signaling on gonadotropin release and gene expression in pituitary gonadotrophs. *Vitam Horm.* (2001) 63:63–90.
34. Stocco DM, Wang X, Jo Y, Manna PR. Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: more complicated than we thought. *Mol Endocrinol.* (2005) 19:2647–59. doi: 10.1210/me.2004-0532
35. Manna PR, Dyson MT, Eubank DW, Clark BJ, Lalli E, Sassone-Corsi P, et al. Regulation of steroidogenesis and the steroidogenic acute regulatory protein by a member of the cAMP response-element binding protein family. *Mol Endocrinol.* (2002) 16:184–99. doi: 10.1210/mend.16.1.0759
36. Payne AH. Hormonal regulation of cytochrome P450 enzymes, cholesterol side-chain cleavage and 17 alpha-hydroxylase/C17-20 lyase in Leydig cells. *Biol Reprod.* (1990) 42:399–404.
37. Keeney DS, Mason JI. Expression of testicular 3 beta-hydroxysteroid dehydrogenase/delta 5—4-isomerase: regulation by luteinizing hormone and forskolin in Leydig cells of adult rats. *Endocrinology* (1992) 130:2007–15. doi: 10.1210/endo.130.4.1312436

38. Kabbaj O, Holm C, Vitale ML, Pelletier RM. Expression, activity, and subcellular localization of testicular hormone-sensitive lipase during postnatal development in the guinea pig. *Biol Reprod.* (2001) 65:601–12. doi: 10.1095/biolreprod65.2.601
39. Kabbaj O, Yoon SR, Holm C, Rose J, Vitale ML, Pelletier R-M. Relationship of the hormone-sensitive lipase-mediated modulation of cholesterol metabolism in individual compartments of the testis to serum pituitary hormone and testosterone concentrations in a seasonal breeder, the mink (Mustela vison). *Biol Reprod.* (2003) 68:722–34.
40. Shen WJ, Azhar S, Kraemer FB. Lipid droplets and steroidogenic cells. *Exp Cell Res.* (2016) 340:209–14. doi: 10.1016/j.yexcr.2015.11.024
41. Venugopal S, Martinez-Arguelles DB, Chebbi S, Hullin-Matsuda F, Kobayashi T, Papadopoulos V. Plasma membrane origin of the steroidogenic pool of cholesterol used in hormone-induced acute steroid formation in leydig cells. *J Biol Chem.* (2016) 291:26109–25. doi: 10.1074/jbc.M116.740928
42. Stocco DM. The role of the StAR protein in steroidogenesis: challenges for the future. *J Endocrinol.* (2000) 164:247–53.
43. Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL. Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipoid adrenal hyperplasia. *Proc Natl Acad Sci USA.* (1997) 94:11540–5.
44. Lin Y, Hou X, Shen WJ, Hanssen R, Khor VK, Cortez Y, et al. SNARE-mediated cholesterol movement to mitochondria supports steroidogenesis in rodent cells. *Mol Endocrinol.* (2016) 30:234–47. doi: 10.1210/me.2015-1281
45. Hu MC, Hsu HJ, Guo IC, Chung BC. Function of Cyp11a1 in animal models. *Mol Cell Endocrinol.* (2004) 215:95–100. doi: 10.1016/j.mce.2003.11.024
46. Conley AJ, Bird IM. The role of cytochrome P450 17 alpha-hydroxylase and 3 beta-hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the delta 5 and delta 4 pathways of steroidogenesis in mammals. *Biol Reprod.* (1997) 56:789–99.
47. Hammar M, Petersson F. Testosterone production *in vitro* in human testicular tissue. *Andrologia* (1986) 18:196–200.
48. Rey R, Campo S, Ayuso S, Nagle C, Chemes H. Testicular steroidogenesis in the Cebus monkey throughout postnatal development. *Biol Reprod.* (1995) 52:997–1002.
49. Wang W, Wei S, Li L, Su X, Du C, Li F, et al. Proteomic analysis of murine testes lipid droplets. *Sci Rep.* (2015) 5:12070. doi: 10.1038/srep12070
50. Tsai EC, Matsumoto AM, Fujimoto WY, Boyko EJ. Association of bioavailable, free, and total testosterone with insulin resistance: influence of sex hormone-binding globulin and body fat. *Diabetes Care* (2004) 27:861–8.
51. Xu Q, Lin HY, Yeh SD, Yu IC, Wang RS, Chen YT, et al. Infertility with defective spermatogenesis and steroidogenesis in male mice lacking androgen receptor in Leydig cells. *Endocrine* (2007) 32:96–106. doi: 10.1007/s12020-007-9015-0
52. Oakes MB, Eyvazzadeh AD, Quint E, Smith YR. Complete androgen insensitivity syndrome–a review. *J Pediatr Adolesc Gynecol.* (2008) 21:305–10. doi: 10.1016/j.jpag.2007.09.006
53. Green S, Walter P, Greene G, Krust A, Goffin C, Jensen E, et al. Cloning of the human oestrogen receptor cDNA. *J Steroid Biochem.* (1986) 24:77–83.
54. Koike S, Sakai M, Muramatsu M. Molecular cloning and characterization of rat estrogen receptor cDNA. *Nucleic Acids Res.* (1987) 15:2499–513.
55. Delbès G, Levacher C, Duquenne C, Racine C, Pakarinen P, Habert R. Endogenous estrogens inhibit mouse fetal Leydig cell development via estrogen receptor alpha. *Endocrinology* (2005) 146:2454–61. doi: 10.1210/en.2004-1540
56. Carreau S, Hess RA. Oestrogens and spermatogenesis. *Philos Trans R Soc Lond B Biol Sci.* (2010) 365:1517–35. doi: 10.1098/rstb.2009.0235
57. Maqdasy S, Baptissart M, Vega A, Baron S, Lobaccaro JMA, Volle DH. Cholesterol and male fertility: what about orphans and adopted? *Mol Cell Endocrinol.* (2013) 368:30–46. doi: 10.1016/j.mce.2012.06.011
58. Maqdasy S, Trousson A, Tauveron I, Volle DH, Baron S, Lobaccaro JMA. Once and for all, LXRα and LXRβ are gatekeepers of the endocrine system. *Mol Aspects Med.* (2016) 49:31–46. doi: 10.1016/j.mam.2016.04.001
59. Chau YM, Crawford PA, Woodson KG, Polish JA, Olson LM, Sadovsky Y. Role of steroidogenic-factor 1 in basal and 3′,5′-cyclic adenosine monophosphate-mediated regulation of cytochrome P450 side-chain cleavage enzyme in the mouse. *Biol Reprod.* (1997) 57:765–71.
60. Leers-Sucheta S, Morohashi K, Mason JI, Melner MH. Synergistic activation of the human type II 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase promoter by the transcription factor steroidogenic factor-1/adrenal 4-binding protein and phorbol ester. *J Biol Chem.* (1997) 272:7960–7.
61. Wang ZN, Bassett M, Rainey WE. Liver receptor homologue-1 is expressed in the adrenal and can regulate transcription of 11 beta-hydroxylase. *J Mol Endocrinol.* (2001) 27:255–8.
62. Sirianni R, Seely JB, Attia G, Stocco DM, Carr BR, Pezzi V, et al. Liver receptor homologue-1 is expressed in human steroidogenic tissues and activates transcription of genes encoding steroidogenic enzymes. *J Endocrinol.* (2002) 174:R13–7.
63. Cao G, Zhao L, Stangl H, Hasegawa T, Richardson JA, Parker KL, et al. Developmental and hormonal regulation of murine scavenger receptor, class B, type 1. *Mol Endocrinol.* (1999) 13:1460–73. doi: 10.1210/mend.13.9.0346
64. Schoonjans K, Annicotte JS, Huby T, Botrugno OA, Fayard E, Ueda Y, et al. Liver receptor homolog 1 controls the expression of the scavenger receptor class B type I. *EMBO Rep.* (2002) 3:1181–7. doi: 10.1093/embo-reports/kvf238
65. Mascaró C, Ortiz JA, Ramos MM, Haro D, Hegardt FG. Sterol regulatory element binding protein-mediated effect of fluvastatin on cytosolic 3-hydroxy-3-methylglutaryl-coenzyme A synthase transcription. *Arch Biochem Biophys.* (2000) 374:286–92. doi: 10.1006/abbi.1999.1600
66. Datta S, Wang L, Moore DD, Osborne TF. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase promoter by nuclear receptors liver receptor homologue-1 and small heterodimer partner: a mechanism for differential regulation of cholesterol synthesis and uptake. *J Biol Chem.* (2006) 281:807–12. doi: 10.1074/jbc.M511050200
67. Volle DH, Mouzat K, Duggavathi R, Siddeek B, Déchelotte P, Sion B, et al. Multiple roles of the nuclear receptors for oxysterols liver X receptor to maintain male fertility. *Mol Endocrinol.* (2007) 21:1014–27. doi: 10.1210/me.2006-0277
68. Robertson KM, Schuster GU, Steffensen KR, Hovatta O, Meaney S, Hultenby K, et al. The liver X receptor-β is essential for maintaining cholesterol homeostasis in the testis. *Endocrinology* (2005) 146:2519–30. doi: 10.1210/en.2004-1413
69. Volle DH, Duggavathi R, Magnier BC, Houten SM, Cummins CL, Lobaccaro JMA et al. The small heterodimer partner is a gonadal gatekeeper of sexual maturation in male mice. *Genes Dev.* (2007) 21:303–15. doi: 10.1101/gad.409307
70. Vassileva G, Golovko A, Markowitz L, Abbondanzo SJ, Zeng M, Yang S, et al. Targeted deletion of Gpbar1 protects mice from cholesterol gallstone formation. *Biochem J.* (2006) 398:423–30. doi: 10.1042/BJ20060537
71. Sèdes L, Martinot E, Baptissart M, Baron S, Caira F, Beaudoin C, et al. Bile acids and male fertility: from mouse to human? *Mol Aspects Med.* (2017) 56:101–9. doi: 10.1016/j.mam.2017.05.004
72. Baptissart M, Vega A, Martinot E, Pommier AJ, Houten SM, Marceau G, et al. Bile acids alter male fertility through G-protein-coupled bile acid receptor 1 signaling pathways in mice. *Hepatology* (2014) 60:1054–65. doi: 10.1002/hep.27204
73. Martinot E, Sèdes L, Baptissart M, Holota H, Rouaisnel B, Damon-Soubeyrand C, et al. The bile acid nuclear receptor FXRα is a critical regulator of mouse germ cell fate. *Stem Cell Rep.* (2017) 9:315–28. doi: 10.1016/j.stemcr.2017.05.036
74. Baptissart M, Martinot E, Vega A, Sédes L, Rouaisnel B, de Haze A, et al. Bile acid-FXRα pathways regulate male sexual maturation in mice. *Oncotarget* (2016) 7:19468–82. doi: 10.18632/oncotarget.7153
75. De França LR, Hess RA, Cooke PS, Russell LD. Neonatal hypothyroidism causes delayed Sertoli cell maturation in rats treated with propylthiouracil: evidence that the Sertoli cell controls testis growth. *Anat Rec.* (1995) 242:57–69. doi: 10.1002/ar.1092420108
76. Tarulli GA, Stanton PG, Meachem SJ. Is the adult Sertoli cell terminally differentiated? *Biol Reprod.* (2012) 87:13, 1–11. doi: 10.1095/biolreprod.111.095091
77. Willems A, Batlouni SR, Esnal A, Swinnen JV, Saunders PTK, Sharpe RM, et al. Selective ablation of the androgen receptor in mouse sertoli cells affects sertoli cell maturation, barrier formation and cytoskeletal development. *PLoS ONE* (2010) 5:e14168. doi: 10.1371/journal.pone.0014168

78. Francavilla F, Santucci R, Barbonetti A, Francavilla S. Naturally-occurring antisperm antibodies in men: interference with fertility and clinical implications. An update. *Front Biosci.* (2007) 12:2890–2911.
79. Skinner MK, Tung PS, Fritz IB. Cooperativity between Sertoli cells and testicular peritubular cells in the production and deposition of extracellular matrix components. *J Cell Biol.* (1985) 100:1941–7.
80. Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocr Rev.* (2004) 25:747–806. doi: 10.1210/er.2003-0022
81. Robinson R, Fritz IB. Metabolism of glucose by Sertoli cells in culture. *Biol Reprod.* (1981) 24:1032–41.
82. Chen SR, Liu YX. Regulation of spermatogonial stem cell self-renewal and spermatocyte meiosis by Sertoli cell signaling. *Reproduction* (2015) 149:R159–67. doi: 10.1530/REP-14-0481
83. Sylvester SR, Griswold MD. The testicular iron shuttle: a "nurse" function of the Sertoli cells. *J Androl.* (1994) 15:381–5.
84. Porter SB, Ong DE, Chytil F, Orgebin-Crist MC. Localization of cellular retinol-binding protein and cellular retinoic acid-binding protein in the rat testis and epididymis. *J Androl.* (1985) 6:197–212.
85. Wiebe JP, Tilbe KS. *De novo* synthesis of steroids (from acetate) by isolated rat Sertoli cells. *Biochem Biophys Res Commun.* (1979) 89:1107–13.
86. Fofana M, Travert C, Carreau S, Le Goff D. Evaluation of cholesteryl ester transfer in the seminiferous tubule cells of immature rats *in vivo* and *in vitro*. *J Reprod Fertil.* (2000) 118:79–83.
87. Rigotti A, Miettinen HE, Krieger M. The role of the high-density lipoprotein receptor SR-BI in the lipid metabolism of endocrine and other tissues. *Endocr Rev.* (2003) 24:357–87. doi: 10.1210/er.2001-0037
88. Landschulz KT, Pathak RK, Rigotti A, Krieger M, Hobbs HH. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J Clin Invest.* (1996) 98:984–95. doi: 10.1172/JCI118883
89. Akpovi CD, Yoon SR, Vitale ML, Pelletier RM. The predominance of one of the SR-BI isoforms is associated with increased esterified cholesterol levels not apoptosis in mink testis. *J Lipid Res.* (2006) 47:2233–47. doi: 10.1194/jlr.M600162-JLR200
90. Nakanishi Y, Shiratsuchi A. [Physiological role and mechanism of phagocytic clearance of apoptotic cells]. *Seikagaku* (2003) 75:1429–37.
91. Selva DM, Hirsch-Reinshagen V, Burgess B, Zhou S, Chan J, McIsaac S, et al. The ATP-binding cassette transporter 1 mediates lipid efflux from Sertoli cells and influences male fertility. *J Lipid Res.* (2004) 45:1040–50. doi: 10.1194/jlr.M400007-JLR200
92. Singaraja RR, Brunham LR, Visscher H, Kastelein JJP, Hayden MR. Efflux and atherosclerosis: the clinical and biochemical impact of variations in the ABCA1 gene. *Arterioscler Thromb Vasc Biol.* (2003) 23:1322–32. doi: 10.1161/01.ATV.0000078520.89539.77
93. Rondanino C, Ouchchane L, Chauffour C, Marceau G, Déchelotte P, Sion B, et al. Levels of liver X receptors in testicular biopsies of patients with azoospermia. *Fertil Steril.* (2014) 102:361–71.e5. doi: 10.1016/j.fertnstert.2014.04.033
94. Maqdasy S, El Hajjaji FZ, Baptissart M, Viennois E, Oumeddour A, Brugnon F, et al. Identification of the functions of liver X receptor-β in sertoli cells using a targeted expression-rescue model. *Endocrinology* (2015) 156:4545–57. doi: 10.1210/en.2015-1382
95. Kennedy MA, Venkateswaran A, Tarr PT, Xenarios I, Kudoh J, Shimizu N, et al. Characterization of the human ABCG1 gene: liver X receptor activates an internal promoter that produces a novel transcript encoding an alternative form of the protein. *J Biol Chem.* (2001) 276:39438–47. doi: 10.1074/jbc.M105863200
96. Dadoune JP. Expression of mammalian spermatozoal nucleoproteins. *Microsc Res Tech.* (2003) 61:56–75. doi: 10.1002/jemt.10317
97. Hecht NB. Regulation of "haploid expressed genes" in male germ cells. *J Reprod Fertil.* (1990) 88:679–93.
98. Potter JE, Millette CF, James MJ, Kandutsch AA. Elevated cholesterol and dolichol synthesis in mouse pachytene spermatocytes. *J Biol Chem.* (1981) 256:7150–4.
99. Osuga J, Ishibashi S, Oka T, Yagyu H, Tozawa R, Fujimoto A, et al. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. *Proc Natl Acad Sci USA.* (2000) 97:787–92.
100. Wang F, Chen Z, Ren X, Tian Y, Wang F, Liu C, et al. Hormone-sensitive lipase deficiency alters gene expression and cholesterol content of mouse testis. *Reproduction* (2017) 153:175–85. doi: 10.1530/REP-16-0484
101. Lenzi A, Picardo M, Gandini L, Dondero F. Lipids of the sperm plasma membrane: from polyunsaturated fatty acids considered as markers of sperm function to possible scavenger therapy. *Hum Reprod Update* (1996) 2:246–56.
102. Ladha S. Lipid heterogeneity and membrane fluidity in a highly polarized cell, the mammalian spermatozoon. *J Membr Biol.* (1998) 165:1–10.
103. Neill AR, Masters CJ. Metabolism of fatty acids by ovine spermatozoa. *J Reprod Fertil.* (1973) 34:279–87.
104. Fleming AD, Yanagimachi R. Evidence suggesting the importance of fatty acids and the fatty acid moieties of sperm membrane phospholipids in the acrosome reaction of guinea pig spermatozoa. *J Exp Zool.* (1984) 229:485–9. doi: 10.1002/jez.1402290317
105. Meizel S, Turner KO. Stimulation of an exocytotic event, the hamster sperm acrosome reaction, by cis-unsaturated fatty acids. *FEBS Lett.* (1983) 161:315–8.
106. Tavilani H, Doosti M, Abdi K, Vaisiraygani A, Joshaghani HR. Decreased polyunsaturated and increased saturated fatty acid concentration in spermatozoa from asthenozoospermic males as compared with normozoospermic males. *Andrologia* (2006) 38:173–8. doi: 10.1111/j.1439-0272.2006.00735.x
107. Andersen JM, Rønning PO, Herning H, Bekken SD, Haugen TB, Witczak O. Fatty acid composition of spermatozoa is associated with BMI and with semen quality. *Andrology* (2016) 4:857–65. doi: 10.1111/andr.12227
108. Boerke A, Brouwers JF, Olkkonen VM, van de Lest CHA, Sostaric E, Schoevers EJ, et al. Involvement of bicarbonate-induced radical signaling in oxysterol formation and sterol depletion of capacitating mammalian sperm during *in vitro* fertilization. *Biol Reprod.* (2013) 88:21. doi: 10.1095/biolreprod.112.101253
109. Bielska AA, Olsen BN, Gale SE, Mydock-McGrane L, Krishnan K, Baker NA, et al. Side-chain oxysterols modulate cholesterol accessibility through membrane remodeling. *Biochemistry* (2014) 53:3042–51. doi: 10.1021/bi5000096
110. Parks JE, Lynch DV. Lipid composition and thermotropic phase behavior of boar, bull, stallion, and rooster sperm membranes. *Cryobiology* (1992) 29:255–66.
111. Rejraji H, Sion B, Prensier G, Carreras M, Motta C, Frenoux JM, et al. Lipid remodeling of murine epididymosomes and spermatozoa during epididymal maturation. *Biol Reprod.* (2006) 74:1104–13. doi: 10.1095/biolreprod.105.049304
112. Whitfield M, Pollet-Villard X, Levy R, Drevet JR, Saez F. Posttesticular sperm maturation, infertility, and hypercholesterolemia. *Asian J Androl.* (2015) 17:742–8. doi: 10.4103/1008-682X.155536
113. Jin SK, Yang WX. Factors and pathways involved in capacitation: how are they regulated? *Oncotarget* (2017) 8:3600–27. doi: 10.18632/oncotarget.12274
114. Jafaroghli M, Abdi-Benemar H, Zamiri MJ, Khalili B, Farshad A, Shadparvar AA. Effects of dietary n-3 fatty acids and vitamin C on semen characteristics, lipid composition of sperm and blood metabolites in fat-tailed Moghani rams. *Anim Reprod Sci.* (2014) 147:17–24. doi: 10.1016/j.anireprosci.2014.03.013
115. Wazzan WC, Gwatkin RB, Thomas AJ. Zona drilling enhances fertilization by mouse caput epididymal sperm. *Mol Reprod Dev.* (1990) 27:332–6. doi: 10.1002/mrd.1080270407
116. Matsuo Y, Nomata K, Eguchi J, Aoki D, Hayashi T, Hishikawa Y, et al. Immunohistochemical analysis of connexin43 expression in infertile human testes. *Acta Histochem Cytochem.* (2007) 40:69–75. doi: 10.1267/ahc.07001
117. Brehm R, Marks A, Rey R, Kliesch S, Bergmann M, Steger K. Altered expression of connexins 26 and 43 in Sertoli cells in seminiferous tubules infiltrated with carcinoma-*in-situ* or seminoma. *J Pathol.* (2002) 197:647–53. doi: 10.1002/path.1140
118. Segretain D, Decrouy X, Dompierre J, Escalier D, Rahman N, Fiorini C, et al. Sequestration of connexin43 in the early endosomes: an early event of Leydig cell tumor progression. *Mol Carcinog.* (2003) 38:179–87. doi: 10.1002/mc.10160

119. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod.* (2007) 22:1506–12. doi: 10.1093/humrep/dem046
120. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. European Association of Urology Working Group on Male Infertility. European Association of Urology guidelines on Male Infertility: the 2012 update. *Eur Urol.* (2012) 62:324–32. doi: 10.1016/j.eururo.2012.04.048
121. Dimopoulou C, Goulis DG, Corona G, Maggi M. The complex association between metabolic syndrome and male hypogonadism. *Metab Clin Exp.* (2018). doi: 10.1016/j.metabol.2018.03.024. [Epub ahead of print].
122. Kalyani RR, Dobs AS. Androgen deficiency, diabetes, and the metabolic syndrome in men. *Curr Opin Endocrinol Diabetes Obes.* (2007) 14:226–34. doi: 10.1097/MED.0b013e32814db856
123. Jensen TK, Andersson AM, Jørgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril.* (2004) 82:863–70. doi: 10.1016/j.fertnstert.2004.03.056
124. Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK, et al. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Hum Reprod Update* (2013) 19:221–31. doi: 10.1093/humupd/dms050
125. Samavat J, Natali I, Degl'Innocenti S, Filimberti E, Cantini G, Di Franco A, et al. Acrosome reaction is impaired in spermatozoa of obese men: a preliminary study. *Fertil Steril.* (2014) 102:1274–81.e2. doi: 10.1016/j.fertnstert.2014.07.1248
126. Dupont C, Faure C, Sermondade N, Boubaya M, Eustache F, Clément P, et al. Obesity leads to higher risk of sperm DNA damage in infertile patients. *Asian J Androl.* (2013) 15:622–5. doi: 10.1038/aja.2013.65
127. Ramírez-Torres MA, Carrera A, Zambrana M. [High incidence of hyperestrogenemia and dyslipidemia in a group of infertile men]. *Ginecol Obstet Mex.* (2000) 68:224–9.
128. Cross NL. Role of cholesterol in sperm capacitation. *Biol Reprod.* (1998) 59:7–11.
129. Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF, Louis GMB. The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Hum Reprod.* (2015) 30:493–4. doi: 10.1093/humrep/deu322
130. Lu JC, Jing J, Yao Q, Fan K, Wang GH, Feng RX, et al. Relationship between Lipids Levels of Serum and Seminal Plasma and Semen Parameters in 631 Chinese Subfertile Men. *PLoS ONE* (2016) 11:e0146304. doi: 10.1371/journal.pone.0146304
131. Filippi S, Vignozzi L, Morelli A, Chavalmane AK, Sarchielli E, Fibbi B, et al. Testosterone partially ameliorates metabolic profile and erectile responsiveness to PDE5 inhibitors in an animal model of male metabolic syndrome. *J Sex Med.* (2009) 6:3274–88. doi: 10.1111/j.1743-6109.2009.01467.x
132. Vignozzi L, Morelli A, Sarchielli E, Comeglio P, Filippi S, Cellai I, et al. Testosterone protects from metabolic syndrome-associated prostate inflammation: an experimental study in rabbit. *J Endocrinol.* (2012) 212:71–84. doi: 10.1530/JOE-11-0289
133. Maneschi E, Vignozzi L, Morelli A, Mello T, Filippi S, Cellai I, et al. FXR activation normalizes insulin sensitivity in visceral preadipocytes of a rabbit model of MetS. *J Endocrinol.* (2013) 218:215–31. doi: 10.1530/JOE-13-0109
134. Vignozzi L, Morelli A, Filippi S, Comeglio P, Chavalmane AK, Marchetta M, et al. Farnesoid X receptor activation improves erectile function in animal models of metabolic syndrome and diabetes. *J. Sex Med.* (2011) 8:57–77. doi: 10.1111/j.1743-6109.2010.02073.x
135. Maneschi E, Morelli A, Filippi S, Cellai I, Comeglio P, Mazzanti B, et al. Testosterone treatment improves metabolic syndrome-induced adipose tissue derangements. *J Endocrinol.* (2012) 215:347–62. doi: 10.1530/JOE-12-0333
136. Marchiani S, Vignozzi L, Filippi S, Gurrieri B, Comeglio P, Morelli A, et al. Metabolic syndrome-associated sperm alterations in an experimental rabbit model: relation with metabolic profile, testis and epididymis gene expression and effect of tamoxifen treatment. *Mol Cell Endocrinol.* (2015) 401:12–24. doi: 10.1016/j.mce.2014.11.005
137. Simón L, Funes AK, Yapur MA, Cabrillana ME, Monclus MA, Boarelli PV, et al. Manchette-acrosome disorders during spermiogenesis and low efficiency of seminiferous tubules in hypercholesterolemic rabbit model. *PLoS ONE* (2017) 12:e0172994. doi: 10.1371/journal.pone.0172994
138. Morgan DH, Ghribi O, Hui L, Geiger JD, Chen X. Cholesterol-enriched diet disrupts the blood-testis barrier in rabbits. *Am J Physiol Endocrinol Metab.* (2014) 307:E1125–30. doi: 10.1152/ajpendo.00416.2014
139. Fan Y, Liu Y, Xue K, Gu G, Fan W, Xu Y, et al. Diet-induced obesity in male C57BL/6 mice decreases fertility as a consequence of disrupted blood-testis barrier. *PLoS ONE* (2015) 10:e0120775. doi: 10.1371/journal.pone.0120775
140. Ghanayem BI, Bai R, Kissling GE, Travlos G, Hoffler U. Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. *Biol Reprod.* (2010) 82:96–104. doi: 10.1095/biolreprod.109.078915
141. Bakos HW, Mitchell M, Setchell BP, Lane M. The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model. *Int J Androl.* (2011) 34:402–10. doi: 10.1111/j.1365-2605.2010.01092.x
142. Fernandez CDB, Bellentani FF, Fernandes GSA, Perobelli JE, Favareto APA, Nascimento AF, et al. Diet-induced obesity in rats leads to a decrease in sperm motility. *Reprod Biol Endocrinol.* (2011) 9:32. doi: 10.1186/1477-7827-9-32
143. Sánchez-Garrido MA, Ruiz-Pino F, Manfredi-Lozano M, Leon S, Garcia-Galiano D, Castaño JP, et al. Obesity-induced hypogonadism in the male: premature reproductive neuroendocrine senescence and contribution of Kiss1-mediated mechanisms. *Endocrinology* (2014) 155:1067–79. doi: 10.1210/en.2013-1584
144. Campos-Silva P, Costa WS, Sampaio FJB, Gregorio BM. Prenatal and/or postnatal high-fat diet alters testicular parameters in adult Wistar Albino rats. *Histol Histopathol.* (2017) 33:407–16. doi: 10.14670/HH-11-941
145. Mu Y, Yan WJ, Yin TL, Zhang Y, Li J, Yang J. Diet-induced obesity impairs spermatogenesis: a potential role for autophagy. *Sci Rep.* (2017) 7:43475. doi: 10.1038/srep43475
146. Fullston T, McPherson NO, Owens JA, Kang WX, Sandeman LY, Lane M. Paternal obesity induces metabolic and sperm disturbances in male offspring that are exacerbated by their exposure to an "obesogenic" diet. *Physiol Rep.* (2015) 3:12336. doi: 10.14814/phy2.12336
147. Rodríguez-González GL, Vega CC, Boeck L, Vázquez M, Bautista CJ, Reyes-Castro LA, et al. Maternal obesity and overnutrition increase oxidative stress in male rat offspring reproductive system and decrease fertility. *Int J Obes.* (2015) 39:549–56. doi: 10.1038/ijo.2014.209
148. Vega A, Martinot E, Baptissart M, De Haze A, Vaz F, Kulik W, et al. Bile acid alters male mouse fertility in metabolic syndrome context. *PLoS ONE* (2015) 10:e0139946. doi: 10.1371/journal.pone.0139946

Conflict of Interest Statement: 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 Endocrinology

ORIGINAL RESEARCH
published: 23 August 2018
doi: 10.3389/fendo.2018.00470

Estrogen Receptor Beta-Mediated Modulation of Lung Cancer Cell Proliferation by 27-Hydroxycholesterol

Shiro Hiramitsu[1,2†‡], Tomonori Ishikawa[3†‡], Wan-Ru Lee[3], Tamor Khan[1,2], Christine Crumbley[1,2], Nimra Khwaja[1,2], Faezeh Zamanian[1,2], Arvand Asghari[1,2], Mehmet Sen[2], Yang Zhang[4], John R. Hawse[5], John D. Minna[6] and Michihisa Umetani[1,2]*

[1] Center for Nuclear Receptors and Cell Signaling, University of Houston, Houston, TX, United States, [2] Department of Biology and Biochemistry, College of Natural Sciences and Mathematics, University of Houston, Houston, TX, United States, [3] Division of Pulmonary and Vascular Biology, Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX, United States, [4] Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX, United States, [5] Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, United States, [6] Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, TX, United States

27-hydroxycholesterol (27HC) is an abundant cholesterol metabolite in human circulation and promotes breast cancer cell proliferation. Although lung is one of the organs that contain high levels of 27HC, the role of 27HC in lung is unknown. In this study, we found that 27HC promotes lung cancer cell proliferation in an estrogen receptor β (ERβ)-dependent manner. The expression of 27HC-generating enzyme CYP27A1 is higher in lung cancer cells than in normal lung cells. Treatment with 27HC increased cell proliferation in ERβ-positive lung cancer cells, but not in ERα-positive or ER-negative cells. The effect on cell proliferation is specific to 27HC and another oxysterol, 25-hydroxycholesterol that has a similar oxysterol structure with 27HC. Moreover, among ligands for nuclear receptors tested, only estrogen had the proliferative effect, and the effect by 27HC and estrogen was inhibited by ERβ-specific, but not ERα-specific, inhibitors. In addition, the effect by 27HC was not affected by membrane-bound estrogen receptor GPR30. Interestingly, despite the high expression of CYP27A1, endogenously produced 27HC was not the major contributor of the 27HC-induced cell proliferation. Using kinase inhibitors, we found that the effect by 27HC was mediated by the PI3K-Akt signaling pathway. These results suggest that 27HC promotes lung cancer cell proliferation via ERβ and PI3K-Akt signaling. Thus, lowering 27HC levels may lead to a novel approach for the treatment of lung cancer.

Keywords: lung cancer, estrogen receptor, ERβ, 27-hydroxycholesterol, cholesterol metabolites

OPEN ACCESS

Edited by:
Rosamaria Lappano, University of Calabria, Italy

Reviewed by:
Marc Poirot, Institut National de la Santé et de la Recherche Médicale (INSERM), France
Andrzej T. Slominski, University of Alabama at Birmingham, United States

***Correspondence:**
Michihisa Umetani
mumetani@uh.edu

[†] These authors have contributed equally to this work

[‡] Present Address:
Shiro Hiramitsu, Department of Comprehensive Reproductive Medicine, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan
Tomonori Ishikawa, Department of Pediatrics, Perinatal and Maternal Medicine (Ibaraki), Graduate School, Tokyo Medical and Dental University, Tokyo, Japan

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *23 May 2018*
Accepted: *30 July 2018*
Published: *23 August 2018*

Citation:
Hiramitsu S, Ishikawa T, Lee W-R, Khan T, Crumbley C, Khwaja N, Zamanian F, Asghari A, Sen M, Zhang Y, Hawse JR, Minna JD and Umetani M (2018) Estrogen Receptor Beta-Mediated Modulation of Lung Cancer Cell Proliferation by 27-Hydroxycholesterol. Front. Endocrinol. 9:470. doi: 10.3389/fendo.2018.00470

INTRODUCTION

Lung cancer is the leading cause of cancer deaths in men and women in the United States (1), and there is accumulating evidence that estrogen is a major driver of lung cancer (2). In addition, the proportional occurrence of histological lung carcinoma subtypes differs between men and women. While squamous cell carcinoma is the most common subtype in men, adenocarcinoma

is the most common subtype in women (1, 3). Estrogen receptors, ERα and ERβ, are members of the nuclear receptor family, and are present both in normal lung tissue and lung tumors with ERβ as a dominant isoform (4–6). Large-scale randomized and observational studies showed that women who used hormone replacement therapy (HRT) including estrogen have a higher risk of adenocarcinoma than women who did not use HRT, and that risk of lung cancer was associated with chronic use of HRT (7). In contrast, breast cancer patients who received anti-estrogen treatment had significantly lower subsequent lung cancer mortality (8). Studies using genetically modified mice showed that both male and female lungs are highly estrogen-responsive (9, 10). Mice deficient in ERβ exhibited significant lung dysfunction both in males and females, indicating the importance of ERβ in the maintenance of lung function (11). Estrogens also stimulate growth and progression of lung tumors, and local production of estrogen in lung tissues by aromatase could affect lung tumor progression in ER(+) malignancies in men and women (12, 13). ER also interacts with epidermal growth factor receptors (EGFRs), and the combination of EGFR tyrosine kinase inhibitors and ER antagonists gives maximal inhibition of tumor cell proliferation *in vitro* and also anti-tumor activity in mouse tumor xenograft models *in vivo* (14, 15). Taken together, estrogen and ERs play important roles in lung cancer pathogenesis and treatment.

Oxysterols are metabolites of cholesterol that are produced in the liver and other peripheral tissues as a means to eliminate cholesterol (16). The most abundant circulating oxysterol is 27-hydroxycholesterol (27HC), and serum concentrations of 27HC correlate well with that of cholesterol. The levels of 27HC also rise progressively with age. The enzyme that generates 27HC, sterol 27-hydroxylase (CYP27A1), is primarily expressed in the liver, but also in peripheral tissues to a lesser extent (17). Using cell-based and *in vitro* assays, we discovered that 27HC is a competitive ER antagonist in the cardiovascular system (18). We further found that 27HC binds directly to ERα ($K_i = 1.32\,\mu M$) and ERβ ($K_i = 0.42\,\mu M$) in their ligand binding pockets, and it inhibits both transcriptional and non-transcriptional estrogen-dependent production of nitric oxide by vascular cells. In mice, elevated 27HC levels decreased ER-dependent expression of vascular nitric oxide synthase and repressed carotid artery reendothelialization after vascular injury. In addition to the anti-estrogenic effects of 27HC in vascular cells, we identified pro-estrogenic actions of 27HC in hepatoma HepG2 and colon cancer Caco-2 cells (18). Combinatorial peptide phage display revealed that 27HC induces a unique active conformation of ERα (19). In contrast to estrogens that have various levels of agonistic activity in all tissues, selective ER modulators (SERMs) are compounds that act as agonists or antagonists depending on the target genes and tissues (16). Although many compounds have been identified as SERMs, all of them were synthetic compounds. Thus, 27HC is the first identified endogenously produced SERM, and has important biological actions *in vitro* and *in vivo*.

In breast cancer, in which ERα is classically involved in the development and progression of many tumors, paradoxically, post-menopausal women with decreased estrogen production are particularly at increased risk of ER-positive breast cancer. This risk occurs at a time when circulating estrogen levels are declining, and endocrine-based therapies against ER-positive breast cancer employing synthetic SERMs or aromatase inhibition are often ineffective or acquire resistance, suggesting other important unknown ER-mediated mechanisms (20). Once we identified 27HC as a novel endogenous SERM and that its abundance increases with age, we tested its potential actions on ER-positive breast cancer cells, and found that 27HC up-regulated ERα target gene expression and increased cell proliferation (19). Also in mice, 27HC promoted tumor growth and metastasis to the lung in an orthotropic breast tumor xenograft model (21). In humans, we found that elevated levels of 27HC in tumors, which are correlated with reduced expression of the 27HC-catabolizing enzyme oxysterol 7α-hydroxylase (CYP7B1), are associated with poorer patient survival (22). Even when age, tumor size, nodal status, and perioperative therapy are taken into consideration, low expression of *CYP7B1* continues to be associated with poor overall outcome. Thus, 27HC is a non-estrogen, locally-modulated, non-aromatized ER ligand that stimulates ER-positive breast tumor growth, and, most importantly, it is abundant in the microenvironment of tumors in women.

In the present study, we investigated how 27HC impacts lung cancer cell proliferation through its modulation of the ER-mediated action. We found that ERβ expression is higher in lung cancer cells than in normal lung cells, and also that 27HC promotes ERβ (+) lung cancer cell proliferation. Although lung cancer cells have elevated gene expression of 27HC-producing enzyme CYP27A1, endogenously produced 27HC was not the major factor involved in the 27HC-induced cell proliferation. We sought to determine the underlying mechanism, and found that the PI3K-Akt pathway is involved in the effect by 27HC on lung cancer cell proliferation.

MATERIALS AND METHODS

Materials

27HC was purchased from Avanti Polar Lipids. T0901317 (T1317) was purchased from Cayman Chemical. 1,3-bis (4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy) phenol]-1H-pyrazole dihydrochloride (MPP), 4-[2-phenyl-5,7-bis(trifluoromethyl) pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP), G1, G15, and iressa were purchased from Tocris Bioscience. Cholestane-3β,5α,6β-triol and 5α-hydroxy-6-ketocholesterol were purchased from Steraloids. 17β-estradiol (E_2), GW3965 (GW), 4β-hydroxycholesterol, 7-ketocholesterol, 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 25-hyd roxycholesterol, cholesterol 5α,6α-epoxide, cholesterol 5β,6β-epoxide, cholesterol, progesterone, 5α-dihydrotestosterone (DHT), dexamethasone, cortisone, Wy-14643, GW501516, troglitazone, EGF, insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), PD0325901, U0126, SB203580, LY294002, and clotrimazole were purchased from Sigma-Aldrich.

Gene Expression Analyses and Assessments of Gene Expression

Expression profiling of *ERα*, *ERβ*, *CYP27A1*, and *CYP7B1* was part of a larger study (Gene Expression Omnibus DataSets accession number GSE32036) that has been previously published (23, 24). Raw data was background corrected with RMA, Log2 transformed, and summarized by medianpolish. Differently expressed genes were called using the LIMMA package and *p*-values were corrected for multiple testing with the Benjamini-Hochberg procedure. The expression heatmap was generated in R and ontology enrichment was performed in HOMER. *CYP27A1*, *CYP7B1*, *ERα*, and *ERβ* transcript abundance were further evaluated by quantitative RT-qPCR (25). Primer sequences used for RT-qPCR are listed in **Supplemental Table 1**.

Cell Culture Assays

MCF-7 (ATCC), A549 (ATCC), Lewis lung carcinoma (LLC, ATCC), H1395, and H596 cells were maintained in RPMI 1640 medium containing 5% FBS. Cell proliferation was assessed by quantifying ^{3}H-thymidine incorporation (22). Cells were maintained in phenol red-free DMEM containing 5% charcoal-stripped FBS for 72 h. Cells were then passed into 24-well plates at a density of 100,000 cells per ml in media containing 5% charcoal-stripped FBS, and 24 h later they were treated with compounds for 24 h, during which ^{3}H-thymidine incorporation was quantified. To provide a complementary approach in H1395 cells, in selected studies cell proliferation was quantified by cell number using FluoReporter Blue Fluorometric dsDNA Quantitation kit (Thermo Fisher Scientific). To evaluate the involvement of ERβ in the 27HC-induced lung cancer cell proliferation, LLC cells were transfected with an ERβ-expression plasmid using Lipofectamine 2000 and used for the cell proliferation assay. To specifically evaluate the impact of silencing of *CYP27A1* or *CYP7B1* on the modulation of lung cancer cell proliferation, their expression was knocked down using dsRNA targeting human *CYP27A1* (TF313602, OliGene Technologies), *CYP7B1* (Dharmacon), or control dsRNA. H1395 cells were transfected with 50 nM RNA as described previously (25) and cell proliferation responses to vehicle or 27HC were evaluated from 48 to 72 h post-transfection.

Immunoblot Analyses

ER protein abundance was assessed by immunoblot analysis using antibodies against ERα (ab75635, Abcam), ERβ (26), and GAPDH (G8796, Sigma-Aldrich) as a control. Phosphorylation of ERK1/2, p38 MAPK, and Akt proteins were assessed by immunoblot with their phosphorylated protein-specific antibodies (Cell Signaling), and their total protein abundance was also assessed.

Statistical Analysis

All data are expressed as mean ± SEM. Two-tailed Student's *t*-test or ANOVA was used to assess differences between two groups or among more than two groups, respectively, with Newman-Keuls *post-hoc* testing following ANOVA. *P*-values <0.05 were considered significant.

RESULTS

Gene Expression of ER in Lung Cancer Cell Lines

To evaluate possible actions of 27HC in lung cancer pathogenesis, we determined the expression levels of *CYP27A1*, *CYP7B1*, *ERα*, and *ERβ* in a variety of lung cancer and normal lung cell lines. Ninety-one human lung tumor cell lines and 30 human normal bronchial epithelial cell lines from the Lung Cancer Program at UT Southwestern Medical Center were analyzed. With relative levels of expression represented as $\log_2$ (signal) in the heat diagram, mRNAs for *CYP27A1* were detectable at varying levels with clear difference among the different cell types (**Figure 1A**). Thirty-one tumor cell lines showed moderate to high expression of 27HC-generating enzyme CYP27A1 while none of the normal cell line showed moderate to high *CYP27A1* expression. The $\log_2$ *CYP27A1* expression ratio of tumor vs. normal lung cells is 0.87 ($P < 0.001$). The mRNA expression for *ERβ* was also higher in tumor cells than normal lung cells ($\log_2$ ratio 0.28, $P = 0.004$). There were also varying levels of mRNA expression for *CYP7B1* and *ERα* transcripts, although there is no significant difference between tumor vs. normal lung cells. We further validated the microarray data and also compared the mRNA expression levels in high ERβ expressed H1395, high ERα expressed H596, and ER-negative A549 cells with those in breast cancer MCF-7 cells by qRT-PCR analysis. As expected, MCF-7 cells have high mRNA expression of *ERα*. Consistent with the microarray results (**Figure 1A**), H1395 and H596 cells have high expression of *ERβ* and *ERα*, respectively (**Figure 1B**). The protein expression of ERα and ERβ was also confirmed in MCF-7 and H1395 cells, respectively (**Figure 1C**). The mRNA expression of *CYP27A1* and *CYP7B1* varied among cell lines, and the gene expression of aromatase, which converts testosterone to estrogen, was not observed in the cells tested (**Supplemental Figure 1** and data not shown). These results suggest that the molecular machinery necessary to synthesize and metabolize 27HC is found in lung cancer cells, and local 27HC production and *ERβ* mRNA expression are higher in lung tumor cells than normal lung cells.

27HC Promotes H1395 Lung Cancer Cell Proliferation

Next, we evaluated whether 27HC has cell proliferative effects in lung cancer cells. We selected H1395 and H596 lung adenocarcinoma cells, which express high levels of *ERβ* and *ERα*, respectively and also A549 cells which have no expression of ER (**Figure 1**). 27HC and E_2 induced cell proliferation of H1395, but not H596 or A549 cells (**Figure 2A**), suggesting that the effect by 27HC is ERβ-dependent. The proliferative effects of 27HC are also dose-dependent (**Figure 2B**) and specific to the 27HC structure, but not to general oxysterols. Among other oxysterols tested, only 27HC, and 25-hydroxycholesterol were able to induce cell proliferation in this model (**Figure 2C**). 25-Hydroxycholesterol has a similar oxysterol structure as 27HC and also acts as an ER ligand. We could not see any promoting effect on the cell proliferation by 4β-Hydroxycholesterol, 7-keto-cholesterol, 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, cholesterol

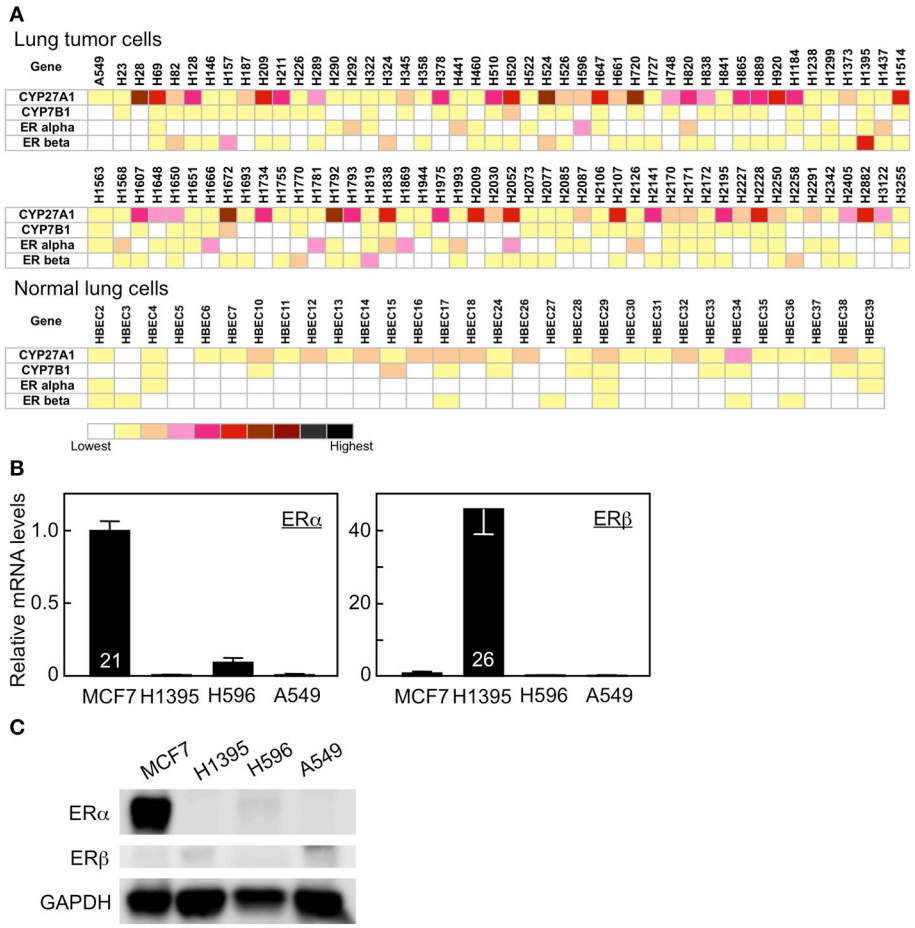

FIGURE 1 | Gene expression in human lung tumor and normal bronchial epithelial cells. **(A)** Gene expression profile of *CYP27A1*, *CYP7B1*, *ERα*, and *ERβ*. **(B)** qRT-PCR analysis of *ERα*, and *ERβ* in MCF7, H1395, H596, and A549 cancer cells (n = 6–7). Cycle time of the highest expressing group for each gene is indicated inside its corresponding bar. **(C)** Immunoblots of ERα, ERβ, and GAPDH proteins.

5α,6α-epoxide, cholesterol 5β,6β-epoxide, cholestane-3β,5α,6β-triol, and 5α-hydroxy-6-ketocholesterol, which are reported to stimulate breast cancer cell proliferation (27). These results indicate that 27HC promotes H1395 lung cancer cell proliferation and that the effect is specific to 27HC and 25-hydroxycholesterol.

Effects of 27HC on Lung Cancer Cell Proliferation Are ERβ-Specific

To examine whether the effect of 27HC on lung cancer cell proliferation was ERβ-specific, ligands for other nuclear receptors were tested on H1395 cells. Ligands for liver X receptor (LXR, T1317, GW), progesterone receptor (progesterone), androgen receptor (5α-dihydrotestosterone), glucocorticoid receptor (dexamethasone), mineralocorticoid receptor (cortisone), or α (Wy-14643), δ (GW501516), and γ (troglitazone) isoforms of PPAR in the concentrations known to activate their receptors (18) did not induce growth promoting effects in H1395 cells (**Figure 3A**). Together with the results in **Figure 2C** that 22(R)-hydroxycholesterol and 24(S)-hydroxycholesterol, which also act as ligands for LXR and retinoic acid related orphan receptors (RORs), respectively (28, 29), did not show any promoting effect on the H1395 cell proliferation, these results indicate that the effect by 27HC is not LXR-, PR-, AR-, MR-, GR-, PPAR-, or ROR-dependent. Next, we used ER isoform-specific inhibitors. The effect by 27HC and E_2 was inhibited by the ERβ-specific inhibitor PHTPP, but not by the ERα-specific inhibitor MPP (**Figure 3B**). To confirm the importance of ERβ in the 27HC-mediated lung cancer cell proliferation, we compared the effect by 27HC on LLC cells, which are ER-negative, and LLC cells that overexpress ERβ following transfection with an ERβ expression plasmid. 27HC and E_2 induced cell proliferation of LLC cells that expressed ERβ, but had no effect on parental LLC cells (**Supplemental Figure 2**), further indicating that the effect by 27HC on lung cancer cell proliferation is ERβ-dependent. In addition to classical ER, estrogen can also activate the G protein-coupled receptor GPR30 (6). Thus, the involvement of GPR30 in the H1395 cell proliferation was tested using its agonist G1 and antagonist G15. Neither of these GPR30 ligands affected H1395 cell

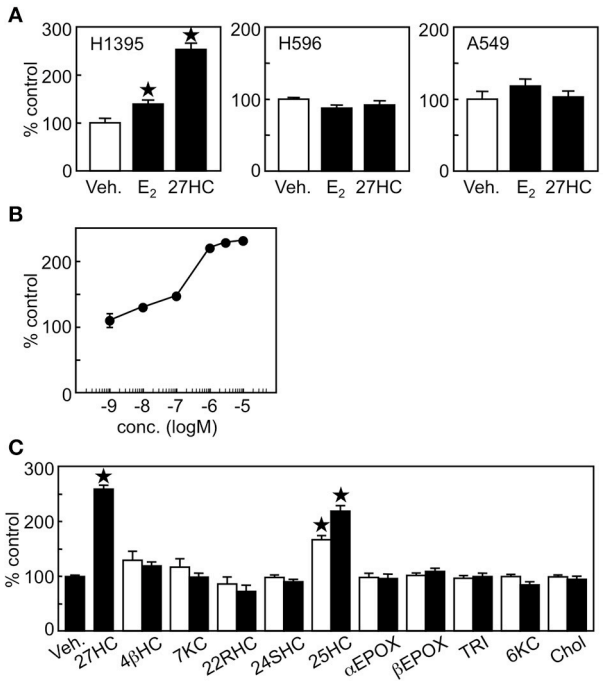

FIGURE 2 | 27HC promotes H1395 lung cancer cell proliferation. **(A)** Cell proliferation assay. Vehicle control, E_2 (10 nM), or 27HC (1 μM) were treated for 24 h ($n = 4$). **(B)** Dose response of 27HC in H1395 cell proliferation ($n = 4$). **(C)** H1395 cell proliferation in the presence of oxysterols for 24 h ($n = 4$). 4βHC, 4β-hydroxycholesterol; 7KC, 7keto-cholesterol; 22RHC, 22(R)-hydroxycholesterol; 24SHC, 24(S)-hydroxycholesterol; 25HC, 25-hydroxycholesterol; αEPOX, cholesterol 5α,6α-epoxide; βEPOX, cholesterol 5β,6β-epoxide; TRI, cholestane-3β,5α,6β-triol; 6KC, 5α-hydroxy-6-ketocholesterol; and Chol, cholesterol at 0.1 μM (open bars) and 1 μM (closed bars). *$p < 0.05$ vs. vehicle control.

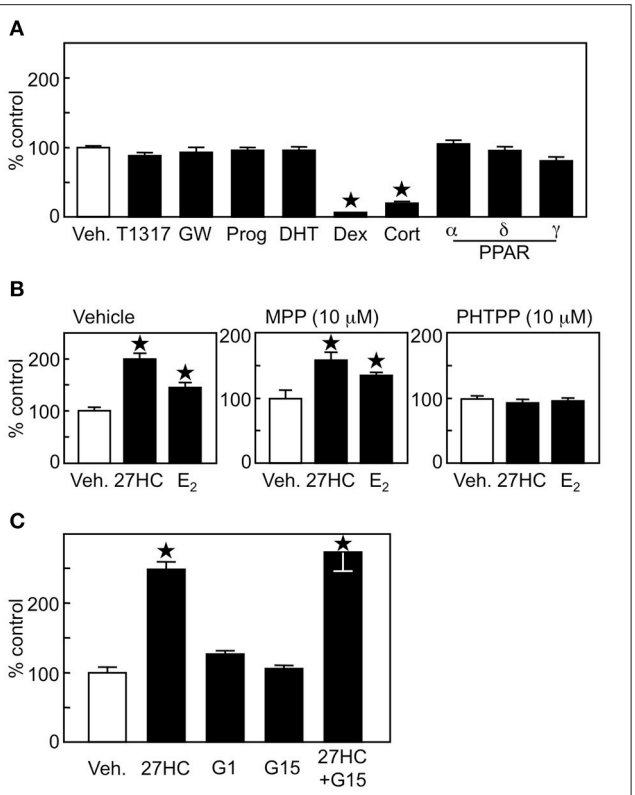

FIGURE 3 | 27HC promotes H1395 cell proliferation in an ERβ-dependent manner. **(A)** Cell proliferation assay of H1395 cells was performed using ligands for nuclear receptors ($n = 4$). T1317 (1 μM), GW (1 μM), Prog, progesterone (100 nM); Dex, dexamethasone (10 nM); Cort; cortisone (10 nM); α, Wy-14643 (1 μM); δ, GW501516 (1 μM); and γ, troglitazone (1 μM). **(B)** Effect of ER isoform-specific antagonist on the 27HC-induced H1395 cell proliferation ($n = 4$). **(C)** Effect of GPR30 on the cell proliferation induced by 27HC. H1395 cells were treated with vehicle, 27HC (1 μM), G1 (100 nM), G15 (100 nM), or 27HC plus G15 for 24 h ($n = 4$). *$p < 0.05$ vs. vehicle control.

proliferation and G15 did not abrogate the inducing effects of 27HC (**Figure 3C**), indicating that GPR30 is not involved in the 27HC-induced H1395 cell proliferation. These results indicate that 27HC promotes lung cancer cell proliferation via ERβ.

Exogenous 27HC Is the Major Effector on ERβ (+) Lung Cancer Cell Proliferation

Since lung tumor cells have higher expression of 27HC-generating CYP27A1 (**Figure 1A**), it is plausible that endogenous 27HC production affects ERβ (+) lung cancer cell proliferation. To examine the involvement of endogenous 27HC production in H1395 cell proliferation, we introduced shRNA against *CYP27A1* into H1395 cells and generated clones A3 and D2. The shRNA decreased the expression of *CYP27A1* significantly in two clones, A3 and D2 (**Figure 4A**); however, the cell proliferation was not altered in the presence of control or shRNA against *CYP27A1* (**Figure 4B**). In a similar fashion, siRNA mediated suppression of *CYP7B1*, #32 and #33, significantly decreased *CYP7B1* mRNA levels in H1395 cells (**Figure 4C**) but did not alter cell proliferation relative to control siRNA cells (**Figure 4D**). We further confirmed our findings using clotrimazole, an inhibitor of P450 enzymes to which both CYP27A1 and CYP7B1 belong, and the treatment of H1395 cells at 1 μM did not alter cell proliferation (**Figure 4E**). These results indicate that although lung cancer cells have high 27HC-generating enzyme expression, endogenous 27HC is not the major effector of ERβ (+) lung cancer cell proliferation.

27HC Promotes Cell Proliferation Through the PI3K-Akt Pathway

In addition to its action as a transcription factor, ER has an action as an extranuclear signaling molecule involving various kinase pathways such as PI3K-Akt and MEK-MAPK pathways. In non-small cell lung cancer, ER activation leads to the induction of the p44/p42 MAPK signaling pathway, which is considered to be an extranuclear action of ER (14). In addition, ER interacts with EGF receptor and activate tyrosine kinase signaling in breast cancer (15), and EGF, but not IGF or VEGF, promotes H1395 cell proliferation (**Supplemental Figure 3A**). To examine whether 27HC is capable of eliciting extranuclear effects, we performed immunoblot analysis using H1395 cell extracts. EGF stimulated the phosphorylation of p38 MAPK,

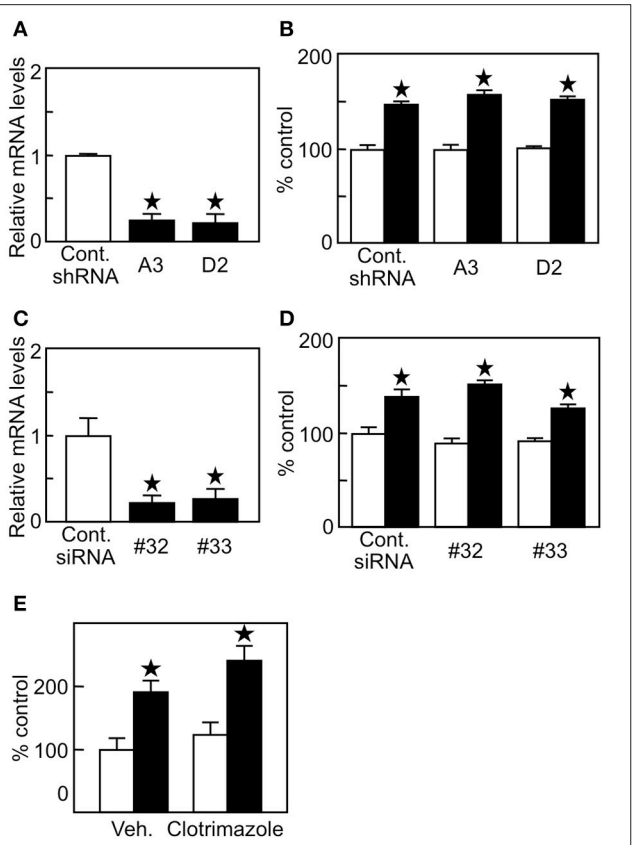

FIGURE 4 | Endogenous production of 27HC is not a major contributor in H1395 cell proliferation. **(A)** *CYP27A1* gene expression ($n = 4$). **(B)** Cell proliferation assay using H1395 cells with shRNA against *CYP27A1* ($n = 4$). Open bars, vehicle treatments; closed bars, 27HC (1 μM) treatments. **(C)** *CYP7B1* gene expression ($n = 4$). **(D)**. cell proliferation assay using H1395 cells with siRNA against *CYP7B1* ($n = 4$). Open bars, vehicle treatments; closed bars, 27HC (1 μM) treatments. $^*p < 0.05$ against vehicle control. **(E)** Effect by clotrimazole on the 27HC-induced cell proliferation. Cells were treated with vehicle or 27HC (1 μM) in the absence (Veh.) or presence of clotrimazole (1 μM) for 24 h ($n = 4$). $^*p < 0.05$ vs. vehicle control.

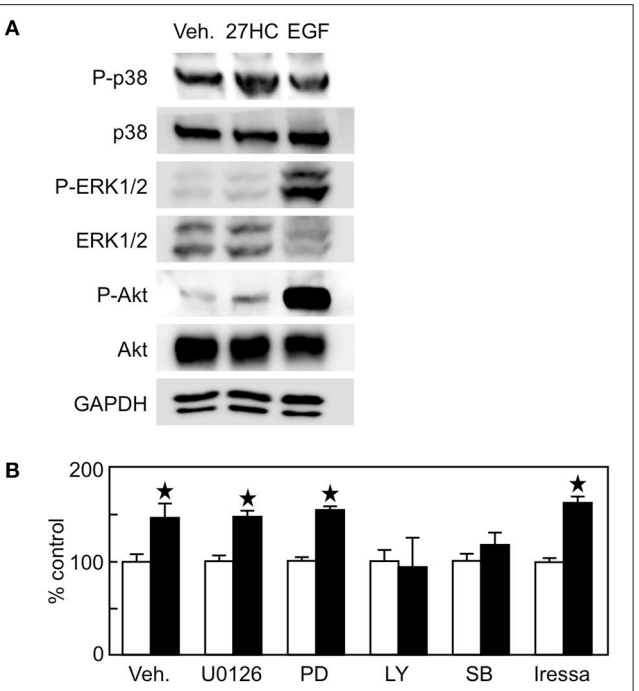

FIGURE 5 | The effect by 27HC on cell proliferation is mediated by PI3K-Akt signaling pathway. **(A)** Immunoblot of phospho-p38 MAPK, total p38 MAPK, phospho-ERK1/2, total ERK1/2, phospho-Akt, and total Akt proteins. H1395 cells were treated with vehicle (Veh.), 10 μM 27HC, or 10 ng/ml EGF for 30 min, and protein abundance of phosphorylated or total proteins in the cell lysates were detected. **(B)** H1395 cell proliferation with kinase inhibitors. U0126 (1 μM), PD0325901 (1 μM), LY294002 (5 μM), SB203580 (1 μM), and iressa (1 μM) were treated 1 h before the 27HC treatment throughout the experiment ($n = 4$). Open bars, vehicle treatments; closed bars, 27HC (1 μM) treatments. $^*p < 0.05$ vs. vehicle control.

ERK1/2, and Akt in H1395 cells (**Figure 5A**). The treatment of 27HC also phosphorylated p38 MAPK and Akt, but not ERK1/2. Next, we performed cell proliferation assays in combination with kinase inhibitors. These inhibitors suppressed basal cell growth (**Supplemental Figure 3C**). The effect by 27HC was not inhibited by MEK/MAPK inhibitors PD0325901 or U0126 (**Figure 5B**). In contrast, the effect by 27HC was suppressed in the presence of the PI3K inhibitor LY294002. Interestingly, another p38MAPK inhibitor SB203580, which also inhibits Akt, suppressed the effect of 27HC. To examine the involvement of the EGFR signaling pathway in the ERβ-mediated 27HC effect on cell proliferation, EGFR inhibitor Irressa was also used. Iressa at 1 μM, which completely blocked the cell proliferation induced by EGF (**Supplemental Figure 3B**), did not alter the effects of 27HC (**Figure 5B**). These results suggest that 27HC promotes H1395 cell proliferation through the activation of PI3K-Akt signaling pathway in ERβ (+) lung cancer cells.

DISCUSSION

Although it is still controversial whether plasma cholesterol levels correlate with lung cancer risks, and also whether higher body mass index (BMI) is protective or harmful to lung cancer depends on cancer types, sex, and treatments (30–32), statins that lower cholesterol levels reduce the risk and mortality of lung cancer in human (33, 34), suggesting that lowering cholesterol levels, which also lowers 27HC levels, has a beneficial effect against lung cancer. Nevertheless, the precise mechanism of how cholesterol affects lung cancer pathogenesis, especially in relation with estrogen receptor status, still remains unknown. In addition, among many risk factors that may contribute to lung cancer incidence or mortality, endocrine factors including estrogen are critical modulators of lung cancer development and progression, and there is accumulating evidence that ERβ expression in conjunction with aromatase expression predicts survival in non-small cell lung cancer both in men and women (35, 36). Despite the poorly understood role of cholesterol in lung cancer pathogenesis, lung tissue has abundant levels of 27HC over a range of concentrations relevant to the modulation of ER function (18, 37). Additionally, protein expression of CYP27A1 is higher in lung tissues from chronic obstructive

pulmonary disease patients than normal lung tissues (38). With the gene expression results showing that 27HC production and ERβ expression is higher in lung tumor cells than in normal lung cells (**Figure 1**), we determined if 27HC modifies lung cancer cell proliferation, and found that 27HC promotes cell proliferation of lung cancer cells that express ERβ. Although some reports suggested that 27HC is a weak agonist for LXR (39), 27HC does not activate LXR in the vascular system and in liver (18). The effect of 27HC on H1395 cells was ERβ-specific, and other nuclear receptor ligands tested did not show proliferation-inducing effects (**Figure 3**). The effect of 27HC was observed not only in H1395 cells, but also in LLC cells that are transfected with ERβ (**Supplemental Figure 1**), indicating the effect of 27HC is not H1395 cell-specific. Recently, a report indicated that 27HC acts as a negative modulator of ERβ (40). In our study, both 27HC and E_2 promoted H1395 cell proliferation. Considering estrogen always activates ER, 27HC likely has an ERβ-mediated, pro-estrogenic function in lung cancer cells. Since 27HC shows both agonistic and antagonistic effects on ERα function depending on target organs in our previous studies (16), it is possible that 27HC may also function as both an agonist and antagonist for ERβ in a cell/tissue type specific manner.

Interestingly, although the expression of *CYP27A1* is higher in lung cancer cells than in normal lung cells (**Figure 1**), endogenously produced 27HC did not alter lung cancer cell proliferation in this study (**Figure 4**). The production of 27HC in lung cancer cells was not be determined in this study, however, there is accumulating evidence that *CYP27A1* mRNA expression correlates with its protein expression and enzymatic activity, and also that CYP27A1 expression correlates with the levels of its product 27HC in various types of cells and also *in vivo* (41–43). In addition, to our knowledge, there is no other enzyme than CYP27A1 known to endogenously produce 27HC in mammals. Thus, it is reasonable to assume that higher CYP27A1 expression reflects higher 27HC production in lung cancer cells, and also there are reports showing effective *CYP27A1* knockdown and consequent decrease of its protein expression (44, 45). In breast cancer, resident macrophages are important for generating and providing 27HC to the microenvironment (21). In addition to the key involvement of ERβ in lung tumor cells, ER activation by 27HC in surrounding stromal cells may also contribute importantly to oncogenesis, such as the regulation of host immunity by estrogen, which is observed in breast tumor models (46). Other possibilities include that the experimental condition in this study was not suitable for detecting the subtle differences caused by endogenously produced 27HC, and also that there are some effects by endogenous 27HC other than those that we examined. Further study will examine the role of endogenously produced 27HC in lung cancer cells and the major source of 27HC involved in lung cancer cell proliferation.

In this study, we found that the effect of 27HC is stronger than that of E_2, even with superphysiological doses of E_2 (**Figure 2** and data not shown). Mechanistic studies indicated that the effect of 27HC is mediated not by EGFR or MAPK, but by the PI3K-Akt signaling pathway (**Figure 5**). We could not find evidence that 27HC acts on pathways other than ER; however, there is still a possibility that 27HC acts on unknown pathways, if any, in addition to the ER signaling pathway. The PI3K-Akt signaling pathway is involved in lung cancer progression (47), and 27HC acts on the cardiovascular system through the activation of PI3K-Akt signaling pathway (48). Further investigation on how 27HC affects the PI3K-Akt signaling pathway are necessary to clarify the role of PI3K-Akt signaling on lung cancer progression.

The novel nature of 27HC modulation of ERβ that we show in this study has revealed a theoretical concept that links cholesterol metabolism with lung cancer progression and treatment failure. Such studies will further our understanding of lung tumor pathogenesis and will further indicate the efficacy of 27HC-directed therapeutic interventions for the treatment of this deadly disease.

AUTHOR CONTRIBUTIONS

SH, TI, and MU designed the experiments. JM provided cell lines. SH, TI, W-RL, TK, CC, NK, FZ, and AA performed the experiments and acquired the data. SH, TI, MS, YZ, JH, JM, and MU analyzed and interpreted the results, and MU wrote the manuscript.

FUNDING

This work was supported by National Institutes of Health grants P03DK079328 (MU) and UH CNRCS Startup Fund (MU).

ACKNOWLEDGMENTS

We thank Drs. Philip Shaul (UT Southwestern), Jan-Ake Gustafsson (University of Houston) and their lab members, Anders Strom, Surendra Chaurasiya, Shivangi Srivastava (University of Houston), and Kendra Carmon (UT-Health) for their technical help, thoughtful discussions, and suggestions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2018.00470/full#supplementary-material

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* (2018) 68:7–30. doi: 10.3322/caac.21442
2. Siegfried JM, Stabile LP, Estrongenic steroid hormones in lung cancer. *Semin Oncol*. (2014) 41:5–16. doi: 10.1053/j.seminoncol.2013.12.009
3. Lortet-Tieulent J, Soerjomataram I, Ferlay J, Rutherford M, Weiderpass E, Bray F. International trends in lung cancer incidence by histological subtype: adenocarcinoma stabilizing in men but still increasing in women. *Lung Cancer* (2014) 84:13–22. doi: 10.1016/j.lungcan.2014.01.009
4. Stabile LP, Dacic S, Land SR, Lenzner DE, Dhir R, Acquafondata M, et al. Combined analysis of estrogen receptor beta-1 and progesterone receptor

expression identifies lung cancer patients with poor outcome. *Clin Cancer Res*. (2011) 17:154–64. doi: 10.1158/1078-0432.CCR-10-0992
5. Schwartz AG, Prysak GM, Murphy V, Lonardo F, Pass H, Schwartz J, et al. Nuclear estrogen receptor beta in lung cancer: expression and survival differences by sex. *Clin Cancer Res.* (2005) 11:7280–7. doi: 10.1158/1078-0432.CCR-05-0498
6. Siegfried JM, Hershberger PA, Stabile LP. Estrogen receptor signaling in lung cancer. *Semin Oncol*. (2009) 36:524–31. doi: 10.1053/j.seminoncol.2009.10.004
7. Slatore CG, Chien JW, Au DH, Satia JA, White E. Lung cancer and hormone replacement therapy: association in the vitamins and lifestyle study. *J Clin Oncol*. (2010) 28:1540–6. doi: 10.1200/JCO.2009.25.9739
8. Bouchardy C, Benhamou S, Schaffar R, Verkooijen HM, Fioretta G, Schubert H, et al. Lung cancer mortality risk among breast cancer patients treated with anti-estrogens. *Cancer* (2011) 117:1288–95. doi: 10.1002/cncr.25638
9. Ciana P, Di Luccio G, Belcredito S, Pollio G, Vegeto E, Tatangelo L, et al. Engineering of a mouse for the *in vivo* profiling of estrogen receptor activity. *Mol Endocrinol.* (2001) 15:1104–13. doi: 10.1210/mend.15.7.0658
10. Lemmen JG, Arends RJ, van Boxtel AL, van der Saag PT, van der Burg B. Tissue- and time-dependent estrogen receptor activation in estrogen reporter mice. *J Mol Endocrinol*. (2004) 32:689–701. doi: 10.1677/jme.0.0320689
11. Morani A, Barros RP, Imamov O, Hultenby K, Arner A, Warner M, et al. Lung dysfunction causes systemic hypoxia in estrogen receptor beta knockout (ERbeta$^{-/-}$) mice. *Proc Natl Acad Sci USA*. (2006) 103:7165–9. doi: 10.1073/pnas.0602194103
12. Pietras RJ, Marquez DC, Chen HW, Tsai E, Weinberg O, Fishbein M. Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells. *Steroids* (2005) 70:372–81. doi: 10.1016/j.steroids.2005.02.017
13. Brodie A, Lu Q, Yue W, Wang J, Liu Y. Intratumoral aromatase model: the effects of letrozole (CGS 20267). *Breast Cancer Res Treat*. (1998) 49:S23–6; discussion S33–7.
14. Stabile LP, Lyker JS, Gubish CT, Zhang W, Grandis JR, Siegfried JM. Combined targeting of the estrogen receptor and the epidermal growth factor receptor in non-small cell lung cancer shows enhanced antiproliferative effects. *Cancer Res.* (2005) 65:1459–70. doi: 10.1158/0008-5472.CAN-04-1872
15. Marquez-Garban DC, Chen HW, Fishbein MC, Goodglick L, Pietras RJ. Estrogen receptor signaling pathways in human non-small cell lung cancer. *Steroids* (2007) 72:135–43. doi: 10.1016/j.steroids.2006.11.019
16. Umetani M. Re-adopting classical nuclear receptors by cholesterol metabolites. *J Steroid Biochem Mol Biol*. (2016) 157:20–6. doi: 10.1016/j.jsbmb.2015.11.002
17. Crisby M, Nilsson J, Kostulas V, Bjorkhem I, Diczfalusy U. Localization of sterol 27-hydroxylase immuno-reactivity in human atherosclerotic plaques. *Biochim Biophys Acta* (1997) 1344:278–85.
18. Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, et al. 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nat Med*. (2007) 13:1185–92. doi: 10.1038/nm1641
19. DuSell CD, Umetani M, Shaul PW, Mangelsdorf DJ, McDonnell DP. 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator. *Mol Endocrinol.* (2008) 22:65–77. doi: 10.1210/me.2007-0383
20. Chen S, Masri S, Wang X, Phung S, Yuan YC, Wu X. What do we know about the mechanisms of aromatase inhibitor resistance? *J Steroid Biochem Mol Biol*. (2006) 102:232–40. doi: 10.1016/j.jsbmb.2006.09.012
21. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, et al. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* (2013) 342:1094–8. doi: 10.1126/science.1241908
22. Wu Q, Ishikawa T, Sirianni R, Tang H, McDonald JG, Yuhanna IS, et al. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Rep.* (2013) 5:637–45. doi: 10.1016/j.celrep.2013.10.006
23. Kim J, McMillan E, Kim HS, Venkateswaran N, Makkar G, Rodriguez-Canales J, et al. XPO1-dependent nuclear export is a druggable vulnerability in KRAS-mutant lung cancer. *Nature* (2016) 538:114–7. doi: 10.1038/nature19771
24. McMillan EA, Ryu MJ, Diep CH, Mendiratta S, Clemenceau JR, Vaden RM, et al. Chemistry-first approach for nomination of personalized treatment in lung cancer. *Cell* (2018) 173:864–78 e29. doi: 10.1016/j.cell.2018.03.028
25. Ishikawa T, Yuhanna IS, Umetani J, Lee WR, Korach KS, Shaul PW, et al. LXRbeta/estrogen receptor-alpha signaling in lipid rafts preserves endothelial integrity. *J Clin Invest*. (2013) 123:3488–97. doi: 10.1172/JCI66533
26. Dey P, Velazquez-Villegas LA, Faria M, Turner A, Jonsson P, Webb P, et al. Estrogen receptor beta2 induces hypoxia signature of gene expression by stabilizing HIF-1alpha in prostate cancer. *PLoS ONE* (2015) 10:e0128239. doi: 10.1371/journal.pone.0128239
27. Voisin M, de Medina P, Mallinger A, Dalenc F, Huc-Claustre E, Leignadier J, et al. Identification of a tumor-promoter cholesterol metabolite in human breast cancers acting through the glucocorticoid receptor. *Proc Natl Acad Sci USA*. (2017) 114:E9346–55. doi: 10.1073/pnas.1707965114
28. Wang Y, Kumar N, Crumbley C, Griffin PR, Burris TP. A second class of nuclear receptors for oxysterols: regulation of RORalpha and RORgamma activity by 24S-hydroxycholesterol (cerebrosterol). *Biochim Biophys Acta* (2010) 1801:917–23. doi: 10.1016/j.bbalip.2010.02.012
29. Jetten AM, Takeda Y, Slominski A, Kang HS. Retinoic acid-related orphan receptor gamma (RORgamma): connecting sterol metabolism to regulation of the immune system and autoimmune disease. *Curr Opin Toxicol.* (2018) 8:66–80. doi: 10.1016/j.cotox.2018.01.005
30. Greenlee H, Unger JM, LeBlanc M, Ramsey S, Hershman DL. Association between body mass index and cancer survival in a pooled analysis of 22 clinical trials. *Cancer Epidemiol Biomarkers Prev*. (2017) 26:21–9. doi: 10.1158/1055-9965.EPI-15-1336
31. Carreras-Torres R, Johansson M, Haycock PC, Wade KH, Relton CL, Martin RM, et al. Obesity, metabolic factors and risk of different histological types of lung cancer: a Mendelian randomization study. *PLoS ONE* (2017) 12:e0177875. doi: 10.1371/journal.pone.0177875
32. Huang X, Shu C, Chen L, Yao B. Impact of sex, body mass index and initial pathologic diagnosis age on the incidence and prognosis of different types of cancer. *Oncol Rep.* (2018) 40:1359–69. doi: 10.3892/or.2018.6529
33. Khurana V, Bejjanki HR, Caldito G, Owens MW. Statins reduce the risk of lung cancer in humans: a large case-control study of US veterans. *Chest* (2007) 131:1282–8. doi: 10.1378/chest.06-0931
34. Cardwell CR, Mc Menamin U, Hughes CM, Murray LJ. Statin use and survival from lung cancer: a population-based cohort study. *Cancer Epidemiol Biomarkers Prev.* (2015) 24:833–41. doi: 10.1158/1055-9965.EPI-15-0052
35. Mah V, Marquez D, Alavi M, Maresh EL, Zhang L, Yoon N, et al. Expression levels of estrogen receptor beta in conjunction with aromatase predict survival in non-small cell lung cancer. *Lung Cancer* (2011) 74:318–25. doi: 10.1016/j.lungcan.2011.03.009
36. Miki Y, Abe K, Suzuki S, Suzuki T, Sasano H. Suppression of estrogen actions in human lung cancer. *Mol Cell Endocrinol.* (2011) 340:168–74. doi: 10.1016/j.mce.2011.02.018
37. Babiker A, Andersson O, Lindblom D, van der Linden J, Wiklund B, Lutjohann D, et al. Elimination of cholesterol as cholestenoic acid in human lung by sterol 27-hydroxylase: evidence that most of this steroid in the circulation is of pulmonary origin. *J Lipid Res.* (1999) 40:1417–25.
38. Kikuchi T, Sugiura H, Koarai A, Ichikawa T, Minakata Y, Matsunaga K, et al. Increase of 27-hydroxycholesterol in the airways of patients with chronic obstructive pulmonary disease: possible role of 27-hydroxycholesterol in tissue fibrosis. *Chest* (2012) 142:329–37. doi: 10.1378/chest.11-2091.
39. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature* (1996) 383:728–31. doi: 10.1038/383728a0
40. Starkey NJE, Li Y, Drenkhahn-Weinaug SK, Liu J, Lubahn DB. 27-hydroxycholesterol is an estrogen receptor beta-selective negative allosteric modifier of 17beta-estradiol binding. *Endocrinology* (2018) 159:1972–81. doi: 10.1210/en.2018-00081
41. Li X, Pandak WM, Erickson SK, Ma Y, Yin L, Hylemon P, et al. Biosynthesis of the regulatory oxysterol, 5-cholesten-3beta,25-diol 3-sulfate, in hepatocytes. *J Lipid Res*. (2007) 48:2587–96. doi: 10.1194/jlr.M700301-JLR200
42. Li J, Daly E, Campioli E, Wabitsch M, Papadopoulos V. De novo synthesis of steroids and oxysterols in adipocytes. *J Biol Chem*. (2014) 289:747–64. doi: 10.1074/jbc.M113.534172
43. Fu X, Menke JG, Chen Y, Zhou G, MacNaul KL, Wright SD, et al. 27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. *J Biol Chem*. (2001) 276:38378–87. doi: 10.1074/jbc.M105805200

44. Bingham TC, Parathath S, Tian H, Reiss A, Chan E, Fisher EA, et al. Cholesterol 27-hydroxylase but not apolipoprotein apoE contributes to A2A adenosine receptor stimulated reverse cholesterol transport. *Inflammation* (2012) 35:49–57. doi: 10.1007/s10753-010-9288-y
45. Fedorova OV, Zernetkina VI, Shilova VY, Grigorova YN, Juhasz O, Wei W, et al. Synthesis of an endogenous steroidal Na pump inhibitor marinobufagenin, implicated in human cardiovascular diseases, is initiated by CYP27A1 via bile acid pathway. *Circ Cardiovasc Genet.* (2015) 8:736–45. doi: 10.1161/CIRCGENETICS.115.001217
46. Curran EM, Judy BM, Duru NA, Wang HQ, Vergara LA, Lubahn DB, et al. Estrogenic regulation of host immunity against an estrogen receptor-negative human breast cancer. *Clin Cancer Res.* (2006) 12:5641–7. doi: 10.1158/1078-0432.CCR-05-1117
47. Chappell WH, Steelman LS, Long JM, Kempf RC, Abrams SL, Franklin RA, et al. Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR inhibitors: rationale and importance to inhibiting these pathways in human health. *Oncotarget* (2011) 2:135–64. doi: 10.18632/oncotarget.240
48. Umetani M, Ghosh P, Ishikawa T, Umetani J, Ahmed M, Mineo C, et al. The cholesterol metabolite 27-hydroxycholesterol promotes atherosclerosis via proinflammatory processes mediated by estrogen receptor alpha. *Cell Metab.* (2014) 20:1–11. doi: 10.1016/j.cmet.2014.05.013

Conflict of Interest Statement: 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 Endocrinology

REVIEW
published: 11 September 2018
doi: 10.3389/fendo.2018.00525

Cholesterol as an Endogenous ERRα Agonist: A New Perspective to Cancer Treatment

Ivan Casaburi†, Adele Chimento†, Arianna De Luca, Marta Nocito, Sara Sculco, Paola Avena, Francesca Trotta, Vittoria Rago, Rosa Sirianni‡ and Vincenzo Pezzi‡*

Department of Pharmacy and Health and Nutritional Science, University of Calabria, Cosenza, Italy

OPEN ACCESS

Edited by:
Antimo Migliaccio, Università degli Studi Della Campania "Luigi Vanvitelli" Naples, Italy

Reviewed by:
Didier Picard, Université de Genève, Switzerland
Roberta Malaguarnera, Università Degli Studi Magna Græcia di Catanzaro, Italy

***Correspondence:**
Vincenzo Pezzi
v.pezzi@unical.it

†These authors share co-first authorship

‡These authors share co-last authorship

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *08 June 2018*
Accepted: *21 August 2018*
Published: *11 September 2018*

Citation:
Casaburi I, Chimento A, De Luca A, Nocito M, Sculco S, Avena P, Trotta F, Rago V, Sirianni R and Pezzi V (2018) Cholesterol as an Endogenous ERRα Agonist: A New Perspective to Cancer Treatment. Front. Endocrinol. 9:525. doi: 10.3389/fendo.2018.00525

The estrogen-related receptors (ERRs) are important members of nuclear receptors which contain three isoforms (α, β, and γ). ERRα is the best-characterized isoform expressed mainly in high-energy demanding tissues where it preferentially works in association with the peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) and PGC-1β. ERRα together with its cofactors modulates cellular metabolism, supports the growth of rapidly dividing cells, directs metabolic programs required for cell differentiation and maintains cellular energy homeostasis in differentiated cells. In cancer cells, the functional association between ERRα and PGC-1s is further influenced by oncogenic signals and induces metabolic programs favoring cell growth and proliferation as well as tumor progression. Recently, cholesterol has been identified as a natural ERRα ligand using a combined biochemical strategy. This new finding highlighted some important physiological aspects related to the use of cholesterol-lowering drugs such as statins and bisphosphonates. Even more meaningful is the link between increased cholesterol levels and certain cancer phenotypes characterized by an overexpressed ERRα such as mammary, prostatic, and colorectal cancers, where the metabolic adaptation affects many cancer processes. Moreover, high-energy demanding cancer-related processes are strictly related to the cross-talk between tumor cells and some key players of tumor microenvironment, such as tumor-associated macrophage that fuels cancer progression. Some evidence suggests that high cholesterol content and ERRα activity favor the inflammatory environment by the production of different cytokines. In this review, starting from the most recent observations on the physiological role of the new signaling activated by the natural ligand of ERRα, we propose a new hypothesis on the suitability to control cholesterol levels as a chance in modulating ERRα activity in those tumors in which its expression and activity are increased.

Keywords: ERRα, cholesterol, cancer metabolism, breast and prostate cancer, colonrectal cancer, adrenocortical carcinoma (ACC), IL-8

GENERAL CONCEPT

Nuclear receptors (NRs) are a large family of transcription factors that are activated by different signal molecules such as steroids, thyroid hormones, vitamins, retinoic acid, oxysterols, and many other metabolites (1).

A distinct subset of the NRs still remains "orphans" (ONRs) waiting for a defined endogenous ligand. Once activated, NRs together with a multitude of co-factors, drive transcription of genes that control cell proliferation, development, reproduction, and different metabolic phenomena upon which those processes are strictly dependent.

A proper functional control of energy pathways within the cells is supported by the coordination of several transcription factors, including NRs, and associated co-factors. Many members of NR superfamily are involved in these processes since they can activate a specific gene expression network in response to hormonal, nutrient, and metabolite signals coming from distinct physiological (or pathological) conditions (2).

ERRs Structure

The estrogen-related receptors (ERRs) are important members of the ONRs family, deeply involved in the control of energy homeostasis. The ERR subfamily comprises three isoforms, namely ERRα (NR3B1), ERRβ (NR3B2), and ERRγ (NR3B3).

ERRα (45.5kDa, 423a.a.) and β (56.2kDa, 508a.a.) were first identified by screening human male gonad cDNA library with a probe synthesized based on the DNA-binding domain sequence of estrogen receptor alpha (ERα) and ERRα cDNA as a probe, respectively (3). The discovery of the third member, ERRγ (51.3kDa, 45a.a.), was made 10 years later by a different methodological approach (4, 5). In addition, several splice variants of human ERRs have been identified although their physiological role is still to be discovered (6). Despite their name and sequence homology with ERs, they do not bind natural estrogens. Moreover, although the existence of functional crosstalk between ERRs and ERs cannot be ruled out, especially in breast cancer pathology, a deeper investigation revealed that ERRs, particularly ERRα, and ERα have a distinct genomic signature and functions (7).

As members of the nuclear receptor superfamily, ERRs are characterized by a conserved structural and functional organization consisting of:

- A N–terminal region (A/B domain) that contains a ligand-independent transcriptional activation function domain (AF-1). The AF-1 domain of all three ERR isoforms contains conserved motifs that allow the control of the transcriptional activity by post-translational modification such as phosphorylation and sumoylation. In particular, phosphorylation-dependent sumoylation of ERRα within the NH-terminal domain of ERRα and ERRγ negatively affects their transcriptional activity without altering DNA binding with cofactors (8). This mechanism becomes particularly important considering the absence of a specific/natural ligand and the constitutively active conformation of this class of receptors that make their functions dependent on the presence of coactivators and corepressor proteins (see below);
- A central C domain, also known as DNA binding domain or DBD, a sequence shared by almost all three ERR members. DBD allows the recognition of DNA sites through the TNAAGGTCA sequence also known as ERR responsive elements or ERREs. In the DBD there are two digitally shaped helical structures, called "zinc fingers," in which a zinc ion is coordinated with 4 cysteines. The P-box (proximal box), placed in the first helical structure, enables the recognition of a specific DNA sequence, while the D-box (distal box), placed in the second structure, is involved in the dimerization process on the DNA sequences. Indeed, ERRs can interact with an ERRE sequence as a monomer, homodimer or as a heterodimer consisting of two distinct ERR isoforms (9–11);
- A region linking C to Ligand Binding Domain (LBD);
- A ligand binding domain or LBD, which adopts a transcriptionally active conformation in the absence of any ligand (12);
- A less conserved C-terminal F region, which is present only in some nuclear receptors, including the ERs.

The sequence analysis of all three receptor isoforms reveals a different sequence homology in their domains: all three members show a high sequence identity in their DBD (93–98%); regarding the LBD, ERRα, and ERRβ are less related (57%) while that of ERRβ and ERRγ are closer (73% sequence identity). The latter domain exhibits a 63% sequence identity between ERRα and ERRγ; high level of sequence identity in the A/B domain (60%) characterizes ERRβ and ERRγ (13).

According to their discovery, ERR isoforms are more strongly related to estrogen receptors (ERα and β) than to any other member of the NR superfamily. In particular, the analysis of each individual domain reveals that the DBDs of ERRα and ERα are 70% homologues, but their LBD is only 36% similar, explaining the reasons for the absence of ERRα response to ER ligands (12).

ERRα: Function, Regulation, and Activity

ERRα discovery determined straightaway questions about its physiological function: ERRα plays a role in embryonic development and its expression level is high in the heart, skeletal muscles, and nervous system. The main physiological role of ERRα is to act as energy sensor to control cellular adaptation to energy demands and to respond to various metabolic stress conditions. Therefore, ERRα is present in high-demand energy tissues, such as muscles and brown adipose tissue. Cells that do not express an activated ERRα cannot produce enough energy at peak demand moments.

In adipose tissue, ERRα promotes the differentiation of mesenchymal stem cells into adipocytes where it regulates energy metabolism. In fact, ERRα increases lipid absorption, β-oxidation, tricarboxylic acid cycle, oxidative phosphorylation, and mitochondrial biogenesis and functions. These metabolic effects are clearly noticeable in all those tissues with high-energy demand including cardiomyocytes and cells of the immune system like macrophages. The prominent role of ERRα in metabolic regulation is underlined by the demonstration

that ERRα gene knockout (ERRα-KO) mice have altered fat absorption and metabolism and are resistant to fat-induced obesity (14). Moreover, these mice are not able to adapt to the cold environment and develop cardiac contraction dysfunction. Stress-induced cardiac hypertrophy in ERRα-KO mice is caused by poor ATP synthesis and reduced phosphocreatine deposition (15).

ERRα influences the differentiation of myocytes, T cells, intestinal epithelial cells and osteoblasts. A study showed that ERRα plays a key role in bone development and metabolism during embryogenesis (16). Its mRNA is expressed in murine bone cells during bone formation by endochondral and intramembranous ossification as well as in primary human osteoblasts. ERRα influences the transcription of the gene coding for osteopontin an essential constituent of the mineralized extracellular bone matrix. In the ERRα-KO mice, the loss of ERRα gene expression modestly increased osteoblastic differentiation and spongy bone mineral density, as well as the differentiation of mesenchymal cells into osteoblasts (17).

The constitutive activity of ERRα is structurally related to the presence of a phenylalanine residue (Phe^{328} on helix H3) within its LBD which maintains a conformational arrangement suitable for the interaction with different cofactors (18). ERRα transcriptional activity is dependent on the presence of co-regulatory proteins, which are differentially expressed in various cells and tissues. ERRα preferentially works in association with the peroxisome proliferator-activated receptor-γ receptor (PPARγ) co-activator-1α (PGC-1α) and PGC-1β (18). The functional interaction between ERRα and PGC-1s is highly specific and it is essential for full ERRα receptor activity in most cells. Moreover, ERRα together with PGC-1s integrates many intracellular signals arising from extrinsic/intrinsic metabolic stresses, as well as from growth factors (18).

The activity of ERRα has been examined by the study on the transcriptional co-activators PGC-1α and PGC-1β, which integrate the signals on nutritional and energetic status and drive expression of genes that control mitochondrial biogenesis, oxidative metabolism, and gluconeogenesis.

The ability of the co-activator PGC-1α to favor the activation of target genes is based on its recruitment at the level of gene-specific regulatory sites through physical interactions with NRs and other transcription factors. Regarding the NRs, the amphipathic propellers of PGC-1α bind the hydrophobic region of LBD domain while PGC-1α has a surface that interacts specifically with ERRα. Moreover, ERRα is a powerful transcriptional activator when PGC-1α or PGC-1β are introduced into the system. The tight dependency between ERRα and PGC-1s as the result of the high affinity between ERRα and PGC-1s, suggests that ERRα activity is mainly controlled by the interactions with PGC-1α and β. In addition, PGC-1s are also able to modulate ERRα gene expression (19). Consistent with the ability of PGC-1 co-activators to induce ERRα expression, ERRα mRNA levels are higher in tissues with elevated levels of PGC-1α and β (19).

Given the predominant role of PGC-1s/ERRα transcriptional complex in controlling cellular metabolism, its involvement in the process of tumorigenesis is evident, considering that metabolic adaptations is an hallmark of cancer cells (20). Nevertheless, there are still many aspects to be clarified. In normal cells, the activity of the PGC-1s/ERRα axis is directed to increase cellular metabolism, to support the growth of rapidly dividing cells, to control metabolic programs during cell differentiation and to preserve energy homeostasis once differentiated (13). In cancer cells, the PGC-1s/ERRα complex is a direct target of oncogenic signals that affect metabolic programs in order to favor or attenuate cell growth and proliferation (18). Moreover, PGC-1α-mediated mitochondrial biogenesis and respiration in cancer cells is functionally related to metastatic dissemination (21). Accordingly, PGC-1α gene suppression, by disabling mitochondrial biogenesis and oxidative phosphorylation, decreases the rate of metastasis (21). These findings are also supported by the observations that:

- ERRα together with its coactivator PGC-1α binds to the promoter and regulates the expression of vascular endothelial growth factor (VEGF), a master regulator of tumor invasion and angiogenesis (22, 23);
- ERRα is able to regulate the expression of HIF (hypoxia-inducible factor) in breast cancer cells and to associate with the HIFα/β heterodimer to promote its transcriptional activity on genes involved in angiogenesis and cell migration (23, 24).
- ERRα cooperates with HIF to induce the cancer metabolic reprogramming toward the metastatic-promoting glycolytic state (25).
- ERRα, together with its coactivator PGC-1α, regulates the expression of WNT11, a mechanism involving ERRα in a transcriptional complex with β-catenin (26). In fact, ablation of either ERRα or β-catenin expression decreases the migratory ability of different types of cancer cells. In addition, functional genomic studies have identified further ERRα target genes playing an important role in the process of invasion, migration and tumor vascularization (27).

All these observations highlight the pivotal role of ERRα in many (altered) processes that characterize tumors especially those signaling driving tumor progression and aggressiveness.

ERRα Agonists

The constitutive activity of ERRα does not exclude the existence of a molecule able to modulate its activity enabling the recruitment of cofactors and playing a critical role in the maintenance of energy homeostasis as well as in disease progression. Recently, several synthetic antagonists have been identified (28–30). Moreover, dietary products, including cholesterol, have been reported as potential agonists (31, 32). Suetsugi and collaborators identified agonists through virtual ligand screening on an ERRα ligand binding model based on the crystalline structure of ERRγ-LBD (33). Thus, four ligands, increasing the transcriptional activity of ERRα, have been identified: isoflavones (genistein, daidzein, and biochanin A) and a flavone (trihydroxyflavone) (33). Later, scientists synthesized the potential molecules able to interact with the ligand-domain, guided by ERRα crystalline structure, but they were not able to demonstrate the activity of the agonists (34). Moreover, Peng and collaborators synthesized a series of pyrid (1,2-α) pyrimidin-4 in

order to produce more powerful ERRα agonists and to confirm the ability to induce the receptor transcriptional activity (35). These compounds have improved glucose and fatty acid uptake from muscle cells (35) and thus, could have a clinical utility for the treatment of metabolic diseases, including metabolic syndrome and diabetes.

Cholesterol: The First Endogenous ERRα Agonist

Recently, an important study investigated the binding ability of ERRα with endogenous lipids (36). To this aim experiments by using chromatography techniques were performed according to previous approaches validated for the study of PPAR with endogenous lipids from the lipidome. The experimental model used is the mouse brain, selected for the high expression of ERRα. The receptor was expressed, purified, and immobilized onto a resin and then incubated with enriching lipidomes. This experimental approach allowed the identification of a single ion that was significantly enriched by the beads bound to ERR-LBD and this ion was identified as cholesterol. Furthermore, to check the specificity of the interaction between ERRα and cholesterol, authors used targeted LC-MS method to increase the detection sensitivity for the lower-abundance sterols (37). The latter results were in agreement with those from lipidomic experiments. Moreover, in order to verify the specificity of ERRα-LBD-cholesterol interaction, a deeper investigation was performed with a competitive binding assay by using diethylstilbestrol (DES), a synthetic ERRα antagonist, that binds to the lipid-binding pocket of ERRα (38). A further confirmation was obtained by the authors with circular dichroism (CD) spectroscopy tests, where cholesterol, DES and the inverse agonist XCT790, all induced a conformational change in ERRα-LBD, while estradiol did not. These results suggested that Cholesterol-ERRα-LBD binding is more than a simple hydrophobic interaction. In addition, dye-labeled cholesterol derivatives were used and, after fluorescence polarization assay, the results showed that cholesterol binds the ligand-binding pocket of ERRα through its hydroxyl group. These findings indicate that cholesterol could exert a functional control of the ERRα activity (36).

Cholesterol Regulates ERRα Transcriptional Activity

The demonstration that cholesterol impacts ERRα activity comes from the investigation during the osteocalciogenesis process. It has been revealed that ERRα-KO mice have a decreased bone resorption and high bone mass indicating that ERRα is able to promote osteoclast differentiation and activity. The suppression of the osteoclast functions was achieved by the use of statins and nitrogen-containing bisphosphonates, two drugs able to inhibit cholesterol biosynthesis, by blocking the HMG-CoA reductase and farnesyl diphosphate synthase (FPPS), respectively (39, 40). Interestingly, in the absence of ERRα, osteoclast differentiation was neither enhanced by cholesterol nor suppressed by statins. In addition, pharmacological inhibition with XCT790 prevented the effects of cholesterol on parental osteoclasts differentiation. All this data strongly supported the ability of cholesterol to promote osteoclastogenesis acting as an ERRα agonist, whereas statins and bisphosphonates suppressed osteoclastogenesis by reducing cholesterol bioavailability.

Moreover, a further demonstration of the ability of cholesterol to modulate the transcriptional activity of ERRα comes out from conventional transactivation assays by using luciferase as the reporter gene. Indeed, the authors decided to modulate cholesterol abundance in the culture medium. To realize this goal, intracellular sterol levels were reduced by: (i) using lipid-free serum, (ii) adding cholesterol bound to hydroxypropyl cyclodextrin, and (iii) adding the hydrophobic statin, lovastatin.

Cyclodextrin and statin, used as a single agent or as combined treatment, were able to reduce ERRα transactivation. Moreover, the addition of exogenous cholesterol to the samples treated with statins and/or cyclodextrin restored the transcriptional activity of the receptor. This data indicated that cholesterol or a downstream sterol, but not a precursor of the cholesterol pathway, affected ERRα activity. An interesting aspect from the results of the research study by Wei et al. (36), is the need for the presence of cofactors to make ERRα functional, even in the presence of an endogenous ligand. In fact, experiments performed with a silenced PGC-1α gene, revealed that luciferase expression was no longer detectable upon cholesterol treatment even in the presence of ectopic ERRα. These results clearly indicate that cholesterol, and most likely other sterols, affects ERRα function in a PGC-1α-dependent fashion. The authors demonstrated also that ERRα is able to recruit PGC-1α and PGC-1β upon cholesterol addition, but upon cholesterol depletion, PGC-1 coactivators are dissociated from ERRα. Specifically, cholesterol enhances the interaction between ERRα and PGC-1β in osteoclast promoting osteoclastogenesis and bone resorption, while promotes ERRα interaction with PGC-1α in myocytes inducing myogenesis and decreasing statins-induced muscle toxicity.

Cholesterol-Lowering Drugs Use and Outcome of Cancer Patients

Many experimental evidence strongly suggest that the inhibition of the mevalonate pathway using statin or bisphosphonate drugs has an impact on oncogenic processes such as cell proliferation, tumor progression, and metastatic potential (41). Statins are inhibitors of HMG-CoA reductase, that block the mevalonate pathway progression limiting the accumulation of the final products such as cholesterol, dolichol, isoprenoids, ubiquinone, and isopentenyladenine (42, 43).

Effects of statins on the outcome of patients affected by different types of cancer, including breast (44, 45), prostate (46–48), ovarian (49), lymphoma (50), renal cell carcinoma (51), and colorectal (52, 53) cancer have been examined in the last years. Some of these studies suggests that statins use is associated with longer survival, while others report no benefits. A recent meta-analysis showed that the average effect of statin use is beneficial for overall survival and cancer-specific survival (54). In particular, the study specified that colorectal, prostate, and

breast cancers, the three largest cancer-type subgroups, showed a benefit from statins use (54). Interestingly, these three tumors are characterized by high expression of ERRα that, depending on the particular tumor phenotype, is associated with tumor progression and/or worst prognosis (55, 56).

Statins may exert their anticancer effect through several molecular mechanisms: via lowering protein prenylation, reducing tumor cell proliferation and migration, inhibiting rat sarcoma (Ras) signaling, inducing apoptosis through inhibition of Akt phosphorylation and consequently mammalian target of rapamycin (mTOR) down-regulation and other pleiotropic effects on cellular level (57, 58).

Bisphosphonates, especially nitrogen-containing bisphosphonates (N-BPs), are widely used to preserve bone health in patients with cancer thank to their ability to negatively regulate osteoclast-mediated bone resorption that is closely associated with metastasis in different cancer types (59). N-BPs work by interfering with two enzymes belonging to the mevalonate pathway called farnesyl pyrophosphate synthase (FPPS) and geranylgeranyl pyrophosphate synthase (GGPPS). The inhibition of mevalonate pathway by N-BPs results in the accumulation of isopentenyl pyrophosphate (IPP), which is then converted to a cytotoxic ATP analog called ApppI. This last event together with the N-BPs-induced inhibition of protein prenylation causes osteoclast dysfunction and reduced bone resorption. Similarly to studies on statins, epidemiological data projecting bisphosphonates as antitumor agents are still controversial. Although further studies are needed to better elucidate the antitumor effects of bisphosphonates, the general concept that is drawn from current available data (60) is that bisphosphonates reduce cancer metastatic lesions in non-solid as well as in solid tumors. Indeed, in addition to their clinical use to preserve bone tissue, emerging *in vitro* and clinical studies suggest that N-BSs have direct effects on cancer cells including induction of apoptosis, inhibition of proliferation, adhesion, invasion and angiogenesis (60). Synergism with chemotherapeutics and enhancement of immune surveillance expand the pleiotropic effects exerted by N-BPs in oncology.

A new regulator of cholesterol metabolism is represented by protein convertase subtilisin/kexin type 9 (PCSK9) (61) an enzyme produced by hepatocytes and secreted into the plasma to bind to the LDL receptor resulting in lysosomal degradation of the receptor. Consequently, PCSK9 reduces the expression of LDL receptors on the cell membrane thereby decreasing the clearance of LDL-cholesterol. PCSK9 inhibitors (monoclonal-antibodies) have become very useful in statin intolerant patients or when statin therapy is unable to reduce LDL (61).

PCSK9 is mostly expressed in the liver (62), which is one of the most common sites for metastasis, thus the ability of PCSK9 and cholesterol to favor a pro-metastatic environment was investigated. Clinical studies are very poor (63, 64), while the majority of the results comes from preclinical experimental models: the PCSK9-KO mice and melanoma cells (65). In the presence of a reduced expression of PCSK9, the number of hepatic metastases was significantly lower. By contrast, PCSK9-KO and wild-type mice fed with a high cholesterol diet showed an increased number of liver metastasis, suggesting a prominent role of cholesterol in tumor-microenvironment interaction. The results obtained on melanoma cells can be extended to other types of cancer.

The identification of cholesterol as ERRα agonist adds a new anticancer mechanism for statins and nitrogen-containing bisphosphonates that could be further investigated to widen the potential therapeutic alternatives in cancers where ERRα is overexpressed. By contrast, the reduction of LDL-R by PCSK9 inhibitors may result in an increased intracellular content of cholesterol within tumor cells that could sustain ERRα activation. Thus, the use of PCSK9 inhibitors in oncology needs further investigations before the preclinical results can be translated into a clinical setting.

ERRα, Cholesterol, and Inflammatory Markers

Cholesterol has been shown to inhibit the expression of chemokines, such as CXCL9 and CXCL10, in macrophages (66) while statins and bisphosphonates enhance CXCL9 and CXCL10 gene expression in wild-type (WT) but not in ERRα-KO macrophages. A similar mechanism was observed for other inflammatory markers such as IL-1β and MMP9. These data suggest that chemokine-suppressive effects of cholesterol in macrophages is dependent on ERRα, revealing an anti-inflammatory role for the NR in a cholesterol-rich environment.

However, a deeper analysis should be performed on the role of cholesterol as the modulator of inflammatory markers produced by macrophages. In fact, an interesting paper (67) reported an induction of IL-8 (CXCL8) in response to cholesterol loading in macrophages foam cells, one of the hallmarks of atherosclerosis (68–70). It has been observed that cholesterol loading, in addition to affecting its own uptake, induces several effects in macrophages including the alteration of the cellular metabolism (71, 72), the increase of phospholipids synthesis (73), the increase of apolipoprotein E synthesis and secretion (74), and the enhancement of lipoprotein and apoprotein internalization and degradation (75). All these phenomena contribute to different phases in the progression of atherosclerosis.

In the paper by Wang et al. (67), macrophages were incubated with or without acetylated LDL (acLDL) in the presence or in the absence of an acyl-CoA:cholesterol-acyltransferase (ACAT) inhibitor before evaluating changes in chemokines mRNA content, growth factors, interleukins, and adhesion molecules. Among these genes, a significative increase in mRNA level was observed only for IL-8. Specifically, cholesterol intake by macrophages through scavenger receptor-mediated endocytosis of acLDL enhanced IL-8 expression both at transcriptional and post-transcriptional level.

These observations arouse some deductions concerning the potential positive effect of cholesterol on the expression of the cytokine IL-8, known to be associated with proliferation, angiogenesis, migration, and chemosensitivity of many cancer cells (76). In fact, IL-8 expression has been detected in numerous cancer types, but its value as a cancer biomarker has been poorly investigated, even though it could be relevant for a good

number of malignant diseases such as thyroid cancers where IL-8 represents the most deeply investigated chemokine in thyroid tumor microenvironment (77).

For these reasons, we can speculate that cholesterol trough ERRα-dependent signaling regulates production of inflammatory markers in cancer cells as well as in macrophages within tumor microenvironment. Therefore, it seems necessary to delve into this last aspect because ERRα could represent a relevant therapeutic target since this receptor is a key functional factor shared by several oncogenic signals belonging to both tumor cells and microenvironment.

ERRα, IL-8, and Colorectal Cancer

Starting from the evidence that ERRα is overexpressed in colorectal (CRC) tumor tissues and cell lines, it was demonstrated that ERRα promotes *in vitro* proliferation and migration (78). Moreover, a close correlation between ERRα protein level and activity and the production of IL-8 in CRC has been found (78). Moreover, chemical inhibition of ERRα activity by treating different CRC cell lines with its inverse agonist, XCT-790, significantly decreased the IL-8 mRNA content, without affecting the expression of other chemokines. These results were further confirmed by ERRα specific silencing experiments. On the other hand, IL-8 expression was upregulated in all CRC cell models characterized by ERRα overexpression.

The same authors, investigating the mechanism by which ERRα regulates the expression of IL-8 in CRC cells revealed that XCT-790 treatment or ERRα gene silencing decreased the promoter activity of IL-8. Moreover, the observation that XCT-790 increased IL-8 mRNA degradation, demonstrated that ERRα regulated IL-8 gene transcription and mRNA stability. Importantly, IL-8 gene silencing suppressed CRC cells proliferation and migration similarly to XCT-790, while a pre-treatment with recombinant IL-8 can rescue the inhibitory effects exerted by XCT-790 on cell proliferation and migration. These results clearly suggest that ERRα activity is directly involved in IL-8-induced CRC cells growth and motility.

Several findings indicate that ERRα activity in CRC could be induced by an excess of cholesterol in the tumor microenvironment. In fact, epidemiologic studies indicate that CRC risk is directly associated with a higher consumption of animal fats and inversely correlated with a diet rich in fruits and vegetables (79, 80). Animal fats diet is also associated with an increased risk of developing chronic intestinal inflammation (81). In fact, an excess of intake in fats of animal origin induces the production of oxidized molecules responsible for lipid oxidation processes able to generate different toxic products for the intestinal epithelial barrier function and the production of pro-inflammatory molecules. All these factors contribute to developing a cancer-prone microenvironment.

The existence of a direct correlation between the levels of cholesterol and the production of IL-8 in the macrophage suggests some interesting hypotheses that could represent the rational basis for further studies: (a) the agonistic action of cholesterol on ERRα in CRC cells could favor the recruitment of co-regulators involved in the enhancement of IL-8 gene expression; (b) a similar mechanism could occur also in macrophages leading the way for new hypotheses on ERRα involvement in the regulation of the inflammatory process within the tumor microenvironment.

Cholesterol and ERRα in Breast, Prostate, and Adrenocortical Cancer

A new potential therapeutic application in a clinical setting controlling cholesterol levels come out from the observations on the role played by ERRα in breast (BC) and prostate (PC) cancers. In BC, high ERRα expression characterizes tumors with poor prognosis (81). Moreover, ERRα mRNA is positively correlated with the oncogene ERBB2 and AIB1 (82) and inversely correlated with that of ERα and progesterone receptor that are good prognostic factors for the anti-hormonal treatment of breast cancer patients. Indeed, depending on the cellular context, ERRα could act promoting or inhibiting transcription (83). Findings suggested that in ER-negative BC, ERRα compensates for the loss of ERα in addition to triggering the expression of ERα-independent genes since it recognizes estrogen response element (ERE) as is the case for vascular endothelial growth factor (VEGF) promoting BC metastasis (23, 24). By contrast, in ER-positive BC cells, ERRα negatively controls ERE transcription by interacting with corepressor such as RIP1. Alternatively, ERRα could promote BC cells growth by enhancing circulating estrogen production. In fact, it has been found that ERRα could activate steroid sulfotransferase (SULT2A1) that works to maintain high level of peripheral dehydroepiandrosteronesulfate (DHEAS), an important metabolite in estrogen synthesis in adrenal tissues. In addition, it has also been evidenced that SULT2A1 inactivates tamoxifen and raloxifene (84). Thus, high ERRα expression in breast cancer by enhancing SULT2A1 activity could also support breast cancer cell resistance to anti-hormonal therapy (84).

The enhanced expression of ERRα has been found also in prostate cancer (PCa) and PCa cell lines (85). A study indicates a positive correlation between ERRα expression and the Gleason score while results from a preclinical study showed that ERRα can promote the hypoxic growth adaptation of prostate cancer cells by interacting with HIF-1α. As above explained, ERRα is also expressed in the bone regulating activity of osteoblasts and osteoclasts, that is implicated into the mixed osteolytic and osteoblastic lesions observed in advanced prostate cancer patients (86).

An increased cholesterol biosynthesis, regulated by sterol regulatory element-binding protein−2 (SREBP-2), is a key player in the initiation and progression of PCa where an enhanced stem cell population was observed (87). Moreover, aberrant cholesteryl ester accumulation in lipid droplets exacerbates cancer invasiveness and characterize high-grade PCa with PTEN loss and consequently, constitutive PI3K/Akt activation promotes metabolic dysregulation where ERRα/PGC-1α, as already mentioned, play a central role (18). In addition, the cholesterol metabolite, 27-hydroxyl-cholesterol (27-OHC) is now recognized as selective estrogen receptor modulator (SERM) which promotes tumorigenesis in ER-positive BC (88). Higher levels of 27-OHC have been reported in ERα-positive breast cancers with respect to normal breast tissue, along with an

observed reduction in the 27-OHC metabolizing enzyme such as CYP7B1 (89). Results from *in vivo* experiments demonstrated that 27-OHC alone is sufficient to support estrogenic activity in ER-dependent breast cancer cells (89). Accordingly, an increased growth and metastasis of ER-positive tumors were observed in a mouse model of breast cancer fed only with a cholesterol-rich diet (89). The function of cholesterol as an ERRα agonist may provide the molecular basis and mechanistic insight into clinical studies suggesting that drugs able to lower cholesterol levels (i.e., statins) can be used to treat or prevent breast and prostate cancer.

A different tumor phenotype where cholesterol could have positive growing effects, over its physiological role, is the adrenocortical cancer (ACC). ACC is a very rare and aggressive disease with very limited therapeutic options (90). The pathogenesis of ACC involves the integration of molecular signals and the interplay of different downstream pathways (i.e., IGFII/IGF1R, β-catenin, Wnt, ERα) (91). Our published results indicate that treatment of ACC cell model with XCT-790, to the purpose of reducing ERRα expression, impaired cancer cell growth, both *in vitro* and *in vivo* (92). Our data well correlate with that reporting an increased ERRα expression in ACC compared to normal adrenal and adenoma (93) underling the involvement of this metabolic receptor in ACC biology. Indeed, our unpublished results revealed that treatment of H295R cells with statins caused a significant reduction in cell growth and motility. Although these data are still preliminary, they suggest that cholesterol may affect various biological processes in ACC through the modulation of ERRα activity. Therefore, cholesterol lowering-drug could extend the therapeutic opportunity to fight this rare tumor.

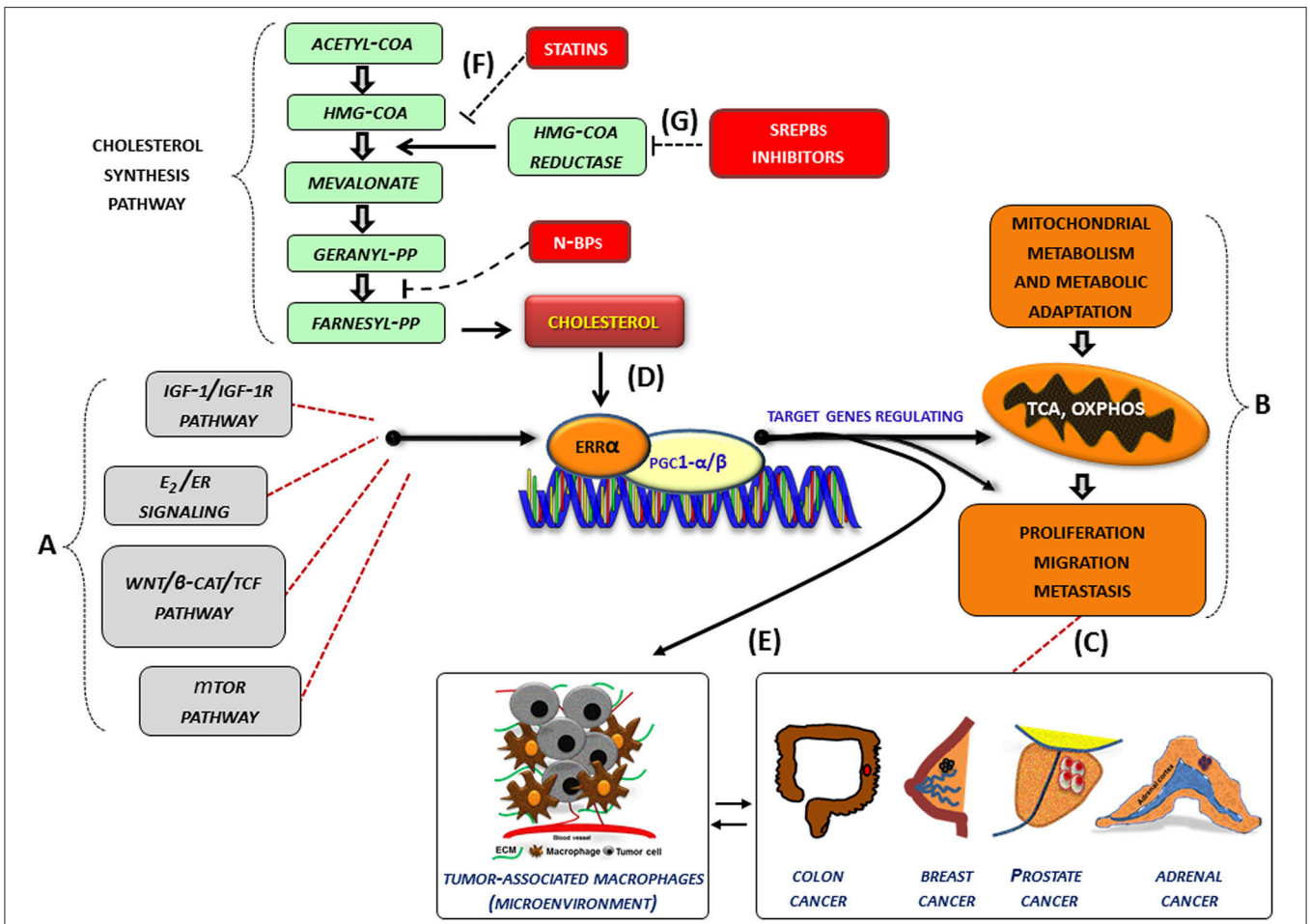

FIGURE 1 | Role of cholesterol as modulator of ERRα action in cancer. **(A–G)** Schematic representation of how cholesterol, as a new ERRα ligand, can contribute to the complex molecular network consisting in the functional cross-talk between oncogenes and oncogenic pathways (IGF-1/IGF-1R, E2/ER, β-catenin/TCF, mTOR) **(A)** that support the overexpression of ERRα. In turn, ERRα, together with its main cofactors (PGC-1α and PGC-1β) and activators, such as cholesterol **(D)**, affects cancer cell metabolism promoting proliferation, migration and, metastasis **(B)** of different tumor phenotypes **(C)**. All these bioenergy-consuming functions are strictly related to **(E)** the cross-talk between tumor cells and some key players of the tumor microenvironment, such as macrophages (tumor-associate macrophages). The use of drugs [statins, **(F)**, N-bisphosphonates, N-BPs, SREBPs inhibitors, **(G)**] able to reduce cholesterol levels and ERRα transcriptional activity could widen the therapeutic opportunities for the treatment of different ERRα overexpressing tumors. More details are explained within the text. E2, estradiol; ER, estrogen receptor; IGF-1/IGF-1R, insulin-like growth factor-1/insulin-like growth factor-1 receptor; WNT, Wingless-type MMTV integration site family member; TCF, T-cell factor; TOR, mammalian target of rapamycin; N-BPs, nitrogen-containing bisphosphonates.

CONCLUSION

The ERRα transcriptional activity in normal cells is directed to modulate cellular metabolism, supporting the growth of rapidly dividing cells and to control metabolic programs required for cellular energy homeostasis in differentiated cells and to satisfy energy request during cell differentiation. The recent identification of cholesterol as an endogenous ERRα agonist evidenced that this sterol enhances the interaction between ERRα and PGC-1β in osteoclasts, promoting osteoclastogenesis and bone resorption. Similarly, cholesterol promotes ERRα interaction with PGC-1α in myocytes inducing myogenesis and decreasing muscle toxicity. The discovery of this new molecular mechanism has elucidated the genesis of two important phenomena with an unexplained mechanism: the statin-induced muscle toxicity and the bisphosphonate suppression of bone resorption.

Moreover, the discovery of cholesterol as an agonist of ERRα demonstrated that this receptor works as a metabolic-sensing nuclear receptor distinguishing it from steroid receptors that respond to an acute and steep rise in hormonal levels. Consequently, ERRα is constitutively active because cholesterol is ubiquitous.

This new mechanism calls fresh thinking about the role of ERRα in cancer cells keeping in mind the key role played by this receptor as modulator of cancer metabolism.

It is well known that the metabolic alterations of lipids, carbohydrates, and proteins are one of the hallmarks of cancers (20). In particular, an increase in the glycolytic rate at the expense of oxidative phosphorylation even in the presence of adequate oxygen concentrations (Warburg effect) (94) allows a rapid adaptation of tumor cells to the continuous metabolic changes that, together with the tumor microenvironment, are the driving forces for cancer survival and its evolution. Given the high interconnection between enzymes that regulate the metabolism and the molecular pathways induced by altered oncogenes, research of the key regulators that behave on metabolic adaptations and proliferative, anti-apoptotic, invasive and metastatic responses, could represent elective targets to break down tumors with a single shot. The ERRα could work for this end due to its location at the intersection of dysregulated metabolism and oncogenic pathways.

In several cancer cells, the expression and the activity of ERRα, together with its cofactors (PGC-1 α/β), is further influenced by oncogenic signals (IGF1-/IGF1R pathway, estrogen signaling, Wnt/β-cat/TCF, mTOR pathway) (**Figure 1A**) and can thus be re-directed to induce metabolic programs (**Figure 1B**) favoring tumor growth and progression. (**Figure 1C**). In this context, an increased level of cholesterol, through the new molecular mechanism, supports all tumor-related processes. (**Figure 1D**). Accordingly, high levels of cholesterol are associated with an increased risk of different type of cancers including breast, prostate (95) and CRC (96).

Although epidemiological data on the correlation between cholesterol and cancer are conflicting, the preclinical results positively highlight different molecular aspects revealing how oncogenic growth signaling meet the bioenergetics and biosynthetic demands of rapidly proliferating tumor cells. In fact, altered cholesterol pathway in cancer could be reached through different mechanisms. One of the most important is the constitutive activity of the oncogenic PI3K/AKT/mTOR signaling pathway that enhances intracellular cholesterol levels by: (i) inducing cholesterol synthesis through the activation of the transcription factor SREBP (sterol regulatory element binding proteins); (ii) inducing LDL receptor–mediated cholesterol import; (iii) inhibiting ABCA1-mediated cholesterol export. Moreover, high-energy demanding cancer related process are strictly related to the cross-talk between tumor cells and some key players of the tumor microenvironment (TME), such as macrophages (TAM, tumor-associated macrophage), that in turn, fuels cancer progression through the formation of an inflammatory milieu characterized by the production of different cytokines such as IL-1, IL-6, and IL-8 among others. The latter, as above reported, could be a target of ERRα action (**Figure 1E**). For most solid tumors, infiltration by inflammatory cells such as macrophages is associated with poor prognosis (97, 98).

The links between inflammation and cholesterol are best exemplified by atherosclerosis, but similar mechanisms may also contribute to other metabolic disorders including cancer. It is noteworthy that cholesterol accumulation in TAM triggers the phenotype switch from M1, antitumorigenic, to M2-like macrophage, protumorigenic (99, 100).

Based on these considerations, the use of therapeutic strategy aimed to reduce cholesterol levels, such as statins (**Figure 1F**) or drugs targeting the SREBP metabolic pathways (**Figure 1G**), could be a promising option to counteract metabolic rewiring in cancer cells where ERRα plays a pivotal role.

In conclusion, identification of cholesterol as an endogenous ERRα agonist has already elucidated the most likely mechanisms underlying the side-effects induced by statins and bisphosphonate, but at the same time, it gives new perspectives to be further investigated in order to explore new therapeutic options for the treatment of ERRα overexpressing tumors. This alternative approach could bring additional benefits to the treatment of tumors that have already adopted successful therapies, but especially for those tumors, such as ACC, which are characterized by a limited or failed therapeutic choice.

AUTHOR CONTRIBUTIONS

IC, AC: literature revision and drafting of the article. ADL, MN, SS, PA, FT, and VR: drafting of the article. RS, VP: critical revision of the article and final approval.

ACKNOWLEDGMENTS

The authors thank the AIRC (Associazione Italiana per la Ricerca sul Cancro, IG2017: Id 20122, PI VP and IG15230, PI RS) for support of their research.

REFERENCES

1. Dhiman VK, Bolt MJ, White KP. Nuclear receptors in cancer - uncovering new and evolving roles through genomic analysis. *Nat Rev Genet.* (2018) 19:160–74. doi: 10.1038/nrg.2017.102
2. Schulman IG. Nuclear receptors as drug targets for metabolic disease. *Adv Drug Deliv Rev.* (2010) 162:307–15. doi: 10.1016/j.addr.2010.07.002
3. Giguere V, Yang N, Segui P, Evans RM. Identification of a new class of steroid hormone receptors. *Nature* (1988) 331:91–4.
4. Hong H, Yang L, Stallcup MR. Hormone-independent transcriptional activation and coactivator binding by novel orphan nuclear receptor ERR3. *J Biol Chem.* (1999) 274:22618–26. doi: 10.1074/jbc.274.32.22618
5. Eudy JD, Yao S, Weston MD, Ma-Edmonds M, Talmadge CB, Cheng JJ, et al. Isolation of a gene encoding a novel member of the nuclear receptor superfamily from the critical region of Usher syndrome type IIa at 1q41. *Genomics* (1998) 50:382–4. doi: 10.1006/geno.1998.5345
6. Tremblay AM, Giguere V. The NR3B subgroup: an ovERRview. *Nucl Recept Signal.* (2007) 5:e009. doi: 10.1621/nrs.05009
7. Tiraby C, Hazen BC, Gantner ML, Kralli A. Estrogen-related receptor gamma promotes mesenchymal-to-epithelial transition and suppresses breast tumor growth. *Cancer Res.* (2011) 71:2518–28. doi: 10.1158/0008-5472.CAN-10-1315
8. Tremblay AM, Wilson BJ, Yang XJ, Giguere V. Phosphorylation-dependent sumoylation regulates estrogen-related receptor-alpha and -gamma transcriptional activity through a synergy control motif. *Molecul Endocrinol.* (2008) 22:570–84. doi: 10.1210/me.2007-0357
9. Gearhart MD, Holmbeck SM, Evans RM, Dyson HJ, Wright PE. Monomeric complex of human orphan estrogen related receptor-2 with DNA: a pseudo-dimer interface mediates extended half-site recognition. *J Molecul Biol.* (2003) 327:819–32. doi: 10.1016/S0022-2836(03)00183-9
10. Giguere V. Transcriptional control of energy homeostasis by the estrogen-related receptors. *Endocr Rev.* (2008) 29:677–96. doi: 10.1210/er.2008-0017
11. Huppunen J, Aarnisalo P. Dimerization modulates the activity of the orphan nuclear receptor ERRgamma. *Biochem Biophys Res Commun.* (2004) 314:964–70. doi: 10.1016/j.bbrc.2003.12.194
12. Kallen J, Schlaeppi JM, Bitsch F, Filipuzzi I, Schilb A, Riou V,et al. Evidence for ligand-independent transcriptional activation of the human estrogen-related receptor alpha (ERRalpha): crystal structure of ERRalpha ligand binding domain in complex with peroxisome proliferator-activated receptor coactivator-1alpha. *J Biol Chem.* (2004) 279:49330–7. doi: 10.1074/jbc.M407999200
13. Huss JM, Garbacz WG, Xie W. Constitutive activities of estrogen-related receptors: transcriptional regulation of metabolism by the ERR pathways in health and disease. *Biochimica et Biophysica Acta* (2015) 1852:1912–27. doi: 10.1016/j.bbadis.2015.06.016
14. Luo J, Sladek R, Carrier J, Bader JA, Richard D, Giguere V. Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. *Molecul Cell Biol.* (2003) 23:7947–56. doi: 10.1128/MCB.23.22.7947-7956.2003
15. Huss JM, Imahashi K, Dufour CR, Weinheimer CJ, Courtois M, Kovacs A, et al. The nuclear receptor ERRalpha is required for the bioenergetic and functional adaptation to cardiac pressure overload. *Cell Metabol.* (2007) 6:25–37. doi: 10.1016/j.cmet.2007.06.005
16. Bonnelye E, Vanacker JM, Dittmar T, Begue A, Desbiens X, Denhardt DT., et al. The ERR-1 orphan receptor is a transcriptional activator expressed during bone development. *Molecul Endocrinol.* (1997) 11:905–16. doi: 10.1210/mend.11.7.9948
17. Delhon I, Gutzwiller S, Morvan F, Rangwala S, Wyder L, Evans G, et al. Absence of estrogen receptor-related-alpha increases osteoblastic differentiation and cancellous bone mineral density. *Endocrinology* (2009) 150:4463–72. doi: 10.1210/en.2009-0121
18. Deblois G, St-Pierre J, Giguere V. The PGC-1/ERR signaling axis in cancer. *Oncogene* (2013) 32:3483–90. doi: 10.1038/onc.2012.529
19. Villena JA, Kralli A. ERRalpha: a metabolic function for the oldest orphan. Trends in endocrinology and metabolism. *Trends Endocrinol Metab.* (2008) 19:269–76. doi: 10.1016/j.tem.2008.07.005
20. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
21. LeBleu VS, O'Connell JT, Gonzalez Herrera KN, Wikman H, Pantel K, Haigis MC, et al. PGC-1alpha mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. *Nat Cell Biol.* (2014) 16:992–1003. doi: 10.1038/ncb3039
22. Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, et al. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. *Nature* (2008) 1451:8–12. doi: 10.1038/nature06613
23. Stein RA, Gaillard S, McDonnell DP. Estrogen-related receptor alpha induces the expression of vascular endothelial growth factor in breast cancer cells. *J Steroid Biochem Molecul Biol.* (2009) 114:106–12. doi: 10.1016/j.jsbmb.2009.02.010
24. Stein RA, Chang CY, Kazmin DA, Way J, Schroeder T, Wergin M, et al. Estrogen-related receptor alpha is critical for the growth of estrogen receptor-negative breast cancer. *Cancer Res.* (2008) 68:8805–12. doi: 10.1158/0008-5472.CAN-08-1594
25. Ao A, Wang H, Kamarajugadda S, Lu J. Involvement of estrogen-related receptors in transcriptional response to hypoxia and growth of solid tumors. *Proc Natl Acad Sci USA.* (2008) 105:7821–6. doi: 10.1073/pnas.0711677105
26. Dwyer MA, Joseph JD, Wade HE, Eaton ML, Kunder RS, Kazmin D, et al. WNT11 expression is induced by estrogen-related receptor alpha and beta-catenin and acts in an autocrine manner to increase cancer cell migration. *Cancer Res.* (2010) 70:9298–308. doi: 10.1158/0008-5472.CAN-10-0226
27. Deblois G, Giguere V. Functional and physiological genomics of estrogen-related receptors (ERRs) in health and disease. *Biochimica et Biophysica Acta* (2011) 1812:1032–40. doi: 10.1016/j.bbadis.2010.12.009
28. Busch BB, Stevens WC Jr, Martin R, Ordentlich P, Zhou S, Sapp DW, et al. Identification of a selective inverse agonist for the orphan nuclear receptor estrogen-related receptor alpha. *J Med Chem.* (2004) 47:5593–6. doi: 10.1021/jm049334f
29. Chisamore MJ, Cunningham ME, Flores O, Wilkinson HA, Chen JD. Characterization of a novel small molecule subtype specific estrogen-related receptor alpha antagonist in MCF-7 breast cancer cells. *PLoS ONE* (2009) 4:e5624. doi: 10.1371/journal.pone.0005624
30. Willy PJ, Murray IR, Qian J, Busch BB, Stevens WC Jr, Martin R, et al. Regulation of PPARgamma coactivator 1alpha (PGC-1alpha) signaling by an estrogen-related receptor alpha (ERRalpha) ligand. *Proc Natl Acad Sci USA.* (2004) 101:8912–7. doi: 10.1073/pnas.0401420101
31. Teng CT, Beames B, Alex Merrick B, Martin N, Romeo C, Jetten AM. Development of a stable cell line with an intact PGC-1alpha/ERRalpha axis for screening environmental chemicals. *Biochem biophys Res Commun.* (2014) 444:177–81. doi: 10.1016/j.bbrc.2014.01.033
32. Teng CT, Hsieh JH, Zhao J, Huang R, Xia M, Martin N, et al. Development of novel cell lines for high-throughput screening to detect estrogen-related receptor alpha modulators. *SLAS Discov.* (2017) 22:720–31. doi: 10.1177/2472555216689772
33. Suetsugi M, Su L, Karlsberg K, Yuan YC, Chen S. Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptors. *Molecul Cancer Res.* (2003) 1:981–91.
34. Hyatt SM, Lockamy EL, Stein RA, McDonnell DP, Miller AB, Orband-Miller LA. et al. On the intractability of estrogen-related receptor alpha as a target for activation by small molecules. *J Med Chem.* (2007) 50:6722–4. doi: 10.1021/jm7012387
35. Peng L, Gao X, Duan L, Ren X, Wu D, Ding K. Identification of pyrido[1,2-alpha]pyrimidine-4-ones as new molecules improving the transcriptional functions of estrogen-related receptor alpha. *J Med Chem.* (2011) 54:7729–33. doi: 10.1021/jm200976s
36. Wei W, Schwaid AG, Wang X, Wang X, Chen S, Chu Q, et al. Ligand activation of ERRalpha by cholesterol mediates statin and bisphosphonate effects. *Cell Metabol.* (2016) 23:479–91. doi: 10.1016/j.cmet.2015.12.010
37. McDonald JG, Thompson BM, McCrum EC, Russell DW. Extraction and analysis of sterols in biological matrices by high performance liquid chromatography electrospray ionization mass spectrometry. *Method Enzymol.* (2007) 432:145–70. doi: 10.1016/S0076-6879(07)32006-5
38. Tremblay GB, Kunath T, Bergeron D, Lapointe L, Champigny C, Bader JA, et al. Diethylstilbestrol regulates trophoblast stem cell differentiation as a ligand of orphan nuclear receptor ERR beta. *Gene Dev.* (2001) 15:833–8. doi: 10.1101/gad.873401

39. Russell RG. Bisphosphonates: the first 40 years. *Bone* (2011) 49:2–19. doi: 10.1016/j.bone.2011.04.022
40. Toledano JE, Partridge NC. Statins: not just for cholesterol? *Trends Endocrinol Metabol.* (2000) 11:255–6. doi: 10.1016/S1043-2760(00)00295-2
41. Cruz PM, Mo H, McConathy WJ, Sabnis N, Lacko AG. The role of cholesterol metabolism and cholesterol transport in carcinogenesis: a review of scientific findings, relevant to future cancer therapeutics. *Front Pharmacol.* (2013) 4:119. doi: 10.3389/fphar.2013.00119
42. Clendening JW, Pandyra A, Boutros PC, El Ghamrasni S, Khosravi F, Trentin GA, et al. Dysregulation of the mevalonate pathway promotes transformation. *Proc Natl Acad Sci USA.* (2010) 107:15051–6. doi: 10.1073/pnas.0910258107
43. Thurnher M, Nussbaumer O, Gruenbacher G. Novel aspects of mevalonate pathway inhibitors as antitumor agents. *Clin Cancer Res.* (2012) 18:3524–31. doi: 10.1158/1078-0432.CCR-12-0489
44. Cardwell CR, Hicks BM, Hughes C, Murray LJ. Statin use after diagnosis of breast cancer and survival: a population-based cohort study. *Epidemiology* (2015) 26:68–78. doi: 10.1097/EDE.0000000000000189
45. Desai P, Lehman A, Chlebowski RT, Kwan ML, Arun M, Manson, JE, et al. Statins and breast cancer stage and mortality in the Women's Health Initiative. *Cancer Causes Control* (2015) 26:529–39. doi: 10.1007/s10552-015-0530-7
46. Yu O, Eberg M, Benayoun S, Aprikian A, Batist G, Suissa S, et al. Use of statins and the risk of death in patients with prostate cancer. *J Clin Oncol.* (2014) 32:5–11. doi: 10.1200/JCO.2013.49.4757
47. Geybels MS, Wright JL, Holt SK, Kolb S, Feng Z, Stanford JL. Statin use in relation to prostate cancer outcomes in a population-based patient cohort study. *Prostate* (2013) 73:1214–22. doi: 10.1002/pros.22671
48. Caon J, Paquette M, Hamm J, Pickles T. Does statin or ASA affect survival when prostate cancer is treated with external beam radiation therapy? *Prostate Cancer* (2014) 2014:184297. doi: 10.1155/2014/184297
49. Habis M, Wroblewski K, Bradaric M, Ismail N, Yamada SD, Litchfield L, et al. Statin therapy is associated with improved survival in patients with non-serous-papillary epithelial ovarian cancer: a retrospective cohort analysis. *PLoS ONE* (2014) 9:e104521. doi: 10.1371/journal.pone.0104521
50. Nowakowski, GS, Maurer MJ, Habermann TM, Ansell SM, Macon WR, Ristow KM, et al. Statin use and prognosis in patients with diffuse large B-cell lymphoma and follicular lymphoma in the rituximab era. *J Clin Oncol.* (2010) 28:412–7. doi: 10.1200/JCO.2009.23.4245
51. Viers BR, Houston Thompson R, Psutka SP, Lohse CM, Cheville JC, Leibovich BC, et al. The association of statin therapy with clinicopathologic outcomes and survival among patients with localized renal cell carcinoma undergoing nephrectomy. *Urol Oncol.* (2015) 33:388 e11–8. doi: 10.1016/j.urolonc.2015.01.009
52. Cardwell CR, Hicks BM, Hughes C, Murray LJ. Statin use after colorectal cancer diagnosis and survival: a population-based cohort study. *J Clin Oncol.* (2014) 32:3177–83. doi: 10.1200/JCO.2013.54.4569
53. Shao YY, Hsu CH, Yeh KH, Chen HM, Yeh YC, Lai CL, et al. Statin use is associated with improved prognosis of colorectal Cancer in Taiwan. *Clin Colorectal Cancer* (2015) 14:177–84 e4. doi: 10.1016/j.clcc.2015.02.003
54. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: Systematic review and meta-analysis of observational studies. *Cancer Treat Rev.* (2015) 41:554–67. doi: 10.1016/j.ctrv.2015.04.005
55. Misawa A, Inoue S. Estrogen-related receptors in breast cancer and prostate cancer. *Front Endocrinol* (2015) 6:83. doi: 10.3389/fendo.2015.00083
56. Bernatchez G, Giroux V, Lassalle T, Carpentier AC, Rivard N, Carrier JC. ERRalpha metabolic nuclear receptor controls growth of colon cancer cells. *Carcinogenesis* (2013) 34:2253-61. doi: 10.1093/carcin/bgt180
57. Matusewicz L, Meissner J, Toporkiewicz M, Sikorski AF. The effect of statins on cancer cells–review. *Tumour Biol.* (2015) 36:4889–904. doi: 10.1007/s13277-015-3551-7
58. Zaleska M, Mozenska O, Bil J. Statins use and cancer: an update. *Future Oncol.* (2018) 14:1497–509. doi: 10.2217/fon-2017-0543
59. Maurizi A, Rucci N. The osteoclast in bone metastasis: player and target. *Cancers* (2018) 10:E218. doi: 10.3390/cancers10070218.
60. Van Acker HH, Anguille S, Willemen Y, Smits EL, Van Tendeloo VF. Bisphosphonates for cancer treatment: mechanisms of action and lessons from clinical trials. *Pharmacol Therapeut.* (2016) 158:24–40. doi: 10.1016/j.pharmthera.2015.11.008
61. Liu ZP, Wang Y. PCSK9 inhibitors: novel therapeutic strategies for lowering LDL-cholesterol. *Mini Rev Med Chem.* (2018) 18:1. doi: 10.2174/1389557518666180423111442
62. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci USA*. (2003) 100:928–33. doi: 10.1073/pnas.0335507100
63. Benn M, Tybjaerg-Hansen A, Stender S, Frikke-Schmidt R, Nordestgaard BG. Low-density lipoprotein cholesterol and the risk of cancer: a mendelian randomization study. *J Natl Cancer Inst*. (2011) 103:508–19. doi: 10.1093/jnci/djr008
64. Folsom AR, Peacock JM, Boerwinkle E. Sequence variation in proprotein convertase subtilisin/kexin type 9 serine protease gene, low LDL cholesterol, and cancer incidence. *Cancer Epidemiol Biomark Prev*. (2007) 16:2455–8. doi: 10.1158/1055-9965.EPI-07-0502
65. Seidah NG. Proprotein convertase subtilisin kexin 9 (PCSK9) inhibitors in the treatment of hypercholesterolemia and other pathologies. *Curr Pharm Design* (2013) 19:3161–72. doi: 10.2174/1381612811319990313
66. Spann NJ, Garmire LX, McDonald JG, Myers DS, Milne SB, Shibata N, et al. Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. *Cell* (2012) 151:138–52. doi: 10.1016/j.cell.2012.06.054
67. Wang N, Tabas I, Winchester R, Ravalli S, Rabbani LE, Tall A. Interleukin 8 is induced by cholesterol loading of macrophages and expressed by macrophage foam cells in human atheroma. *J Biol Chem.* (1996) 271:8837–42. doi: 10.1074/jbc.271.15.8837
68. Schaffner T, Taylor K, Bartucci EJ, Fischer-Dzoga K, Beeson JH, Glagov S, et al. Arterial foam cells with distinctive immunomorphologic and histochemical features of macrophages. *Am J Pathol.* (1980) 100:57–80.
69. Klurfeld DM. Identification of foam cells in human atherosclerotic lesions as macrophages using monoclonal antibodies. *Arch Pathol Lab Med.* (1985) 109:445–9.
70. Faggiotto A, Ross R. Studies of hypercholesterolemia in the nonhuman primate. II. Fatty streak conversion to fibrous plaque. *Arteriosclerosis* (1984) 4:341–56.
71. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* (1986) 232:34–47.
72. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem.* (1983) 52:223–61.
73. Shiratori Y, Okwu AK, Tabas I. Free cholesterol loading of macrophages stimulates phosphatidylcholine biosynthesis and up-regulation of CTP: phosphocholine cytidylyltransferase. *J Biol Chem.* (1994) 269:11337–48.
74. Kayden HJ, Maschio F, Traber MG. The secretion of apolipoprotein E by human monocyte-derived macrophages. *Arch Biochem Biophys*. (1985) 239:388–95. doi: 10.1016/0003-9861(85)90704-0
75. Bottalico LA, Keesler GA, Fless GM, Tabas I. Cholesterol loading of macrophages leads to marked enhancement of native lipoprotein(a) and apoprotein(a) internalization and degradation. *J Biol Chem.* (1993) 268:8569–73.
76. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. *Clin Cancer Res.* (2008) 14:6735–41. doi: 10.1158/1078-0432.CCR-07-4843
77. Rotondi M, Coperchini F, Latrofa F, Chiovato L. Role of chemokines in thyroid cancer microenvironment: is CXCL8 the main player? *Front Endocrinol.* (2018) 9:314. doi: 10.3389/fendo.2018.00314
78. Ding S, Tang Z, Jiang Y, Huang H, Luo P, Qing B, et al. IL-8 Is involved in estrogen-related receptor alpha-regulated proliferation and migration of colorectal cancer cells. *Digest Diseas Sci.* (2017) 62:3438–46. doi: 10.1007/s10620-017-4779-4
79. Rossin D, Calfapietra S, Sottero B, Poli G, Biasi F. HNE and cholesterol oxidation products in colorectal inflammation and carcinogenesis. *Free Radic Biol Med.* (2017) 111:186–95. doi: 10.1016/j.freeradbiomed.2017.01.017
80. Lewis JD, Abreu MT. Diet as a trigger or therapy for inflammatory bowel diseases. *Gastroenterology* (2017) 152:398–414 e6. doi: 10.1053/j.gastro.2016.10.019

81. Ariazi EA, Clark GM, Mertz JE. Estrogen-related receptor alpha and estrogen-related receptor gamma associate with unfavorable and favorable biomarkers, respectively, in human breast cancer. *Cancer Res.* (2002) 62:6510-8.
82. Surowiak P, Materna V, Gyorffy B, Matkowski R, Wojnar A, Maciejczyk A, et al. Multivariate analysis of oestrogen receptor alpha, pS2, metallothionein and CD24 expression in invasive breast cancers. *Br J Cancer* (2006) 95:339–46. doi: 10.1038/sj.bjc.6603254
83. Kraus RJ, Ariazi EA, Farrell ML, Mertz JE. Estrogen-related receptor alpha 1 actively antagonizes estrogen receptor-regulated transcription in MCF-7 mammary cells. *J Biol Chem.* (2002) 277:24826–34. doi: 10.1074/jbc.M202952200
84. Apak TI, Duffel MW. Interactions of the stereoisomers of alpha-hydroxytamoxifen with human hydroxysteroid sulfotransferase SULT2A1 and rat hydroxysteroid sulfotransferase STa. *Drug Metabol Dispos.* (2004) 32:1501–8. doi: 10.1124/dmd.104.000919
85. Cheung CP, Yu S, Wong KB, Chan LW, Lai FM, Wang X, et al. Expression and functional study of estrogen receptor-related receptors in human prostatic cells and tissues. *J Clin Endocrinol Metabol.* (2005) 90:1830–44. doi: 10.1210/jc.2004-1421
86. Bonnelye E, Aubin JE. An energetic orphan in an endocrine tissue: a revised perspective of the function of estrogen receptor-related receptor alpha in bone and cartilage. *J Bone Miner Res.* (2013) 28:225–33. doi: 10.1002/jbmr.1836
87. Li X, Wu JB, Li Q, Shigemura K, Chung LW, Huang,WC. SREBP-2 promotes stem cell-like properties and metastasis by transcriptional activation of c-Myc in prostate cancer. *Oncotarget* (2016) 7:12869–84. doi: 10.18632/oncotarget.7331
88. Warner M, Gustafsson JA. On estrogen, cholesterol metabolism, and breast cancer. *N Engl J Med.* (2014) 370:572–3. doi: 10.1056/NEJMcibr1315176
89. Wu Q, Ishikawa T, Sirianni R, Tang H, McDonald JG, Yuhanna IS, et al. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Rep.* (2013) 5:637–45. doi: 10.1016/j.celrep.2013.10.006
90. Glover AR, Ip JC, Zhao JT, Soon PS, Robinson BG, Sidhu SB. Current management options for recurrent adrenocortical carcinoma. *OncoTarget Ther.* (2013) 6:635–43. doi: 10.2147/OTT.S34956
91. Sirianni R, Zolea F, Chimento A, Ruggiero C, Cerquetti L, Fallo F, et al. Targeting estrogen receptor-alpha reduces adrenocortical cancer (ACC) cell growth *in vitro* and *in vivo*: potential therapeutic role of selective estrogen receptor modulators (SERMs) for ACC treatment. *J Clin Endocrinol Metabol.* (2012) 97:E2238–50. doi: 10.1210/jc.2012-2374
92. Casaburi I, Avena P, De Luca A, Chimento A, Sirianni R, Malivindi R, et al. Estrogen related receptor alpha (ERRalpha) a promising target for the therapy of adrenocortical carcinoma (ACC). *Oncotarget* (2015) 6:25135–48. doi: 10.18632/oncotarget.4722
93. Felizola SJ, Nakamura Y, Hui XG, Satoh F, Morimoto R, Midorikawa S, et al. Estrogen-related receptor alpha in normal adrenal cortex and adrenocortical tumors: involvement in development and oncogenesis. *Molecul Cell Endocrinol.* (2013) 365:207–11. doi: 10.1016/j.mce.2012.10.020
94. Warburg O. On the origin of cancer cells. *Science* (1956) 123:309–14.
95. Gutierrez-Pajares JL, Ben Hassen C, Chevalier S, Frank PG. SR-BI: linking cholesterol and lipoprotein metabolism with breast and prostate cancer. *Front Pharmacol.* (2016) 7:338. doi: 10.3389/fphar.2016.00338
96. Wang C, Li P, Xuan J, Zhu C, Liu J, Shan L, et al. Cholesterol enhances colorectal cancer progression via ROS elevation and MAPK signaling pathway activation. *Cell Physiol Biochem.* (2017) 42:729–42. doi: 10.1159/000477890
97. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol.* (2017) 14:399–416. doi: 10.1038/nrclinonc.2016.217
98. Illemann M, Laerum OD, Hasselby JP, Thurison T, Hoyer-Hansen G, Nielsen HJ, et al. Urokinase-type plasminogen activator receptor (uPAR) on tumor-associated macrophages is a marker of poor prognosis in colorectal cancer. *Cancer Med.* (2014) 3:855–64. doi: 10.1002/cam4.242
99. Leitinger N, Schulman IG. Phenotypic polarization of macrophages in atherosclerosis. *Arterioscler Thromb Vascul Biol.* (2013) 33:1120–6. doi: 10.1161/ATVBAHA.112.300173
100. Rhee I. Diverse macrophages polarization in tumor microenvironment. *Arch Pharmacal Res.* (2016) 139:1588–96. doi: 10.1007/s12272-016-0820-y

Conflict of Interest Statement: 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 Endocrinology

REVIEW
published: 19 September 2018
doi: 10.3389/fendo.2018.00509

Sterol Metabolism and Transport in Atherosclerosis and Cancer

Yoshio Yamauchi[1,2]* *and Maximillian A. Rogers*[3]

[1] *Nutri-Life Science Laboratory, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan,* [2] *AMED-CREST, Japan Agency for Medical Research and Development, Tokyo, Japan,* [3] *Division of Cardiovascular Medicine, Center for Interdisciplinary Cardiovascular Sciences, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States*

Cholesterol is a vital lipid molecule for mammalian cells, regulating fluidity of biological membranes, and serving as an essential constituent of lipid rafts. Mammalian cells acquire cholesterol from extracellular lipoproteins and from *de novo* synthesis. Cholesterol biosynthesis generates various precursor sterols. Cholesterol undergoes metabolic conversion into oxygenated sterols (oxysterols), bile acids, and steroid hormones. Cholesterol intermediates and metabolites have diverse and important cellular functions. A network of molecular machineries including transcription factors, protein modifiers, sterol transporters/carriers, and sterol sensors regulate sterol homeostasis in mammalian cells and tissues. Dysfunction in metabolism and transport of cholesterol, sterol intermediates, and oxysterols occurs in various pathophysiological settings such as atherosclerosis, cancers, and neurodegenerative diseases. Here we review the cholesterol, intermediate sterol, and oxysterol regulatory mechanisms and intracellular transport machineries, and discuss the roles of sterols and sterol metabolism in human diseases.

Keywords: ABC transporters, cholesterol, lanosterol, oxysterols, cholesterol efflux, intracellular cholesterol transport, atherosclerosis, cancer

OPEN ACCESS

Edited by:
Michihisa Umetani,
University of Houston, United States

Reviewed by:
Alan Remaley,
National Heart, Lung, and Blood Institute (NHLBI), United States
Verena M. Dirsch,
Universität Wien, Austria
Verena Hiebl,
Universität Wien, Austria,
in collaboration with reviewer VD

***Correspondence:**
Yoshio Yamauchi
a-yoshio@mail.ecc.u-tokyo.ac.jp

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *23 May 2018*
Accepted: *14 August 2018*
Published: *19 September 2018*

Citation:
Yamauchi Y and Rogers MA (2018) Sterol Metabolism and Transport in Atherosclerosis and Cancer. Front. Endocrinol. 9:509. doi: 10.3389/fendo.2018.00509

INTRODUCTION

Sterol biosynthesis is thought to have evolved 2.31 billion years ago (1). In eukaryotes, various complex sterols exist among plants, yeast, and mammals, and a few bacteria are also capable of synthesizing simpler sterols. Cholesterol is a vital lipid molecule for all mammals, and plays diverse and important roles in a number of biological processes, physiology, and disease (2). Mammalian cells can acquire cholesterol from two sources; uptake from extracellular milieu (exogenous source) and *de novo* synthesis (endogenous source). In addition to cholesterol, cells produce a variety of sterols. In the process of cholesterol synthesis, a series of intermediate sterols are generated as cholesterol precursors. Cholesterol is metabolized to cholesteryl ester (CE), oxysterols, bile acids, and steroid hormones. All these non-cholesterol sterols have important physiological functions in cells and in tissues.

Cholesterol is an important constituent of cellular membranes, regulating membrane fluidity and functionality. Cellular cholesterol distribution is highly heterogeneous among organelles [reviewed in (3–5)]. The plasma membrane (PM) contains 60–90% of total cellular cholesterol, which accounts for 25–40 mol% of PM lipids. The endocytic compartments and *trans*-Golgi network (TGN) are also cholesterol-rich organelles; whereas, the endoplasmic reticulum (ER) and the mitochondria contain only ∼1% of total cellular cholesterol, which accounts for ∼5 mol%

of ER lipids. Cholesterol is an essential constituent of membrane domains known as lipid rafts, which are small domains enriched in cholesterol and sphingolipids [both sphingomyelin (SM) and glycosphingolipids] [reviewed in (6)]. Lipid rafts are involved in key cellular functions like endocytosis, cellular signaling, and cell motility (6). In cells, cholesterol and other sterol molecules move dynamically among organelles to maintain proper distribution.

Aberrant accumulation of unesterified cholesterol in membranes is toxic to cells; therefore, various intrinsic and elaborate systems cooperatively regulate cellular sterol homeostasis. Cellular cholesterol content is tightly controlled by regulating *de novo* synthesis, extracellular uptake, export to extracellular milieu, and metabolic conversion (7, 8). Impairments in sterol homeostasis can cause various congenital and acquired diseases in humans, and pathophysiological conditions can also affect sterol homeostasis (2).

In this review, we provide an up-to-date assessment of the cell-intrinsic regulatory mechanisms for biosynthesis, intracellular transport, and efflux of cholesterol, intermediate sterols, and oxysterols. Additionally, we describe the roles of these sterol molecules in human diseases. This review contains four broad topics; (1) sterol biosynthesis and regulation, (2) intracellular sterol transport, (3) cellular sterol export, and (4) the roles of sterols in human diseases. Finally, we highlight several areas of research where mechanistic clarification is needed for sterol-related disease therapeutic development.

ROLES OF STEROLS

Cholesterol, intermediate sterols, and oxysterols have various important functions; **Table 1** summarizes some of the functions further described in this review.

Role of Cholesterol

Cholesterol is a membrane lipid that is indispensable for integrity of biological membranes. It is vital for forming lipid rafts, membrane nano-domains enriched in cholesterol, and sphingolipids, which play a variety of important roles in mammalian cells (6). Cholesterol can regulate functions of biological membranes including endocytosis, membrane trafficking, and signaling. Cholesterol is the precursor of steroid hormones, bile acids, and oxysterols. In addition, cholesterol modifies select proteins: it is covalently attached to Hedgehog proteins (15) and to smoothened (16), both of which work in concert in Hedgehog singling, a signaling pathway playing a critical role in embryonic development and tumorigenesis. As described in more detail below, cholesterol partly regulates cholesterol biosynthesis.

TABLE 1 | Roles of intermediate sterols, cholesterol, and oxysterols.

Sterols	Biological activities	References
Lanosterol	Inhibition of lens protein aggregation	(9)
Dihydrolanosterol	Stimulation of HMGCR ubiquitination	(10, 11)
FF-MAS	Meiosis activation	(12)
T-MAS	Meiosis activation	(12)
Desmosterol	Binding and regulation of SCAP, LXR ligand	(13, 14)
Cholesterol	Lipid raft formation	(6)
	Binding and regulation of SCAP	(13)
	Modification of Hedgehogs and Smoothend	(15, 16)
7α-OHC	Major bile acid precursor	(17)
24(S)-OHC	INSIG ligand, LXR ligand	(18, 19)
25-OHC	INSIG ligand, LXR ligand, anti-viral effect	(18–20)
27-OHC	INSIG ligand, LXR ligand, SERM	(18, 19, 21)
7α,25-di-OHC	EBI2 ligand	(22, 23)

Role of Intermediate Sterols

Sterol intermediates are not just precursors of cholesterol but can also act as biologically active agents. Lanosterol, the first biosynthesized sterol in the cholesterol biosynthetic pathway, can prevent lens protein aggregation and cataracts (9). Lanosterol treatment partially corrects cataracts in animal models. Zhao et al. (9) demonstrated that missense mutations in the lanosterol synthase *LSS* gene cause congenital cataracts. Dihydrolanosterol promotes ubiquitination and degradation of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase (HMGCR), inactivating cholesterol biosynthesis rapidly (10, 11). 4,4-dimethylcholesta-8,14,24-trien-3β-ol (follicular fluid meiosis-activating sterol, FF-MAS) and 4,4-dimethylcholesta-8,24-dien-3β-ol (testis-MAS, T-MAS) are implicated as meiosis-activating substances in oocyte maturation (12). In addition to cholesterol as described in the following section, desmosterol binds to sterol regulatory element binding protein (SREBP) cleavage activating protein (SCAP), and blocks SREBP activation to regulate cholesterol homeostasis (13). Desmosterol is also known to act as an endogenous ligand for the nuclear receptor liver X receptor (LXR) (14). Defects in enzymes involved in conversion of lanosterol to cholesterol cause severe malformation observed in Smith-Lemli-Optiz syndrome, desmosterolosis, X-linked dominant chondrodysplasia punctate type 2 (CDPX2), CHILD syndrome, Greenberg dysplasia, and Antley-Bixler syndrome (24, 25). In these patients, intermediate sterols accumulate in tissues and plasma.

Role of Oxysterols

Oxysterols are one of the most potent negative regulators of cholesterol biosynthesis. Side-chain oxysterols including 25-hydroxycholesterol (25-OHC), 27-OHC, and 24(*S*)-OHC bind INSIG proteins (18), which are ER-resident proteins involved in negative feedback regulation of cholesterol homeostasis. Recent work established virus infection activates conversion of cholesterol to 25-OHC, which inhibits the SREBP pathway and down-regulates cholesterol biosynthesis (20). 25-OHC formation-dependent suppression of cholesterol synthesis plays an important role in viral infection and replication inhibition. In addition, oxysterols act as LXR ligands (19) that induce expression of genes involved in cholesterol efflux, including ATP-binding cassette (ABC) transporters ABCA1 and ABCG1, and apolipoprotein E (apoE) [reviewed in (26)]. Furthermore, recent studies demonstrated that 27-OHC can bind to estrogen receptors and act as an endogenous selective estrogen receptor modulator (SERM) (21). Side-chain oxysterols, but not sterol

ring modified oxysterols, show membrane-disordering effects that result in alternations in membrane functions (27).

STEROL SYNTHESIS AND ITS REGULATION

Biosynthesis of Intermediate Sterols and Cholesterol

In mammals, virtually all cells are capable of synthesizing cholesterol *de novo*. In humans, the liver is the most active organ in cholesterol synthesis, synthesizing as much as 1 g of cholesterol per day. Biosynthesis of cholesterol from the simple, two-carbon acetyl-CoA is a complex, multi-step process involving over 30 enzymes, and consumes 18 ATP a cholesterol molecule. Cholesterol biosynthesis begins with condensation of acetyl-CoA and acetoacetyl-CoA, which results in formation of HMG-CoA (28). A key rate-limiting enzyme in cholesterol biosynthesis is the ER membrane-bound enzyme HMGCR, which catalyzes reduction of HMG-CoA to mevalonate. This reaction uses NADPH as the reducing agent. Mevalonate is then phosphorylated by mevalonate kinase and phosphomevalonate kinase to yield 5-pyrophosphomevalonate. Subsequent isopentenyl pyrophosphate (IPP) production, a five-carbon (C5) isoprene unit, serves as precursor for all isoprenoids. IPP undergoes isomerization to 3,3-dimethylallyl pyrophosphate (DPP). DPP then condenses with IPP, yielding C10 geranyl pyrophosphate (GPP). Condensation of GPP and IPP produces C15 farnesyl pyrophosphate (FPP). Two molecules of FPP are then condensed and reduced to yield C30 squalene, the final non-sterol precursor of cholesterol. Squalene synthase catalyzes this reacting using NADPH as the reducing agent. Oxidation and cyclization of squalene yield C30 lanosterol, the first sterol in the biosynthetic pathway, via 2,3-epoxysqualene; squalene monooxygenase (also known as squalene epoxidase) that converts squalene to 2,3-epoxysqualene using NADPH and molecular oxygen, and lanosterol synthase then catalyzes the cyclization of 2,3-epoxysqualene to lanosterol.

Lanosterol undergoes extensive modifications en route to the final product, cholesterol (**Figure 1**). Conversion of lanosterol to C27 cholesterol involves at least 18 different enzymatic reactions including removal of the three methyl groups, reduction of the side chain, and rearrangement of the double bonds within sterol rings by consuming NADPH and O_2. All of the enzymes responsible for converting squalene to cholesterol localize in the ER membrane. Conversion of lanosterol to cholesterol proceeds through either one of two pathways known as Bloch pathway and Kandutsch-Russell pathway (**Figure 1**). Desmosterol and 7-dehydrocholesterol are the final precursors in the Bloch pathway and the Kandutsch-Russell pathway, respectively. The sterol Δ24-reductase, DHCR24, which catalyzes reduction of the side chain at position 24 using NADPH as the reducing agent, is a key branching enzyme for the two pathways. 14α-methyl group of lanosterol and dihydrolanosterol is first removed by lanosterol 14α-demethylase (CYP51A1), yielding C29 sterols with two methyl groups at C4 position. The two methyl groups are then sequentially trimmed by a series of complex reactions involving three enzymes, methylsterol monooxygenase 1 (encoded by *MSMO1*/*SC4MOL*), sterol-4α-carboxylate 3-dehydrogenase (NSDHL), and 3-keto-steroid reductase (HSD17B7), producing C27 zymosterol or zymostenol. Conversion of zymosterol or zymostenol to cholesterol involves rearrangements of the double bonds within the sterol rings. A detailed review describing cholesterol biosynthetic reactions is available (30).

Recent work by Mitsche et al. shows that utilization of the Bloch pathway or Kandutsch-Russell pathway is cell and tissue type-dependent (29). No tissues use canonical Kandutsch-Russell pathway, but instead may use a proposed "modified" Kandutsch-Russell pathway, in which DHCR24 mediates the entry of zymosterol into Kandutsch-Russell pathway (**Figure 1**). In mice, the Bloch pathway mediates 90% or more cholesterol biosynthesis in the testis, spleen, and adrenal, and the modified Kandutsch-Russell pathway is used for more than 70% of cholesterol biosynthesis in the brain, skin, and preputial (29).

Regulation of Sterol Synthesis

Mammalian cells acquire cholesterol from two sources: endogenous synthesis and uptake from exogenous sources. These two processes are tightly controlled for cellular cholesterol content maintenance by multiple modes of regulation at transcriptional and post-transcriptional levels.

SREBP transcription factors serve as master regulators of cholesterol synthesis [reviewed in (31)]. There are two SREBP genes (*SREBF1* and *SREBF2*) and three SREBP proteins, SREBP-1a, SREBP-1c, and SREBP-2. *SREBF1* encodes SREBP-1a and SREBP-1c through alternative splicing, and *SREBF2* encodes SREBP-2 protein [reviewed in (32)]. SREBP-2 regulates expression of virtually all genes involved in cholesterol synthesis. SREBP-1a regulates both cholesterol and fatty acid synthesis, whereas SREBP-1c participates in the regulation of fatty acid synthesis [reviewed in (32)]. SREBPs are unique, membrane-bound transcription factors. When cellular cholesterol is at sufficient levels, SREBPs locate at the ER as inactive forms by forming a complex with SCAP and INSIG. INSIG1 and INSIG2 are ER resident proteins, and act as retention factors of the SCAP-SREBP complex in the ER. INSIG proteins are stabilized by side-chain oxysterols including 25-OHC and 27-OHC through direct interaction with the oxysterols (18). Upon cellular cholesterol reduction, the SCAP-SREBP complex is transported to the Golgi via the COP-II pathway, and SREBPs are sequentially cleaved by two proteases, Site-1 protease (S1P) and Site-2 protease (S2P), to liberate the transcriptionally active domain from membranes. SCAP possesses a sterol-sensing domain (SSD) and can bind cholesterol. Cholesterol binding to SCAP leads to a conformational change, which prevents the SCAP-SREBP complex from its incorporation into the COP-II vesicles (13, 33). Moreover, small changes in ER cholesterol levels regulate this translocation: SCAP-SREBP-2 complex leaves the ER for the Golgi when the ER cholesterol concentration is below 5% of total ER lipids (34). In addition, polyunsaturated fatty acids regulate proteolytic processing of SREBP-1, but not SREBP-2 (35).

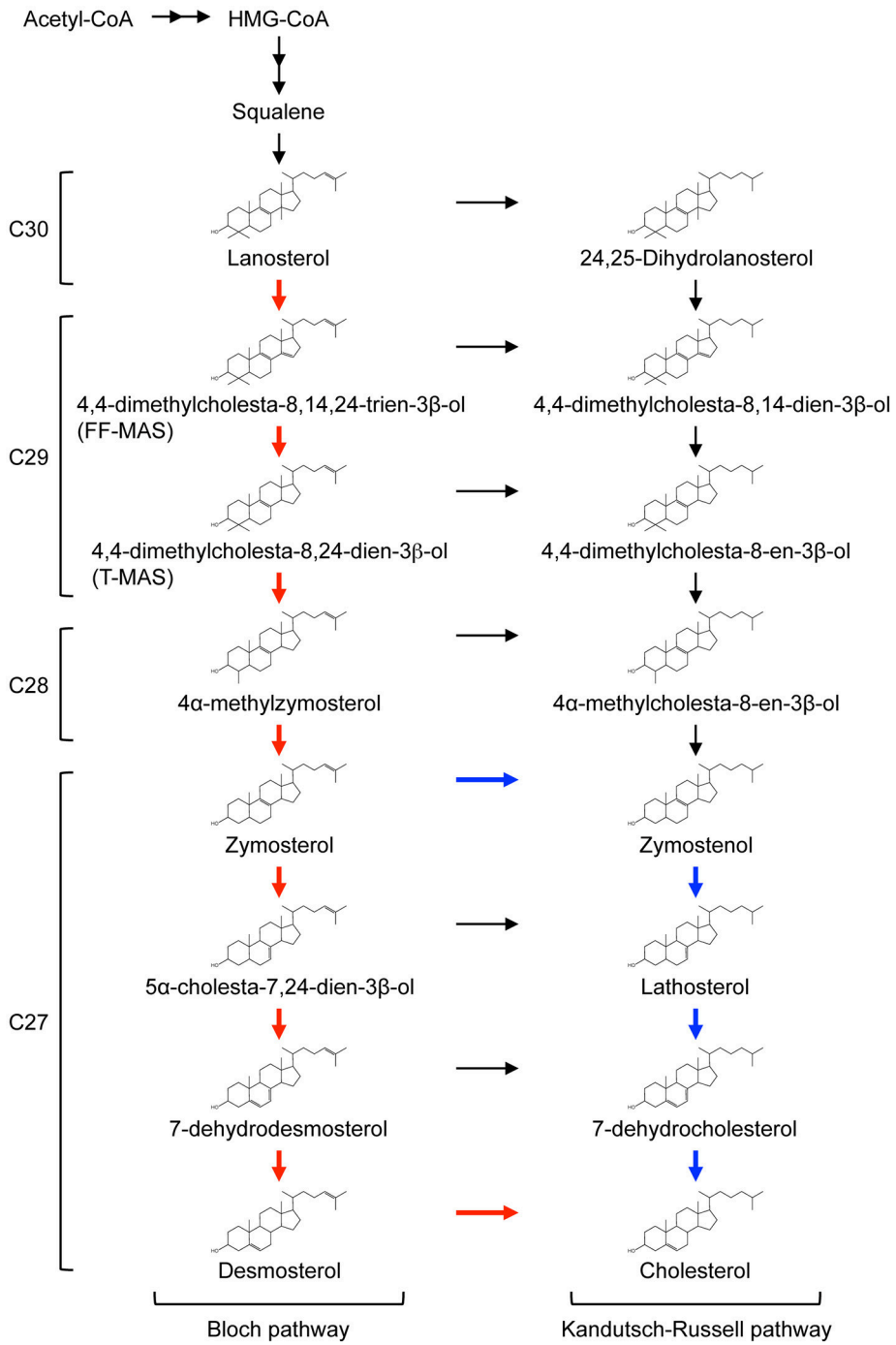

FIGURE 1 | Cholesterol biosynthetic pathway.The conversion of lanosterol to cholesterol proceeds through either Bloch pathway (red line) or Kandutsch-Russell pathway. Recently, modified Kandutsch-Russell pathway (blue line) has been proposed (29). See text for more details. The number of carbons in each sterol is presented on the left of the pathway.

In addition to transcriptional regulation, several important enzymes are post-transcriptionally regulated to control cholesterol synthesis. The best-characterized enzyme is HMGCR, an ER-bound protein with eight transmembrane domains and a catalytic domain that projects into the cytosol. As the rate-limiting cholesterol synthesis enzyme, HMGCR has multiple modes of regulation. In addition to SREBP-dependent transcriptional control, HMGCR is regulated post-transcriptionally. HMGCR is a short-lived protein with a half-life of 1 h when cellular cholesterol is at sufficient levels (36).

HMGCR is degraded by the ubiquitin-proteasome pathway; ubiquitination of HMGCR requires INSIG-1 or-2 (37). INSIG proteins bind not only SCAP but also HMGCR via their SSD (38). INSIG binding to HMGCR facilitates the ubiquitination and degradation. HMGCR ubiquitination is promoted by the intermediate sterol dihydrolanosterol, and the side-chain oxysterols 25-OHC and 27-OHC, but not by cholesterol itself (10, 11, 39). Geranylgeraniol further accelerates degradation of HMGCR in the presence of sterols, but has little effect on the degradation by itself, showing a synergistical effect with sterols (39). Side-chain oxysterols stabilize INSIG proteins that promote HMGCR degradation. Three membrane-bound E3 ligases for HMGCR, gp78 (40), TRC8 (41), and RNF145 (42) have been identified. Importantly, all of these E3 ligases can interact with INSIG-1 and/or INSIG-2 (gp78 binds only INSIG-1), which stimulate HMGCR ubiquitination and degradation. However, involvement of gp78 and TRC8 in sterol-dependent HMGCR degradation may need further clarification since conflicting results have been reported (43). Additionally, another E3 ligase MARCH6 may also be involved in the regulation of HMGCR protein abundance (44).

In addition, phosphorylation regulates enzymatic activity of HMGCR. Serine-872 in human HMGCR (or serine-871 in mouse and hamster HMGCRs) is phosphorylated by AMP kinase (AMPK), a protein serine/threonine kinase regulated by cellular AMP levels (45). This phosphorylation inactivates the enzyme activity. Phosphorylation-dependent inactivation and sterol-dependent degradation are independently regulated to meet cellular demands (46).

In addition to HMGCR, recent studies identified squalene monooxygenase (47) and 7-dehydrocholesterol reductase (48) as enzymes highly regulated at post-transcriptional levels. Both enzymes are rapidly degraded by the ubiquitin-proteasome pathway in response to cholesterol loading (47, 48). The E3 ligase MARCH6 mediates the degradation of squalene monooxygenase (44).

Esterification and Hydroxylation of Cholesterol

Because cholesterol cannot simply be degraded, excess cholesterol undergoes enzymatic esterification and hydroxylation within cells (**Figure 2**). To prevent toxic accumulation of free cholesterol, excess cholesterol is esterified, and stored in lipid droplets [reviewed in (49)]. This esterification is catalyzed by the ER-resident enzyme acyl-CoA:cholesterol acyltransferase 1 (ACAT1, also known as sterol O-acyltransferase 1, SOAT1), which transfers various long chain fatty acids such as oleic acid to the 3β-position of cholesterol (49). ACAT1 is ubiquitously expressed, while ACAT2, another form of ACAT, is mainly expressed in the intestine (enterocytes) and the liver (hepatocytes). In plasma, lecithin:cholesterol acyltransferase (LCAT) esterifies cholesterol in high-density lipoprotein (HDL). Cholesterol esterification is reversible; cholesteryl ester hydrolase located in the ER converts CE to cholesterol (8, 50), while acid lipase mediates hydrolysis of lysosomal CE derived from low-density lipoprotein (LDL). In addition to cholesterol, oxysterols (such as 24(*S*)-OHC, 25-OHC, and 27-OHC), pregnenolone, and phytosterols are substrates of ACAT1 [reviewed in (51)]. Intermediate sterols containing gem-dimethyl moieties at C4 position cannot act as ACAT substrates because the dimethyl moieties sterically hinder the 3β-OH group (52).

Enzymatic and non-enzymatic processes oxidize cholesterol [reviewed in (53)]. It is converted to oxysterols by the addition of one or more hydroxyl groups, keto groups, or epoxy groups. Side-chain oxysterols act as a potent negative regulator of cholesterol biosynthesis. Recent studies showed that cholesterol can also be glucosylated by β-glucocerebrosidase (GBA1 and GBA2), forming β-cholesteryl glucoside (**Figure 2**) in response to heat shock [reviewed in (54)]. In this review, we focus on the enzymatic production of the cholesterol metabolites, oxysterols, and discuss the roles of several major oxysterols and related enzymes. In mammals, many enzymes mediate cholesterol hydroxylation [reviewed in (53)]. With one exception, cholesterol 25-hydroxylase CH25H, cholesterol hydroxylases belong to the cytochrome P450 family. CH25H is a member of proteins that use diiron-oxygen as a cofactor (55). Hydroxylation of cholesterol occurs on its side-chain and/or on its steroid B ring. Here we review several major oxysterols that play important roles in human health and diseases.

25-OHC is biosynthesized from cholesterol via CH25H, a membrane-bound enzyme localized to the ER orienting the catalytic domain to the ER lumen. 25-OHC is also enzymatically synthesized by cholesterol 27-hydroxylase (CYP27A1) and cytochrome P450 3A4 (CYP3A4) and can be generated by free radical oxidation (53). 25-OHC can be metabolized by CYP7B1 (25-hydroxycholesterol 7α-hydroxylase), an enzyme that adds an OH-group to the steroid ring (at C7 position) in the ER, generating 7α, 25-dihydroxycholesterol (7α,25-di-OHC), a bile acid precursor (17) as well as a ligand for the G-protein-coupled receptor EBI2 (22, 23).

27-OHC is generated via cholesterol 27-hydroxylase (CYP27A1), which resides in the mitochondria. CYP27A1 can also convert cholesterol to 25-OHC. PM cholesterol is transported to the mitochondria where it serves as a major substrate for this hydroxylase (56). 27-OHC is the most abundant oxysterol in the plasma. CYP27A1 is expressed in many tissues including the liver, lung, and small intestine. Like 25-OHC, 27-OHC is further hydroxylated by CYP7B1 at the C7 position in the ER, forming 7α, 27-di-OHC that serves as a bile acid precursor (17).

Cholesterol 24-hydroxylase (CYP46A1) catalyzes biosynthesis of 24(*S*)-OHC [reviewed in (57)]. This enzyme is highly expressed in the brain, particularly in neurons. In the human brain, 24(*S*)-OHC is present at high concentration (up to 15 ng/mg wet weight) (58). Excess cholesterol in the brain is converted to 24(*S*)-OHC, which is then released into the plasma across the blood-brain barrier. Thus the conversion of cholesterol to 24(*S*)-OHC is an important step for eliminating excess cholesterol from the brain [reviewed in (57, 59)].

Hydroxylation of the cholesterol C7 position is catalyzed by CYP7A1 (cholesterol 7α-monooxygenase or cholesterol 7α-hydroxylase), which is almost exclusively expressed in the liver. CYP7A1 produces 7α-OHC, the major precursor of bile acids,

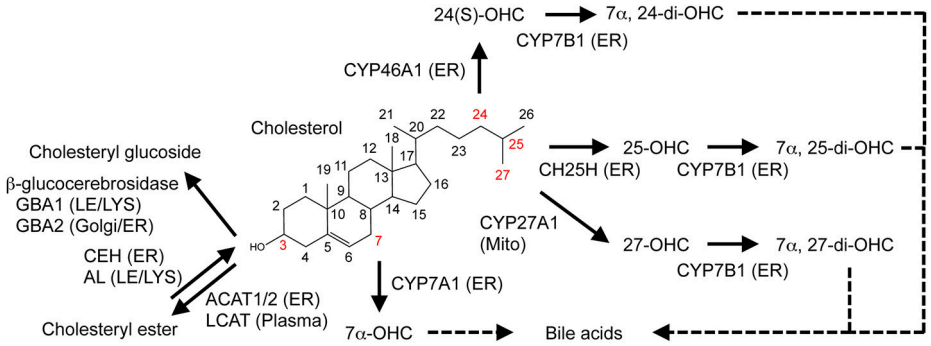

FIGURE 2 | Metabolic conversion of cholesterol. Enzymatic conversion of cholesterol into oxysterols, cholesteryl ester, and cholesteryl glucoside is shown. Enzymes responsible for the conversion and their localization are also indicated. See text for more details. ER, endoplasmic reticulum; LE, late endosome; LYS, lysosome; Mito, mitochondria.

and is a rate-limiting enzyme for synthesizing bile acids [reviewed in (17)].

Regulation of Oxysterol Synthesis

Although much less is known about regulation of oxysterol synthesis, a growing body of evidence has shown that enzymes involved in hydroxylation of cholesterol is also subjected to tight regulation. Expression of CH25H is highly transcriptionally regulated. In macrophages and hepatocytes, virus infection leads to upregulation of CH25H mRNA levels and to marked increases in 25-OHC (60–63). Furthermore, lipopolysaccharide (component of gram-negative bacteria) induces its expression through toll-like receptor 4 in macrophages (64, 65). Signal transducer and activator of transcription factor 1 (STAT1), a transcription factor activated by type I interferon signaling, induces Ch25h mRNA expression (61). The resultant product 25-OHC has an anti-viral role by suppressing virus infection and replication.

CYP27A1 expression is not associated with cellular cholesterol levels in macrophages, but its expression is transcriptionally regulated by several nuclear hormone receptors. In human macrophages, retinoid X receptor (RXR) and peroxisome proliferator-activated receptor-γ (PPARγ) cooperatively induce expression of CYP27A1 mRNA (66). In HepG2 or Huh7 human hepatoblastoma cells, CYP27A1 expression is increased by glucocorticoids, growth hormone, and insulin-like growth factor 1, and decreased by cholic acid and thyroid hormones (67, 68). In human intestinal Caco2 cells (a colon adenocarcinoma cell line) but not in human hepatocytes, pregnane X receptor (PXR) induces CYP27A1 expression (69). Collectively, CYP27A1 expression is differentially regulated in a cell type-dependent manner.

Expression of CYP7A1, the rate-limiting enzyme of bile acid synthesis, is positively regulated by cholesterol and negatively by bile acids in the liver. Several nuclear receptors including LXRα, farnesoid X receptor (FXR), small heterodimer protein (SHP-1), and liver receptor homolog-1 (LRH-1) cooperatively control CYP7A1 transcription (70, 71). Upon an increase in intake of dietary cholesterol, LXRα activates CYP7A1 gene expression and facilitates bile acid synthesis for excretion in rodents (72). LXR does not activate CYP7A1 expression in humans due to lack of an LXR response element in the human *CYP7A1* promoter (73, 74). For transcriptional repression, FXR binds bile acids, inducing the expression of SHP-1, a nuclear receptor with promoter-specific repressor activity that suppresses CYP7A1 expression by inhibiting LRH-1, a nuclear receptor that positively regulates CYP7A1 expression in humans and rodents (70, 71).

INTRACELLULAR STEROL TRANSPORT AND CHOLESTEROL HOMEOSTASIS

Cholesterol moves dynamically within a cell (**Figure 3**), with this movement being essential to control cellular cholesterol homeostasis and to regulate heterogeneous cholesterol distribution among organelles (7, 8). The PM contains most of the cellular cholesterol. Cholesterol is also abundant in the endocytic compartments and the *trans*-Golgi network (TGN). In contrast, the ER and the mitochondria contain only 1% (or less) of total cellular cholesterol. How mammalian cells maintain uneven cholesterol distribution among organelles remains largely unknown. Differences in lipid compositions of each organelle may affect cholesterol-lipid interaction in membranes, allowing for organelle-specific cholesterol contents (4). As sterol is a highly hydrophobic substance, its transport between organelles, probably even within an organelle, requires transport carriers. Both vesicular (membrane-based) and non-vesicular (protein-based) transport are involved in intracellular transport of sterols.

Sterol Transport Proteins With Sterol Binding Ability

Several proteins directly interact with sterols and transport them within cells. In patients with Niemann-Pick type C (NPC) disease, a lysosomal storage disorder with fatal neurodegeneration, either NPC1 or NPC2 protein is defective (75). Defects in NPC1 or NPC2 cause the aberrant accumulation of unesterified cholesterol and sphingolipids in the late endosome (LE) and the lysosome (LYS) (75). NPC1 and NPC2 both locate to the LE/LYS, but their localization within the organelles is

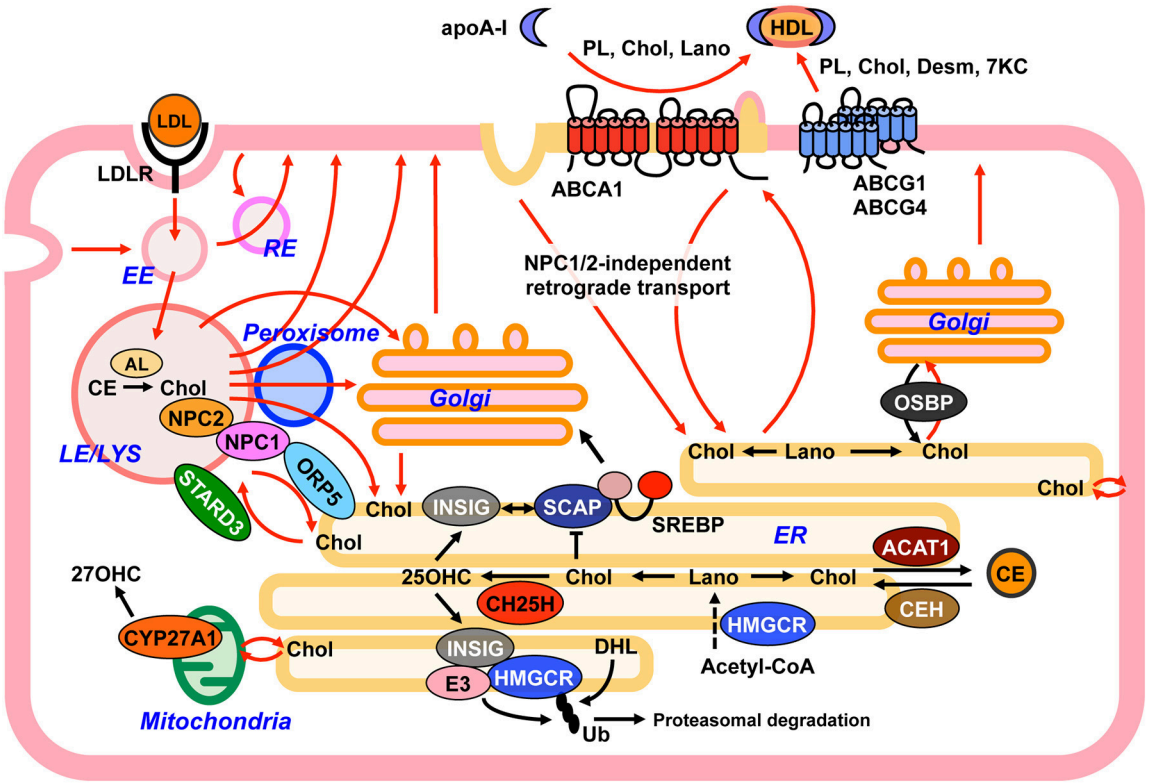

FIGURE 3 | Model depicting intracellular sterol movement. Within cells, sterols dynamically move among organelles (red lines) to maintain cholesterol homeostasis. See text for more details. 7KC, 7-ketocholesterol; 25OHC, 25-hydroxycholeterol; 27OHC, 27-hydroxycholeterol; AL, acid lipase; CE, cholesteryl ester; Chol, cholesterol; Desm, desmosterol; DHL, dihydrolanosterol; EE, early endosome; ER, endoplasmic reticulum; Lano, lanosterol; LE, late endosome; LYS, lysosome; PL, phospholipid; RE, recycling endosome; Ub, ubiquitin.

different: NPC1 has multiple transmembrane domains and localizes to LE/LYS membranes, while NPC2 is a soluble protein and localizes to the lumen. Both NPC1 and NPC2 can directly bind cholesterol, and cooperatively contribute to exporting cholesterol from the LE/LYS as described below.

Oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) constitute a family of proteins that have the ability to bind sterol and other lipids [reviewed in (76, 77)]. In humans, 12 members, OSBP and ORP1-11, belong to this family, producing 16 different proteins through alternative splicing. In yeast, there are seven OSBP/ORP proteins, Osh1-7. The OSBP-related domain (ORD that consists of a hydrophobic pocket) is conserved in all OSBP/ORP proteins. Only two members, ORP5 and ORP8 contain transmembrane domain at their C-terminal region. Other functionally important domains found in most, but not all OSBP/ORPs include a pleckstrin homology (PH) domain and a FFAT (diphenyl alanine in an acidic tract) domain. OSBP was originally found as a protein that has high affinity to 25-OHC, in 1980s (78). Recent studies demonstrated that OSBP transports cholesterol (79, 80). A growing body of evidence [reviewed in (77)] has revealed that OSBP/ORPs transport not only sterols but also other lipids including phosphatidylserine and phosptidylinositol 4-phosphate at membrane contact sites (MCSs), which are proximal regions of two organelle membranes, through non-vesicular process.

Other sterol transport proteins belong to steroidogenic acute regulatory (StAR) protein-related lipid transfer (START) domain family, which consists of 15 members, STARD1-15 [reviewed in (81)]. The START domain forms a hydrophobic cavity that can accommodate one lipid molecule. STARD proteins also mediate non-vesicular lipid transport, and some of these proteins can bind sterols.

Anterograde Transport of Cholesterol and Intermediate Sterols

The enzymes responsible for converting squalene to cholesterol are all located at the ER, indicating that intermediate sterols and cholesterol are synthesized in this organelle. Upon synthesis, cholesterol leaves the ER, and rapidly reaches the PM with a half-time of 10–20 min (82, 83). In addition to cholesterol, cells contain small but significant amounts of intermediate sterols as cholesterol precursors. A significant portion of intermediate sterols including lanosterol (C30 sterol), dimethylsterols (C29 sterols), monomethysterols (C28 sterols), zymosterol (C27 sterol), and desmosterol (C27 sterol) is transported to the PM immediately after synthesis but prior to conversion to cholesterol (84–87). The ER-to-PM anterograde sterol transport is not

impaired in NPC cells (87), indicating that an NPC1/NPC2-independent pathway transports sterols from the ER to the PM. Involvement of OSBP in the anterograde sterol transport has been recently suggested (79). Upon binding to 25-OHC, OSBP translocates to the Golgi apparatus from cytoplasm (88). Recent findings show that OSBP counter-exchanges cholesterol in the ER and phosphatidylinositol 4-phosphate (PI4P) in the Golgi (80). On the other hand, an earlier study showed that there are Golgi-dependent and Golgi-independent routes that transport newly synthesized cholesterol from the ER to the PM, and that the Golgi-independent route plays a major role in this anterograde transport (83). How much newly synthesized cholesterol relies on OSBP for reaching the PM is unknown. Additionally unknown and of interest is whether OSBP can transfer intermediate sterols from the ER to the Golgi. In addition to OSBP, several other proteins including ORP2 (89) and sterol carrier protein-2 (90) are suggested to participate in the anterograde transport; however, this involvement needs further investigation.

Active Cholesterol Hypothesis, Sterol Sensing, and Sterol Homeostasis

At the PM, cholesterol forms stoichiometric complexes with phospholipids, particularly with SM and glycerophospholipids containing long saturated acyl chains (91). Cholesterol pools that exceed the binding capacity of phospholipids could become more chemically active and more mobile. This mobile cholesterol is recognized as "active" cholesterol, and is expected to act as regulatory cholesterol for cellular cholesterol homeostasis (92). The ER contains a series of sterol sensing proteins. For storage, excess cellular cholesterol is esterified by the ER resident enzyme ACAT1 (49). ACAT1 enzymatic activity is stimulated by cholesterol and oxysterols. As described above, SREBP activation depends on the transport of SCAP-SREBP complex form the ER to the Golgi via COPII pathway. This transport is sensitive to ER cholesterol levels (34). A novel ER sterol sensing protein was recently identified; nuclear factor erythroid 2 related factor-1 (Nrf1, also known as Nfe2L1) is an ER-membrane bound transcription factor, which is cleaved near its N-terminus to be released from the ER. Through its ability to bind cholesterol, cholesterol blocks the cleavage and translocation of Nrf1 to the nucleus, and enhances LXR target gene expression including *Abca1* and *Abcg1* as a means to respond to excess cellular cholesterol (93). Thus, delivering active cholesterol to the ER is considered an essential step to sense and respond to excess cellular cholesterol (94, 95). Retrograde sterol transport from the PM to the ER involves both vesicular and non-vesicular transport. Here we describe the current understanding of these two transport processes and the proteins involved.

Retrograde Sterol Transport

Cells can acquire exogenous cholesterol from lipoproteins. The best-characterized, major lipoprotein uptake process is LDL receptor (LDLR)-mediated internalization of LDL (**Figure 3**). LDL contains a large amount of cholesterol in an esterified form. LDL is first bound at the cell surface by LDLR, and internalized by endocytosis (96). LDLR-mediated endocytosis is classified as clathrin-mediated endocytosis (CME). CE in LDL is hydrolyzed by acid lipase (**Figure 2**) in endocytic compartments, yielding free cholesterol (97). Endogenously synthesized cholesterol and a portion of intermediate sterols are also internalized from the PM and reach the LE/LYS (87, 98). Egress of free cholesterol from the LE/LYS requires the two cholesterol-binding proteins, NPC1 and NPC2. NPC1 is a large membrane-bound protein with 10 transmembrane domains (TMDs), whereas NPC2 is relatively small, 154-amino acid protein localized to luminal side of the LE/LYS. NPC1 has a cholesterol-binding pocket in its N-terminal luminal side (99). NPC1 also contains a SSD. Although whether the SSD is also able to directly bind cholesterol is not known, mutations within this domain lose cholesterol-transporting and cholesterol-binding activities (100). A potential model by which NPC1 and NPC2 act in concert to export cholesterol from the LE/LYS has been proposed (99, 101). In this "hand-off" model, at luminal side of the LE/LYS, NPC2 binds cholesterol and transfers it to the N-terminal, luminal cholesterol-binding domain of NPC1. Cholesterol is then incorporated into late endosomal/lysosomal membranes, and moves to various organelles including the ER, TGN (102), PM, mitochondria (103), and peroxisome (104) in a manner dependent of NPC1 and NPC2. In response to LDL uptake, delays in SREBP inactivation and in cholesterol re-esterification by ACAT1 both at the ER are observed in NPC cells. In addition, deficiency in either NPC1 or NPC2 impairs the conversion of LDL-derived cholesterol to 25-OHC at the ER and 27-OHC at the mitochondria (103). LDL-derived cholesterol also reaches the PM in a manner dependent on NPC1/NPC2 activity and becomes available for ABCA1-dependent release (105). As such, NPC1/NPC2 proteins play a crucial role in redistribution of internalized cholesterol from the LE/LYS to other organelles.

Both vesicular and non-vesicular transport processes participate in cholesterol transport from the LE/LYS to other organelles. Vesicular cholesterol transport to the ER involves several TGN-specific SNARE proteins including syntaxin 6, syntaxin 16, and VAMP4, indicating that LDL-derived cholesterol is at least partially delivered to the TGN from the LE/LYS before arriving at the ER (102). Transport of LDL-derived cholesterol from the LE/LYS to the PM is mediated by Rab8-dependent vesicular trafficking (106). Non-vesicular cholesterol transport is at least partly mediated by MCS. At the LE/LYS-ER MCS, ORP5 binds to NPC1, and transport cholesterol from the LE/LYS to the ER (107). On the other hand, at the same MCS, STARD3 mediates cholesterol transport from the ER to the LE/LYS (108). Chu et al. found that significant amounts of cholesterol in the LE/LYS are transported to peroxisomes through LE/LYS-peroxisome MCS, which precedes its arrival at the ER and PM (104). The LE/LYS physically interacts with peroxisomes, and the MCS formation is mediated by synaptotagmin VII at the LE/LYS and PI(4,5)P2 at peroxisomes (104). Unknown cytosolic factors are suggested to facilitate cholesterol movement from the LE/LYS to peroxisomes (104).

Retrograde sterol transport from the PM to the ER is an important element of sterol sensing at the ER (95). Aside from NPC1/NPC2-dependent pathway, recent works show that mammalian cells utilize a PM-to-ER sterol trafficking route that does not require NPC proteins (87, 109). The nature

of this NPC1/NPC2-independent sterol transport is not well understood, but it has been suggested that both vesicular and non-vesicular mechanisms participate in it. ER membranes are partly elongated to proximal regions of the PM and form the MCSs where non-vesicular lipid transport can occur. The ORP proteins ORP1S and ORP2 are implicated in the PM-to-ER cholesterol transport through a non-vesicular pathway (110). Other studies show that STARD4 mediates cholesterol transport from the PM to the endocytic recycling compartments and to the ER (111). In addition to a non-vesicular process, involvement of endocytic sterol internalization is also proposed as a mechanism for the delivery of PM sterol to the ER (87, 94). Inhibition of clathrin-independent endocytosis (CIE), but not CME, significantly impairs PM-to-ER transport of cholesterol and intermediate sterols (87, 94). This CIE is suggested to require dynamin, a GTPase that plays a key role in scission of endocytic structure from the PM. Importantly, blocking dynamin activity results in a marked increase in SREBP-2 processing and in a reduction in esterification of PM cholesterol, indicating a decrease in ER cholesterol levels (94).

ABCA1, which plays an essential role in HDL formation (discussed in more detail in the following section), mediates transport of phospholipids and sterols. Although ABCA1 transports lipids to apoA-I for formation of HDL, its activity is independent of availability of lipid acceptors such as apoA-I (112, 113). In absence of a lipid acceptor, ABCA1 deficiency represses retrograde cholesterol transport (94), causes accumulation of cholesterol in the PM (94, 114), and impairs internalization of cholera toxin B subunit, a marker of CIE (94). It is therefore plausible that ABCA1 contributes to the regulation of PM lipid composition and functionality of PM through its lipid transport activity. How and whether lipid compositions of the PM are modulated by ABCA1 and other lipid transporters, and whether this affects PM functionality as well as non-vesicular sterol transport are important issues yet to be determined.

Select tissues including the liver and steroidogenic tissues also acquire cholesterol from plasma HDL for biliary secretion and steroidogenesis, respectively (115). Scavenger receptor class B type I (SR-BI) serves as a HDL receptor, and mediates the selective uptake of HDL-cholesterol (both CE and free cholesterol) (116). In contrast to the endocytic internalization of LDL, SR-BI-mediated uptake does not involve lysosomal degradation of HDL. How HDL-cholesterol is internalized and transported into cell interior through SR-BI remain to be clarified (117).

Intracellular Transport of Oxysterols

Oxysterols are synthesized in the ER and mitochondria depending on hydroxylase localization. Oxysterols play various roles in different organelles. Oxysterols bind LXR, and regulate the transcription of LXR target genes in the nucleus. At the ER, side-chain oxysterols bind INSIGs, leading to their stabilization (18), and also become ACAT1 substrates (51). At the PM, oxysterols can affect membrane organization (27). Oxysterols are also released from cells (118). Furthermore, several proteins with sterol transporting activity, including OSBP (78) and NPC1 (119) have the ability to bind oxysterols. Together these findings strongly suggest that, like cholesterol, oxysterol distribution is tightly regulated. However, little is known about intracellular transport of oxysterols due to their extremely low cellular contents (∼0.1% of cholesterol), in addition to difficulties in their handling.

Recent work developed an intrinsically fluorescent oxysterol, 25-hydroxycholestatrienol (25-HCTL) that mimics 25-OHC (120). 25-HCTL is a hydroxylated derivative of cholestatrienol (CTL), a fluorescent cholesterol analog without fluorophore that may affect the physical properties of cholesterol. Both 25-HCTL and CTL contain two additional double bonds at the steroid B and C rings, thereby exhibiting intrinsic fluorescence with excitation max at 325 nm. When cells are incubated with 25-HCTL in the presence of LDL, 25-HCTL enters cells via LDLR-mediated endocytosis, and is transported to the LE/LYS. Like cholesterol, egress of 25-HCTL from the intracellular compartments requires functional NPC1 protein (120), which is consistent with 25-OHC-binding ability of NPC1 protein (119). Furthermore, 25-HCTL can be transported to the ER, and is also redistributed to the PM and the recycling endosome (120). It remains to be determined how endogenously synthesized oxysterols are transported from the sites where they are synthesized: the ER and mitochondria.

STEROL EXPORT TO EXTRACELLULAR MILIEU

Because mammalian cells cannot break down the sterol backbone, and conversion of cholesterol into bile acids is restricted to only the liver, efflux of excess cholesterol and other sterols from cells is crucial for cellular cholesterol homeostasis. Cholesterol can be removed from cells by two processes; active export and passive diffusion. Passive diffusion largely depends on a cholesterol gradient between the cell surface and cholesterol acceptors such as HDL. Active export is energy-dependent and involves several ABC transporter proteins that use ATP as driving force.

ABC Transporters

The human genome encodes 48 ABC transporters, classified into seven subfamilies, ABCA-G families (121). ABC transporters bind and hydrolyze ATP for energy-dependent transport of chemically diverse substrates across biological membranes. The substrates vary greatly for the various ABC transporters. Studies have revealed that several ABC transporters play a role in exporting cellular sterols including cholesterol, intermediate sterols, oxysterols, and phytosterols (122), as described in further detail in this review.

ABCA1 and HDL Assembly

It was first reported in 1991 by Hara and Yokoyama (123) that interaction of lipid-free apoA-I with macrophages resulted in formation of HDL with cellular phospholipids and cholesterol. It was subsequently demonstrated that fibroblasts isolated from patients with Tangier disease, a severe HDL deficiency, lack the apoA-I-mediated phospholipid and cholesterol release and HDL formation (124). In 1999, *ABCA1* was identified as the gene

mutated in Tangier disease patients (125–127). These reports and numerous other studies established that ABCA1 plays an essential role in the release of cellular phospholipids and cholesterol and in the assembly of nascent HDL [reviewed in (128)]. Further studies with conditional *Abca1* knockout mice revealed that liver ABCA1 and intestinal ABCA1 contribute to produce 70–80 and 30% of total plasma HDL, respectively (129, 130). The physiological acceptor for phospholipids and cholesterol is apoA-I, a major HDL apolipoprotein. HDL is a key lipoprotein that transports excess cholesterol from peripheral tissues to the liver where cholesterol is converted to bile acids for excretion. This cholesterol transport system is often referred to as "reverse cholesterol transport." Because Tangier disease patients largely lack serum HDL, they exhibit accumulation of cholesterol (both unesterfied and esterified cholesterol) in peripheral tissues and have increased risk of cardiovascular disease (131–133).

How does ABCA1 mediate HDL formation? ABCA1 localizes to both the PM and the endocytic compartments. ABCA1 that resides in the PM mediates the assembly of nascent HDL (134), while ABCA1 internalized into the endocytic compartments is subjected to degradation or recycles back to the PM (135). ABCA1 creates membrane deformation sites at the PM, where apoA-I binds (136). This could be related with the finding that overexpression of ABCA1 disrupts lipid rafts (137). In contrast, ABCA1 deficiency increases lipid rafts and cholesterol contents in the PM (94, 114, 138). ApoA-I can directly bind to ABCA1, and this interaction plays a role in ABCA1-dependent lipid release and HDL formation; therefore, lipid compositions of nascent HDL may reflect those of membrane domains where ABCA1 mediates HDL assembly. A study using recombinant ABCA1 reconstituted in liposomes shows that ABCA1 can export phosphatidylcholine (PC), phosphatidylserine (PS), and SM (139). Cholesterol reduces ATPase activity of ABCA1 (112, 139), suggesting that cholesterol may not be a direct substrate of this transporter. These *in vitro* observations are consistent with a cell-based study showing that ABCA1 primarily mediates phospholipid release to apoA-I. Recent work showed that ABCA1 also flops and releases phosphatidylinositol (4,5) bis-phosphate (PIP2) (140). Increases in cell surface PIP2 levels enhances apoA-I binding to cell surface and ABCA1-dependent HDL biogenesis (140). In certain cell models, ABCA1 primarily releases phospholipids and can generate cholesterol-poor nascent HDL particles (141). ABCA1-dependent cholesterol release can thus uncouple phospholipid release and HDL formation. Although the mechanisms by which ABCA1 mediates cholesterol incorporation into nascent HDL remain obscure, cholesterol availability for ABCA1-dependent HDL formation is dependent on cellular cholesterol pool sizes regulated by ACAT1 (142). In cholesterol-loaded macrophages, autophagy-dependent lysosomal degradation of lipid droplets, called lipophagy, hydrolyzes lipid droplet-associated CE by lysosomal acid lipase, generating free cholesterol available for ABCA1-dependent HDL assembly (143).

Our recent work determined sterol specificity of ABCA1-mediated sterol release (144). We showed that in addition to cholesterol, ABCA1 preferentially releases intermediate sterols with extra methyl groups including lanosterol, which accounts for very minor quantity of cellular sterol (0.1% or less of cellular total cholesterol) and its half-life is <1 h in cells. Lanosterol and other methylated intermediate sterols synthesized at the ER are immediately delivered to the PM (87). Therefore it is postulated that these minor sterols become constituents of certain PM domains where ABCA1 preferentially release these sterols along with cholesterol and phospholipid for HDL formation (144). There are significant structural differences between lanosterol and cholesterol, and lanosterol has the ability to induce membrane curvature formation in model membranes (145). Whether lanosterol affects membrane structure/organization in living cells remains to be investigated. In the absence of acceptors, lanosterol and other intermediate sterols immediately leave the PM domains and are transported back to the ER for cholesterol synthesis (87). ABCA1 participates in this retrograde transport (94). ABCA1 deficiency thus associates with accumulation of cholesterol and other sterols in the PM of various cell types including macrophages and fibroblasts (94, 114). In addition to endogenously synthesized sterols, LE/LYS-derived cholesterol is a significant source for ABCA1-dependent HDL formation (105, 146).

Recently, the structure of human ABCA1 in digitonin micelles but not in phospholipid bilayer was determined by cryoelectron microscopy (cryo-EM) (147). The structure reveals that an elongated hydrophobic tunnel is present within the two large extracellular domains. Whether this hydrophobic tunnel serves as a route for delivering lipids to apoA-I needs further investigation. Although the molecular mechanisms by which ABCA1 transports lipid and mediates HDL biogenesis remain obscure, the cryo-EM structure supports that structure-based studies of ABCA1 can provide further insights into its function and HDL assembly.

Regulation of ABCA1

ABCA1 expression is tightly regulated at transcriptional and post-transcriptional levels. LXR serves as major transcription factor that activate *ABCA1* gene expression (148, 149). Oxysterols and intermediate sterols, including desmosterol, activate LXR and induce ABCA1 expression (14, 148). LXR forms a heterodimer with another nuclear receptor, RXR. 9-*cis* retinoic acid, an RXR ligand, also induces ABCA1 expression (148). Recent studies found that many microRNAs (miRNAs), such as miR-33a/b and miR-148 are involved in *ABCA1* gene expression [reviewed in (150)]. These miRNAs suppress *ABCA1* gene expression and reduce plasma HDL levels in mice and non-human primates (151–153). MiR-33a and miR-33b are encoded by introns of *SREBF2* and *SREBF1* genes, respectively (151, 152).

ABCA1 protein is a short-live protein with half-life of about 1–2 h (154, 155). ABCA1 is degraded by the calcium-dependent cysteine protease, calpain (154, 156), and in proteasomes or in the LYS through ABCA1 ubiquitination by an unknown ubiquitin ligase (157–159). Unsaturated fatty acids accelerate ABCA1 degradation (160). Free cholesterol loading enhances the ubiquination and degradation of ABCA1 (158, 161). Importantly, in addition to its lipid acceptor function, apoA-I has an ability to protect ABCA1 from calpain-mediated degradation, thereby

increasing cell surface ABCA1 and further inducing lipid release (154, 156). It has been suggested that the ABCA1-apoA-I interaction at the cell surface leads ABCA1 to recycle back to the PM from the endocytic compartments (135) through a Rab8-dependent trafficking pathway (162). It would be interesting to determine whether the interaction between ABCA1 and apoA-I causes modifications of ABCA1 before endocytic internalization, to prevent its degradation.

ABCA1 has several important motifs/domains that modulate its stability. It has been demonstrated that the PDZ domain located in the C-terminal cytosolic region binds to several proteins including α1-syntrophin and β1-syntrophin (155, 163). Interaction of these two proteins with ABCA1 leads to its stabilization. LXRβ also binds to the C-terminal region of ABCA1 and stabilizes ABCA1 (164). The cytosolic region between the two transmembrane domains contains the PEST sequence that plays an important role in calpain-mediated degradation (154). Near the PEST sequence, ABCA1 contains calmodulin binding site. Calmodulin binding to ABCA1 inhibits ABCA1 degradation (165).

Several protein kinases including protein kinase A (PKA), PKC, and Janus kinase 2 (JAK2) modulate ABCA1 activity and stability. PKA phosphorylates ABCA1 and modulates its lipid efflux activity (166, 167). PKC, activated by apoA-I-cell interaction or by apoA-I-dependent lipid removal, is involved in the stability of ABCA1 (168, 169). JAK2 is activated by apoA-I and modulates ABCA1 activity without affecting its stability (170, 171). Other factors regulating ABCA1 protein activity and/or stability are reviewed elsewhere (172).

ABCG1 and ABCG4

In addition to ABCA1, the ABCG subfamily proteins ABCG1 and ABCG4 are well-recognized members of ABC transporters that facilitate cholesterol efflux to HDL (173). ABCG transporters are half-transporters that contain one nucleotide-binding domain in the N-terminal region. ABCG1 and ABCG4 transporters form either homodimer or heterodimer to function as an active transporter. ABCG1 is expressed in numerous tissues. ABCG1 localizes to both the PM and the endocytic compartments when overexpressed (174, 175). At physiological expression levels, however, this transporter largely resides in the intracellular compartments and acts as intracellular cholesterol transporter (176). Regardless of its localization, deficiency of ABCG1 impairs cholesterol efflux to HDL in macrophages (177). Importantly, ABCG1 also exports 7-ketocholesterol, an oxysterol abundant in oxidized LDL and atherosclerotic lessions, to HDL, whereas ABCA1 does not (118). Because 7-KC can induce apoptosis in macrophages, ABCG1-mediated 7-KC efflux to HDL protects macrophages from its cytotoxicity (118). Furthermore, in addition to sterols, ABCG1 preferentially exports SM over PC (174). While how ABCG1 mediates efflux of sterols and phospholipids is poorly characterized, its ATPase activity is stimulated by cholesterol and SM *in vitro* (178). These findings suggest that the activities of ABCA1 and ABCG1 are differentially regulated. ABCA1 and ABCG1 cooperatively promote cholesterol export pathway, and as such act synergistically to prevent cholesterol accumulation in macrophages (179).

ABCG4 is highly expressed in the brain (180). ABCG1 and ABCG4 are expressed in both neurons and astrocytes. Both of these transporters have the ability to export cholesterol as well as the intermediate sterol desmosterol to HDL (180). Deficiency in ABCG1 and/or ABCG4 results in the accumulation of intermediate sterols including lanosterol, desmosterol, and lathosterol, but not cholesterol, in mouse brains, with greater accumulation in double knockout mice (180). In ABCG1/ABCG4 double knockout mice, 27-OHC but not 24(*S*)-OHC is also accumulated in the brain (180).

ABCG5 and ABCG8

ABCG5 and ABCG8 form a heterodimer as an active transporter and are expressed in the apical membranes of enterocytes and hepatocytes. In the small intestine, ABCG5/G8 promote efflux of cholesterol and plant sterols absorbed through NPC1 like 1 (NPC1L1), to the lumen, thus limiting the absorption of dietary sterols (181). In the liver, the heterodimer exports cholesterol and phytosterols into the bile, thus enhancing sterol excretion from the body (182). Deficiency in either one the two transporters causes β-sitosterolemia, which is characterized by the robust accumulation of dietary sterols in plasma and tissues (183, 184). This accumulation is caused by increased intestinal absorption and decreased biliary excretion, and individuals with β-sitosterolemia develop premature atherosclerosis [reviewed in (185)].

Other ABC Transporters With Potential Sterol Exporting Activity

ABCA7, a close homolog of ABCA1, is highly expressed in the brain, lung, spleen, and adrenal in mice. ABCA7 is associated with Alzheimer's disease [reviewed in (186)]. The exact functions of ABCA7 are largely unknown, but it is capable of exporting lipids (phospholipid and cholesterol) to extracellular apoA-I and generates HDL-like particles when overexpressed in mammalian cells (187, 188). The ability of ABCA7 to export cholesterol is much less compared to that of ABCA1 (189). Consistent with the cell-based studies, an *in vitro* reconstitution assay shows that ABCA7 exports PS and PC with preference for PS (139). Although ABCA7 has similar lipid transporting functions as ABCA1, it plays a minimum, if any, role in lipid efflux and HDL formation in macrophages and in mice (190). At physiological expression levels, ABCA7 is mainly intracellular localized in resting macrophages, and following stimulation with apoptoic cells, ABCA7 translocates to the PM and plays a role in phagocytosis (191).

Additional ABCA family proteins are involved in cholesterol efflux. ABCA8 facilitates cholesterol efflux to apoA-I and modulates HDL-cholesterol levels (192). In humans, mutations in this gene are associated with low HDL-cholesterol levels (192). In addition, ABCA12 also regulates cholesterol efflux (193). It has been reported that ABCA12 interacts with ABCA1 and regulates its stability in macrophages, thereby modulating ABCA1-dependent lipid release (193). ABCA12 was originally identified as a protein defective in one of the most severe

skin disorders, Harlequin ichthyosis that is characterized by severe skin barrier defects and by abnormal keratinocyte lamellar granules (194). It is suggested that ABCA12 is involved in the transport of glucosylceramide in the lamellar granules (194). Unlike other ABC transporters described above, ABCA2 attenuates cholesterol efflux to extracellular cholesterol acceptors through poorly characterized pathway (195).

Certain ABCB family proteins also exhibit cholesterol transport activities. ABCB1 (also known as multidrug resistant protein 1, MDR1, or P-glycoprotein) translocates cholesterol from the inner leaflet to the outer leaflet of the PM (196). ABCB4 (also known as MDR3 in humans or MDR3 in mice), is expressed on the apical canalicular membranes of hepatocytes and exports PC and cholesterol to bile acids, as such ABCB4 along with ABCG5/ABCG8 contributes to biliary cholesterol excretion (197).

Passive Diffusion

Cholesterol can be removed from cells through passive diffusion, which is driven by a cholesterol gradient between cell surface and acceptors [reviewed in (198, 199)], with HDL serving as the major cholesterol acceptor. LCAT has phospholipase A2 and acyltransferase activities; LCAT transfers an acyl-chain from PC to 3-OH position of cholesterol, resulting in the formation of CE. LCAT-mediated cholesterol esterification plays a role in HDL maturation (200). Cholesterol esterification by LCAT in HDL causes the expansion of the cores in HDL particles and the reduction of free cholesterol at HDL surface, further facilitating net cholesterol efflux from cells to HDL. Serum albumin acts as a shuttle to facilitate passive cholesterol efflux from cells (199). Although the HDL receptor SR-BI is known to enhance cholesterol efflux from cells to HDL, SR-BI-mediated cholesterol efflux may not contribute to net cholesterol removal from cells, as it mediates bidirectional transport of cholesterol between the cell surface and HDL [reviewed in (115)]. As previously described, SR-BI rather plays an important role in selective uptake of HDL-cholesterol in the liver and steroidogenic tissues (115).

STEROLS AND HUMAN DISEASES

Cholesterol, sterol intermediates, and oxysterols play diverse and important roles in the body, in addition to contributing to various human diseases including cancer, cardiovascular disease, neurodegenerative disorders such as Alzheimer's disease and infection. This review focuses on discussing the connection between sterol metabolism and atherosclerosis as well as cancers. For a detailed discussion between cholesterol metabolism and neurodegenerative diseases, a recent review by Chang et al. (201) is available. Detailed reviews on the roles of sterols in immune responses are available elsewhere (20, 202). Pathophysiological roles of SREBPs in various human diseases are extensively discussed by Shimano and Sato (203).

Sterols and Atherosclerosis

Cholesterol along with inflammation regulation are major therapeutic targets for atherosclerosis, a condition in which plaque buildup in the artery wall can lead to a multitude of vascular complications, including heart attack and stroke. Large-scale trials have demonstrated the effectiveness of HMGCR inhibitors, statins, but have also shown residual risk with many statin-taking patients suffering from cardiovascular events (204). Further LDL cholesterol lowering by combining statin with the Niemann-Pick C1-like 1 intestinal cholesterol absorption inhibitor, ezetimibe provides modest benefit (205). Monoclonal antibody inhibition of proprotein convertase subtilisin-kexin type 9 (PCSK9), which reduces LDL cholesterol by about 60% via reducing LDL receptor degradation, improves clinical outcomes in patients with cardiovascular disease (206). While outcomes are not known, inhibition of the lipoprotein lipase inhibitor, angiopoitein-like 3 reduces circulating cholesterol, notably in homozygous familial hypercholesterolemia patients that only have limited responses to statin and PCSK9 therapies, and who typically require arduous LDL apheresis treatment (207). Supporting a key role of SR-BI-mediated HDL-cholesterol uptake in human reverse cholesterol transport, a loss-of-function variant (P376L) in SR-BI was recently identified in people with extremely high HDL cholesterol that also have increased risk of coronary heart disease (208). Much is known about the impact of reducing cholesterol in cardiovascular disease; however, less is known about the contributions of sterol metabolites to atherosclerosis that may provide insight into novel risk factors and therapies to target residual cardiovascular risk.

Macrophage lipid accumulation leads to foam cell formation and inflammation, contributing to atherosclerosis (209). In macrophages, the cholesterol precursor desmosterol exhibits several beneficial properties including inhibiting expression of cholesterol biosynthesis and proinflammatory genes, along with induction of LXR genes and cholesterol efflux (210). LXR-deficiency in mice exacerbates atherosclerosis disease pathology, and LXR activation generally reduces cell and animal model pathology through a variety of mechanisms [reviewed in (211)]; however, LXR activation can also have an accompanying unacceptable side effect of raising liver triglyceride synthesis (212).

Oxysterols are associated with nearly every atherosclerosis-contributing pathway, as such they present a promising area of future therapeutic research. Oxidized forms of cholesterol produced by LDL oxidation exhibit pro-atherosclerotic properties [reviewed in (213, 214)]. Like cholesterol, oxysterols can be stored as fatty acid esters [reviewed in (51)]. In mice, ACAT1 small molecule inhibition (215), genetic knockout (216), and macrophage-specific deletion (217) reduce atherosclerosis pathology. Additionally, ACAT1 was recently associated as a causal modifier variant explaining strain differences in mouse atherosclerosis pathology (218); although a cardiovascular benefit of ACAT inhibition has not been demonstrated in human ACAT inhibitor trials (219).

Oxysterols can regulate atherosclerosis-related pathologies, but the extent to which oxysterols drive atherosclerosis is unclear. Multi-omics mapping of oxysterols and associated genes and proteins in healthy and diseased tissue could clarify the complex roles of these lipids in cardiovascular disease. In primary mouse macrophages, 22-OHC, 24-OHC, and the sterol

24, 25 epoxycholesterol repress activation of the inflammation-related factor, inducible nitric oxide synthase, while also inducing ABCA1 expression, and 25-OHC and 27-OHC activate ABCA1 but do not repress inducible nitric oxide synthase (220). On top of plaque formation, calcification is frequently observed in atherosclerosis, and may contribute to plaque rupture (221). In bovine aortic and mouse smooth muscle cells, 25-OHC stimulates mineralization pathways leading to vascular calcification, including through LXR-independent mechanisms (222, 223). 27-OHC is increased in human atherosclerotic arteries and functions in macrophage cholesterol elimination (224); however, the role of 27-OHC in atherosclerosis is complex. Elevated 27-OHC via deletion of CYP7B1 that metabolizes 27-OHC promotes atherosclerosis via a proinflammatory process involving estrogen receptor alpha in apoE-deficient mice (225). Knockout of *Cyp27A1* in apolipoprotein E-deficient mice revealed a gene dose effect with a 10-fold reduction in atherosclerosis severity observed in double knockout mice, and *Cyp27A1* heterozygosity leads to accelerated atherosclerosis (226). Cerebrotendinous xanthomatosis develops in the absence of CYP27A1 in humans, while mice lacking CYP27A1 do not develop xanthomas. Further complicating matters are that loss of 27-hydroxylase may have athero-protective effects including increased cholesterol degradation, in addition to pro-atherogenic effects, such as reduced cholesterol efflux, and the balance of this may vary between patients (227). Raising a confounding factor, sex-specific effects in bile acids are observed in CYP27A1 overexpressing mice (227).

Sterol Metabolism and Cancer

Altered metabolism is a hallmark of cancer cells, with a major metabolic alteration being elevated glucose uptake and glycolysis (228). In addition to this "glycolytic phenotype," biosynthesis of lipids (including cholesterol), which are essential building blocks of cell membranes, is increased in cancer cells to support proliferation (229). Elevated lipogenesis is referred to as "lipogenic phenotype." In human melanomas, increased expression of various cholesterol biosynthetic genes is found in more than 60% of patients (230). SREBPs serve as master drivers for lipogenic phenotype of cancer cells. Activation of SREBPs and/or upregulation of SREBP-target gene expression is observed in a wide variety of human cancers including glioblastoma (231), prostate cancer (232), breast cancer (233), melanoma (230, 234), and is often associated with poor prognosis/survival. Cholesterol is an indispensable component of lipid rafts, which serve as platforms for various oncogenic signals including Akt activation, and therefore cholesterol synthesis in cancer cells links to the integrity of these membrane domains. SREBPs are activated by PI3K-Akt-mTORC1 signaling (235, 236), which is hyperactivated in human cancers. Consequently, a positive feedback loop between PI3K-Akt-mTORC1 signaling and SREBP-dependent lipogenesis is formed to sustain malignant properties of cancer cells (234, 237). Several mechanisms are involved in the activation of SREBP by PI3K-Akt-mTORC1 signaling: (1) Phosphorylation of SREBP-1 mature form by the protein kinase GSK3β results in ubiquitination by the ubiquitin ligase Fbw7, leading to the proteasomal degradation of the mature form (238). Independently of mTORC1, Akt phosphorylates and inactivates GSK3β, thereby increasing SREBP transcriptional activity. (2) PI3K-Akt-mTORC1 axis regulates processing of both SREBP-1 and SREBP-2 (234). mTOR phosphorylates cytosolic CREB regulated transcription coactivator 2 (CRTC2) and attenuates its inhibitory effect on COPII-dependent transport of SREBP-1 from the ER to the Golgi, which facilitates SREBP-1 processing (239), and this pathway is at least partly involved in PI3K-Akt-mTORC1 regulation of SREBP processing. Whether this axis also regulates SREBP-2 processing is unknown at present. In addition, the mTOR-CRTC2 axis could be involved in general COP-II transport. Additional mechanisms may also participate in SREBP processing as the protein kinase S6K also regulates SREBP processing downstream of mTORC1 (240). (3) mTORC1 phosphorylates lipin1 and regulates nuclear entry of SREBP mature form through an unknown mechanism (241). While cholesterol plays crucial roles in malignant properties of cancer cells, whether blocking cholesterol biosynthesis by statins, HMGCR inhibitor, reduce the risk of cancer onset or mortality is controversial at present; the statin effects could be dependent on types of cancer (242–244). To plausibly explain this, inhibiting HMGCR increases LDL uptake through upregulation of LDLR expression, which could enable cancer cells to acquire sufficient amounts of cholesterol. LDL levels are much higher in humans than mice. Therefore, inhibiting SREBP could be a more direct target to treat a broad range of cancers.

An aberrant accumulation of CE in lipid droplets via ACAT1 is found in prostate (245) and pancreatic cancer tissues (246). Pharmacological or genetic ACAT1 inhibition attenuates cancer cell proliferation and invasive activity *in vitro* and tumor growth and metastasis in xenograft models (245, 246). Blocking ACAT1 causes an increase in free cholesterol at the ER, which inactivates SREBP-1 but not SREBP-2, reducing cholesterol synthesis and uptake (245). Increased ER free cholesterol levels may induce ER stress and cancer cell apoptosis (246). Furthermore, ACAT inhibition blocks activation of Akt partly though a reduction of cellular arachidonic acid (245), and reduced SREBP activity and cholesterol biosynthesis could also impair the integrity of lipid rafts and suppress Akt activity. Therefore, ACAT1 is an attractive therapeutic target for certain types of cancer where large accumulations of CE are observed.

Cancer immunotherapy targeting PD-1 expressed on T cells or PD-L1 expressed on cancer cells is clinically used. In these therapies, $CD8^+$ T cell activity is crucial for successful achievement. Recent work by Yang et al. (247) showed that blocking ACAT1 activates T cells; ACAT blockage increases PM cholesterol levels and enhances T-cell receptor clustering and signaling, which in turn potentiates effector function and proliferation of these cells to suppress tumor growth in mice. Inhibiting ACAT1 further improved anti-tumor efficacy of anti-PD1 antibody (247). Given the additional suppressive effects of ACAT1 inhibitor on malignant properties of cancer cells, ACAT1 blockage thus has dual beneficial effects in cancer therapy. However, proliferation of $CD8^+$ T cell depends upon SREBPs and cholesterol biosynthesis (248), creating difficulties in targeting SREBP pathway to combat cancer. Developing specific drug delivery systems could overcome this barrier.

Oxysterols and Cancer

Serum cholesterol levels are positively correlated with serum 27-OHC, the most abundant oxysterol (213). Hypercholesterolemia is a risk factor of breast cancer, and 27-OHC is involved in the pathophysiology of human breast cancer (249). Expression of CYP27A1, which converts cholesterol to 27-OHC is positively correlated to tumor grade in human breast cancer (250). In aggressive tumor tissues, high CYP27A1 expression was found in both tumor cells and tumor-associated macrophages (TAMs) (250). 27-OHC administration increases the growth of human breast cancers, and CYP27A1 inhibition suppresses tumor growth in xenograft models (250, 251). Further, abrogation of Cyp7B1, which catalyzes the conversion of 27-OHC to 7α, 27-di-OHC resulting in 27-OHC accumulation in plasma and tumor tissues, accelerates tumor growth in a mouse estrogen receptor-positive mammary adenocarcinoma model (250). Reduced expression of CYP7B1 is correlated with estrogen receptor-positive breast cancer aggressiveness (251). 27-OHC exerts pro-tumor effects on breast cancer cells by acting as an estrogen receptor agonist (as a SERM). Additionally, 27-OHC promotes metastases of not only estrogen receptor-positive and -negative breast cancer cells but also of melanoma, lung cancer cells, and pancreatic cancer cells (252). The pro-metastatic roles of 27-OHC depend partly on LXR activity of tumor cells (250) and on immune cells within tumor microenvironment (252). 27-OHC affects several types of immune cells, increasing the number of polymorphonuclear-neutrophils and γδ-T cells, and decreasing $CD8^+$ T cells (252).

In contrast to breast cancer, CYP27A1 expression is negatively associated with aggressiveness of prostate cancer; patients whose tumors express higher *CYP27A1* mRNA exhibit lower tumor grade and longer disease-free survival (253). 27-OHC suppresses growth of human prostate cancer cells at least partially by inactivating SREBP-2 pathway (253).

Sterol Transporters and Cancer

Lipid transport proteins can modulate malignant properties of cancer cells. ABCA1 has an anti-cancer activity dependent on lipid transport activity (254). ABCA1 expression levels are inversely correlated with tumor aggressiveness in prostate cancer. The *ABCA1* gene promotor is hypermethylated in the tissues of prostate cancer, suppressing its expression (255). ABCA1 deficiency causes an increase in mitochondrial cholesterol content, which promotes survival of cancer cells (254).

Studies using LXR agonists showed that activation of LXR reduces cellular cholesterol content in glioblastoma cells through increased cholesterol export and decreased cholesterol uptake, which suppresses the growth of glioblastoma cells *in vitro* and in xenograft models (256, 257). LXR agonists also suppress metastases of melanoma and prolong animal survival, at least partially though LXRβ-dependent upregulation of apoE expression, in several *in vivo* models (258). Furthermore, accumulation of endogenous 4α-monomethylsterols by genetic manipulation leads to LXR activation and inhibits tumorigenesis in a mouse model (259).

Several ABCA family transporters associate with overall survival of serous ovarian cancer patients. Patients with high expression of ABCA1, ABCA6, ABCA8, or ABCA9 in primary tumors exhibit reduced survival (260). Furthermore, combined expression of ABCA1, ABCA5, and either ABCA8 or ABCA9 leads to poorer survival (260). In this cancer type, ABCA1 seems to have an opposite effect on malignancy by unknown mechanisms. The roles of ABCA5, ABCA8, and ABCA9 are not well-understood.

Independently of LXR/RXR-mediated transcription, ABCA1 expression is regulated by cell density in A431 human epidermal carcinoma cells (261). In cells at low density, focal adhesion kinase (FAK) inactivates the transcription factors Foxo3 and TAL1, resulting in suppression of ABCA1 expression. In contrast, at high density, FAK activity is suppressed, and ABCA1 expression is upregulated (261). Cell density-dependent ABCA1 expression regulates PM lipid compositions (261); cells expressing ABCA1 at high levels contain less cholesterol and ganglioside in the PM compared to those expressing ABCA1 at low levels (94, 114, 261). ABCA1 modulates trans-bilayer distribution of cholesterol at the PM (262). Collectively, in addition to a well-known role in HDL biogenesis, ABCA1 acts to fine-tune membrane lipid compositions to modulate PM functionality that may alter cancer cell phenotypes in a cell type-dependent manner.

Macrophage ABCA1 and ABCG1 also play roles in tumor growth. Deficiency in either ABCA1 or ABCG1 in macrophages attenuates tumor growth in xenograft models (263, 264). Deletion of these genes in macrophages converts the tumor-promoting M2 phenotype to the anti-tumor M1 phenotype TAMs within the tumor microenvironment (263, 264).

Additional lipid/sterol transporter is also involved in human cancer. Higher expression of ORP5, an ER membrane anchored ORP, associates with poor prognosis of patients with pancreatic cancer (265). ORP5 expression levels are positively correlated with cell proliferation and invasive activity. Of mechanistic interest, ORP5 interacts with mTOR, and enhances mTORC1 activity (266).

CONCLUDING REMARKS

Cells are highly compartmentalized at both the cellular and organelle levels. Cholesterol, oxysterols, and intermediate sterols move dynamically in cells with correct cellular distribution, being paramount to regulating cellular functions. Cellular sterol transport must be spatiotemporally regulated; however, the mechanisms of this are not fully understood. Compared to cholesterol, much less is known about the transport of oxysterols and intermediate sterols. Additionally, elucidating sterol metabolism and transport mechanisms in disease via pathophysiologically relevant models, such as patient-derived iPS- cell systems and organoid culture, could help identify novel therapies for human diseases. State-of-the-art technologies including genome editing, imaging, and multi-omics may provide new insights into how cells handle sterols in physiological and pathophysiological conditions.

Experimental evidence demonstrates that sterols and sterol metabolism play crucial roles in various aspects of human

diseases, not only in atherosclerosis and cancer, but also those not reviewed here, including Alzheimer's disease and other neurodegenerative disorders, and infection among others. Sterol metabolism is an attractive target in disease treatment. Several drugs targeting sterol metabolism are clinically used to treat patients currently, including statins to block cholesterol synthesis and enhance LDL uptake, Ezetimibe to block NPC1L1-mediated absorption of dietary cholesterol, and the anti-PCSK9 monoclonal antibodies (Evolocumab and Alirocumab) to block LDLR degradation and reduce plasma LDL. New potential therapeutic target molecules include SREBPs and SREBP regulators, LXR, ABC transporters, ACAT, and sterol hydroxylases, as a growing number of preclinical and clinical studies targeting these molecules are ongoing. Given the importance of target localization, development of novel drug delivery systems to address associated issues will also be key.

AUTHOR CONTRIBUTIONS

YY designed and wrote the article. MR contributed to writing and revising the article. Both authors approved the final version.

ACKNOWLEDGMENTS

We thank Dr. Ta-Yuan Chang (Geisel School of Medicine at Dartmouth, USA) and Dr. Ryuichiro Sato (University of Tokyo, Japan) for discussion and critical reading of this manuscript. YY is supported by AMED-CREST Grant 18gm0910008h0103 from Japan Agency for the Medical Research and Development (AMED) and by KAKENHI Grant 16K08618 from Japan Society for Promotion of Sciences. MR is supported by an NIH fellowship.

REFERENCES

1. Gold DA, Caron A, Fournier GP, Summons RE. Paleoproterozoic sterol biosynthesis and the rise of oxygen. *Nature* (2017) 543:420–3. doi: 10.1038/nature21412
2. Maxfield F, Tabas I. Role of cholesterol and lipid organization in disease. *Nature* (2005) 438:612–21. doi: 10.1038/nature04399
3. Liscum L, Munn NJ. Intracellular cholesterol transport. *Biochim Biophys Acta* (1999) 1438:19–37. doi: 10.1016/S1388-1981(99)00043-8
4. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol.* (2008) 9:112–24. doi: 10.1038/nrm2330
5. Maxfield FR, Wüstner D. Intracellular cholesterol transport. *J Clin Invest.* (2002) 110:891–8. doi: 10.1172/JCI0216500
6. Simons K, Gerl MJ. Revitalizing membrane rafts: new tools and insights. *Nat Rev Mol Cell Biol.* (2010) 11:688–99. doi: 10.1038/nrm2977
7. Ikonen E. Cellular cholesterol trafficking and compartmentalization. *Nat Rev Mol Cell Biol.* (2008) 9:125–38. doi: 10.1038/nrm2336
8. Chang TY, Chang CCY, Ohgami N, Yamauchi Y. Cholesterol sensing, trafficking, and esterification. *Annu Rev Cell Dev Biol.* (2006) 22:129–57. doi: 10.1146/annurev.cellbio.22.010305.104656
9. Zhao L, Chen XJ, Zhu J, Xi YB, Yang X, Hu LD, et al. Lanosterol reverses protein aggregation in cataracts. *Nature* (2015) 523:607–11. doi: 10.1038/nature14650
10. Lange Y, Ory D, Ye J, Lanier M, Hsu F, Steck T. Effectors of rapid homeostatic responses of endoplasmic reticulum cholesterol and 3-hydroxy-3-methylglutaryl-CoA reductase. *J Biol Chem.* (2008) 283:1445–55. doi: 10.1074/jbc.M706967200
11. Song BL, Javitt NB, DeBose-Boyd RA. Insig-mediated degradation of HMG CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. *Cell Metab.* (2005) 1:179–89. doi: 10.1016/j.cmet.2005.01.001
12. Byskov AG, Andersen CY, Nordholm L, Thogersen H, Xia G, Wassmann O, et al. Chemical structure of sterols that activate oocyte meiosis. *Nature* (1995) 374:559–62. doi: 10.1038/374559a0
13. Radhakrishnan A, Sun L, Kwon H, Brown M, Goldstein J. Direct binding of cholesterol to the purified membrane region of SCAP: mechanism for a sterol-sensing domain. *Mol Cell* (2004) 15:259–68. doi: 10.1016/j.molcel.2004.06.019
14. Yang C, McDonald JG, Patel A, Zhang Y, Umetani M, Xu F, et al. Sterol intermediates from cholesterol biosynthetic pathway as liver X receptor ligands. *J Biol Chem.* (2006) 281:27816–26. doi: 10.1074/jbc.M603781200
15. Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. *Science* (1996) 274:255–9. doi: 10.1126/science.274.5285.255
16. Xiao X, Tang JJ, Peng C, Wang Y, Fu L, Qiu ZP, et al. Cholesterol modification of smoothened is required for hedgehog signaling. *Mol Cell* (2017) 66:154–62 e10. doi: 10.1016/j.molcel.2017.02.015
17. Russell DW. Fifty years of advances in bile acid synthesis and metabolism. *J Lipid Res.* (2009) 50 Suppl.):S120–5. doi: 10.1194/jlr.R800026-JLR200
18. Radhakrishnan A, Ikeda Y, Kwon H, Brown M, Goldstein J. Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig. *Proc Natl Acad Sci USA.* (2007) 104:6511–8. doi: 10.1073/pnas.0700899104
19. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature* (1996) 383:728–31. doi: 10.1038/383728a0
20. Cyster JG, Dang EV, Reboldi A, Yi T. 25-Hydroxycholesterols in innate and adaptive immunity. *Nat Rev Immunol.* (2014) 14:731–43. doi: 10.1038/nri3755
21. Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, et al. 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nat Med.* (2007) 13:1185–92. doi: 10.1038/nm1641
22. Hannedouche S, Zhang J, Yi T, Shen W, Nguyen D, Pereira JP, et al. Oxysterols direct immune cell migration via EBI2. *Nature* (2011) 475:524–7. doi: 10.1038/nature10280
23. Liu C, Yang XV, Wu J, Kuei C, Mani NS, Zhang L, et al. Oxysterols direct B-cell migration through EBI2. *Nature* (2011) 475:519–23. doi: 10.1038/nature10226
24. Kelley RI, Herman GE. Inborn errors of sterol biosynthesis. *Annu Rev Genomics Hum Genet.* (2001) 2:299–341. doi: 10.1146/annurev.genom.2.1.299
25. Porter FD. Malformation syndromes due to inborn errors of cholesterol synthesis. *J Clin Invest.* (2002) 110:715–24. doi: 10.1172/JCI0216386
26. Calkin AC, Tontonoz P. Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. *Nat Rev Mol Cell Biol.* (2012) 13:213–24. doi: 10.1038/nrm3312
27. Bielska AA, Schlesinger P, Covey DF, Ory DS. Oxysterols as non-genomic regulators of cholesterol homeostasis. *Trends Endocrinol Metab.* (2012) 23:99–106. doi: 10.1016/j.tem.2011.12.002
28. Bloch K. Sterol molecule: structure, biosynthesis, and function. *Steroids* (1992) 57:378–83. doi: 10.1016/0039-128X(92)90081-J
29. Mitsche MA, McDonald JG, Hobbs HH, Cohen JC. Flux analysis of cholesterol biosynthesis *in vivo* reveals multiple tissue and cell-type specific pathways. *Elife* (2015) 4:e07999 doi: 10.7554/eLife.07999
30. Nes WD. Biosynthesis of cholesterol and other sterols. *Chem Rev.* (2011) 111:6423–51. doi: 10.1021/cr200021m
31. Brown M, Goldstein J. Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. *J Lipid Res.* (2009) 50(Suppl.):S15–27. doi: 10.1194/jlr.R800054-JLR200

32. Horton J, Goldstein J, Brown M. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest.* (2002) 109:1125–31. doi: 10.1172/JCI0215593
33. Brown A, Sun L, Feramisco J, Brown M, Goldstein J. Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. *Mol Cell* (2002) 10:237–45. doi: 10.1016/S1097-2765(02)00591-9
34. Radhakrishnan A, Goldstein J, McDonald J, Brown M. Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. *Cell Metab.* (2008) 8:512–21. doi: 10.1016/j.cmet.2008.10.008
35. Takeuchi Y, Yahagi N, Izumida Y, Nishi M, Kubota M, Teraoka Y, et al. Polyunsaturated fatty acids selectively suppress sterol regulatory element-binding protein-1 through proteolytic processing and autoloop regulatory circuit. *J Biol Chem.* (2010) 285:11681–91. doi: 10.1074/jbc.M109.096107
36. Gil G, Faust J, Chin D, Goldstein J, Brown M. Membrane-bound domain of HMG CoA reductase is required for sterol-enhanced degradation of the enzyme. *Cell* (1985) 41:249–58. doi: 10.1016/0092-8674(85)90078-9
37. Gong Y, Lee J, Lee P, Goldstein J, Brown M, Ye J. Sterol-regulated ubiquitination and degradation of Insig-1 creates a convergent mechanism for feedback control of cholesterol synthesis and uptake. *Cell Metab.* (2006) 3:15–24. doi: 10.1016/j.cmet.2005.11.014
38. Sever N, Yang T, Brown M, Goldstein J, DeBose-Boyd R. Accelerated degradation of HMG CoA reductase mediated by binding of insig-1 to its sterol-sensing domain. *Mol Cell* (2003) 11:25–33. doi: 10.1016/S1097-2765(02)00822-5
39. Sever N, Song B, Yabe D, Goldstein J, Brown M, DeBose-Boyd R. Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methylglutaryl-CoA reductase stimulated by sterols and geranylgeraniol. *J Biol Chem.* (2003) 278:52479–90. doi: 10.1074/jbc.M310053200
40. Song BL, Sever N, DeBose-Boyd RA. Gp78, a membrane-anchored ubiquitin ligase, associates with Insig-1 and couples sterol-regulated ubiquitination to degradation of HMG CoA reductase. *Mol Cell* (2005) 19:829–40. doi: 10.1016/j.molcel.2005.08.009
41. Jo Y, Lee PC, Sguigna PV, DeBose-Boyd RA. Sterol-induced degradation of HMG CoA reductase depends on interplay of two Insigs and two ubiquitin ligases, gp78 and Trc8. *Proc Natl Acad Sci USA*. (2011) 108:20503–8. doi: 10.1073/pnas.1112831108
42. Jiang LY, Jiang W, Tian N, Xiong YN, Liu J, Wei J, et al. Ring finger protein 145 (RNF145) is a ubiquitin ligase for sterol-induced degradation of HMG-CoA reductase. *J Biol Chem.* (2018) 293:4047–55. doi: 10.1074/jbc.RA117.001260
43. Tsai YC, Leichner GS, Pearce MM, Wilson GL, Wojcikiewicz RJ, Roitelman J, et al. Differential regulation of HMG-CoA reductase and Insig-1 by enzymes of the ubiquitin-proteasome system. *Mol Biol Cell* (2012) 23:4484–94. doi: 10.1091/mbc.e12-08-0631
44. Zelcer N, Sharpe LJ, Loregger A, Kristiana I, Cook EC, Phan L, et al. The E3 ubiquitin ligase MARCH6 degrades squalene monooxygenase and affects 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and the cholesterol synthesis pathway. *Mol Cell Biol.* (2014) 34:1262–70. doi: 10.1128/MCB.01140-13
45. Clarke PR, Hardie DG. Regulation of HMG-CoA reductase: identification of the site phosphorylated by the AMP-activated protein kinase *in vitro* and in intact rat liver. *EMBO J.* (1990) 9:2439–46.
46. Sato R, Goldstein J, Brown M. Replacement of serine-871 of hamster 3-hydroxy-3-methylglutaryl-CoA reductase prevents phosphorylation by AMP-activated kinase and blocks inhibition of sterol synthesis induced by ATP depletion. *Proc Natl Acad Sci USA*. (1993) 90:9261–5. doi: 10.1073/pnas.90.20.9261
47. Gill S, Stevenson J, Kristiana I, Brown AJ. Cholesterol-dependent degradation of squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA reductase. *Cell Metab.* (2011) 13:260–73. doi: 10.1016/j.cmet.2011.01.015
48. Prabhu AV, Luu W, Sharpe LJ, Brown AJ. Cholesterol-mediated degradation of 7-dehydrocholesterol reductase switches the balance from cholesterol to vitamin D synthesis. *J Biol Chem.* (2016) 291:8363–73. doi: 10.1074/jbc.M115.699546
49. Chang T, Li B, Chang C, Urano Y. Acyl-coenzyme A:cholesterol acyltransferases. *Am J Physiol Endocrinol Metab.* (2009) 297:E1–9. doi: 10.1152/ajpendo.90926.2008
50. Sekiya M, Osuga J, Igarashi M, Okazaki H, Ishibashi S. The role of neutral cholesterol ester hydrolysis in macrophage foam cells. *J Atheroscler Thromb.* (2011) 18:359–64. doi: 10.5551/jat.7013
51. Rogers MA, Liu J, Song BL, Li BL, Chang CC, Chang TY. Acyl-CoA:cholesterol acyltransferases (ACATs/SOATs): Enzymes with multiple sterols as substrates and as activators. *J Steroid Biochem Mol Biol.* (2015) 151:102–7. doi: 10.1016/j.jsbmb.2014.09.008
52. Tavani DM, Nes WR, Billheimer JT. The sterol substrate specificity of acyl CoA: cholesterol acyltransferase from rat liver. *J Lipid Res.* (1982) 23:774–81.
53. Mutemberezi V, Guillemot-Legris O, Muccioli GG. Oxysterols: From cholesterol metabolites to key mediators. *Prog Lipid Res.* (2016) 64:152–69. doi: 10.1016/j.plipres.2016.09.002
54. Akiyama H, Hirabayashi Y. A novel function for glucocerebrosidase as a regulator of sterylglucoside metabolism. *Biochim Biophys Acta* (2017) 1861:2507–14. doi: 10.1016/j.bbagen.2017.06.003
55. Lund EG, Kerr TA, Sakai J, Li WP, Russell DW. cDNA cloning of mouse and human cholesterol 25-hydroxylases, polytopic membrane proteins that synthesize a potent oxysterol regulator of lipid metabolism. *J Biol Chem.* (1998) 273:34316–27. doi: 10.1074/jbc.273.51.34316
56. Lange Y, Steck T, Ye J, Lanier M, Molugu V, Ory D. Regulation of fibroblast mitochondrial 27-hydroxycholesterol production by active plasma membrane cholesterol. *J Lipid Res.* (2009) 50:1881–8. doi: 10.1194/jlr.M900116-JLR200
57. Russell DW, Halford RW, Ramirez DM, Shah R, Kotti T. Cholesterol 24-hydroxylase: an enzyme of cholesterol turnover in the brain. *Annu Rev Biochem.* (2009) 78:1017–40. doi: 10.1146/annurev.biochem.78.072407.103859
58. Lutjohann D, Breuer O, Ahlborg G, Nennesmo I, Siden A, Diczfalusy U, et al. Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation. *Proc Natl Acad Sci USA*. (1996) 93:9799–804. doi: 10.1073/pnas.93.18.9799
59. Dietschy JM, Turley SD. Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J Lipid Res.* (2004) 45:1375–97. doi: 10.1194/jlr.R400004-JLR200
60. Liu SY, Aliyari R, Chikere K, Li G, Marsden MD, Smith JK, et al. Interferon-inducible cholesterol-25-hydroxylase broadly inhibits viral entry by production of 25-hydroxycholesterol. *Immunity* (2013) 38:92–105. doi: 10.1016/j.immuni.2012.11.005
61. Blanc M, Hsieh WY, Robertson KA, Kropp KA, Forster T, Shui G, et al. The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. *Immunity* (2013) 38:106–18. doi: 10.1016/j.immuni.2012.11.004
62. Xiang Y, Tang JJ, Tao W, Cao X, Song BL, Zhong J. Identification of cholesterol 25-hydroxylase as a novel host restriction factor and a part of the primary innate immune responses against hepatitis C virus infection. *J Virol.* (2015) 89:6805–16. doi: 10.1128/JVI.00587-15
63. Anggakusuma, Romero-Brey I, Berger C, Colpitts CC, Boldanova T, Engelmann M, et al. Interferon-inducible cholesterol-25-hydroxylase restricts hepatitis C virus replication through blockage of membranous web formation. *Hepatology* (2015) 62:702–14. doi: 10.1002/hep.27913
64. Diczfalusy U, Olofsson KE, Carlsson AM, Gong M, Golenbock DT, Rooyackers O, et al. Marked upregulation of cholesterol 25-hydroxylase expression by lipopolysaccharide. *J Lipid Res.* (2009) 50:2258–64. doi: 10.1194/jlr.M900107-JLR200
65. Bauman DR, Bitmansour AD, McDonald JG, Thompson BM, Liang G, Russell DW. 25-Hydroxycholesterol secreted by macrophages in response to Toll-like receptor activation suppresses immunoglobulin A production. *Proc Natl Acad Sci USA*. (2009) 106:16764–9. doi: 10.1073/pnas.0909142106
66. Quinn CM, Jessup W, Wong J, Kritharides L, Brown AJ. Expression and regulation of sterol 27-hydroxylase (CYP27A1) in human macrophages: a role for RXR and PPARgamma ligands. *Biochem J.* (2005) 385(Pt 3):823–30. doi: 10.1042/BJ20041776

67. Segev H, Honigman A, Rosen H, Leitersdorf E. Transcriptional regulation of the human sterol 27-hydroxylase gene (CYP27) and promoter mapping. *Atherosclerosis* (2001) 156:339–47. doi: 10.1016/S0021-9150(00)00654-7
68. Araya Z, Tang W, Wikvall K. Hormonal regulation of the human sterol 27-hydroxylase gene CYP27A1. *Biochem J.* (2003) 372(Pt 2):529–34. doi: 10.1042/bj20021651
69. Li T, Chen W, Chiang JY. PXR induces CYP27A1 and regulates cholesterol metabolism in the intestine. *J Lipid Res.* (2007) 48:373–84. doi: 10.1194/jlr.M600282-JLR200
70. Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, et al. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell* (2000) 6:507–15. doi: 10.1016/S1097-2765(00)00050-2
71. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* (2000) 6:517–26. doi: 10.1016/S1097-2765(00)00051-4
72. Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, et al. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell* (1998) 93:693–704. doi: 10.1016/S0092-8674(00)81432-4
73. Chiang JY, Kimmel R, Stroup D. Regulation of cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRalpha). *Gene* (2001) 262(1–2):257–65. doi: 10.1016/S0378-1119(00)00518-7
74. Agellon LB, Drover VA, Cheema SK, Gbaguidi GF, Walsh A. Dietary cholesterol fails to stimulate the human cholesterol 7alpha-hydroxylase gene (CYP7A1) in transgenic mice. *J Biol Chem.* (2002) 277:20131–4. doi: 10.1074/jbc.C200105200
75. Vanier MT. Complex lipid trafficking in Niemann-Pick disease type C. *J Inherit Metab Dis.* (2015) 38:187–99. doi: 10.1007/s10545-014-9794-4
76. Olkkonen VM, Li S. Oxysterol-binding proteins: sterol and phosphoinositide sensors coordinating transport, signaling and metabolism. *Prog Lipid Res.* (2013) 52:529–38. doi: 10.1016/j.plipres.2013.06.004
77. Pietrangelo A, Ridgway ND. Bridging the molecular and biological functions of the oxysterol-binding protein family. *Cell Mol Life Sci.* (2018) 75:3079–98. doi: 10.1007/s00018-018-2795-y
78. Taylor FR, Kandutsch AA. Oxysterol binding protein. *Chem Phys Lipids* (1985) 38:187–94. doi: 10.1016/0009-3084(85)90066-0
79. Mesmin B, Bigay J, Polidori J, Jamecna D, Lacas-Gervais S, Antonny B. Sterol transfer, PI4P consumption, and control of membrane lipid order by endogenous OSBP. *EMBO J.* (2017) 36:3156–74. doi: 10.15252/embj.201796687
80. Mesmin B, Bigay J, von Filseck JM, Lacas-Gervais S, Drin G, Antonny B. A Four-step cycle driven by PI(4)P hydrolysis directs sterol/PI(4)P exchange by the ER-golgi tether OSBP. *Cell* (2013) 155:830–43. doi: 10.1016/j.cell.2013.09.056
81. Alpy F, Tomasetto C. Give lipids a START: the StAR-related lipid transfer (START) domain in mammals. *J Cell Sci.* (2005) 118(Pt 13):2791–801. doi: 10.1242/jcs.02485
82. DeGrella RF, Simoni RD. Intracellular transport of cholesterol to the plasma membrane. *J Biol Chem.* (1982) 257:14256–62.
83. Heino S, Lusa S, Somerharju P, Ehnholm C, Olkkonen V, Ikonen E. Dissecting the role of the golgi complex and lipid rafts in biosynthetic transport of cholesterol to the cell surface. *Proc Natl Acad Sci USA.* (2000) 97:8375–80. doi: 10.1073/pnas.140218797
84. Echevarria F, Norton R, Nes W, Lange Y. Zymosterol is located in the plasma membrane of cultured human fibroblasts. *J Biol Chem.* (1990) 265:8484–9.
85. Johnson WJ, Fischer RT, Phillips MC, Rothblat GH. Efflux of newly synthesized cholesterol and biosynthetic sterol intermediates from cells. Dependence on acceptor type and on enrichment of cells with cholesterol. *J Biol Chem.* (1995) 270:25037–46. doi: 10.1074/jbc.270.42.25037
86. Lusa S, Heino S, Ikonen E. Differential mobilization of newly synthesized cholesterol and biosynthetic sterol precursors from cells. *J Biol Chem.* (2003) 278:19844–51. doi: 10.1074/jbc.M212503200
87. Yamauchi Y, Reid PC, Sperry JB, Furukawa K, Takeya M, Chang CCY, et al. Plasma membrane rafts complete cholesterol synthesis by participating in retrograde movement of precursor sterols. *J Biol Chem.* (2007) 282:34994–5004. doi: 10.1074/jbc.M703653200
88. Ridgway N, Dawson P, Ho Y, Brown M, Goldstein J. Translocation of oxysterol binding protein to Golgi apparatus triggered by ligand binding. *J Cell Biol.* (1992) 116:307–19. doi: 10.1083/jcb.116.2.307
89. Hynynen R, Laitinen S, Käkelä R, Tanhuanpää K, Lusa S, Ehnholm C, et al. Overexpression of OSBP-related protein 2 (ORP2) induces changes in cellular cholesterol metabolism and enhances endocytosis. *Biochem J.* (2005) 390(Pt 1):273–83. doi: 10.1042/BJ20042082
90. Puglielli L, Rigotti A, Greco AV, Santos MJ, Nervi F. Sterol carrier protein-2 is involved in cholesterol transfer from the endoplasmic reticulum to the plasma membrane in human fibroblasts. *J Biol Chem.* (1995) 270:18723–6. doi: 10.1074/jbc.270.32.18723
91. Radhakrishnan A, Anderson TG, McConnell HM. Condensed complexes, rafts, and the chemical activity of cholesterol in membranes. *Proc Natl Acad Sci USA.* (2000) 97:12422–7. doi: 10.1073/pnas.220418097
92. Lange Y, Steck TL. Active membrane cholesterol as a physiological effector. *Chem Phys Lipids* (2016) 199:74–93. doi: 10.1016/j.chemphyslip.2016.02.003
93. Widenmaier SB, Snyder NA, Nguyen TB, Arduini A, Lee GY, Arruda AP, et al. NRF1 is an ER membrane sensor that is central to cholesterol homeostasis. *Cell* (2017) 171:1094–109.e15. doi: 10.1016/j.cell.2017.10.003
94. Yamauchi Y, Iwamoto N, Rogers MA, Abe-Dohmae S, Fujimoto T, Chang CCY, et al. Deficiency in the lipid exporter ABCA1 impairs retrograde sterol movement and disrupts sterol sensing at the endoplasmic reticulum. *J Biol Chem.* (2015) 290:23464–77. doi: 10.1074/jbc.M115.662668
95. Infante RE, Radhakrishnan A. Continuous transport of a small fraction of plasma membrane cholesterol to endoplasmic reticulum regulates total cellular cholesterol. *Elife* (2017) 6:e25466. doi: 10.7554/eLife.25466
96. Brown M, Goldstein J. A receptor-mediated pathway for cholesterol homeostasis. *Science* (1986) 232:34–47. doi: 10.1126/science.3513311
97. Sugii S, Reid P, Ohgami N, Du H, Chang T. Distinct endosomal compartments in early trafficking of low density lipoprotein-derived cholesterol. *J Biol Chem.* (2003) 278:27180–9. doi: 10.1074/jbc.M300542200
98. Cruz J, Chang T. Fate of endogenously synthesized cholesterol in Niemann-Pick type C1 cells. *J Biol Chem.* (2000) 275:41309–16. doi: 10.1074/jbc.M008272200
99. Kwon H, Abi-Mosleh L, Wang M, Deisenhofer J, Goldstein J, Brown M, et al. Structure of N-terminal domain of NPC1 reveals distinct subdomains for binding and transfer of cholesterol. *Cell* (2009) 137:1213–24. doi: 10.1016/j.cell.2009.03.049
100. Ohgami N, Ko D, Thomas M, Scott M, Chang C, Chang T. Binding between the Niemann-Pick C1 protein and a photoactivatable cholesterol analog requires a functional sterol-sensing domain. *Proc Natl Acad Sci USA.* (2004) 101:12473–8. doi: 10.1073/pnas.0405255101
101. Wang M, Motamed M, Infante R, Abi-Mosleh L, Kwon H, Brown M, et al. Identification of surface residues on Niemann-Pick C2 essential for hydrophobic handoff of cholesterol to NPC1 in lysosomes. *Cell Metab.* (2010) 12:166–73. doi: 10.1016/j.cmet.2010.05.016
102. Urano Y, Watanabe H, Murphy S, Shibuya Y, Geng Y, Peden A, et al. Transport of LDL-derived cholesterol from the NPC1 compartment to the ER involves the trans-Golgi network and the SNARE protein complex. *Proc Natl Acad Sci USA.* (2008) 105:16513–8. doi: 10.1073/pnas.0807450105
103. Frolov A, Zielinski S, Crowley J, Dudley-Rucker N, Schaffer J, Ory D. NPC1 and NPC2 regulate cellular cholesterol homeostasis through generation of low density lipoprotein cholesterol-derived oxysterols. *J Biol Chem.* (2003) 278:25517–25. doi: 10.1074/jbc.M302588200
104. Chu BB, Liao YC, Qi W, Xie C, Du X, Wang J, et al. Cholesterol transport through lysosome-peroxisome membrane contacts. *Cell* (2015) 161:291–306. doi: 10.1016/j.cell.2015.02.019
105. Choi HY, Karten B, Chan T, Vance JE, Greer WL, Heidenreich RA, et al. Impaired ABCA1-dependent lipid efflux and hypoalphalipoproteinemia in human Niemann-Pick type C disease. *J Biol Chem.* (2003) 278:32569–77. doi: 10.1074/jbc.M304553200
106. Kanerva K, Uronen RL, Blom T, Li S, Bittman R, Lappalainen P, et al. LDL cholesterol recycles to the plasma membrane via a Rab8a-Myosin5b-actin-dependent membrane transport route. *Dev Cell* (2013) 27:249–62. doi: 10.1016/j.devcel.2013.09.016
107. Du X, Kumar J, Ferguson C, Schulz TA, Ong YS, Hong W, et al. A role for oxysterol-binding protein-related protein 5 in endosomal cholesterol trafficking. *J Cell Biol.* (2011) 192:121–35. doi: 10.1083/jcb.201004142

108. Wilhelm LP, Wendling C, Vedie B, Kobayashi T, Chenard MP, Tomasetto C, et al. STARD3 mediates endoplasmic reticulum-to-endosome cholesterol transport at membrane contact sites. *EMBO J.* (2017) 36:1412–33. doi: 10.15252/embj.201695917
109. Abi-Mosleh L, Infante R, Radhakrishnan A, Goldstein J, Brown M. Cyclodextrin overcomes deficient lysosome-to-endoplasmic reticulum transport of cholesterol in Niemann-Pick type C cells. *Proc Natl Acad Sci USA.* (2009) 106:19316–21. doi: 10.1073/pnas.0910916106
110. Jansen M, Ohsaki Y, Rita Rega L, Bittman R, Olkkonen VM, Ikonen E. Role of ORPs in sterol transport from plasma membrane to ER and lipid droplets in mammalian cells. *Traffic* (2011) 12:218–31. doi: 10.1111/j.1600-0854.2010.01142.x
111. Mesmin B, Pipalia NH, Lund FW, Ramlall TF, Sokolov A, Eliezer D, et al. STARD4 abundance regulates sterol transport and sensing. *Mol Biol Cell* (2011) 22:4004–15. doi: 10.1091/mbc.e11-04-0372
112. Takahashi K, Kimura Y, Kioka N, Matsuo M, Ueda K. Purification and ATPase activity of human ABCA1. *J Biol Chem.* (2006) 281:10760–8. doi: 10.1074/jbc.M513783200
113. Vaughan AM, Oram JF. ABCA1 redistributes membrane cholesterol independent of apolipoprotein interactions. *J Lipid Res.* (2003) 44:1373–80. doi: 10.1194/jlr.M300078-JLR200
114. Koseki M, Hirano K, Masuda D, Ikegami C, Tanaka M, Ota A, et al. Increased lipid rafts and accelerated lipopolysaccharide-induced tumor necrosis factor-alpha secretion in Abca1-deficient macrophages. *J Lipid Res.* (2007) 48:299–306. doi: 10.1194/jlr.M600428-JLR200
115. Rigotti A, Miettinen HE, Krieger M. The role of the high-density lipoprotein receptor SR-BI in the lipid metabolism of endocrine and other tissues. *Endocr Rev.* (2003) 24:357–87. doi: 10.1210/er.2001-0037
116. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* (1996) 271:518–20. doi: 10.1126/science.271.5248.518
117. Shen WJ, Azhar S, Kraemer FB. SR-B1: A unique multifunctional receptor for cholesterol influx and efflux. *Annu Rev Physiol.* (2018) 80:95–116. doi: 10.1146/annurev-physiol-021317-121550
118. Terasaka N, Wang N, Yvan-Charvet L, Tall A. High-density lipoprotein protects macrophages from oxidized low-density lipoprotein-induced apoptosis by promoting efflux of 7-ketocholesterol via ABCG1. *Proc Natl Acad Sci USA.* (2007) 104:15093–8. doi: 10.1073/pnas.0704602104
119. Infante R, Abi-Mosleh L, Radhakrishnan A, Dale J, Brown M, Goldstein J. Purified NPC1 protein. I. Binding of cholesterol and oxysterols to a 1278-amino acid membrane protein. *J Biol Chem.* (2008) 283:1052–63. doi: 10.1074/jbc.M707943200
120. Iaea DB, Gale SE, Bielska AA, Krishnan K, Fujiwara H, Jiang H, et al. A novel intrinsically fluorescent probe for study of uptake and trafficking of 25-hydroxycholesterol. *J Lipid Res.* (2015) 56:2408–19. doi: 10.1194/jlr.D064287
121. Dean M, Annilo T. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annu Rev Genomics Hum Genet.* (2005) 6:123–42. doi: 10.1146/annurev.genom.6.080604.162122
122. Tarling EJ, de Aguiar Vallim TQ, Edwards PA. Role of ABC transporters in lipid transport and human disease. *Trends Endocrinol Metab.* (2013) 24:342–50. doi: 10.1016/j.tem.2013.01.006
123. Hara H, Yokoyama S. Interaction of free apolipoproteins with macrophages. Formation of high density lipoprotein-like lipoproteins and reduction of cellular cholesterol. *J Biol Chem.* (1991) 266:3080–6.
124. Francis GA, Knopp RH, Oram JF. Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier Disease. *J Clin Invest.* (1995) 96:78–87. doi: 10.1172/JCI118082
125. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet.* (1999) 22:336–45. doi: 10.1038/11905
126. Bodzioch M, Orsó E, Klucken J, Langmann T, Böttcher A, Diederich W, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Genet.* (1999) 22:347–51. doi: 10.1038/11914
127. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Genet.* (1999) 22:352–5. doi: 10.1038/11921
128. Phillips MC. Is ABCA1 a lipid transfer protein? *J Lipid Res.* (2018) 59:749–63. doi: 10.1194/jlr.R082313
129. Timmins JM, Lee JY, Boudyguina E, Kluckman KD, Brunham LR, Mulya A, et al. Targeted inactivation of hepatic Abca1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. *J Clin Invest.* (2005) 115:1333–42. doi: 10.1172/JCI200523915
130. Brunham LR, Kruit JK, Iqbal J, Fievet C, Timmins JM, Pape TD, et al. Intestinal ABCA1 directly contributes to HDL biogenesis *in vivo*. *J Clin Invest.* (2006) 116:1052–62. doi: 10.1172/JCI27352
131. Schaefer EJ, Zech LA, Schwartz DE, Brewer HB. Coronary heart disease prevalence and other clinical features in familial high-density lipoprotein deficiency (Tangier disease). *Ann Intern Med.* (1980) 93:261–6. doi: 10.7326/0003-4819-93-2-261
132. van Dam MJ, de Groot E, Clee SM, Hovingh GK, Roelants R, Brooks-Wilson A, et al. Association between increased arterial-wall thickness and impairment in ABCA1-driven cholesterol efflux: an observational study. *Lancet* (2002) 359:37–42. doi: 10.1016/S0140-6736(02)07277-X
133. Assmann G, von Eckardstein A, Brewer HB. Familial analphalipoproteinemia: Tangier disease. In: Scriver RC, Beaydet LA, Sly SW, Valle D, editors. *The Metabolic & Molecular Bases of Inherited Disease. II.* 8th ed. New York, NY: McGraw-Hill (2001). p. 2937–60.
134. Denis M, Landry YD, Zha X. ATP-binding cassette A1-mediated lipidation of apolipoprotein A-I occurs at the plasma membrane and not in the endocytic compartments. *J Biol Chem.* (2008) 283:16178–86. doi: 10.1074/jbc.M709597200
135. Lu R, Arakawa R, Ito-Osumi C, Iwamoto N, Yokoyama S. ApoA-I facilitates ABCA1 recycle/accumulation to cell surface by inhibiting its intracellular degradation and increases HDL generation. *Arterioscler Thromb Vasc Biol.* (2008) 28:1820–4. doi: 10.1161/ATVBAHA.108.169482
136. Vedhachalam C, Duong PT, Nickel M, Nguyen D, Dhanasekaran P, Saito H, et al. Mechanism of ATP-binding cassette transporter A1-mediated cellular lipid efflux to apolipoprotein A-I and formation of high density lipoprotein particles. *J Biol Chem.* (2007) 282:25123–30. doi: 10.1074/jbc.M704590200
137. Landry YD, Denis M, Nandi S, Bell S, Vaughan AM, Zha X. ATP-binding cassette transporter A1 expression disrupts raft membrane microdomains through its ATPase-related functions. *J Biol Chem.* (2006) 281:36091–101. doi: 10.1074/jbc.M602247200
138. Zhu X, Lee JY, Timmins JM, Brown JM, Boudyguina E, Mulya A, et al. Increased cellular free cholesterol in macrophage-specific Abca1 knock-out mice enhances pro-inflammatory response of macrophages. *J Biol Chem.* (2008) 283:22930–41. doi: 10.1074/jbc.M801408200
139. Quazi F, Molday RS. Differential phospholipid substrates and directional transport by ATP-binding cassette proteins ABCA1, ABCA7, and ABCA4 and disease-causing mutants. *J Biol Chem.* (2013) 288:34414–26. doi: 10.1074/jbc.M113.508812
140. Gulshan K, Brubaker G, Conger H, Wang S, Zhang R, Hazen SL, et al. PI(4,5)P2 is translocated by ABCA1 to the cell surface where it mediates apolipoprotein A1 binding and nascent HDL assembly. *Circ Res.* (2016) 119:827–38. doi: 10.1161/CIRCRESAHA.116.308856
141. Yamauchi Y, Abe-Dohmae S, Yokoyama S. Differential regulation of apolipoprotein A-I/ATP binding cassette transporter Al-mediated cholesterol and phospholipid release. *Biochim Biophys Acta Mol Cell Biol Lipids* (2002) 1585:1–10. doi: 10.1016/S1388-1981(02)00304-9
142. Yamauchi Y, Chang CCY, Hayashi M, Abe-Dohmae S, Reid PC, Chang TY, et al. Intracellular cholesterol mobilization involved in the ABCA1/apolipoprotein-mediated assembly of high density lipoprotein in fibroblasts. *J Lipid Res.* (2004) 45:1943–51. doi: 10.1194/jlr.M400264-JLR200
143. Ouimet M, Franklin V, Mak E, Liao X, Tabas I, Marcel YL. Autophagy regulates cholesterol efflux from macrophage foam cells via lysosomal acid lipase. *Cell Metab.* (2011) 13:655–67. doi: 10.1016/j.cmet.2011.03.023
144. Yamauchi Y, Yokoyama S, Chang T-Y. ABCA1-dependent sterol release: sterol molecule specificity and potential membrane domain for HDL biogenesis. *J Lipid Res.* (2016) 57:77–88. doi: 10.1194/jlr.M063784
145. Bacia K, Schwille P, Kurzchalia T. Sterol structure determines the separation of phases and the curvature of the liquid-ordered phase in model membranes. *Proc Natl Acad Sci USA.* (2005) 102:3272–7. doi: 10.1073/pnas.0408215102
146. Chen W, Sun Y, Welch C, Gorelik A, Leventhal A, Tabas I, et al. Preferential ATP-binding cassette transporter A1-mediated cholesterol

efflux from late endosomes/lysosomes. *J Biol Chem.* (2001) 276:43564–9. doi: 10.1074/jbc.M107938200
147. Qian H, Zhao X, Cao P, Lei J, Yan N, Gong X. Structure of the human lipid Exporter ABCA1. *Cell* (2017) 169:1228–39 e10. doi: 10.1016/j.cell.2017.05.020
148. Costet P, Luo Y, Wang N, Tall A. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J Biol Chem.* (2000) 275:28240–5. doi: 10.1074/jbc.M003337200
149. Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA, et al. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. *Proc Natl Acad Sci USA.* (2000) 97:12097–102. doi: 10.1073/pnas.200367697
150. Feinberg MW, Moore KJ. MicroRNA regulation of atherosclerosis. *Circ Res.* (2016) 118:703–20. doi: 10.1161/CIRCRESAHA.115.306300
151. Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, et al. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. *Science* (2010) 328:1566–9. doi: 10.1126/science.1189123
152. Rayner KJ, Suarez Y, Davalos A, Parathath S, Fitzgerald ML, Tamehiro N, et al. MiR-33 contributes to the regulation of cholesterol homeostasis. *Science* (2010) 328:1570–3. doi: 10.1126/science.1189862
153. Rayner KJ, Esau CC, Hussain FN, McDaniel AL, Marshall SM, van Gils JM, et al. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. *Nature* (2011) 478:404–7. doi: 10.1038/nature10486
154. Wang N, Chen W, Linsel-Nitschke P, Martinez L, Agerholm-Larsen B, Silver D, et al. A PEST sequence in ABCA1 regulates degradation by calpain protease and stabilization of ABCA1 by apoA-I. *J Clin Invest.* (2003) 111:99–107. doi: 10.1172/JCI200316808
155. Munehira Y, Ohnishi T, Kawamoto S, Furuya A, Shitara K, Imamura M, et al. Alpha1-syntrophin modulates turnover of ABCA1. *J Biol Chem.* (2004) 279:15091–5. doi: 10.1074/jbc.M313436200
156. Arakawa R, Yokoyama S. Helical apolipoproteins stabilize ATP-binding cassette transporter A1 by protecting it from thiol protease-mediated degradation. *J Biol Chem.* (2002) 277:22426–9. doi: 10.1074/jbc.M202996200
157. Ogura M, Ayaori M, Terao Y, Hisada T, Iizuka M, Takiguchi S, et al. Proteasomal inhibition promotes ATP-binding cassette transporter A1 (ABCA1) and ABCG1 expression and cholesterol efflux from macrophages *in vitro* and *in vivo*. *Arterioscler Thromb Vasc Biol.* (2011) 31:1980–7. doi: 10.1161/ATVBAHA.111.228478
158. Feng B, Tabas I. ABCA1-mediated cholesterol efflux is defective in free cholesterol-loaded macrophages. Mechanism involves enhanced ABCA1 degradation in a process requiring full NPC1 activity. *J Biol Chem.* (2002) 277:43271–80. doi: 10.1074/jbc.M207532200
159. Mizuno T, Hayashi H, Naoi S, Sugiyama Y. Ubiquitination is associated with lysosomal degradation of cell surface-resident ATP-binding cassette transporter A1 (ABCA1) through the endosomal sorting complex required for transport (ESCRT) pathway. *Hepatology* (2011) 54:631–43. doi: 10.1002/hep.24387
160. Wang Y, Oram JF. Unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ATP-binding cassette transporter A1. *J Biol Chem.* (2002) 277:5692–7. doi: 10.1074/jbc.M109977200
161. Mizuno T, Hayashi H, Kusuhara H. Cellular cholesterol accumulation facilitates ubiquitination and lysosomal degradation of cell surface-resident ABCA1. *Arterioscler Thromb Vasc Biol.* (2015) 35:1347–56. doi: 10.1161/ATVBAHA.114.305182
162. Linder M, Mäyränpää M, Peränen J, Pietilä T, Pietiäinen V, Uronen R, et al. Rab8 regulates ABCA1 cell surface expression and facilitates cholesterol efflux in primary human macrophages. *Arterioscler Thromb Vasc Biol.* (2009) 29:883–8. doi: 10.1161/ATVBAHA.108.179481
163. Okuhira K, Fitzgerald ML, Sarracino DA, Manning JJ, Bell SA, Goss JL, et al. Purification of ATP-binding cassette transporter A1 and associated binding proteins reveals the importance of beta1-syntrophin in cholesterol efflux. *J Biol Chem.* (2005) 280:39653–64. doi: 10.1074/jbc.M510187200
164. Hozoji M, Munehira Y, Ikeda Y, Makishima M, Matsuo M, Kioka N, et al. Direct interaction of nuclear liver X receptor-beta with ABCA1 modulates cholesterol efflux. *J Biol Chem.* (2008) 283:30057–63. doi: 10.1074/jbc.M804599200
165. Iwamoto N, Lu R, Tanaka N, Abe-Dohmae S, Yokoyama S. Calmodulin interacts with ATP binding cassette transporter A1 to protect from calpain-mediated degradation and upregulates high-density lipoprotein generation. *Arterioscler Thromb Vasc Biol.* (2010) 30:1446–52. doi: 10.1161/ATVBAHA.110.203927
166. See RH, Caday-Malcolm RA, Singaraja RR, Zhou S, Silverston A, Huber MT, et al. Protein kinase A site-specific phosphorylation regulates ATP-binding cassette A1 (ABCA1)-mediated phospholipid efflux. *J Biol Chem.* (2002) 277:41835–42. doi: 10.1074/jbc.M204923200
167. Haidar B, Denis M, Marcil M, Krimbou L, Genest J Jr, Apolipoprotein AI activates cellular cAMP signaling through the ABCA1 transporter. *J Biol Chem.* (2004) 279:9963–9. doi: 10.1074/jbc.M313487200
168. Yamauchi Y, Hayashi M, Abe-Dohmae S, Yokoyama S. Apolipoprotein A-I activates protein kinase C alpha signaling to phosphorylate and stabilize ATP binding cassette transporter A1 for the high density lipoprotein assembly. *J Biol Chem.* (2003) 278:47890–7. doi: 10.1074/jbc.M306258200
169. Arakawa R, Hayashi M, Remaley A, Brewer B, Yamauchi Y, Yokoyama S. Phosphorylation and stabilization of ATP binding cassette transporter a1 by synthetic Amphiphilic helical peptides. *J Biol Chem.* (2004) 279:6217–20. doi: 10.1074/jbc.C300553200
170. Tang C, Vaughan AM, Oram JF. Janus kinase 2 modulates the apolipoprotein interactions with ABCA1 required for removing cellular cholesterol. *J Biol Chem.* (2004) 279:7622–8. doi: 10.1074/jbc.M312571200
171. Tang C, Vaughan AM, Anantharamaiah GM, Oram JF. Janus kinase 2 modulates the lipid-removing but not protein-stabilizing interactions of amphipathic helices with ABCA1. *J Lipid Res.* (2006) 47:107–14. doi: 10.1194/jlr.M500240-JLR200
172. Liu Y, Tang C. Regulation of ABCA1 functions by signaling pathways. *Biochim Biophys Acta* (2012) 1821:522–9. doi: 10.1016/j.bbalip.2011.08.015
173. Wang N, Lan D, Chen W, Matsuura F, Tall A. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc Natl Acad Sci USA.* (2004) 101:9774–9. doi: 10.1073/pnas.0403506101
174. Kobayashi A, Takanezawa Y, Hirata T, Shimizu Y, Misasa K, Kioka N, et al. Efflux of sphingomyelin, cholesterol, and phosphatidylcholine by ABCG1. *J Lipid Res.* (2006) 47:1791–802. doi: 10.1194/jlr.M500546-JLR200
175. Wang N, Ranalletta M, Matsuura F, Peng F, Tall A. LXR-induced redistribution of ABCG1 to plasma membrane in macrophages enhances cholesterol mass efflux to HDL. *Arterioscler Thromb Vasc Biol.* (2006) 26:1310–6. doi: 10.1161/01.ATV.0000218998.75963.02
176. Tarling EJ, Edwards PA. ATP binding cassette transporter G1 (ABCG1) is an intracellular sterol transporter. *Proc Natl Acad Sci USA.* (2011) 108:19719–24. doi: 10.1073/pnas.1113021108
177. Kennedy MA, Barrera GC, Nakamura K, Baldán A, Tarr P, Fishbein MC, et al. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. *Cell Metab.* (2005) 1:121–31. doi: 10.1016/j.cmet.2005.01.002
178. Hirayama H, Kimura Y, Kioka N, Matsuo M, Ueda K. ATPase activity of human ABCG1 is stimulated by cholesterol and sphingomyelin. *J Lipid Res.* (2013) 54:496–502. doi: 10.1194/jlr.M033209
179. Yvan-Charvet L, Ranalletta M, Wang N, Han S, Terasaka N, Li R, et al. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. *J Clin Invest.* (2007) 117:3900–8. doi: 10.1172/JCI33372
180. Wang N, Yvan-Charvet L, Lütjohann D, Mulder M, Vanmierlo T, Kim T, et al. ATP-binding cassette transporters G1 and G4 mediate cholesterol and desmosterol efflux to HDL and regulate sterol accumulation in the brain. *FASEB J.* (2008) 22:1073–82. doi: 10.1096/fj.07-9944com
181. Iqbal J, Hussain MM. Intestinal lipid absorption. *Am J Physiol Endocrinol Metab.* (2009) 296:E1183–94. doi: 10.1152/ajpendo.90899.2008
182. Yu L, Hammer RE, Li-Hawkins J, Von Bergmann K, Lutjohann D, Cohen JC, et al. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci USA.* (2002) 99:16237–42. doi: 10.1073/pnas.252582399
183. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* (2000) 290:1771–5. doi: 10.1126/science.290.5497.1771

184. Lee MH, Lu K, Hazard S, Yu H, Shulenin S, Hidaka H, et al. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat Genet.* (2001) 27:79–83. doi: 10.1038/83799
185. Kidambi S, Patel SB. Sitosterolaemia: pathophysiology, clinical presentation and laboratory diagnosis. *J Clin Pathol.* (2008) 61:588–94. doi: 10.1136/jcp.2007.049775
186. Zhao QF, Yu JT, Tan MS, Tan L. ABCA7 in Alzheimer's disease. *Mol Neurobiol.* (2015) 51:1008–16. doi: 10.1007/s12035-014-8759-9
187. Wang N, Lan D, Gerbod-Giannone M, Linsel-Nitschke P, Jehle A, Chen W, et al. ATP-binding cassette transporter A7 (ABCA7) binds apolipoprotein A-I and mediates cellular phospholipid but not cholesterol efflux. *J Biol Chem.* (2003) 278:42906–12. doi: 10.1074/jbc.M307831200
188. Abe-Dohmae S, Ikeda Y, Matsuo M, Hayashi M, Okuhira K, Ueda K, et al. Human ABCA7 supports apolipoprotein-mediated release of cellular cholesterol and phospholipid to generate high density lipoprotein. *J Biol Chem.* (2004) 279:604–11. doi: 10.1074/jbc.M309888200
189. Hayashi M, Abe-Dohmae S, Okazaki M, Ueda K, Yokoyama S. Heterogeneity of high density lipoprotein generated by ABCA1 and ABCA7. *J Lipid Res.* (2005) 46:1703–11. doi: 10.1194/jlr.M500092-JLR200
190. Kim WS, Fitzgerald ML, Kang K, Okuhira K, Bell SA, Manning JJ, et al. Abca7 null mice retain normal macrophage phosphatidylcholine and cholesterol efflux activity despite alterations in adipose mass and serum cholesterol levels. *J Biol Chem.* (2005) 280:3989–95. doi: 10.1074/jbc.M412602200
191. Jehle AW, Gardai SJ, Li S, Linsel-Nitschke P, Morimoto K, Janssen WJ, et al. ATP-binding cassette transporter A7 enhances phagocytosis of apoptotic cells and associated ERK signaling in macrophages. *J Cell Biol.* (2006) 174:547–56. doi: 10.1083/jcb.200601030
192. Trigueros-Motos L, van Capelleveen JC, Torta F, Castano D, Zhang LH, Chai EC, et al. ABCA8 Regulates Cholesterol Efflux and High-Density Lipoprotein Cholesterol Levels. *Arterioscler Thromb Vasc Biol.* (2017) 37:2147–55. doi: 10.1161/ATVBAHA.117.309574
193. Fu Y, Mukhamedova N, Ip S, D'Souza W, Henley KJ, DiTommaso T, et al. ABCA12 regulates ABCA1-dependent cholesterol efflux from macrophages and the development of atherosclerosis. *Cell Metab.* (2013) 18:225–38. doi: 10.1016/j.cmet.2013.07.003
194. Akiyama M, Sugiyama-Nakagiri Y, Sakai K, McMillan JR, Goto M, Arita K, et al. Mutations in lipid transporter ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer. *J Clin Invest.* (2005) 115:1777–84. doi: 10.1172/JCI24834
195. Davis W, Tew KD. ATP-binding cassette transporter-2 (ABCA2) as a therapeutic target. *Biochem Pharmacol.* (2018) 151:188–200. doi: 10.1016/j.bcp.2017.11.018
196. Garrigues A, Escargueil AE, Orlowski S. The multidrug transporter, P-glycoprotein, actively mediates cholesterol redistribution in the cell membrane. *Proc Natl Acad Sci USA.* (2002) 99:10347–52. doi: 10.1073/pnas.162366399
197. Morita SY, Kobayashi A, Takanezawa Y, Kioka N, Handa T, Arai H, et al. Bile salt-dependent efflux of cellular phospholipids mediated by ATP binding cassette protein B4. *Hepatology* (2007) 46:188–99. doi: 10.1002/hep.21591
198. Rothblat GH, de la Llera-Moya M, Atger V, Kellner-Weibel G, Williams DL, Phillips MC. Cell cholesterol efflux: integration of old and new observations provides new insights. *J Lipid Res.* (1999) 40:781–96.
199. Phillips MC. Molecular mechanisms of cellular cholesterol efflux. *J Biol Chem.* (2014) 289:24020–9. doi: 10.1074/jbc.R114.583658
200. Czarnecka H, Yokoyama S. Regulation of cellular cholesterol efflux by lecithin:cholesterol acyltransferase reaction through nonspecific lipid exchange. *J Biol Chem.* (1996) 271:2023–8. doi: 10.1074/jbc.271.4.2023
201. Chang TY, Yamauchi Y, Hasan MT, Chang C. Cellular cholesterol homeostasis and Alzheimer's disease. *J Lipid Res.* (2017) 58:2239–54. doi: 10.1194/jlr.R075630
202. Spann NJ, Glass CK. Sterols and oxysterols in immune cell function. *Nat Immunol.* (2013) 14:893–900. doi: 10.1038/ni.2681
203. Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology - divergent pathophysiology. *Nat Rev Endocrinol.* (2017) 13:710–30. doi: 10.1038/nrendo.2017.91
204. Ridker PM. Residual inflammatory risk: addressing the obverse side of the atherosclerosis prevention coin. *Eur Heart J.* (2016) 37:1720–2. doi: 10.1093/eurheartj/ehw024
205. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, et al. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med.* (2015) 372:2387–97. doi: 10.1056/NEJMoa1410489
206. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med.* (2017) 376:1713–22. doi: 10.1056/NEJMoa1615664
207. Gaudet D, Gipe DA, Pordy R, Ahmad Z, Cuchel M, Shah PK, et al. ANGPTL3 inhibition in homozygous familial hypercholesterolemia. *N Engl J Med.* (2017) 377:296–7. doi: 10.1056/NEJMc1705994
208. Zanoni P, Khetarpal SA, Larach DB, Hancock-Cerutti WF, Millar JS, Cuchel M, et al. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. *Science* (2016) 351:1166–71. doi: 10.1126/science.aad3517
209. Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2012) 32:2045–51. doi: 10.1161/ATVBAHA.108.179705
210. Spann NJ, Garmire LX, McDonald JG, Myers DS, Milne SB, Shibata N, et al. Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. *Cell* (2012) 151:138–52. doi: 10.1016/j.cell.2012.06.054
211. Calkin AC, Tontonoz P. Liver x receptor signaling pathways and atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2010) 30:1513–8. doi: 10.1161/ATVBAHA.109.191197
212. Repa JJ, Mangelsdorf DJ. The liver X receptor gene team: potential new players in atherosclerosis. *Nat Med.* (2002) 8:1243–8. doi: 10.1038/nm1102-1243
213. Brown AJ, Jessup W. Oxysterols and atherosclerosis. *Atherosclerosis* (1999) 142:1–28. doi: 10.1016/S0021-9150(98)00196-8
214. Zmyslowski A, Szterk A. Current knowledge on the mechanism of atherosclerosis and pro-atherosclerotic properties of oxysterols. *Lipids Health Dis.* (2017) 16:188. doi: 10.1186/s12944-017-0579-2
215. Rong JX, Blachford C, Feig JE, Bander I, Mayne J, Kusunoki J, et al. ACAT inhibition reduces the progression of preexisting, advanced atherosclerotic mouse lesions without plaque or systemic toxicity. *Arterioscler Thromb Vasc Biol.* (2013) 33:4–12. doi: 10.1161/ATVBAHA.112.252056
216. Yagyu H, Kitamine T, Osuga J, Tozawa R, Chen Z, Kaji Y, et al. Absence of ACAT-1 attenuates atherosclerosis but causes dry eye and cutaneous xanthomatosis in mice with congenital hyperlipidemia. *J Biol Chem.* (2000) 275:21324–30. doi: 10.1074/jbc.M002541200
217. Huang LH, Melton EM, Li H, Sohn P, Rogers MA, Mulligan-Kehoe MJ, et al. Myeloid Acyl-CoA:cholesterol acyltransferase 1 deficiency reduces lesion macrophage content and suppresses atherosclerosis progression. *J Biol Chem.* (2016) 291:6232–44. doi: 10.1074/jbc.M116.713818
218. Hai Q, Ritchey B, Robinet P, Alzayed AM, Brubaker G, Zhang J, et al. Quantitative trait locus mapping of macrophage cholesterol metabolism and CRISPR/Cas9 editing implicate an ACAT1 truncation as a causal modifier variant. *Arterioscler Thromb Vasc Biol.* (2018) 38:83–91. doi: 10.1161/ATVBAHA.117.310173
219. Nissen SE, Tuzcu EM, Brewer HB, Sipahi I, Nicholls SJ, Ganz P, et al. Effect of ACAT inhibition on the progression of coronary atherosclerosis. *N Engl J Med.* (2006) 354:1253–63. doi: 10.1056/NEJMoa054699
220. Ghisletti S, Huang W, Ogawa S, Pascual G, Lin ME, Willson TM, et al. Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. *Mol Cell* (2007) 25:57–70. doi: 10.1016/j.molcel.2006.11.022
221. Vengrenyuk Y, Carlier S, Xanthos S, Cardoso L, Ganatos P, Virmani R, et al. A hypothesis for vulnerable plaque rupture due to stress-induced debonding around cellular microcalcifications in thin fibrous caps. *Proc Natl Acad Sci USA.* (2006) 103:14678–83. doi: 10.1073/pnas.0606310103
222. Watson KE, Bostrom K, Ravindranath R, Lam T, Norton B, Demer LL. TGF-beta 1 and 25-hydroxycholesterol stimulate osteoblast-like vascular cells to calcify. *J Clin Invest.* (1994) 93:2106–13. doi: 10.1172/JCI117205
223. Hsu JJ, Lu J, Huang MS, Geng Y, Sage AP, Bradley MN, et al. T0901317, an LXR agonist, augments PKA-induced vascular cell calcification. *FEBS Lett.* (2009) 583:1344–8. doi: 10.1016/j.febslet.2009.03.039
224. Bjorkhem I. Are side-chain oxidized oxysterols regulators also *in vivo*? *J Lipid Res.* (2009) 50(Suppl.):S213–8. doi: 10.1194/jlr.R800025-JLR200

225. Umetani M, Ghosh P, Ishikawa T, Umetani J, Ahmed M, Mineo C, et al. The cholesterol metabolite 27-hydroxycholesterol promotes atherosclerosis via proinflammatory processes mediated by estrogen receptor alpha. *Cell Metab.* (2014) 20:172–82. doi: 10.1016/j.cmet.2014.05.013
226. Zurkinden L, Solca C, Vogeli IA, Vogt B, Ackermann D, Erickson SK, et al. Effect of Cyp27A1 gene dosage on atherosclerosis development in ApoE-knockout mice. *FASEB J.* (2014) 28:1198–209. doi: 10.1096/fj.13-233791
227. Meir K, Kitsberg D, Alkalay I, Szafer F, Rosen H, Shpitzen S, et al. Human sterol 27-hydroxylase (CYP27) overexpressor transgenic mouse model. Evidence against 27-hydroxycholesterol as a critical regulator of cholesterol homeostasis. *J Biol Chem.* (2002) 277:34036–41. doi: 10.1074/jbc.M201122200
228. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab.* (2016) 23:27–47. doi: 10.1016/j.cmet.2015.12.006
229. Rohrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. *Nat Rev Cancer* (2016) 16:732–49. doi: 10.1038/nrc.2016.89
230. Kuzu OF, Noory MA, Robertson GP. The role of cholesterol in cancer. *Cancer Res.* (2016) 76:2063–70. doi: 10.1158/0008-5472.CAN-15-2613
231. Guo D, Prins R, Dang J, Kuga D, Iwanami A, Soto H, et al. EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to antilipogenic therapy. *Sci Signal.* (2009) 2:ra82. doi: 10.1126/scisignal.2000446
232. Ettinger S, Sobel R, Whitmore T, Akbari M, Bradley D, Gleave M, et al. Dysregulation of sterol response element-binding proteins and downstream effectors in prostate cancer during progression to androgen independence. *Cancer Res.* (2004) 64:2212–21. doi: 10.1158/0008-5472.CAN-2148-2
233. Pitroda S, Khodarev N, Beckett M, Kufe D, Weichselbaum R. MUC1-induced alterations in a lipid metabolic gene network predict response of human breast cancers to tamoxifen treatment. *Proc Natl Acad Sci USA*. (2009) 106:5837–41. doi: 10.1073/pnas.0812029106
234. Yamauchi Y, Furukawa K, Hamamura K, Furukawa K. Positive feedback loop between PI3K-Akt-mTORC1 signaling and the lipogenic pathway boosts Akt signaling: induction of the lipogenic pathway by a melanoma antigen. *Cancer Res.* (2011) 71:4989–97. doi: 10.1158/0008-5472.CAN-10-4108
235. Porstmann T, Santos C, Griffiths B, Cully M, Wu M, Leevers S, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab.* (2008) 8:224–36. doi: 10.1016/j.cmet.2008.07.007
236. Düvel K, Yecies J, Menon S, Raman P, Lipovsky A, Souza A, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* (2010) 39:171–83. doi: 10.1016/j.molcel.2010.06.022
237. Wang H, Altomare D, Skele K, Poulikakos P, Kuhajda F, Di Cristofano A, et al. Positive feedback regulation between AKT activation and fatty acid synthase expression in ovarian carcinoma cells. *Oncogene* (2005) 24:3574–82. doi: 10.1038/sj.onc.1208463
238. Sundqvist A, Bengoechea-Alonso MT, Ye X, Lukiyanchuk V, Jin J, Harper JW, et al. Control of lipid metabolism by phosphorylation-dependent degradation of the SREBP family of transcription factors by SCF(Fbw7). *Cell Metab.* (2005) 1:379–91. doi: 10.1016/j.cmet.2005.04.010
239. Han J, Li E, Chen L, Zhang Y, Wei F, Liu J, et al. The CREB coactivator CRTC2 controls hepatic lipid metabolism by regulating SREBP1. *Nature* (2015) 524:243–6. doi: 10.1038/nature14557
240. Owen JL, Zhang Y, Bae SH, Farooqi MS, Liang G, Hammer RE, et al. Insulin stimulation of SREBP-1c processing in transgenic rat hepatocytes requires p70 S6-kinase. *Proc Natl Acad Sci USA*. (2012) 109:16184–9. doi: 10.1073/pnas.1213343109
241. Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, Balderas E, et al. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. *Cell* (2011) 146:408–20. doi: 10.1016/j.cell.2011.06.034
242. Demierre M, Higgins P, Gruber S, Hawk E, Lippman S. Statins and cancer prevention. *Nat Rev Cancer* (2005) 5:930–42. doi: 10.1038/nrc1751
243. Nielsen SF, Nordestgaard BG, Bojesen SE. Statin use and reduced cancer-related mortality. *N Engl J Med*. (2012) 367:1792–802. doi: 10.1056/NEJMoa1201735
244. Ahern TP, Lash TL, Damkier P, Christiansen PM, Cronin-Fenton DP. Statins and breast cancer prognosis: evidence and opportunities. *Lancet Oncol.* (2014) 15(10):e461–8. doi: 10.1016/S1470-2045(14)70119-6
245. Yue S, Li J, Lee SY, Lee HJ, Shao T, Song B, et al. Cholesteryl ester accumulation induced by PTEN loss and PI3K/AKT activation underlies human prostate cancer aggressiveness. *Cell Metab.* (2014) 19:393–406. doi: 10.1016/j.cmet.2014.01.019
246. Li J, Gu D, Lee SS, Song B, Bandyopadhyay S, Chen S, et al. Abrogating cholesterol esterification suppresses growth and metastasis of pancreatic cancer. *Oncogene* (2016) 35:6378–88. doi: 10.1038/onc.2016.168
247. Yang W, Bai Y, Xiong Y, Zhang J, Chen S, Zheng X, et al. Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. *Nature* (2016) 531:651–5. doi: 10.1038/nature17412
248. Kidani Y, Elsaesser H, Hock MB, Vergnes L, Williams KJ, Argus JP, et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat Immunol.* (2013) 14:489–99. doi: 10.1038/ni.2570
249. McDonnell DP, Park S, Goulet MT, Jasper J, Wardell SE, Chang CY, et al. Obesity, cholesterol metabolism, and breast cancer pathogenesis. *Cancer Res.* (2014) 74:4976–82. doi: 10.1158/0008-5472.CAN-14-1756
250. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, et al. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* (2013) 342:1094–8. doi: 10.1126/science.1241908
251. Wu Q, Ishikawa T, Sirianni R, Tang H, McDonald JG, Yuhanna IS, et al. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Rep.* (2013) 5:637–45. doi: 10.1016/j.celrep.2013.10.006
252. Baek AE, Yu YA, He S, Wardell SE, Chang CY, Kwon S, et al. The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. *Nat Commun.* (2017) 8:864. doi: 10.1038/s41467-017-00910-z
253. Alfaqih MA, Nelson ER, Liu W, Safi R, Jasper JS, Macias E, et al. CYP27A1 loss dysregulates cholesterol homeostasis in prostate cancer. *Cancer Res.* (2017) 77:1662–73. doi: 10.1158/0008-5472.CAN-16-2738
254. Smith B, Land H. Anticancer activity of the cholesterol exporter ABCA1 gene. *Cell Rep.* (2012) 2:580–90. doi: 10.1016/j.celrep.2012.08.011
255. Lee BH, Taylor MG, Robinet P, Smith JD, Schweitzer J, Sehayek E, et al. Dysregulation of cholesterol homeostasis in human prostate cancer through loss of ABCA1. *Cancer Res.* (2013) 73:1211–8. doi: 10.1158/0008-5472.CAN-12-3128
256. Guo D, Reinitz F, Youssef M, Hong C, Nathanson D, Akhavan D, et al. An LXR agonist promotes glioblastoma cell death through inhibition of an EGFR/AKT/SREBP-1/LDLR-dependent pathway. *Cancer Discov.* (2011) 1:442–56. doi: 10.1158/2159-8290.CD-11-0102
257. Villa GR, Hulce JJ, Zanca C, Bi J, Ikegami S, Cahill GL, et al. An LXR-cholesterol axis creates a metabolic co-dependency for brain cancers. *Cancer Cell* (2016) 30:683–93. doi: 10.1016/j.ccell.2016.09.008
258. Pencheva N, Buss CG, Posada J, Merghoub T, Tavazoie SF. Broad-spectrum therapeutic suppression of metastatic melanoma through nuclear hormone receptor activation. *Cell* (2014) 156:986–1001. doi: 10.1016/j.cell.2014.01.038
259. Gabitova L, Restifo D, Gorin A, Manocha K, Handorf E, Yang DH, et al. Endogenous sterol metabolites regulate growth of EGFR/KRAS-dependent tumors via LXR. *Cell Rep.* (2015) 12:1927–38. doi: 10.1016/j.celrep.2015.08.023
260. Hedditch EL, Gao B, Russell AJ, Lu Y, Emmanuel C, Beesley J, et al. ABCA transporter gene expression and poor outcome in epithelial ovarian cancer. *J Natl Cancer Inst.* (2014) 106:dju149. doi: 10.1093/jnci/dju149
261. Frechin M, Stoeger T, Daetwyler S, Gehin C, Battich N, Damm EM, et al. Cell-intrinsic adaptation of lipid composition to local crowding drives social behaviour. *Nature* (2015) 523:88–91. doi: 10.1038/nature14429
262. Liu SL, Sheng R, Jung JH, Wang L, Stec E, O'Connor MJ, et al. Orthogonal lipid sensors identify transbilayer asymmetry of plasma membrane cholesterol. *Nat Chem Biol.* (2017) 13:268–74. doi: 10.1038/nchembio.2268
263. Sag D, Cekic C, Wu R, Linden J, Hedrick CC. The cholesterol transporter ABCG1 links cholesterol homeostasis and tumour immunity. *Nat Commun.* (2015) 6:6354. doi: 10.1038/ncomms7354
264. Zamanian-Daryoush M, Lindner DJ, DiDonato JA, Wagner M, Buffa J, Rayman P, et al. Myeloid-specific genetic ablation of ATP-binding cassette

transporter ABCA1 is protective against cancer. *Oncotarget* (2017) 8:71965–80. doi: 10.18632/oncotarget.18666

265. Koga Y, Ishikawa S, Nakamura T, Masuda T, Nagai Y, Takamori H, et al. Oxysterol binding protein-related protein-5 is related to invasion and poor prognosis in pancreatic cancer. *Cancer Sci.* (2008) 99:2387–94. doi: 10.1111/j.1349-7006.2008.00987.x
266. Du X, Zadoorian A, Lukmantara IE, Qi Y, Brown AJ, Yang H. Oxysterol-binding protein-related protein 5 (ORP5) promotes cell proliferation by activation of mTORC1 signaling. *J Biol Chem.* (2018) 293:3806–18. doi: 10.1074/jbc.RA117.001558

Conflict of Interest Statement: 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 Endocrinology

MINI REVIEW
published: 30 November 2018
doi: 10.3389/fendo.2018.00715

HDL in Endocrine Carcinomas: Biomarker, Drug Carrier, and Potential Therapeutic

Emily E. Morin[1,2]*, *Xiang-An Li*[3] and *Anna Schwendeman*[1,2]*

[1] Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI, United States, [2] BioInterfaces Institute, University of Michigan, Ann Arbor, MI, United States, [3] Department of Physiology, Saha Cardiovascular Research Center, College of Medicine, University of Kentucky, Lexington, KY, United States

High-density lipoprotein (HDL) have long been studied for their protective role against cardiovascular diseases, however recently relationship between HDL and cancer came into focus. Several epidemiological studies have shown an inverse correlation between HDL-cholesterol (HDL-C) and cancer risk, and some have even implied that HDL-C can be used as a predictive measure for survival prognosis in for specific sub-population of certain types of cancer. HDL itself is an endogenous nanoparticle capable of removing excess cholesterol from the periphery and returning it to the liver for excretion. One of the main receptors for HDL, scavenger receptor type B-I (SR-BI), is highly upregulated in endocrine cancers, notably due to the high demand for cholesterol by cancer cells. Thus, the potential to exploit administration of cholesterol-free reconstituted or synthetic HDL (sHDL) to deplete cholesterol in endocrine cancer cell and stunt their growth of use chemotherapeutic drug loaded sHDL to target payload delivery to cancer cell has become increasingly attractive. This review focuses on the role of HDL and HDL-C in cancer and application of sHDLs as endocrine cancer therapeutics.

Keywords: High-density lipoprotein (HDL), Apolipoprotein A-I (ApoA-I), endocrine cancer, cholesterol, cancer therapy

OPEN ACCESS

Edited by:
Vincenzo Pezzi,
Università della Calabria, Italy

Reviewed by:
Paolo Magni,
Università degli Studi di Milano, Italy
Eva Surmacz,
Temple University, United States

***Correspondence:**
Emily E. Morin
emmorin@med.umich.edu
Anna Schwendeman
annaschw@med.umich.edu

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *22 September 2018*
Accepted: *12 November 2018*
Published: *30 November 2018*

Citation:
Morin EE, Li X-A and Schwendeman A (2018) HDL in Endocrine Carcinomas: Biomarker, Drug Carrier, and Potential Therapeutic. Front. Endocrinol. 9:715. doi: 10.3389/fendo.2018.00715

INTRODUCTION

Endocrine cancers are defined as those affecting the hormone secreting tissues of our body, including cancers of the adrenal, thyroid, parathyroid, prostate, pancreatic, and reproductive tissues. A rare subset of endocrine cancers, called neuroendocrine tumors (NETs), are neoplasms originating in endocrine tissue that migrate to form hormone-secreting tumors in other organs of the body, including intestine, lung, and pancreas (1). While specific molecular signatures may vary among the different types of endocrine cancers, they all share a common modality which is essential for tumor cell proliferation and overall survival: a high demand for cholesterol (2–4).

Cholesterol is a precursor molecule for steroid synthesis and bile acid production, making it essential for hormone production by endocrine tissue (5). Cholesterol is also an important component of cellular membranes, offering structure and rigidity to the plasma membrane as well as clustering with sphingolipids and glycerophospholipids to form highly-stable membrane microdomains or "lipid rafts" that host a number of proteins and lipids involved in key cell signaling pathways (6). In cancer, rapid cellular division is needed for the growth and survival of the tumor. Hence, a large demand for cholesterol is needed to facilitate the rapid formation of new membranes (3). Endocrine cancers in particular, display an even higher demand for cholesterol due to increased hormone and steroid production by these cells (7, 8).

A hydrophobic molecule, cholesterol has very poor aqueous solubility and thus is transported throughout the body by lipoproteins. Under normal conditions, HDL is a key participant in the reverse cholesterol transport (RCT) pathway, a process by which excess cholesterol from peripheral tissue is taken up by HDL and transported back to the liver for secretion in the bile or for redistribution to endocrine tissue for steroid production. Historically, HDL-C has been the focus of lipid metabolism modulating therapeutics for cardiovascular diseases, as high HDL-C or "the good cholesterol" and low LDL-C "bad cholesterol" have been well established as markers of cardiovascular health. Specifically, nascent HDL has the ability to reduce the burden of atherosclerosis by depleting foam-cell macrophages of their cholesterol and reducing inflammation and oxidation in the surrounding atheroma environment (9, 10). In this review, we will summarize what is known about the association between HDL-C levels and cancer and examine the utility of reconstituted or synthetic HDL as a potential therapeutic and drug delivery vehicle for endocrine cancers.

HDL-C IN CANCER

HDL is an endogenous, nanosized particle composed apolipoproteins, and lipids (11). Naturally, these particles range in shape, size, density, and charge depending on their lipid composition, protein cargo, and degree of maturation (11). The main protein component of HDL, apolipoprotein A-I (ApoA-I), is initially synthesized in the liver where it is subsequently secreted into the circulation. Once secreted, ApoA-I picks up a small amount of lipid to form pre-β HDL particles. These nascent, cholesterol-poor discs can then further interact with cholesterol-rich cells of the periphery to take up and deliver that cholesterol back to the liver where it is taken up via scavenger receptor type B-I (SR-BI) for secretion or further processing. Once picked up by HDL, cholesterol is esterified by lecithin:acyl cholesterol transferase (LCAT) to form cholesterol ester. Cholesterol ester is then buried within HDL's hydrophobic lipid core, inducing the maturation and formation of larger, spherical HDL particles. Spherical HDL particles contain not only ApoA-I, but also ApoE, which facilitates the growing load of CE into the hydrophobic core since ApoA-I can only facilitate a limited amount of CE in the HDL core. ApoE is also useful in that it is a substrate for low-density lipoprotein receptor (LDLR) and can deliver HDL cargo to hepatic LDLR for biliary excretion or to endocrine tissue expressing LDLR or SR-BI for use in steroid production (12, 13).

HDL is highly heterogeneous and is present in a variety of different forms depending on its size, shape, density, and lipid/protein composition. This is a result of HDL remodeling, which is a continuous process involving several endogenous enzymes (14). Put simply, HDL can be continually and reversibly recycled between lipid-poor apoA1, discoidal HDL, and small/large/larger spherical HDLs. These subsets of HDL are classified into two groups, HDL_2 and HDL_3, based on their densities (11, 15). HDL_2 is lipid-rich and less dense (1.063–1.125 g/mL) than its HDL_3 counterpart, which is dense (1.125–1.21 g/mL) protein-rich in comparison (11). Both HDL_2 and HDL_3 can be further divided into 2 and 3 subclasses, respectively, based on their size; HDL_3 ranges in size from roughly 7–9 nm in diameter while HDL_2 ranges from about 9–12 nm (11). To further complicate things, HDL can also be classified according to its surface charge and shape. Spherical, more neutral HDL particles are classified as α-HDL, while nascent, discoidal HDL particles, known as β-HDL, are poorly lipidated and more negative in overall charge.

In addition to the existing variety of subpopulations in healthy individuals, HDL particle makeup can vary significantly among patients of different disease states (16). Particularly, recent studies have identified changes in the diverse proteome of HDL particles in the various disease states (17, 18). While ApoA-I is the main protein in HDL, other proteins including ApoA-II, ApoC, paraoxanase (PON), ApoM, and serum amyloid A (SAA) have been identified and can be altered under disease conditions (19–21). The lipid composition of HDL particles can also vary with disease (22), and chronic changes in the HDL lipidome have been attributed to the high inflammatory state of various diseases, including the presence of lysophosphatidic acid (LPA), a phospholipid implicated in the progression of several endocrine cancers (23–25). Under such conditions, including atherosclerosis and lupus, HDL isolated from patients is said to be dysfunctional or proinflammatory, and its abilities to carry out cholesterol efflux and exert anti-inflammatory properties are lost (26–31). Similarly, studies have shown that HDL can promote breast cancer metastasis, which is attributed to the alterations in HDL's lipid and protein compositions under inflammatory and oxidative conditions (32, 33).

Epidemiology

A number of observational studies and retrospective study analyses have shown that plasma HDL-C and ApoA-I levels are significantly reduced in cancer patients, including those with breast, ovarian, colon, prostate, and pancreatic carcinomas (34–50). These studies are summarized in **Table 1**. A number of studies also sought to investigate the predictive power of HDL-C or ApoA-I levels in subsets of cancers and found that, when combined with other traditional cancer biomarkers cancer antigen 125 (CA125) and transthyretin (TTR), either ApoA-I or HDL-C levels significantly increased the power of these panels to predict patient prognosis (52, 54–58). In some cases, however, there were no significant associations between HDL-C, ApoA-I, and cancer risk (51). This is likely due to differences in study design and evaluation as well as the methods used to quantify HDL-C and ApoA-I. For example, direct measurements of HDL-C are generally performed by mass precipitation and can be confounded by the presence of ApoE and other proteins. Other methods directly measure HDL particles via size and charge separation using density gradient ultracentrifugation, gel filtration, high performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR) among others. Each of these techniques has its drawbacks, and is generally bias toward one or more subpopulation of HDL or risks chemical modification of

TABLE 1 | Clinical relationships between HDL-C and endocrine cancers.

Cancer	Study design	Major findings	References
Breast	• Preoperative serum lipid profile (TC, TG, HDL-C, LDL-C, ApoAI, ApoB) and the clinical data were retrospectively collected for 1,044 breast cancer patients undergoing operation • Kaplan-Meier method and the Cox proportional hazards regression model were used in analyzing the OS and DFS	• Preoperative lower TG and HDL-C level were risk factors of breast cancer patients • Decreased HDL-C associated with lower OS rate • Decreased TG associated with lower DFS rate	(49)
Multiple	• Twenty-six studies including 24,655 individuals identified via PubMed and EMBASE • Meta-analysis to investigate the prognostic significance of serum blood TC, TG, HDL-C, and LDL-C for cancer	• Patients with higher HDL-C had a 37% reduced risk of death compared with lower HDL-C • DFS patients with higher HDL-C level had the risk of disease relapse reduced by 35% compared with patients with lower levels.	(50)
Breast	• Examined the possible association of low HDL-C with incidence of breast cancer using data from the Atherosclerosis Risk in Communities Study (ARIC) cohort • Among 7,575 female members of the ARIC cohort, 359 cases of incident breast cancer were ascertained during the follow-up from 1987 through 2000	• No association of low baseline HDL-cholesterol (<50 mg/dL) with incident breast cancer in the total sample and a modest association among women who were pre- menopausal at baseline. No association was observed among women who were post-menopausal at baseline • Low HDL-cholesterol among pre-menopausal women may be a marker of increased breast cancer risk	(51)
Multiple	• Assess the relationships of TC, TG, HDL-C, ApoA, ApoB-100, Lp(a) with risk of common cancer forms, and total cancer mortality in comparison to incidence and mortality of CVD • Case-cohort sample out of the prospective EPIC–Heidelberg study, including a random subcohort ($n = 2,739$), and cases of cancer ($n = 1,632$), cancer mortality ($n = 761$), CVD ($n = 1,070$), and CVD mortality ($n = 381$).	• High TC, HDL-C, ApoA, and Lp(a) levels were associated with a reduction in total cancer mortality • High levels of apoB-100 and TG were inversely associated, and high HDL-C levels were positively associated with breast cancer risk • Higher levels of Lp(a) were associated with an increase in prostate cancer risk	(48)
Multiple	• Serum TC, LDL-C, HDL-C, and TG were analyzed in 530 patients with newly diagnosed cancer (97 with hematological malignancies, 92 with tumor of the lung, 108 of the upper diges- tive system, 103 of colon, 32 of breast, and 98 of the genitourinary system) and in 415 non-cancer subjects	• TC, LDL-C, HDL-C, SA, and BMI were significantly lower in cancer than in non-cancer subjects; similar trend for metastatic vs. non-metastatic cancer patients • Lowest values of TC, LDL-C, and HDL-C recorded in patients with hematological malignancies • Highest values of TC, LDL-C, and HDL-C in patients with breast tumor	(36)
Renal cell carcinoma	• Preoperative serum lipid-profile (TC, TG, HDL-C, LDL-C, ApoA- I, and ApoB) were retrospectively performed in 786 patients with RCC	• Patients with low ApoA-I (<1.04) had significantly lower OS than the high ApoA-I • In the 755 patients with nonmetastasis, the low ApoA-I group was also associated with shortened DFS time compared to the high ApoA-I group	(47)
Pancreatic	• Identify and validate new biomarkers in PCa patient serum samples • 96 serum samples from patients undergoing PCa surgery was compared with sera from 96 healthy volunteers as controls.	• Apolipoprotein A-II, transthyretin, and apolipoprotein A-I were identified as markers • These identified proteins were decreased at least 2-fold in PCa serum compared with the control group.	(52)
Multiple	• A retrospective cohort study of 14,169 men and 23,176 women with type 2 diabetes to investigate the relationship between HDL cholesterol (HDL-C) and cancer risk among type 2 diabetic patients • During a mean follow-up period of 6.4 years, 3,711 type 2 diabetic patients had a cancer diagnosis	• A significant inverse association between HDL-C and the risk of cancer was found among men and women • Suggests an inverse association of HDL-C with cancer risk among men and women with type 2 diabetes, whereas the effect of HDL-C was partially mediated by reverse causation • Each 15 mg/dL increase in baseline HDL-C was associated with an 8–10% decreased risk of cancer in men and a 1–7% decreased risk of cancer in women with type 2 diabetes	(44)
Breast	• Review and meta-analysis of prospective studies investigating associations between TC, HDL-C, and LDL-C levels and the risk of breast cancer	• Evidence of a modest inverse association between TC and more specifically HDL-C and the risk of breast cancer • No association observed between LDL-C and the risk of breast cancer	(45)

(Continued)

TABLE 1 | Continued

Cancer	Study design	Major findings	References
Multiple	• Evaluated the prospective association of total, breast, colorectal, and lung cancers and cancer mortality with lipid biomarkers in 15,602 female health professionals in the Women's Health Study (aged ≥45 y, free of cardiovascular disease and cancer, and without hormone replacement therapy or lipid-lowering medications at baseline) • Included 2,163 incident cancer cases (864 breast, 198 colorectal, and 190 lung cancers) and 647 cancer deaths	• Total cancer risk significantly lower for the highest quartile of ApoA-1 • Significant associations included colorectal and lung cancer risk with HDL cholesterol • LDL cholesterol was not significantly associated with risk of total cancer or any site-specific cancers	(46)
Multiple	• Prospective examination of the association between TC and cancer incidence among 1,189,719 Korean adults enrolled in the National Health Insurance Corporation • Over follow-up, 53,944 men and 24,475 women were diagnosed with a primary cancer	• High TC (≥240 mg/dL) was positively associated with prostate cancer and colon cancer in men and breast cancer in women • Higher TC was associated with a lower incidence of liver cancer, stomach cancer, and, in men, lung cancer • TC was inversely associated with all-cancer incidence in both men and women • TC was associated with the risk of several different cancers, although these relationships differed markedly by cancer site	(42)
Multiple	• Examined the relationship between serum HDL-C and risk of overall and site-specific cancers among 29,093 Finnish male smokers in the Alpha-Tocopherol Beta-Carotene (ATBC) study cohort • 7,545 incident cancers were identified during up to 18 years of follow-up	• Higher serum TC inversely associated with cancer risk • Greater HDL-C levels associated with decreased risk of cancer of the lung, prostate, liver, and hematopoietic system • Largely explained by reverse causation	(40)
Multiple	• Systematic analysis of 24 lipid intervention randomized controlled trials (76,265 intervention patients and 69,478 control patients) • Examined association between baseline and on-treatment HDL-C levels and cancer risk	• Significant inverse association between HDL-C and cancer risk • For every 10 mg/dL increase in HDL-C, 28–36% lower risk of developing cancer	(41)
Ovarian, breast, prostate, colon	• A five-center case-control study, involving a retrospective sample of 645 serum specimen • Serum proteomic expressions were analyzed on 153 patients with invasive epithelial OC, 42 with other OC, 166 with benign pelvic masses, and 142 healthy women • Utilized a ProteinChip Biomarker System and SELDI-TOF-MS	• Three biomarkers identified as biomarkers for OC: ApoA-1 (↓in cancer); TT (↓); and a cleavage fragment of ITIH4 (↑)	(53)
Ovarian	• Serum analysis from 31 healthy individuals and 43 from patients with ovarian tumors • Use of micro-LC-MS/MS followed by Western/ELISA to identify five serum protein biomarkers previously reported using SELDI-TOF-MS (54)	• TT (↓), beta-hemoglobin (↑), ApoA-1 (↓), and transferrin (↓)in early-stage OC • When combined with CA125, biomarkers should significantly improve the detection of early stage ovarian cancer	(55)
Ovarian	• Evaluated markers identified by Zhang et al. (53) in an independent study population • Sera from 42 women with OC, 65 with benign tumors, and 76 with digestive diseases • Measured levels of various posttranslationally forms of TTR, apolipoprotein A1, and CA125 using SELDI-TOF-MS • Examined power of markers to discriminate sera from women with ovarian cancer from sera from women with other diseases	• Confirmed findings by Zhang et al. (53) • ApoA-I and TT levels were lower in disease states compared to controls • Markers used alone improved detection of controls with CA125 levels ≥35 units/mL but lost sensitivity for late-stage cases.	(56)
Ovarian	• Evaluated multiplexed bead-based immunoassay of OC-associated biomarkers (TTR and ApoA-1, together with CA125) using serum of 61 healthy individuals, 84 patients with benign ovarian disease, and 118 patients with OC	• Panel of ApoA-I, TT, and CTAPIII combined with CA125 increased sensitivity for detection of early stage OC • Combination of three markers offered maximum separation between non-cancer and stage I/II or all stages of disease	(57)

(Continued)

TABLE 1 | Continued

Cancer	Study design	Major findings	References
Ovarian	• Development of multiplexed bead-based immunoassay for detection of known serum biomarkers of cancer (118 OC, 84 benign ovarian disease, 61 healthy controls)	• Combination of transthyretin, and ApoA-I with CA125 improved sensitivity and specificity of OC diagnosis	(58)
Ovarian, breast	• Measured ApoA-I and GPX3 mRNA levels via qRT-PCR in 121 effusions (101 OC, 20 BC) and 85 solid OC specimens (43 primary carcinomas, 42 metastases)	• APOA1 and GPX3 transcript levels were higher in ovarian carcinoma compared with breast carcinoma effusions • APOA1 and GPX3 mRNA levels can effectively differentiate ovarian from breast cancer	(59)
Breast	• Fasting serum samples analyzed for lipid fatty acid and lipoprotein levels • Malignant breast tissue analyzed for hormone receptor binding • 100 women with breast masses (50 malignant, 50 benign)	• Serum lipid and apolipoprotein components of LDL were increased in fibrocystic disease and early stage cancer but decreased in women with early recurrence • Ratio of serum ApoA-1/ApoB levels at time of biopsy was the best predictor of cancer recurrence	(34)
Breast	• Nested case-control study to examine association between HDL-C and breast cancer risk • Serum lipid profiles from 200 age-matched (100 diagnosed before age 50 and 100 at age 50 or older) case-control BC patients	• No difference in HDL-C between BC and control samples • Pre-menopausal cases had significantly lower HDL-C levels than controls • In pre-menopausal cases, each 1 mg/dL increase in HDL-C is associated with a 4% reduction in risk of BC	(35)
Breast	• Estimated the relative risk of breast cancer associated with HDL-C levels using serum samples of 38 823 Norwegian women aged 17–54 years at time of entry • 708 BC cases identified over median follow up time of 17.2 years	• Low HDL-C, as part of the metabolic syndrome, is associated with increased postmenopausal BC risk	(37)
Breast	• Examined relationship between breast cancer and lipid profiles in Taiwanese women • Lipid profiles in fasting serum of 150 BC patients before treatment and 71 healthy controls	• BC patients had significantly lower HDL-C and apoA-I, lower apoA-I/apoB ratios, and higher VLDL-C levels than controls • Lower ApoA-1 and HDL-C levels associated with higher incidence of BC	(38)
Breast	• Nested case-control study from trial containing 4,690 women with extensive mammographic density • Examined whether serial measures of serum lipids and lipoproteins were associated with risk of BC • Measured lipids in an average of 4.2 blood samples for 279 invasive breast cancer case subjects and 558 matched control subjects	• HDL-C and apoA-I were positively associated with BC risk only when HRT was not used	(60)
Endometrial	• Case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) • Examined the relation between prediagnostic plasma lipids, lipoproteins, and glucose, metabolic syndrome, and EC risk in 284 women with EC and 546 matched controls	• HDL-C levels were inversely correlated with the risk of developing EC • Metabolic abnormalities and obesity may act synergistically to increase risk of developing EC	(39)
Prostate	• Examined the association between serum lipids and prostate cancer risk • A cohort ($n = 69,735$) of all men aged 35 years or older were selected from the Apolipoprotein MOrtality RISk (AMORIS), • Levels of TG, TC, glucose, LDL-C, HDL-C, ApoB, and ApoA-I were measured at baseline, was database • 2,008 men developed prostate cancer	• ApoA-I and HDL levels were inversely associated with prostate cancer risk • Low HDL and ApoA-I as well as increased lipid ratios are related to increased risk of prostate cancer • No association between ApoB, LDL, and non-HDL with prostate cancer risk	(43)

ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II; ApoB, apolipoprotein B; BC, breast cancer; CA125, cancer antigen 125; CTAPIII, connective tissue activating protein III; CTC, circulating tumor cells; DFS, disease-free survival; DMFS, distant-metastasis-free survival; EC, endometrial cancer; HDL-C, High-density lipoprotein cholesterol; IMRT, intensity-modulated radiation therapy; ITIH4, inter-trypsin inhibitor heavy chain H4; LDL-C, low-density lipoprotein cholesterol; OC, ovarian cancer; OS, overall survival; PCa, pancreatic cancer; SELDI-TOF-MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; TC, total cholesterol; TF, transferrin; TG, triglycerides; TT, truncated transthyretin; TTR, transthyretin; VLDL-C, very low-density lipoprotein cholesterol.

the particles during sample preparation (61). In addition other confounding factors such as lifestyle factors, co-morbidities, and physiological factors (i.e., pre- vs. post-menopausal women), all contributed to heterogeneity of the results since most of the analyses were done retrospectively using the existing body of publicly available clinical trial data. On the other hand, there are studies describing positive correlations between HDL-C and cancer risk, namely in breast cancers (60). However, given the high heterogeneity in HDL proteome, lipidome, and subclass distribution between patients in different disease settings, it is reasonable that such variability exists between studies. While the verdict is still out on the utility of HDL-C and ApoA-I as predictive biomarkers in cancers, there is clearly a role for HDL in this complex disease which will be discussed in more detail to follow.

Mechanism

Whether decreased levels of HDL-C are a causal or consequential factor to cancer progression is yet to be elucidated, however we are logically drawn to the latter. It is known that cancer cells, in particular prostate, adrenal, and breast cancer cells, highly express the SR-BI on their plasma membrane (62–64). Because of their high-demand for cholesterol, cancer cell upregulation of SR-BI is likely a survival mechanism to increase HDL-C recruitment and, thus, increase cholesterol uptake needed for proliferation and hormone production while consequently decreasing circulating HDL-C. That being said, this argument could also be used to explain why, in some reports, HDL-C is associated with an increased risk of cancer, as it continues to provide additional cholesterol to and fuel the growth of the tumor. Regardless, we can take away several key points from these findings: (i) SR-BI is overexpressed in cancer cells, (ii) HDL-C levels are significantly affected by the presence and development of cancer, and (iii) the high affinity between SR-BI and HDL facilitates the transport of cholesterol to/from HDL and the cancer cell. In addition, HDL is known to have potent antioxidant activity and both endogenous and reconstituted HDL particles were shown to inhibit oxidative-stress induced proliferation of pancreatic cells *in vitro* (65). And although the details of the epidemiology can be disputed, there are clear opportunities for therapeutic intervention by utilizing the HDL/SR-BI axis, of which will be discussed below.

SYNTHETIC HDL PRODUCTS

As previously mentioned, the main focus of HDL research over the past several decades has been surrounding its role in cardiovascular disease. Because of its role in facilitating RCT, several "HDL mimetics" have been developed and tested clinically in humans for their ability to reduce the burden of atherosclerosis and number of events following an acute coronary event (66–69). These HDL-mimicking particles, termed reconstituted HDL (rHDL) or synthetic HDL (sHDL), are cholesterol-free HDL particles prepared from plasma purified or recombinantly expressed ApoA-I or short synthetic ApoA-I mimetic peptides complexed with phospholipids. Since they lack cholesterol, these "empty" particles are highly effective in effluxing cholesterol from lipid-laiden cells both *in vitro* and *in vivo* (9, 70, 71). In addition to their augmented efflux capacity, these particles offer a natural ability to target SR-BI-expressing cells. When combined, the ability of rHDL/sHDL to deplete cellular cholesterol, target SR-BI expressing cells, along with the biocompatibility of the individual components and proven clinical safety make the application of sHDL for cancer therapy increasingly attractive.

Clinically Tested sHDL Products

The concept of utilizing sHDL and ApoA-I mimetic peptides as a cholesterol depletion therapy has been around for decades, but focused primarily in the context of cardiovascular diseases. In fact, several sHDL therapies have been developed and tested in various stages of human clinical trials (66, 69, 72). The purpose of such sHDL infusion therapies was to efflux cholesterol and reduce plaque size and vulnerability following an initial coronary event, in order to decrease the occurance of secondary events. Early sHDL clinical trials utilized lipid-free ApoA-I protein or mimetic peptides, such as ApoA-I milano, D-4F, and L-4F, however it was shown that the naked proteins and peptides themselves had a very short plasma half-life, and their pharmacological effect suffered as a consequence (73, 74). Formulation of peptide or full-length ApoA-I protein with phospholipid, forming sHDL, was shown to markedly improve plasma half-life and thus its overall therapeutic effect (75). Moreover, studies have shown that the phospholipid component of sHDL therapies is a driving determinant of the overall pharmacokinetic and pharmacodynamic effect (71).

Measurable improvements in the pharmacokinetic and pharmacodynamic effects of sHDL therapies has led to their progression from bench to bedside in both early and late stage clinical trials. Such sHDL products include peptide-based sHDLs, including ETC-642 (22A peptide/dipalmitoylphosphatidylcholine/sphingomyelin), and ApoA-I protein based sHDLs, including ETC-216 (recombinant ApoA-I/palmitoyloleoylphosphatidylcholine) and CER-001 (recombinant ApoA-I/sphingomyelin/dipalmitoylphosphatidylglycerol), among others. These products were all shown to be safe at high doses of up to 100 mg/kg in humans and possess potent cholesterol efflux abilities (76, 77). More recently CSL-112, reconstituted ApoA-I/soybean phosphatidylcholine, has advanced to a 17,000 patient Phase III clinical trial after showing promising ability to reduce atheroma burden and decrease secondary coronary events in earlier trials (78–80). Given their proven clinical safety and ability to facilitate cholesterol removal, sHDL products could be easily translated for use as cholesterol depleting therapies in cancer.

sHDL FOR CANCER THERAPY

Cholesterol Depletion

Given the dependence of endocrine cancers on cholesterol, cholesterol-targeting therapies have gained increasing attention.

TABLE 2 | Experimental studies utilizing HDL for endocrine cancer therapy.

Cancer	Treatment	Model	Major findings	References
Ovarian	• L-4F peptide or sc-4F ctrl - 10 mg/kg/day S.Q. for 5 wks, starting either the day of ID8 cell injection or 2 wks post-ID8 injection • L-5F peptide - 10 mg/kg/day S.Q. for 5 wks (for S.Q. tumors) or 9 wks (for I.P. tumors) • Compared to buffer treated control mice • D-4F peptide - 300 μg/mL (129.8 μM) in drinking water, starting immediately post-ID8 injection - Compared to normal drinking water	• Female C57BL6/J mice, 9 wk old • ID8 cells given: - S.Q. (5 wk flank tumor model) - I.P. (9 wk tumor model)	• L-4F: - Smaller flank tumor volumes when given both immediately following or 2 wks post-ID8 injection (5 wk S.Q. tumor model) • L-5F: - Flank tumor size decreased (5 wk S.Q. tumor model) - Number of tumor nodules decreased (9 wk I.P tumor model) • D-4F: - Smaller flank tumor volume (5 wk S.Q. tumor model) - Fewer number of tumor nodules (9 wk I.P. tumor model)	(86)
Ovarian	• L-5F peptide - 10 mg/kg/day S.Q. for 5 wks, starting immediately after ID8 cell injections	• Female C57BL/6 mice • ID8 cells injected S.Q.	• Decreased the number of perfused vessels within tumors • Decreased size of total vessels within tumor • Decreased VEGF levels in both serum and tumor tissue	(87)
Ovarian	• L-4F peptide - 10 mg/kg/day S.Q. for 3 wks, starting 2 weeks post-ID8 injection - Compared to sc-4F peptide treated mice	• Female C57BL/6 mice, 9 wk old • ID8 cells injected S.Q.	• Decreased expression and activity of HIF-1α in tumors • Reduced the number of vessels and overall angiogenesis within tumors	(88)
Breast	• L-4F peptide • 10 mg/kg S.Q. daily from weaning to 45 days, and 3x per wk until 19 wks of age	• Mammary PyMT transgenic mice	• Significantly increased tumor latency and inhibited tumor development • Decreased plasma levels of oxLDL	(89)
Pancreatic	• L-4F peptide - 10 mg/kg/day I.P. for 1 week, starting immediately post-H7 injection - Compared to sc-4F peptide treated mice	• Female C57BL/6 mice, 6–8 w/o • H7 cells injected directly into pancreas	• Reduced tumor size and weight • Reduced number of inflammatory tumor infiltrating cells, including Th17 and Th1 lymphocytes • Decreased mRNA expression of inflammatory cytokines in tumors • Decreased % of M2 macrophage polarization in tumors	(90)

D-4F, D-amino acid version of L-4F; I.P., intraperitoneal; L-4F, DWFKAFYDKVAEKFKEAF; L-5F, DWLKAFYDKVFEKFKEFF; oxLDL, oxidized low-density lipoprotein; PyMT, mammary tumor virus-polyoma middle T-antigen; sc-4F, scrambled-4F peptide; S.Q., subcutaneous; Th1, T-helper cell 1; Th17, T-helper cell 17; VEGF, vascular endothelial growth factor; wk, week.

One approach is to directly deplete cholesterol from cells using cholesterol scavenging therapies. In addition to cholesterol being essential for the formation of new membranes during cell division, it is also vital for the formation of lipid-rafts in the plasma membrane. These lipid rafts are rich in cholesterol and sphingolipids and house many proteins and transporters involved in key signaling pathways, including the Akt signaling pathway implicated in the migration, proliferation, and survival of cancer cells (81). By depleting cholesterol from cells, lipid rafts are disrupted and the proteins they house internalized, drastically reducing the cell's ability to carry out its functions and often triggering cell death (4). In cancer cell lines, treatment with cyclodextrins induced marked cell death, and that cells with a higher abundance of lipid rafts were more susceptible to such treatments (82–84). More recently, Taylor et al. showed that HAC15 adrenal carcinoma cells treated with ETC-642, a clinically tested sHDL, displayed marked reduction in cellular cholesterol levels in addition to inhibition of aldosterone, cortisol, and androstenedione production (85). Thus, the application of cholesterol-depleting therapies, namely sHDL, for endocrine cancer deserves further investigation.

sHDL Peptides

Several recent studies have investigated the use of HDL-mimetics, including sHDL, ApoA-I protein, and ApoA-I mimetic peptides, for cancer treatment, outlined in **Table 2**. In addition, treatment of cells with both sHDL and chemotherapeutic drugs was able to reduce the overall effective dose (91). Other studies utilizing ApoA-I protein or mimetic peptides L-4F, L-5F, and D-4F have shown that treatment of tumor-bearing mice with either protein or peptide can reduce both tumor volume and angiogenesis in tumor tissues when compared to control mice (87–90, 92–94). For example, in a mouse model of ovarian cancer, Gao et. al.

show that L-4F peptide, when administered subcutaneously at 10 mg/kg/day for 3 weeks, could reduce overall angiogenesis and vessel number within the tumor tissue, which was connected to a decrease in expression levels of hypoxia inducible factor-1α (HIF-1α) (88). In a similar study, they also show that L-5F peptide could exert similar anti-angiogensis effects and led to decreased levels of serum vascular endothelial growth factor (VEGF) (87). In a separate study utilizing a similar ovarian cancer mouse model, Su et. al. demonstrated that both L-4F and L-5F peptides could decrease the overall volume of tumors in both flank and intraperitoneal tumors when given 10 mg/kg/day over the course of 5 or 9 weeks, respectively (86). They postulate that the antitumorogenic effect could be related to peptides' abilities to reduce circulating levels of LPA, and they were found to have significantly greater binding affinity for LPA when compared to full length ApoA-I protein. In a study by Peng et. al. L-4F peptide given at 10 mg/kg/day for 1 week was able to not only reduce size and weight of H7 pancreatic tumors in mice, but also significantly reduce several markers of inflammation within the tissue as well (90).

While the above studies also included extensive screening of ApoA-I and mimetic peptides for viability and anti-proliferative activity in a broad range of cancer cell lines *in vitro*, there are also reports describing the ability of HDL to induce proliferation, migration, and survival in cancer cell cultures (32, 33, 95, 96). Consistent with previously mentioned clinical findings showing a positive association between HDL-C and cancer risk, these studies support the notion that HDL-C may promote the progression of cancer by supplying the tumor cells with their increasing demand for cholesterol. However, a distinction should be made between the epidemiology of HDL and cancer and the utility of HDL in cancer treatment: namely, that the use of HDL in cancer therapy referred to in this review involves the administration of "empty" cholesterol-free particles. These particles are the nascent, discoidal HDL particles with high cholesterol efflux activity as proven both in basic and clinical research. Of course, studies utilizing plasma purified HDLs should be considered differently. Plasma HDLs contain a variety of different components, including signaling lipids responsible for many of HDL's pro-angiogenic and Akt-activating properties (22, 97), and namely cholesterol capable of being delivered to cells (98, 99). While such studies are integral to understanding the role of endogenous HDL in cancer pathogenesis, they should not be confused with therapeutic implications utilizing sHDL or mimetic peptides with a defined molecular makeup and superior cholesterol efflux capacity.

Targeted Drug Delivery

Given the very poor solubility of many chemotherapeutic drugs, the hydrophobic lipid core of HDL presents an attractive environment and alternative strategy for delivery and formulation of this class of drugs. Not only is it possible to lower the overall dose of drug given by improving its solubility, but the SR-BI targeting ability of these sHDL nanoparticles affords the additional benefit of site-specific, cytosolic drug delivery to SR-BI over-expressing tumor cells while subsequently reducing systemic toxicity (72, 94, 100). Others have, with varying success, shown anti-tumorigenic by introducing HDL surface modifications to augment the targeting capacity and to extend particle half-life (101). The use of HDL-mimetics for targeted drug delivery has been extensively reviewed elsewhere (72, 102) and is beyond the scope of this review, however, its importance and growing relevance warrant mentioning.

SUMMARY AND PERSPECTIVE

Decades of epidemiological evidence suggests that , notably -C, plays a role in the incidence and progression of cancer. Whether or not this role is causal or consequential, or whether the risk association is positive or negative under specific conditions is still left for debate. Despite, we know from years of clinical and basic cardiovascular research that is an intimate player in the RCT process and has specific and potent cholesterol efflux ability both *in vitro* and *in vivo*. We also know that cholesterol is a vital resource for cancer cells, which require a constant supply to maintain and facilitate their rapid proliferation and overall survival. Endocrine cancers, in particular, are at an increased demand for cholesterol given their additional need for steroid production making them even more susceptible to cholesterol depletion interventions and targeting by due to upregulation of SR-BI.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This publication was made possible by Grant Number R01GM113832 (to AS and X-AL) from NIGMS/NIH and NIH T32-HL125242 (EM). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIGMS or NIH.

REFERENCES

1. Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S. The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas* (2010) 39:707–12. doi: 10.1097/MPA.0b013e3181ec124e
2. Hager MH, Solomon KR, Freeman MR. The role of cholesterol in prostate cancer. *Curr Opin Clin Nutr Metab Care* (2006) 9:379–85. doi: 10.1097/01.mco.0000232896.66791.62
3. Cruz PM, Mo H, McConathy WJ, Sabnis N, Lacko AG. The role of cholesterol metabolism and cholesterol transport in carcinogenesis: a review of scientific findings, relevant to future cancer therapeutics. *Front Pharmacol.* (2013). 4:119. doi: 10.3389/fphar.2013.00119

4. Beloribi-Djefaflia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. *Oncogenesis* 5:e189. doi: 10.1038/oncsis.2015.49
5. Hu J, Zhang Z, Shen WJ, Azhar S. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. *Nutri Metab.* (2010) 7:47. doi: 10.1186/1743-7075-7-47
6. Pike LJ. Lipid rafts: bringing order to chaos. *J Lipid Res.* (2003) 44:655–67. doi: 10.1194/jlr.R200021-JLR200
7. Foster PA. Steroid metabolism in breast cancer. *Minerva Endocrinol.* (2008) 33:27–37. Available online at: http://cebp.aacrjournals.org/content/13/7/1185
8. Purohit A, Foster PA. Steroid sulfatase inhibitors for estrogen- and androgen-dependent cancers. *J Endocrinol.* (2012) 212:99–110. doi: 10.1530/JOE-11-0266
9. von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis: role of cholesterol efflux and reverse cholesterol transport. *Arterioscl Thromb Vasc Biol.* (2001) 21:13–27. doi: 10.1161/01.ATV.21.1.13
10. Rye KA, Barter PJ. Cardioprotective functions of HDLs. *J Lipid Res.* (2014) 55:168–79. doi: 10.1194/jlr.R039297
11. Kontush A, Lindahl M, Lhomme M, Calabresi L, Chapman MJ, Davidson WS. Structure of HDL: particle subclasses and molecular components. In: von Eckardstein A, Kardassis D, editors. *High Density Lipoproteins,* vol. 224. Cham; Heidelberg; New York, NY; Dordrecht; London: Springer International Publishing (2015). p. 3–51.
12. Lund-Katz S, Phillips MC. High density lipoprotein structure–function and role in reverse cholesterol transport. In: Harris JR, editor. *Cholesterol Binding and Cholesterol Transport Proteins,* vol. 51. Netherlands: Springer (2010). p. 183–227.
13. Favari E, Chroni A, Tietge UJ, Zanotti I, Escolà-Gil JC, Bernini F. Cholesterol Efflux and Reverse Cholesterol Transport. In: von Eckardstein A, Kardassis D, editors. *High Density Lipoproteins,* vol. 224. Cham; Heidelberg; New York, NY; Dordrecht; London: Springer International Publishing. (2015). p. 181–206.
14. Zannis VI, Fotakis P, Koukos G, Kardassis D, Ehnholm C, Jauhiainen M, et al. HDL biogenesis, remodeling, and Catabolism. In: von Eckardstein A, Kardassis D, editors. *High Density Lipoproteins,* vol. *224*. Cham; Heidelberg; New York, NY; Dordrecht; London: Springer International Publishing (2015). p. 53–111.
15. Kontush A, Chapman MJ. *High-Density Lipoproteins: Structure, Metabolism, Function and Therapeutics*. Hoboken, NJ: John Wiley & Sons (2011).
16. Kratzer A, Giral H, Landmesser U. High-density lipoproteins as modulators of endothelial cell functions: alterations in patients with coronary artery disease. *Cardiovasc Res.* (2014) 103:350–61. doi: 10.1093/cvr/cvu139
17. Heinecke JW. The HDL proteome: a marker–and perhaps mediator–of coronary artery disease. *J Lipid Res.* (2009) 50(Suppl.):S167–71. doi: 10.1194/jlr.R800097-JLR200
18. Alwaili K, Bailey D, Awan Z, Bailey SD, Ruel I, Hafiane A, et al. The HDL proteome in acute coronary syndromes shifts to an inflammatory profile. *Biochim Biophys Acta* (2012) 1821:405–15. doi: 10.1016/j.bbalip.2011.07.013
19. Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, et al. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. *J Clin Invest.* (1995) 96:2758–67. doi: 10.1172/JCI118345
20. Shah AS, Tan L, Long JL, Davidson WS. Proteomic diversity of high density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. *J Lipid Res.* (2013) 54:2575–85. doi: 10.1194/jlr.R035725
21. Birner-Gruenberger R, Schittmayer M, Holzer M, Marsche G. Understanding high-density lipoprotein function in disease: recent advances in proteomics unravel the complexity of its composition and biology. *Prog Lipid Res.* (2014) 56:36–46. doi: 10.1016/j.plipres.2014.07.003
22. Kontush A, Lhomme M, Chapman MJ. Unraveling the complexities of the HDL lipidome. *J Lipid Res.* (2013) 54:2950–63. doi: 10.1194/jlr.R036095
23. Baker DL, Morrison P, Miller B, Riely CA, Tolley B, Westermann AM, et al. Plasma lysophosphatidic acid concentration and ovarian cancer. *JAMA* (2002) 287:3081–2. doi: 10.1001/jama.287.23.3081
24. Sutphen R, Xu Y, Wilbanks GD, Fiorica J, Grendys EC, LaPolla JP, et al. Lysophospholipids are potential biomarkers of ovarian cancer. *Cancer Epidemiol Biomarkers Prev.* (2004) 13:1185–91.
25. Willier S, Butt E, Grunewald TG. Lysophosphatidic acid (LPA) signalling in cell migration and cancer invasion: a focussed review and analysis of LPA receptor gene expression on the basis of more than 1700 cancer microarrays. *Biol Cell* (2013) 105:317–33. doi: 10.1111/boc.201300011
26. McMahon M, Grossman J, FitzGerald J, Dahlin-Lee E, Wallace DJ, Thong BY, et al. Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum.* (2006) 54:2541–9. doi: 10.1002/art.21976
27. Navab M, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Fogelman AM. HDL as a biomarker, potential therapeutic target, and therapy. *Diabetes* (2009) 58:2711–7. doi: 10.2337/db09-0538
28. Namiri-Kalantari R, Gao F, Chattopadhyay A, Wheeler AA, Navab KD, Farias-Eisner R, et al. The dual nature of HDL: anti-inflammatory and pro-inflammatory. *Biofactors* (2015) 41:153–9. doi: 10.1002/biof.1205
29. Rosenson RS, Brewer HB Jr, Ansell BJ, Barter P, Chapman MJ, Heinecke JW. Dysfunctional HDL and atherosclerotic cardiovascular disease. Nat Rev Cardiol. (2015) 13:48–60. doi: 10.1038/nrcardio.2015.124
30. Vaisar T, Tang C, Babenko I, Hutchins P, Wimberger J, Suffredini AF, et al. Inflammatory remodeling of the HDL proteome impairs cholesterol efflux capacity. *J Lipid Res.* (2015) 56:1519–30. doi: 10.1194/jlr.M059089
31. Smith CK, Seto NL, Vivekanandan-Giri A, Yuan W, Playford MP, Manna Z, et al. Lupus high-density lipoprotein induces proinflammatory responses in macrophages by binding lectin-like oxidised low-density lipoprotein receptor 1 and failing to promote activating transcription factor 3 activity. *Ann Rheum Dis.* (2016). 76:602–11. doi: 10.1136/annrheumdis-2016-209683
32. Pan B, Ren H, He Y, Lv X, Ma Y, Li J, et al. HDL of patients with type 2 diabetes mellitus elevates the capability of promoting breast cancer metastasis. *Clin Cancer Res.* (2012) 18:1246–56. doi: 10.1158/1078-0432.CCR-11-0817
33. Pan B, Ren H, Lv X, Zhao Y, Yu B, He Y, et al. Hypochlorite-induced oxidative stress elevates the capability of HDL in promoting breast cancer metastasis. *J Transl Med.* (2012) 10:65. doi: 10.1186/1479-5876-10-65
34. Lane DM, Boatman KK, McConathy WJ. Serum lipids and apolipoproteins in women with breast masses. *Breast Cancer Res Treat.* (1995) 34:161–9. doi: 10.1007/BF00665788
35. Moorman PG, Hulka BS, Hiatt RA, Krieger N, Newman B, Vogelman JH, et al. Association between high-density lipoprotein cholesterol and breast cancer varies by menopausal status. *Cancer Epidemiol Biomark Prev.* (1998) 7:483–8.
36. Fiorenza AM, Branchi A, Sommariva D. Serum lipoprotein profile in patients with cancer. A comparison with non-cancer subjects. *Int J Clin Lab Res.* (2000) 30:141–5. doi: 10.1007/s005990070013
37. Furberg AS, Veierød MB, Wilsgaard T, Bernstein L, Thune I. Serum high-density lipoprotein cholesterol, metabolic profile, and breast cancer risk. *JNCI* (2004) 96:1152–60. doi: 10.1093/jnci/djh216
38. Chang SJ, Hou MF, Tsai SM, Wu SH, Hou LA, Ma H, et al. The association between lipid profiles and breast cancer among Taiwanese women. *Clin Chem Lab Med*. 45:1219–23. doi: 10.1515/CCLM.2007.263
39. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Tjønneland A, et al. Metabolic syndrome, plasma lipid, lipoprotein and glucose levels, and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocrine Relat Cancer* (2007) 14:755–67. doi: 10.1677/ERC-07-0132
40. Ahn J, Lim U, Weinstein SJ, Schatzkin A, Hayes RB, Virtamo J, et al. Prediagnostic total and high-density lipoprotein cholesterol and risk of cancer. *Cancer Epidemiol Biomark Prev.* (2009) 18:2814–21. doi: 10.1158/1055-9965.EPI-08-1248
41. Jafri H, Alsheikh-Ali AA, Karas RH. Baseline and on-treatment high-density lipoprotein cholesterol and the risk of cancer in randomized controlled trials of lipid-altering therapy. *J Am College Cardiol.* (2010) 55:2846–54. doi: 10.1016/j.jacc.2009.12.069
42. Kitahara CM, Berrington de González A, Freedman ND, Huxley R, Mok Y, Jee SH, et al. Total cholesterol and cancer risk in a large prospective study in Korea. *J Clin Oncol.* (2011) 29:1592–8. doi: 10.1200/JCO.2010.31.5200

43. Van Hemelrijck M, Walldius G, Jungner I, Hammar N, Garmo H, Binda E, et al. Low levels of apolipoprotein A-I and HDL are associated with risk of prostate cancer in the Swedish AMORIS study. *Cancer Causes Control* (2011) 22:1011–9. doi: 10.1007/s10552-011-9774-z
44. Zhao W, Guan J, Horswell R, Li W, Wang Y, Wu X, et al. HDL cholesterol and cancer risk among patients with type 2 diabetes. *Diabetes Care* (2014) 37:3196–203. doi: 10.2337/dc14-0523
45. Touvier M, Fassier P, His M, Norat T, Chan DS, Blacher J, et al. Cholesterol and breast cancer risk: a systematic review and meta-analysis of prospective studies. *Br J Nutr.* (2015) 114:347–57. doi: 10.1017/S00071145150 0183X
46. Chandler PD, Song Y, Lin J, Zhang S, Sesso HD, Mora S, et al. Lipid biomarkers and long-term risk of cancer in the Women's Health Study. *Am J Clin Nutr.* (2016) 103:1397–407. doi: 10.3945/ajcn.115.1 24321
47. Guo S, He X, Chen Q, Yang G, Yao K, Dong P, et al. The effect of preoperative apolipoprotein A-I on the prognosis of surgical renal cell carcinoma: a retrospective large sample study. *Medicine* (2016) 95:e3147. doi: 10.1097/MD.0000000000003147
48. Katzke VA, Sookthai D, Johnson T, Kühn T, Kaaks R. Blood lipids and lipoproteins in relation to incidence and mortality risks for CVD and cancer in the prospective EPIC–Heidelberg cohort. *BMC Med.* (2017) 15:218. doi: 10.1186/s12916-017-0976-4
49. Li X, Tang H, Wang J, Xie X, Liu P, Kong Y, et al. The effect of preoperative serum triglycerides and high-density lipoprotein-cholesterol levels on the prognosis of breast cancer. *Breast* (2017) 32:1–6. doi: 10.1016/j.breast.2016.11.024
50. Zhou P, Li B, Liu B, Chen T, Xiao J. Prognostic role of serum total cholesterol and high-density lipoprotein cholesterol in cancer survivors: a systematic review and meta-analysis. *Clin Chim Acta* (2018) 477:94–104. doi: 10.1016/j.cca.2017.11.039
51. Kucharska-Newton AM, Rosamond WD, Mink PJ, Alberg AJ, Shahar E, Folsom AR. HDL-cholesterol and incidence of breast cancer in the ARIC cohort study. *Ann Epidemiol.* (2008) 18:671–7. doi: 10.1016/j.annepidem.2008.06.006
52. Ehmann M, Felix K, Hartmann D, Schnölzer M, Nees M, Vorderwülbecke S, et al. Identification of potential markers for the detection of pancreatic cancer through comparative serum protein expression profiling. *Pancreas* (2007) 34:205–14. doi: 10.1097/01.mpa.0000250128.57026.b2
53. Zhang Z, Bast RC, Yu Y, Li J, Sokoll LJ, Rai AJ, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res.* (2004) 64:5882–90. doi: 10.1158/0008-5472.CAN-04-0746
54. Kozak KR, Amneus MW, Pusey SM, Su F, Luong MN, Luong SA, et al. Identification of biomarkers for ovarian cancer using strong anion-exchange ProteinChips: potential use in diagnosis and prognosis. *Proc Natl Acad Sci USA.* (2003) 100:12343–8. doi: 10.1073/pnas.2033602100
55. Kozak KR, Su F, Whitelegge JP, Faull K, Reddy S, Farias-Eisner R. Characterization of serum biomarkers for detection of early stage ovarian cancer. *Proteomics* (2005) 5:4589–96. doi: 10.1002/pmic.2005 00093
56. Moore LE, Fung ET, McGuire M, Rabkin CC, Molinaro A, Wang Z, et al. Evaluation of apolipoprotein A1 and posttranslationally modified forms of transthyretin as biomarkers for ovarian cancer detection in an independent study population. *Cancer Epidemiol Biomark Prev.* (2006) 15:1641–6. doi: 10.1158/1055-9965.EPI-05-0980
57. Clarke CH, Yip C, Badgwell D, Fung ET, Coombes KR, Zhang Z, et al. Proteomic biomarkers apolipoprotein A1, truncated transthyretin and connective tissue activating protein III enhance the sensitivity of CA125 for detecting early stage epithelial ovarian cancer. *Gynecol Oncol.* (2011) 122:548–53. doi: 10.1016/j.ygyno.2011.06.002
58. Kim YW, Bae SM, Lim H, Kim YJ, Ahn WS. Development of multiplexed bead-based immunoassays for the detection of early stage ovarian cancer using a combination of serum biomarkers. *PLoS ONE* (2012) 7:e44960. doi: 10.1371/journal.pone.0044960
59. Tuft Stavnes H, Nymoen DA, Hetland Falkenthal TE, Kærn J, Tropé CG, Davidson B. (APOA1 mRNA expression in ovarian serous carcinoma effusions is a marker of longer survival. *Am J Clin Pathol.* 2014) 142:51–7. doi: 10.1309/AJCPD8NBSHXRXQL7
60. Martin LJ, Melnichouk O, Huszti E, Connelly PW, Greenberg CV, Minkin S, et al. Serum lipids, lipoproteins, and risk of breast cancer: a nested case-control study using multiple time points. *JNCI J Natl Cancer Institute* (2015) 107:djv032. doi: 10.1093/jnci/djv032
61. Hafiane A, Genest J. High density lipoproteins: measurement techniques and potential biomarkers of cardiovascular risk. *BBA Clin.* (2015) 3:175–88. doi: 10.1016/j.bbacli.2015.01.005
62. Gutierrez-Pajares JL, Ben Hassen C, Chevalier S, Frank PG. SR-BI: linking cholesterol and lipoprotein metabolism with breast and prostate cancer. *Front Pharmacol.* (2016) 7:338. doi: 10.3389/fphar.2016.00338
63. Li J, Wang J, Li M, Yin L, Li XA, Zhang TG. Up-regulated expression of scavenger receptor class B type 1 (SR-B1) is associated with malignant behaviors and poor prognosis of breast cancer. *Pathol Res Pract.* (2016) 212:555–9. doi: 10.1016/j.prp.2016.03.011
64. Yuan B, Wu C, Wang X, Wang D, Liu H, Guo L, et al. High scavenger receptor class B type I expression is related to tumor aggressiveness and poor prognosis in breast cancer. *Tumour Biol.* (2016) 37:3581–8. doi: 10.1007/s13277-015-4141-4
65. Ruscica M, Botta M, Ferri N, Giorgio E, Macchi C, Franceschini G, et al. High density lipoproteins inhibit oxidative stress-induced prostate cancer cell proliferation. *Sci Rep.* (2018) 8:2236. doi: 10.1038/s41598-018-19568-8
66. Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, et al. Effect of recombinant apoa-i milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA* (2003) 290:2292–300. doi: 10.1001/jama.290.17.2292
67. Remaley AT, Amar M, Sviridov D. HDL-replacement therapy: mechanism of action, types of agents and potential clinical indications. *Exp Rev Cardiovasc Ther.* (2008) 6:1203–15. doi: 10.1586/14779072.6.9.1203
68. Dasseux JL, Schwendeman AS, Zhu L. *Apolipoprotein A-I Mimics*. USPTO. United States, Cerenic Therapeutics Holding SA (2013).
69. Krause BR, Remaley AT. Reconstituted HDL for the acute treatment of acute coronary syndrome. *Curr Opin Lipidol.* (2013) 24:480–6. doi: 10.1097/MOL.0000000000000020
70. Di Bartolo BA, Nicholls SJ, Bao S, Rye KA, Heather AK, Barter PJ, et al. The apolipoprotein A-I mimetic peptide ETC-642 exhibits anti-inflammatory properties that are comparable to high density lipoproteins. *Atherosclerosis* (2011) 217:395–400. doi: 10.1016/j.atherosclerosis.2011.04.001
71. Schwendeman A, Sviridov DO, Yuan W, Guo Y, Morin EE, Yuan Y, et al. The effect of phospholipid composition of reconstituted HDL on its cholesterol efflux and anti-inflammatory properties. *J Lipid Res.* (2015) 56:1727–37. doi: 10.1194/jlr.M060285
72. Kuai R, Li D, Chen YE, Moon JJ, Schwendeman A. High-density lipoproteins: nature's multifunctional nanoparticles. *ACS Nano* (2016) 10:3015–41. doi: 10.1021/acsnano.5b07522
73. Nanjee MN, Crouse JR, King JM, Hovorka R, Rees SE, Carson ER, et al. Effects of intravenous infusion of lipid-free apo A-I in humans. *Arterioscler Thromb Vasc Biol.* (1996) 16:1203–14. doi: 10.1161/01.ATV.16.9.1203
74. Li D, Gordon S, Schwendeman A, Remaley A. Apolipoprotein mimetic peptides for stimulating cholesterol efflux. In: Anantharamaiah GM and Goldberg D, editors. *Apolipoprotein Mimetics in the Management of Human Disease.* Cham; Heidelberg; New York, NY; Dordrecht; London: Springer International Publishing (2015). p. 29–42.
75. Tang J, Li D, Drake L, Yuan W, Deschaine S, Morin EE, et al. Influence of route of administration and lipidation of apolipoprotein A-I peptide on pharmacokinetics and cholesterol mobilization. *J Lipid Res.* (2017) 58:124–36. doi: 10.1194/jlr.M071043
76. Khan MLN, Drake SL, Crockatt JG, Dasseux JLH. Single-dose intravenous infusion of ETC-642, a 22-Mer ApoA-I analogue and phospholipids complex, elevates HDL-C in atherosclerosis patients. *Circulation* (2003) 108(Suppl. IV):563–4.
77. Miles J, Khan M, Painchaud C, Lalwani N, Drake S, Dasseux J. Single-dose tolerability, pharmacokinetics, and cholesterol mobilization in HDL-C fraction following intravenous administration of ETC-642, a 22-mer ApoA-I analogue and phospholipids complex, in atherosclerosis patients. *Arterioscler Thromb Vasc Biol.* (2004), E19–E19.

78. CSL (2018). *CSL Behring Announces First Patient Enrollment in Phase 3 Clinical Trial of CSL112 to Assess Reduction of Early Recurrent Cardiovascular Events in Heart Attack Survivors*. King of Prussia, PA: PR Newswire.
79. Easton R, Gille A, D'Andrea D, Davis R, Wright SD, Shear C. A multiple ascending dose study of CSL112, an infused formulation of ApoA-I. *J Clin Pharmacol.* (2014) 54:301–10. doi: 10.1002/jcph.194
80. Gille A, D'Andrea D, Tortorici, MA, Hartel G, Wright SD. CSL112 (apolipoprotein A-I [human]) enhances cholesterol efflux similarly in healthy individuals and stable atherosclerotic disease patients. *Arterioscler Thromb Vasc Biol.* (2018) 38:953–63. doi: 10.1161/ATVBAHA.118.310538
81. Badana A, Chintala M, Varikuti G, Pudi N, Kumari S, Kappala VR, et al. Lipid raft integrity is required for survival of triple negative breast cancer cells. *J Breast Cancer* (2016) 19:372–84. doi: 10.4048/jbc.2016.19.4.372
82. Li YC, Park MJ, Ye SK, Kim CW, Kim YN. Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents. *Am J Pathol.* (2006) 168:1107–18; quiz 1404–1105. doi: 10.2353/ajpath.2006.050959
83. Resnik N, Repnik U, Kreft ME, Sepčić K, Maček P, Turk B, et al. Highly selective anti-cancer activity of cholesterol-interacting agents methyl-beta-cyclodextrin and ostreolysin A/pleurotolysin b protein complex on urothelial cancer cells. *PLoS ONE* (2015) 10:e0137878. doi: 10.1371/journal.pone.0137878
84. Yamaguchi R, Perkins G, Hirota K. Targeting cholesterol with beta-cyclodextrin sensitizes cancer cells for apoptosis. *FEBS Lett.* (2015) 589 (24 Pt B):4097–105. doi: 10.1016/j.febslet.2015.11.009
85. Taylor MJ, Sanjanwala AR, Morin EE, Rowland-Fisher E, Anderson K, Schwendeman A, et al. Synthetic high-density lipoprotein (sHDL) inhibits steroid production in HAC15 adrenal cells. *Endocrinology* (2016) 157:3122–9. doi: 10.1210/en.2014-1663
86. Su F, Kozak KR, Imaizumi S, Gao F, Amneus MW, Grijalva V, et al. Apolipoprotein A-I (apoA-I) and apoA-I mimetic peptides inhibit tumor development in a mouse model of ovarian cancer. *Proc Natl Acad Sci USA.* (2010) 107:19997–20002. doi: 10.1073/pnas.1009010107
87. Gao F, Vasquez SX, Su F, Roberts S, Shah N, Grijalva V, et al. L-5F, an apolipoprotein A-I mimetic, inhibits tumor angiogenesis by suppressing VEGF/basic FGF signaling pathways(). *Integr Biol.* (2011) 3:479–89. doi: 10.1039/c0ib00147c
88. Gao F, Chattopadhyay A, Navab M, Grijalva V, Su F, Fogelman AM, et al. Apolipoprotein A-I mimetic peptides inhibit expression and activity of hypoxia-inducible factor-1α in human ovarian cancer cell lines and a mouse ovarian cancer model. *J Pharmacol Exp Ther.* (2012) 342:255–62. doi: 10.1124/jpet.112.191544
89. Cedó L, García-León A, Baila-Rueda L, Santos D, Grijalva V, Martínez-Cignoni MR, et al. ApoA-I mimetic administration, but not increased apoA-I-containing HDL, inhibits tumour growth in a mouse model of inherited breast cancer. *Sci Rep.* (2016) 6:36387. doi: 10.1038/srep36387
90. Peng M, Zhang Q, Cheng Y, Fu S, Yang H, Guo X, et al. Apolipoprotein A-I mimetic peptide 4F suppresses tumor-associated macrophages and pancreatic cancer progression. *Oncotarget* (2017) 8:99693–706. doi: 10.18632/oncotarget.21157
91. Subramanian C, Kuai R, Zhu Q, White P, Moon J, Schwendeman A, et al. sHDL nanoparticles: a novel therapeutic strategy for adrenocortical carcinomas. *Surgery* (2016) 159:284–95. doi: 10.1016/j.surg.2015.08.023
92. Su F, Grijalva V, Navab K, Ganapathy E, Meriwether D, Imaizumi S, et al. HDL mimetics inhibit tumor development in both induced and spontaneous mouse models of colon cancer. *Mol Cancer Ther.* (2012) 11:1311–9. doi: 10.1158/1535-7163.MCT-11-0905
93. Zamanian-Daryoush M, Lindner D, Tallant TC, Wang Z, Buffa J, Klipfell E, et al. The cardioprotective protein apolipoprotein a1 promotes potent anti-tumorigenic effects. *J Biol Chem.* (2013) 288:21237–52. doi: 10.1074/jbc.M113.468967
94. Zheng Y, Liu Y, Jin H, Pan S, Qian Y, Huang C, et al. Scavenger receptor B1 is a potential biomarker of human nasopharyngeal carcinoma and its growth is inhibited by HDL-mimetic nanoparticles. *Theranostics* (2013) 3:477–86. doi: 10.7150/thno.6617
95. Sekine Y, Demosky SJ, Stonik JA, Furuya Y, Koike H, Suzuki K, et al. High-density lipoprotein induces proliferation and migration of human prostate androgen-independent cancer cells by an ABCA1-dependent mechanism. *Mol Cancer Res.* (2010) 8:1284–94. doi: 10.1158/1541-7786.MCR-10-0008
96. Danilo C, Gutierrez-Pajares JL, Mainieri MA, Mercier I, Lisanti MP, Frank PG. Scavenger receptor class B type I regulates cellular cholesterol metabolism and cell signaling associated with breast cancer development. *Breast Cancer Res.* (2013) 15:R87. doi: 10.1186/bcr3483
97. Nofer JR, Kehrel B, Fobker M, Levkau B, Assmann G, von Eckardstein A. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis* (2002) 161:1–16. doi: 10.1016/S0021-9150(01)00651-7
98. Pussinen PJ, Karten B, Wintersperger A, Reicher H, McLean M, Malle E, et al. The human breast carcinoma cell line HBL-100 acquires exogenous cholesterol from high-density lipoprotein via CLA-1 (CD-36 and LIMPII analogous 1)-mediated selective cholesteryl ester uptake. *Biochem J.* (2000) 349 (Pt 2):559–66. doi: 10.1042/bj3490559
99. Connelly MA, Williams DL. SR-BI and cholesterol uptake into steroidogenic cells. *Trends Endocrinol Metab.* (2003) 14:467–72. doi: 10.1016/j.tem.2003.10.002
100. Mooberry LK, Sabnis NA, Panchoo M, Nagarajan B, Lacko AG. Targeting the SR-B1 receptor as a gateway for cancer therapy and imaging. *Front Pharmacol.* (2016) 7:466. doi: 10.3389/fphar.2016.00466
101. Tang J, Kuai R, Yuan W, Drake L, Moon JJ, Schwendeman A. Effect of size and pegylation of liposomes and peptide-based synthetic lipoproteins on tumor targeting. *Nanomedicine* (2017) 13:1869–78. doi: 10.1016/j.nano.2017.04.009
102. Foit L, Giles FJ, Gordon LI, Thaxton CS. Synthetic high-density lipoprotein-like nanoparticles for cancer therapy. *Expert Rev Anticancer Ther.* (2015) 15:27–34. doi: 10.1586/14737140.2015.990889

Conflict of Interest Statement: 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 Endocrinology

REVIEW
published: 21 January 2019
doi: 10.3389/fendo.2018.00807

Cholesterol and Its Metabolites in Tumor Growth: Therapeutic Potential of Statins in Cancer Treatment

Adele Chimento†, Ivan Casaburi†, Paola Avena, Francesca Trotta, Arianna De Luca, Vittoria Rago, Vincenzo Pezzi‡ and Rosa Sirianni‡*

Department of Pharmacy and Health and Nutritional Sciences, University of Calabria, Cosenza, Italy

Cholesterol is essential for cell function and viability. It is a component of the plasma membrane and lipid rafts and is a precursor for bile acids, steroid hormones, and Vitamin D. As a ligand for estrogen-related receptor alpha (ESRRA), cholesterol becomes a signaling molecule. Furthermore, cholesterol-derived oxysterols activate liver X receptors (LXRs) or estrogen receptors (ERs). Several studies performed in cancer cells reveal that cholesterol synthesis is enhanced compared to normal cells. Additionally, high serum cholesterol levels are associated with increased risk for many cancers, but thus far, clinical trials with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have had mixed results. Statins inhibit cholesterol synthesis within cells through the inhibition of HMG-CoA reductase, the rate-limiting enzyme in the mevalonate and cholesterol synthetic pathway. Many downstream products of mevalonate have a role in cell proliferation, since they are required for maintenance of membrane integrity; signaling, as some proteins to be active must undergo prenylation; protein synthesis, as isopentenyladenine is an essential substrate for the modification of certain tRNAs; and cell-cycle progression. In this review starting from recent acquired findings on the role that cholesterol and its metabolites fulfill in the contest of cancer cells, we discuss the results of studies focused to investigate the use of statins in order to prevent cancer growth and metastasis.

Keywords: cholesterol, statins, cancer treatment, lipid raft, oxysterols, steroids, vitamin D, ERRalpha

OPEN ACCESS

Edited by:
Gabriella Castoria, Università degli Studi della Campania Luigi Vanvitelli Naples, Italy

Reviewed by:
Erika Di Zazzo, Università degli Studi della Campania Luigi Vanvitelli Caserta, Italy
Maria Marino, Università degli Studi Roma Tre, Italy
Pia Giovannelli, Università degli Studi della Campania Luigi Vanvitelli, Italy

***Correspondence:**
Rosa Sirianni
rosa.sirianni@unical.it

†Co-first authors

‡Co-last authors

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *19 September 2018*
Accepted: *21 December 2018*
Published: *21 January 2019*

Citation:
Chimento A, Casaburi I, Avena P, Trotta F, De Luca A, Rago V, Pezzi V and Sirianni R (2019) Cholesterol and Its Metabolites in Tumor Growth: Therapeutic Potential of Statins in Cancer Treatment. Front. Endocrinol. 9:807. doi: 10.3389/fendo.2018.00807

INTRODUCTION

Cholesterol homeostasis is required for the normal growth of eukaryotic cells. Cholesterol is needed within cell membranes where it regulates membrane fluidity, signaling initiation, and cell adhesion to the extracellular matrix (1). In mammals, cholesterol is a precursor for bile acid and steroid hormone synthesis, cholesterol can be derived from food or synthesized *de novo* by specialized cells. The low-density lipoprotein (LDL) receptor (LDLR) is the primary pathway for removal of cholesterol from the circulation (2) and its activity is accurately controlled by intracellular cholesterol levels (3). The biosynthetic pathway of cholesterol is highly conserved, from yeast to humans. In the mitochondria, citrate, derived from the tricarboxylic acid (TCA) cycle, is converted to acetyl-coenzyme A (acetyl-CoA) beginning cholesterol synthesis. In the endoplasmic reticulum, acetyl-CoA is converted to lanosterol through a cascade of enzymatic reactions known as the mevalonate pathway. This series of reactions is regulated by a rate-limiting step catalyzed by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which converts

HMG-CoA to mevalonate. Downstream products of mevalonate, include cholesterol, ubiquinone, dolichol, and the isoprenoids geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP) that bind to several small GTP-binding proteins such as Ras and Rho. Protein prenylation facilitates protein translocation from the cytosol to the membrane, promoting protein-protein and protein-membrane interactions and regulating protein function (4).

Several studies reported that cholesterol plays critical roles in the progression of numerous cancers (5), suggesting how cholesterol accumulation represents another general feature of tumors. HMG-CoA reductase and LDL receptor activities are increased in proliferating cancer cells, causing a rise in cholesterol content and consumption (6, 7). Cholesterol synthesis is under feedback control (8, 9), which is operated at the level of mevalonic acid production (10–12). When exogenous cholesterol is not available, the synthesis of mevalonate is increased in order to maintain levels of cholesterol and isoprenoids. When exogenous cholesterol levels are elevated a negative feedback mechanism inhibits the synthesis of mevalonic acid (10–12), this is particularly true for the liver (9), but in some species (13) for all tissues, blocking the overall *de novo* synthesis of endogenous cholesterol. In malignant cells this feedback mechanism is lost (10).

Statins are cholesterol-lowering drugs, which inhibit the rate-limiting step of conversion of HMG-CoA to mevalonate (14). Statins can be differentiated into two types, based on their solubility: hydrophilic (pravastatin, rosuvastatin) and lipophilic (simvastatin, lovastatin, fluvastatin, atorvastatin) statins. Several reports propose a promising role for statins in cancer treatment (15). Observational studies have tried to evaluate the effect of statins on patients with several cancer types such as prostate (16), colorectal (17, 18), renal cell carcinoma (19), breast (20), ovarian (21), and lymphoma (22). Results are variable, with some studies suggesting longer survival, and others reporting no benefit. Epidemiological evidences are also variable, depending on the cancer particular type as well as on the statin class used (23). Several data suggest that lipophilic statins may be preferable over the hydrophilic ones as anticancer agents (24). Statins anticancer properties could be explained through their pleiotropic effects such as lowering protein prenylation (17), reducing tumor cell proliferation and migration (20, 25), inhibiting Ras signaling (26) inducing apoptosis through inactivation of Akt and down-regulation of mammalian target of rapamycin (mTOR) (27).

Statins can interfere with cholesterol activities, which include:

- signal molecule on membrane rafts;
- substrate for steroids, oxysterols and Vitamin D3 synthesis;
- ligand for estrogen-related receptor alpha (ERRα).

In this review, we will discuss the results of studies focused on the use of statins to the purpose of interfering with cholesterol activities, in order to prevent cancer growth and metastasis.

CHOLESTEROL AS SIGNAL MOLECULE ON MEMBRANE RAFTS

Cholesterol can act as a signaling molecule at the membrane rafts (28). It is well established that cholesterol-rich microdomains in the plasma membrane and lipid rafts constitute centers of organization for signal transduction and intracellular transport (29). Rafts are small functional areas of the plasma membrane, rich in sphingolipids and cholesterol. These regions are fluid but more ordered and tightly packed than the surrounding bilayer, because of saturated fatty acids. Rafts and related membrane microdomains, such as caveolae characterized by high caveolin-1 expression, have been proposed to play important roles in sorting of membrane molecules and in signal transduction in animal cells (30). Glycophosphatidylinositol-anchored (GPI-anchored) proteins; doubly acylated proteins, such as tyrosine kinases of the Src family, Gα subunits of heterotrimeric G proteins, endothelial nitric oxide synthase (eNOS), and cholesterol-linked and palmitate-anchored proteins are examples of molecules that can be found at membrane rafts (31).

Growth factors signaling are often deregulated in cancer cells. Insulin-like growth factor-I (IGF-I) through its receptor (IGF1R) is one of the most potent natural activators of the phosphatidylinositol 3-kinase (PI3K) and its downstream target Akt, which participate in cell survival pathway. The PI3K/Akt pathway is compartmentalized within plasma membrane raft domains (32). Activated PI3K/Akt control of cell proliferation, apoptosis and tumorogenesis, and aberrant activation of PI3K/Akt pathway contributes to the development and invasiveness of cancer cells (33, 34).

Other studies suggest that, within lipid rafts localize both epidermal growth factor receptor (EGFR) and Human Epidermal growth factor Receptor 2 (HER2) and their signaling events are dependent on cholesterol content of the lipid-rafts (35). Once again, the disruption of the lipid rafts, via depletion of circulating cholesterol levels, interferes with the receptor activation, inhibiting cell growth and development (35–37).

Metastatic events are characterized by cell adhesion decrease and cell migration promotion. Integrins and cell surface glycoproteins such as CD44, have a central role in adhesion mechanisms. CD44 is an adhesion molecule associated with lipid rafts and expressed in several cancers (38, 39). It has been demonstrated that ligand-binding ability of CD44 to hyaluronan, is governed by its cholesterol-dependent localization to cell membrane microdomains (40).

Several cancer cell types, including breast and prostate, have higher membrane cholesterol levels and are richer in membrane rafts (probably as a result of cholesterol accumulation) than their normal counterparts. For this reason, cholesterol-depleting agents are more effective in inducing apoptosis on these cancer cells than on normal cells (41, 42). Rafts/caveolae are rich in various signaling molecules and they have been associated with a number of functions, including cell survival, proliferation, and migration (28, 43).

It has been reported that apoptotic pathways, both extrinsic (death receptor pathway) and intrinsic (mitochondrial), are

associated with lipid rafts because changes in cholesterol content within specific membrane regions regulate apoptotic signaling events (41, 44). Signalings from death receptors Fas receptor (FasR) and TNF-related apoptosis-inducing ligand (TRAIL) receptors 1 and 2 are strictly dependent on translocation into lipid rafts (44, 45). In fact, TRAIL- and Fas-mediated apoptosis is down-regulated by lowering membrane cholesterol in non-small cell lung carcinoma (46) and human Jurkat T leukemia cells (44).

Very recently, disruption of lipid rafts on breast cancer (BC) cell lines MDA-MB231 and MDA-MB468, using the cholesterol depleting agent methyl-β-cyclodextrin, resulted in reduced proliferation and migration, induction of apoptosis evidenced by cell shrinkage, membrane blebbing, nuclear condensation, and chromatin cleavage (47). It was already demonstrated for BC, that lipid rafts disruption causes decreased migration and invasion downregulating caveolin-1 along with urokinase-type plasminogen activator receptor (uPAR) and matrix metallopeptidase 9 (MMP-9) (48). In general, proteins identified in cancer lipid rafts include those involved in endocytosis, Src signaling, cytoskeletal remodeling, chaperones, extracellular matrix (ECM) remodeling (49).

Statins and Lipid Rafts

Based on the importance of cholesterol at the raft/caveola, cholesterol depletion from the plasma membrane would disrupt intracellular signaling triggered by cell surface receptors (50). Statins lower cellular cholesterol content and thus are useful in the analysis of lipid-raft function. However, the effect on raft/caveola formation in cancer cells after statins treatment are not completely defined.

Menter et al. (51), evidenced that simvastatin inhibited the growth of several tumor cell lines with a time-dependent behavior. A significant reduction in cellular cholesterol level were observed after simvastatin treatment, starting at 24 h and up to 72 h. During this time frame, authors observed a reduction in cholesterol content at membrane rafts, caveolin-1 phosphorylation inhibition, disruption of caveolae and loss of membrane integrity. However, cholesterol depletion affected membrane signaling also in caveolin-negative cells (52). Specifically, using the prostate cancer (PCa) cells LNCaP, which do not express caveolin, simvastatin lowered raft cholesterol content, inhibited Akt signaling and induced apoptosis. In addition, using the same cells grown as xenografts, authors demonstrated that elevation of circulating cholesterol using a cholesterol enriched diet, promoted tumor growth and survival, as a consequence of activated Akt signaling via cholesterol-rich lipid rafts (52).

In another study, four head-and-neck squamous cell carcinomas (HNSCCs), four cervical carcinomas, five non small cell lung cancers (NSCLCs), four colon carcinomas, the epidermoid carcinoma cell line A431, and the breast adenocarcinoma MCF-7 were treated with lipophilic lovastatin, which inhibited EGF-induced EGFR autophosphorylation and its downstream signaling cascades (53).

Similarly, another hydrophobic statin, simvastatin caused in A431 cell line anoikis-like apoptosis, characterized by decreased raft levels, Bcl-xL down-regulation, caspase-3 activation, and Akt inactivation (41).

In PC-3 cells simvastatin treatment down-regulated IGF1R expression (54) and inhibited both basal and IGF-1-induced ERK and Akt activation (55). Raft modulating agents are more effective in cells containing a higher content of lipid rafts. In fact, breast (MCF-7, MDA-MB231) and prostate (PC-3, LNCaP) cancer cell lines were more sensitive to cholesterol depletion-induced cell death than normal breast and prostate cell lines (MCF-10A and PZ-HPV7, respectively) (41).

Garnett et al. examined the effects of MβCD and the hydrophilic paravastatin on cholesterol-rich rafts and caveolae and on gene transcription in MDA-MB231 and CaLu-1, lung adenocarcinoma. Both treatments caused a downregulation of genes involved in signal transduction, chemokine and anti-apoptotic pathways. Pravastatin increased expression of caveolin-1, but caveolae density was decreased, because of caveolin-1 inability to properly complex with cholesterol in an altered sterol environment. Similarly, MβCD caused an increase in caveolin-1 expression and reduced both rafts and caveolae, however, it had less effects on gene transcription. Indeed, signaling are more profoundly affected by statins than by the cholesterol-sequestering drug, indicating that not only cholesterol but also some intermediates of cholesterol synthesis downstream of mevalonate, play an important role in signaling pathways at the caveolae (56).

It has been reported that primary cells, HEL, SET-2, and UKE-1, derived from myeloproliferative neoplasms (MPNs) patients require mutated Janus kinase 2 (JAK2), responsible for increased growth signaling (57, 58). Simvastatin disrupts lipid raft and has a negative effect on mutated JAK2-dependent signaling. More specifically, in MPNs cells, simvastatin, lovastatin and atorvastatin inhibited mutated JAK2 localization to lipid rafts reducing cell viability, inducing apoptosis and inhibiting colony formation (59). Colony formation assay is considered a 3D cell culture assay where cells grow independently of a substrate (it is also known as anchorage-independent growth). This assay is particularly useful when studying long-term effects of drugs on anchorage-independent growth of cancer cells. Colony formation evaluates the ability of tumor cells, escaped from the primary tumor site, to exit the blood flow and initiate the post-intravasation phases of metastasis. CD44, as adhesion molecule, plays a central role in the progression of metastasis. The modulation of cholesterol either by statin or MβCD causes dissociation of CD44 from the lipid rafts (40) suggesting that membrane cholesterol may impact metastasis formation. Similar to the results on CD44 cholesterol depletion triggers the shedding of several molecules involved in cancer cell adhesion, including CD30 (60), L1-CAM (61) and collagen types XVII (62) and XXIII (63).

Activity of Ras-Related C3 Botulinum Toxin Substrate 1 (Rac1), a member of the Rho family of Small GTPase, is dependent upon its localization in membrane rafts and its activation is correlated with invasion and metastasis. Cholangiocarcinoma cells treated with simvastatin lose Rac1 rafts localization, because of decreased total cellular cholesterol and disruption of membrane rafts. In normal human cholangiocytes,

simvastatin reduced cholesterol level, but did not affect Rac1 localization. In addition, simvastatin inhibited cell proliferation but, differently from cancer cells, it did not lead to apoptosis (64).

All these data unequivocally suggest that statins have a direct impact on membrane rafts, reducing signaling transduction and adhesion mechanisms in several cancer cell types, thus interfering with cell proliferation and metastasis.

CHOLESTEROL AS SUBSTRATE FOR STEROIDS AND OXYSTEROLS SYNTHESIS

Steroid hormones are synthesized from cholesterol in gonads, adrenal cells and placenta (65). The *de novo* synthesis of some steroid hormones occurs also in the nervous system (66, 67), in cardiac tissue (68) and other peripheral sites (69).

Based on their physiological function, steroid hormones are divided into five groups: mineralocorticoids, which act on the kidney to retain sodium; glucocorticoids, involved in the regulation of glucose metabolism; estrogens, which induce female secondary sexual traits; progestins, which are essential for reproduction; and androgens, which induce male secondary sexual characteristics. These classes of hormones contain the cyclopenta-phenanthrene nucleus and arise from reactions catalyzed by enzymes that belong mainly to the family of cytochrome P450 (CYP). They bind to specific steroid hormone receptors, which act as transcription factors. The active hormone/receptor complexes regulate transcription of distinct set of genes in a tissue-specific manner.

Oxysterols represent 27 carbon-atom molecules derived from cholesterol oxidization through enzymatic processes, or by-products of the cholesterol biosynthetic pathway. Considering the shorter biologic half-life when compared to cholesterol, oxysterols can be considered a way to route cholesterol for catabolism. Specific CYP, localized within the mitochondria or endoplasmic reticulum, are responsible for oxysterol synthesis (70, 71). Among them, the most abundant in human serum are 27, 24(S), 7α, and 4β hydroxycholesterol (HC). The 24(S)HC is synthesized by cholesterol hydroxylase encoded by CYP46A1 in neurons of the central nervous system (72). 7α and 27HC are synthesized in the liver by CYP7A1 and CYP27A1, and represent, respectively, the first intermediates of classic and acidic bile acid synthetic pathways (73). However, 27HC and its synthesizing enzyme CYP27A1 are found also in other cell types (74). Lastly, 4βHC is generated by CYP3A4, a hepatic drug metabolizing enzyme.

Oxysterols act as ligands of Liver X Receptors (LXR) α (NR1H3) and β (NR1H2) (75) to regulate transcription of specific genes. LXRα is expressed primarily in liver, intestine, adipose tissue and macrophages (76), and adrenal (77), whereas LXRβ is expressed in many cell types (78).

LXRα is also involved in the regulation of the adenosine triphosphate-binding cassette (ABC) proteins A1 and G1, cholesterol transporters involved in the flux of cholesterol from enterocytes and macrophages, respectively (79–82). LXRs also seem to have a role in the regulation of human cholesterol ester transfer protein (CETP), which translocates cholesterol esters between lipoproteins (83). Either steroid hormones or oxysterols activate proliferative and metastatic pathways in cancer cells.

Steroid Dependent-Cancer Growth and Progression

It is well known that estrogens exert their biological effects interacting with two members of the nuclear receptor (NR) family, estrogen receptor α (ERα) or estrogen receptor β (ERβ) (84) and with a G-protein coupled receptor namely GPER (85). All three receptors can act at the cell membrane to activate signaling transduction pathways that ultimately regulate gene expression (rapid signaling). Additionally, ERα and ERβ can bind promoter regions of target genes, modulating their transcription (nuclear action). They directly bind DNA at estrogen response elements (EREs) located within promoters of estrogen-regulated target genes. Alternatively, they indirectly bind DNA through the interaction with transcription factors (TFs) that directly bind gene promoters. These TFs are stimulating protein 1 (Sp1) (86), activator protein (AP)-1 (87), nuclear factor-κB (NF-κB) (88). The oncogenic role of estrogens is well characterized in carcinomas of hormone-sensitive tissues including breast, prostate, endometrium and ovary, as well as in non-classical estrogen target tissues such as, adrenal, colon, and lung (89).

Genes involved in cell survival and proliferation and regulated by ERs, through direct or indirect binding to DNA, include myelocytomatosis viral oncogene homolog (MYC), cyclin D1 (CCND1), member RAS oncogene family 17 (RAB17), eukaryotic translation initiation factor 3 subunit A (EIF3A), and tumor protein D52-like 1 (TPD52L1) (90–92). Additionally, membrane ERs engage a functional crosstalk with growth factor receptors, including epidermal growth factor receptor (EGFR), insulin receptor (IR), insulin-like growth factor receptor (IGFR). Growth factor signaling can activate ERα in a ligand independent fashion through phosphorylation, and at the same time, estrogens can regulate IGF signaling (93). Treatment of ERα positive BC with selective estrogen receptor modulators (SERMs) such as tamoxifen, often leads to resistance. EGFR and/or IGFR are critical for the resistance to endocrine therapies (94). Additionally, transactivation of EGFR has been observed in MCF7 breast cancer cells via tamoxifen-dependent activation of GPER (95). The use of tamoxifen on patients with initial GPER-positive tumors increased GPER protein expression, and survival of these BC patients was markedly reduced (95).

Currently, a hot topic in the field of BC research is the definition of the role of androgens and the androgen receptor (AR), with studies revealing both tumor promotion and inhibition (96–98). Expression of AR is associated with favorable prognosis depending on the BC subtype and on whether ER is expressed or not (99, 100). Recently, dihydroxytestosterone (DHT) bound to AR has been shown to directly mediate epigenetic modifications of the chromatin, regulating expression of target genes (101). AR binds to ARE on target genes and, with the help of Lysine-specific demethylase 1A (LSD1), regulates histone modifications, demethylation by LSD1 at H3K4 of the E-cadherin promoter represses gene expression; similarly, LSD1 demethylation at H3K9 activates vimentin gene expression.

Importantly, LSD1 is crucial for epithelial to mesenchimal transition (EMT) induction in several cancer cells (102, 103).

Prostate cancer relays on distinct proliferative pathways, including the PI3K and RAS/RAF pathways downstream of membrane AR activation; dysregulation of these pathways in both early and late stage prostate cancer was demonstrated through genomic profiling (104). In prostate cancer, androgens, testosterone (T), and DHT stimulate proliferation and inhibit apoptosis. Androgen ablation using anti-androgens such as bicalutamide favors cancer regression. This event is related to a lower rate of cell proliferation and to an increased rate of cell death (105). However, many patients do not respond to this therapy and die of recurrent androgen-independent prostate cancer (AIPC), characterized by a high metastatic rate. A crosstalk between androgen-sensitive PCa cells, androgen-independent PCa cells, and PCa-derived stromal cells has been very recently highlighted (106). Adrenal dehydroepiandrosterone (DHEA) is metabolized to DHT in androgen-independent PCa cells (AR negative cells, AR-) as well as in stromal cells. DHT is able to activate AR in androgen sensitive PCa cells (AR positive cells, AR+). Crosstalk among these cells may increase the migration and invasion potential of androgen independent PCa cells via EMT, evidenced by induction of N-cadherin, Snail and vimentin (106). GPER seems to have a role in tumor growth and progression of triple negative breast cancers (TNBC), tumors that lack expression for ERα, progesterone receptor (PR) and HER2 (107, 108). AR directly binds to GPER promoter and treatment with DHT decreases its transcription, possibly by competitively blocking the binding of positive regulators of GPER transcription (109). This reduced GPER expression following DHT treatment, is associated with increased tumor growth (110). Indeed, GPER role in TNBC is still controversial, with some studies indicating GPER involvement in increased tumor growth and worse overall survival (OS) (108) and some others a positive correlation between GPER and OS (111).

Estrogens exert carcinogenic effects on the prostatic epithelium. Combination of estradiol with low-doses of testosterone increased the incidence of prostate carcinomas in a rat model of PCa (112). Similar effects were observed in a mouse model of PCa. When ERα was knocked out in those mice, chronic treatment with testosterone combined with estradiol was unable to induce PCa. Additionally, mice had reduced PCa incidence when aromatase was knocked out. All together these data indicate that autocrine-produced estradiol working through ERα is determinant in PCa development (113). In agreement with animal studies, in the human prostatic epithelium ERα is overexpressed during the malignant transformation, supporting its role as an oncogene (114). On the contrary, ERβ is considered a tumor suppressor; in fact, its expression is decreased or lost in about 40% of PCa (115).

Inhibitory functions of GPER activation in prostate cancers has been demonstrated both *in vitro* and *in vivo* (116).

Statins and Steroid Production

Statins, by decreasing cholesterol synthesis, will also affect the production of steroid hormones. Most steroid hormones are produced by the gonads and adrenal cortex from cholesterol, which is uptaken from the circulating LDL and HDL (117, 118). Since steroidogenesis requires an efficient intracellular pool of cholesterol, by reducing its synthesis, statin therapy could affect steroid production. Cortisol is a steroid hormone produced by the adrenal gland, is mainly released at times of stress, but in normal conditions, its production has a circadian rhythm (119). The effects of statin treatment on cortisol synthesis or cortisol levels were evaluated in several studies, that, however, did not demonstrate any significant effect of statins on cortisol levels (120, 121). An increase in plasma cortisol concentrations was highlighted by a recent meta-analysis study of data from seven randomized controlled trials with various statins (122). In general, the study demonstrated a higher impact of lipophilic statins (atorvastatin, lovastatin, and simvastatin), when compared to hydrophilic statins (pravastatin and rosuvastatin) (123). The increase in cortisol after statin treatment might explain the previously demonstrated anti-inflammatory effects of these drugs. The precise mechanism underling the rise in cortisol after statin use is not known. Studies evaluating the effect on hypothalamic-pituitary system or adrenal cortex itself should be performed to explain the mechanism activated by statins and responsible for the increase in cortisol levels. Based on the observation that only liphophilic statins, which have a greater non-hepatic distribution, affect cortisol levels, it can be speculated that statins have a direct effect on the adrenal gland. Enhancement of LDL-receptor expression, following the inhibition of adrenal HMG-CoA reductase, is responsible for increased cholesterol uptake allowing higher substrate availability for cortisol production. The effect of statins on the expression of steroidogenic enzymes involved in cortisol production is unknown.

A systematic review and meta-analysis of randomized controlled trials demonstrated that among 501 hypercholesterolemic men statins lowered testosterone; similarly, testosterone was reduced in a trial of 368 young women with polycystic ovary syndrome (PCOS) (124).

The direct effect of statins on HMG-CoA reductase in tumor cells is responsible for decreased substrate availability, lowering estrogens and androgens production that drive BC and PCa respectively. Recently, it has been found that statins and dehydroepiandrosterone sulfate (DHEAS) compete for the same transporter, SLCO2B1. Statin administration competitively reduces uptake of DHEAS and consequently tumor cell proliferation of PCa cell lines. The authors demonstrated that statin use at the time of androgen deprivation therapy initiation was associated with delayed tumor progression (125).

In physiological conditions, the prostate is not a steroidogenic site; but steroids, particularly testis-derived testosterone and DHT, regulate its function. In the context of a tumor, prostatic cells become capable of autonomous steroidogenesis (126). Evaluation of statin effects on the expression of steroidogenic enzymes in PC3 cells, demonstrated no effects on steroidogenic acute regulatory protein (StAR), cytochrome P450 family 11 subfamily A member 1 (CYP11A1), cytochrome P450 family 17 subfamily A member 1 (CYP17A1), hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2 (HSD3B2),

steroid 5 alpha-reductase 2 (SRD5A2), and aldo-keto reductase family 1 member C2 (AKR1C2). Conversely, the expression of 17β-hydroxysteroid dehydrogenase type 5 (AKR1C3) was increased and 3β-hydroxysteroid dehydrogenase 1 (HSD3B1) was decreased. These changes in gene expression are responsible for the increase in DHT and T observed following simvastatin treatment (55).

All these studies suggest that steroidogenic tissues are potential sites for statins effect. On the adrenal gland there is no clear modulation of cortisol production, with only lipophilic statins having a direct effect on the adrenal and increasing cortisol synthesis. On the testis and PCOS statins reduce T production. In the context of tumors, there are discording data, reporting both decrease and increase in T production.

Oxysterols Dependent-Cancer Growth and Progression

Epidemiological studies evidenced that high levels of dietary cholesterol would increase the risk of BC in postmenopausal women and the risk of cancers of the stomach, colon, rectum, pancreas, lung, kidney, bladder and non-Hodgkin lymphoma (127). This is probably due to increased oxysterol production which parallels hypercholesterolemia. Elevated concentrations of oxysterols have been associated with colon (128), lung (129), breast (130, 131), skin (132), prostate (133), and bile duct (134) cancers.

Among oxysterols, 27HC is synthesized by CYP27A1, which has a broad substrate specificity and is present in most tissues and not only in the liver. However, 27HC is not an efficient activator of human LXRs (75), instead has been identified to bind ERs (135). 27HC, binds the ERα on epithelial cells of the mammary gland and promotes BC growth (130, 131), while binding LXRα increases metastasis in the MMTV-PyMT mouse model of BC. Similarly, 25HC enhances cell proliferation of a breast cancer cell line via the activation of ERα target genes (136). Both 25HC and 27HC increased the transcription of ER target genes in long-term estrogen deprived BC cell lines, suggesting that these oxysterols replace estrogens and activate ER-mediated gene expression. This event can explain a mechanism involved in the development of resistance to aromatase inhibitors (137). Interestingly, BC patients treated with aromatase inhibitors had significantly increased plasma levels of 27HC and moderately increased levels of 25HC after 28 days of treatment (138), supporting a potential role of 25HC and 27HC level in patient outcome.

More recently, effects of 27HC have been studied in both androgen-responsive LNCaP (AR+) cells and androgen-irresponsive PC3 (AR-) prostate cancer cells. Both cell types increased proliferation in response to the oxysterol binding to ERβ (133).

Oxysterol-binding protein (OSBP) (139) and OSBP-related proteins (ORPs) were originally isolated because of their ability to bind oxysterols, and later cholesterol (140). They comprise a 12-member mammalian gene family, characterized by a conserved OSBP homology domain (OHD) that binds sterols and lipids, as well as the pleckstrin homology (PH) domain and two phenylalanines in an acidic tract (FFAT) motif that mediate interaction with organelle membranes. Upon binding to cholesterol, OSBP promotes ERK (extracellular signal regulated kinase) activity and hence cellular proliferation (140). Among them ORP4, also known as OSBP2, is expressed constitutively in brain, heart and testis, where is expressed as three variants, ORP4L, ORP4M, and ORP4S. Recently, cell growth regulatory activity has been evidenced for ORP4 (141). ORP4 binds sterols and phosphatidylinositol 4-phosphate (PI4P), and binding activity is required for ORP4 to promote cell proliferation and survival (141). Silencing of all ORP4 variants (ORP4L, ORP4M, ORP4S) in HEK293 and HeLa cells inhibited cell proliferation and promoted growth arrest without inducing cell death (141). ORP4L promoted proliferation of three different cervical carcinoma cell lines. (142). ORP4 has been identified as high-affinity cellular receptor for a group of natural products named ORPphilins that potently inhibit the growth of human cancer cell lines (143). Administration of 25HC, a high-affinity ligand for ORP4, suppressed ORPphilin activity.

Statins and Oxysterols Production

A recent phase II clinical trial aimed to investigate effects of statins on BC growth related to a reduction in 27HC levels. Patients were treated with 80 mg/day of atorvastatin to investigate the impact of statin treatment on serum 27HC and on tumor-specific CYP27A1 expression. Atorvastatin exhibited an anti-proliferative effect evidenced by changes in Ki67 index, which did not significantly correlate with changes in either serum 27HC or changes in intratumoral CYP27A1 protein expression. Collectively these data indicate that the anti-proliferative responses to statin treatment do not depend on 27HC reduction (144).

However, 27HC can still affect proliferation of BC resistant to aromatase inhibitors (AI). It has been suggested that AI-resistant tumors can still proliferate in response to 27HC, which similarly to E2 activates ERs (131). In fact, despite lower estrogen levels, aromatase inhibitors resistant tumors have extensive ERα binding to target genes. This is due to ERα activation by 27HC synthesized consequently to stable up-regulation of the entire cholesterol biosynthetic pathways, including genes involved in 27HC biosynthesis. Statins, reducing cholesterol, reduce 27HC, and decrease ERα binding to DNA, abrogating cell invasion (145).

Statin treatments do not seem to have any beneficial effect on the rate of appearance of prostate cancer, but definitively has an effect on the incidence of advanced PCa (146–148). Moreover, in the PC3 prostate cancer cell line, statins prevent the cell migration potential therefore reducing the formation of metastatic prostate colonies; however, the mechanism relies on inhibition of prenylated proteins, not on inhibition of oxysterol formation (149).

These observations suggest that the reduction of oxysterol production by statins treatment could have effect on specific tumors preventing cell migration and invasion.

CHOLESTEROL AS SUBSTRATE FOR VITAMIN D SYNTHESIS

Cholesterol is the precursor molecule for vitamin D. There are two major isoforms of vitamin D: vitamin D_2 (ergocalciferol), and vitamin D_3 (cholecalciferol) (150, 151). UVB radiations are needed to synthesize Vitamin D_2 from ergosterol in plants, yeasts, and fungi and can be ingested from a diet containing food products of plant origin. In humans, vitamin D_3 is synthesized from 7-dehydrocholesterol by UVB radiation in the epidermis of skin and can also be derived from the diet containing food products of animal origin.

Vitamin D is a prohormone that undergoes two-step metabolism in the liver by CYP27A1 to produce the 25(OH)D (calcidiol) and in the kidney by CYP27B1 to produce the biologically active metabolite 1α,25(OH)2D3 (calcitriol) which binds to the vitamin D receptor (VDR) regulating expression of diverse genes (152). CYP27B1 is also expressed in multiple extra-renal sites, including cancer cells (153). Thus, calcitriol can function in an endocrine (systemic) or autocrine manner when it is locally synthesized. Serum level of 30 to 50 ng/mL is normal for healthy people. Vitamin D deficiency and insufficiency is defined as serum 25-hydroxyvitamin D [25(OH)D] levels <20 and 21 to 29 ng/mL, respectively.

Recent studies have revealed that vitamin D can also be metabolized and activated through a CYP11A1-driven non-canonical metabolic pathway (154). The products of CYP11A1, such as 20(OH)D and its hydroxy-metabolites, produce differentiation, have anti-proliferative and anti-inflammatory effects in skin cells comparable or superior to calcitriol (155). Low vitamin D levels increase cancer risk, as evidenced by epidemiological, preclinical and cellular studies (154, 156). In particular, many *in vitro* studies, performed in several malignant cell lines, showed that the anti-cancer activity of this molecule is related to the inhibition of proliferation and angiogenesis and induction of apoptosis (157).

Epidemiological studies showed that serum levels of 25(OH)D adversely correlate with prostate cancer risk (158). In men living at high latitude, as in Scandinavia, 25(OH)D blood serum levels are below 16 ng/mL and the incidence of prostate cancer is high (159). It was observed that 1α,25(OH)2D3 inhibited proliferation and stimulated apoptosis of VDR-positive prostate cancer cells and, interestingly had an anti-inflammatory effect toward this subtype of prostate tumors (160).

In BC cells 1α,25(OH)2D3 caused cell cycle arrest, by interfering with cyclin-dependent kinases activity (161). Additionally, apoptosis can be activated by reducing bcl-2 and up-regulating p53 levels (162). Cell proliferation can be inhibited by 1α,25(OH)2D3, interfering with ER function. Specifically, 1α,25(OH)2D3 and its analogs down-regulate the expression of ERα, which in turn reduced estrogen-dependent activation of mitogenic signal (163). Another action of 1α,25(OH)2D3 against breast cancer cells is the down-regulation of aromatase expression (164).

Proliferation of colon cancer cells was inhibited by 1α,25(OH)2D3 and its analogs, that caused a cell cycle arrest at the G0/G1 phase. This was consequent to enhanced expression of p21 and p27, two cyclin-dependent kinase inhibitors, and to reduced expression of cyclin D1 and cyclin E (165). In addition, following colon cancer cells treatment with 1α,25(OH)2D3, genes with a pro-apoptotic function were increased, while those anti-apoptotic were downregulated (166). It was observed that

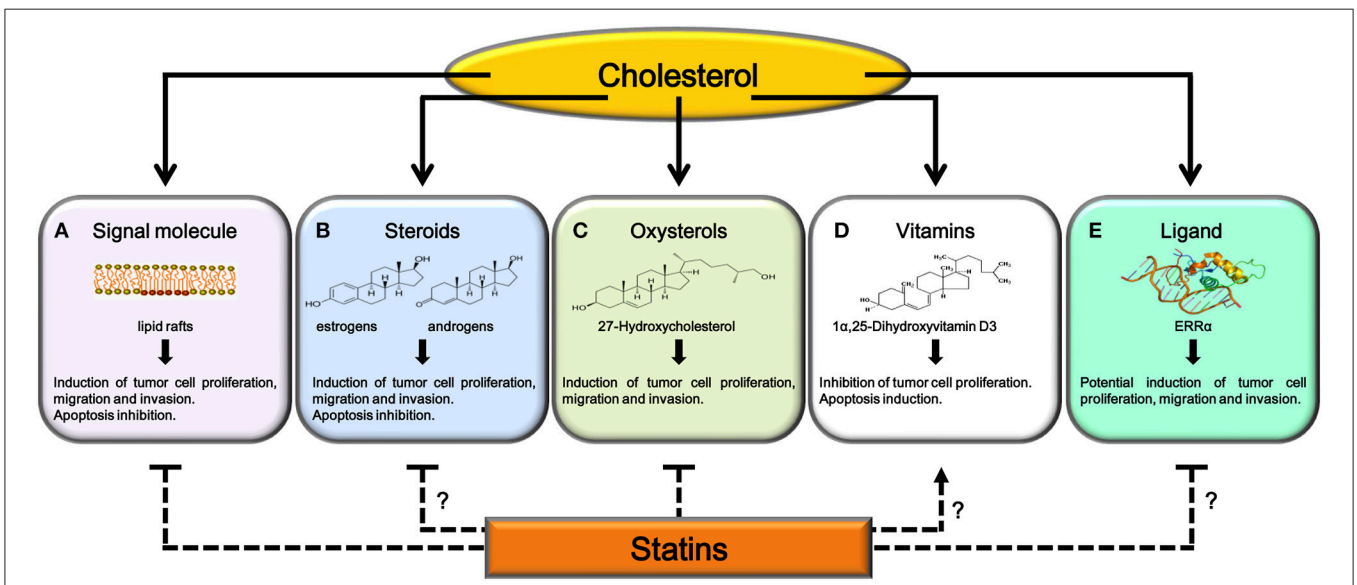

FIGURE 1 | Potential mechanisms explaining antitumoral effects of statins. Cholesterol, after its utilization as signaling molecule **(A)**, as substrate for steroids **(B)**, oxysterols **(C)**, and 1α,25(OH)2D3 synthesis **(D)** or as ligand for ERRα **(E)**, regulates tumor growth and progression. Statins, inhibiting cholesterol synthesis, interfere with **(A)** and **(C)**. Further studies are needed to confirm statins ability to: reduce production of steroid hormones involved in cancer progression **(B)**, increase serum or intratumor 1α,25(OH)2D3 levels **(D)** or interfere with ERRα activity **(E)**.

in differentiated human colon tumors CYP27B1 expression is enhanced compared to untransformed colon mucosa. It was observed a parallel increase of VDR and CYP27B1 mRNA during early tumor progression. This suggests that 1α,25(OH)2D3 synthesized in colonocytes and bound to its receptor could exert its anti-mitotic function in both an autocrine and a paracrine fashion to prevent intestinal tumor formation and progression during early phases of colon tumorigenesis. In fact, in high-grade undifferentiated tumors, expression of CYP27B1 is decreased (167). However, other reports did not find a rise in CYP27B1 expression in tumors, possibly because there was not a distinction for the biological grade of the tumor (168). With a very similar pattern of expression, when compared with normal colon mucosa, VDR expression is increased in early stages of tumorigenesis, but declines in late-stage tumors (169–171). Alternatively, 1α,25(OH)2D3 produced during early phases of transformation could interact with other receptors such as thyroid receptors (TR) (172) and induce cell proliferation. In fact, the expression of TRβ1 was found associated with polypoid growth and with higher histological grade and advanced stage (173). To confirm a protective role for 1α,25(OH)2D3, CYP24A1, the degrading enzyme, has enhanced expression in the majority of colon adenocarcinomas, keeping low 1α,25(OH)2D3 levels (174). Adrenocortical cancers express VDR, its activation by slightly supra-physiological concentrations of 1α,25(OH)2D3 has a moderate anti-proliferative effect, that is related to cell cycle arrest, promoting accumulation of cells in G1 phase, without inducing apoptosis. Additionally, VDR activation decreases cortisol, aldosterone, and DHEA-S production (175).

A very recent study demonstrated no impact of statin therapy on plasma vitamin D levels (176). However, a meta-analysis report indicated opposite data, with statins causing an increase in vitamin D serum levels (177), this effect was observed with lovastatin (178), simvastatin (179), atorvastatin (180), and especially rosuvastatin (181, 182). If further confirmed, these data might help explaining the anti-neoplastic effect exerted by statins on colon cancer (183–185). Currently, there are no studies investigating the effects of statins on intratumor vitamin D synthesis despite vitamin D can act in an autocrine manner to regulate cancer growth. For these reasons further studies on specific tumor are necessary to establish a direct effect of vitamin D on cell tumor proliferation and consequently if statins could induce anti-tumoral effects modulating intra-tumoral vitamin D levels.

CHOLESTEROL AS ESTROGEN-RELATED RECEPTOR ALPHA (ERRα) LIGAND

The Estrogen-related receptor (ERR) family is known to comprise three members [ERRα (NR3B1), ERRβ (NR3B2), and ERRγ (NR3B3)] (186). ERRα is ubiquitously expressed in adult tissues; ERRβ is detected at low levels in the liver, skeletal muscle, stomach, heart, and kidney; ERRγ is widely expressed and can be detected in brain, lung, bon marrow, adrenal and thyroid glands, trachea and spinal cord. The ERRs, like most NRs, are organized in functional domains for ligand (LBD) and DNA binding (DBD), in addition to an activation function 1 (AF-1) involved in cofactors binding. ERα and ERRα LBD share only 37% amino acid homology, indicating low affinity for common ligands, and in fact estradiol fails in activating ERRα (186). While ERRβ and ERRγ are still orphan receptors since their natural ligands have not been identified, ERRα is an adopted orphan receptor, for which ligand has been identified to be cholesterol (187). This finding implies cholesterol involvement in mitochondrial metabolism and biogenesis. In fact, ERRα regulates the expression of a broad range of genes driving mitochondrial biogenesis, the tricarboxylic acid (TCA) cycle, and substrate oxidation.

ERR monomers preferentially recognize the consensus site referred to as the ERR-response element (ERRE). ERRα and ERα share 68% amino acid identity in the DBD, indicating that the two receptors can potentially regulate common genes. Indeed ERR dimers can bind to the ERE, and ERα dimers can also recognize ERRE sites (188). ERα and the ERRs compete for common coactivators such as steroid receptor coactivator (SRC) proteins in transfected cells (189). In addition, another coactivator, the small heterodimer partner (SHP), a coregulator of ER, interacts with all members of the ERR family inhibiting their transcriptional activity. Thus, ERRs and ERs have the potential to differentially regulate common target genes.

ERRα transcriptional activity in normal cells has important roles in cellular metabolism, this is particularly relevant in rapidly dividing cells such as tumor cells. Cholesterol interaction with ERRα (187) allows recruitment of coactivators PGC1α/β and increases ERRα transcriptional activity. ERRα interaction with PGC1α favors osteoclastogenesis (190) and bone reabsorption in osteoclasts, myogenesis (191) and decreases muscle toxicity in myocytes (187). Differently from other nuclear receptors, ERRα is constitutively active because cholesterol is ubiquitous, meaning that it does not require any spike in ligand concentration, as is the case for steroid hormone receptors. ERRα antagonists have been found to induce cancer cell death (192, 193), inhibit tumor growth (194) and improve insulin sensitivity and glucose tolerance (195).

The use of statins or drugs targeting the SREBP metabolic pathways could be a promising option to counteract ERRα-dependent metabolic rearrangement. Identification of cholesterol as ERRα ligand is relatively new, so far no studies have investigated statins effects on ERRα activity in tumor cells. However, the discovery of cholesterol as ERRα ligand has elucidated the mechanism behind statin-induced muscle toxicity (187).

CONCLUSIONS

Statins are widely used drugs for their ability to lower cholesterol levels in hypercholesterolemic patients. Their mechanism of action consists in the inhibition of HMG-CoA reductase, the main enzyme involved in cholesterol biosynthesis.

Aim of this review was to discuss the results of studies focused on the use of statins to the purpose of interfering with cholesterol activities, in order to prevent cancer growth and metastasis.

Cholesterol plays an important function as part of membrane rafts where is involved in the modulation of signaling transduction related to cell proliferation and migration (**Figure 1A**). Data discussed herein unequivocally suggest that statins have a direct impact on the function of membrane rafts, inhibiting, in tumor cells, pathways regulating growth, and metastasis. Cholesterol represents a precursor for estrogens and androgens, hormones involved in modulating cell proliferation, migration, invasion and apoptosis in different cancers (**Figure 1B**). Even though steroidogenic tissues are potential sites for statins effects, there are discording data on a direct role for statins in decreasing steroid production in hormone-dependent cancers. Furthermore, using cholesterol as substrate for specific metabolizing enzymes it is also possible to produce oxysterols, such as 27HC, which has been shown to act as an endogenous selective estrogen receptor modulator able to increase cancer growth and metastasis (**Figure 1C**). Data discussed in this review suggest that the reduction of oxysterol production caused by statins could have a strong effect on specific tumors (i.e., breast cancer) preventing cell migration and invasion.

Cholesterol is also precursor of vitamin 1α,25(OH)2D3, which is involved in modulating VDR-responsive genes, encoding for proteins involved in anti-proliferative signaling (**Figure 1D**). However, the analysis of data published in this field suggest that further studies are necessary to establish a direct effect (anti-proliferative or proliferative) of vitamin D on different cell tumors and consequently if statins could induce specific effects modulating intra-tumoral vitamin D levels.

In conclusion, while the anti-tumor effects produced by statins on cholesterol-mediated transduction mechanisms at the membrane raft or on oxysterols synthesis, appear to be a promising therapeutic strategy, further studies are needed to determine if cholesterol depletion is a valid strategy to limit the effects of steroid hormones on endocrine-dependent tumors. The ability of statins to increase 1α,25(OH)2D3 serum levels need to be confirmed, in order to define another antitumor mechanism for these drugs. Recently, the discovery of cholesterol as ERRα ligand has elucidated the mechanism behind statin-induced muscle toxicity; however, no studies have investigated statins effects on ERRα activity in tumor cells (**Figure 1E**). This last aspect has opened a new field of investigation, in fact, strategies aimed to reduce cholesterol levels, such as the use of statins or drugs targeting SREBP metabolic pathways, could be a promising option to counteract metabolic rewiring in cancer cells where ERRα plays a pivotal role.

Preclinical studies support the potential use of statins as anticancer agents. Epidemiological studies indicate that statin use is associated with a reduction in the incidence of some tumor types. The few clinical trials of statins as monotherapy do not provide convincing results; however, in combination therapy with other agents, statins have shown more promising data. Conclusion of clinical trials not yet completed and publication of data from closed trials will provide a wider picture on the effectiveness of this class of drugs as anticancer therapy.

AUTHOR CONTRIBUTIONS

AC, IC: literature search and drafting of the article. FT, PA, AD, VR: drafting of the article. VP, RS: critical revision of the article and final approval.

FUNDING

This work was supported by AIRC (Associazione Italiana per la Ricerca sul Cancro), projects n. IG15230 and IG20122.

REFERENCES

1. Simons K, Ikonen E. How cells handle cholesterol. *Science* (2000) 290:1721–6. doi: 10.1126/science.290.5497.1721
2. Slater HR, McKinney L, Packard CJ, Shepherd J. Contribution of the receptor pathway to low density lipoprotein catabolism in humans. New methods for quantitation. *Arteriosclerosis* (1984) 4:604–13. doi: 10.1161/01.ATV.4.6.604
3. Brown MS, Goldstein JL. Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. *J Lipid Res.* (2009) 50(Suppl):S15–27. doi: 10.1194/jlr.R800054-JLR200
4. Casey PJ. Mechanisms of protein prenylation and role in G protein function. *Biochem Soc Trans.* (1995) 23:161–6. doi: 10.1042/bst0230161
5. Kuzu OF, Noory MA, Robertson GP. The role of cholesterol in cancer. *Cancer Res.* (2016) 76:2063–70. doi: 10.1158/0008-5472.CAN-15-2613
6. Lee SS, Ho KJ. Cholesterol fatty kidney: morphological changes in the course of its development in rabbits. *Exp Mol Pathol.* (1978) 29:412–25. doi: 10.1016/0014-4800(78)90082-5
7. Azrolan NI, Coleman PS. A discoordinate increase in the cellular amount of 3-hydroxy-3-methylglutaryl-CoA reductase results in the loss of rate-limiting control over cholesterogenesis in a tumour cell-free system. *Biochem J.* (1989) 258:421–5. doi: 10.1042/bj2580421
8. Gould RG. Lipid metabolism and atherosclerosis. *Am J Med.* (1951) 11:209–27. doi: 10.1016/0002-9343(51)90107-6
9. Tomkins GM, Sheppard H, Chaikoff IL. Cholesterol synthesis by liver. IV. Suppression by steroid administration. *J Biological Chem.* (1953) 203:781–6.
10. Siperstein MD, Guest MJ. Studies on the site of the feedback control of cholesterol synthesis. *J Clin Investig.* (1960) 39:642–52. doi: 10.1172/JCI104079
11. Siperstein MD, Fagan VM. Feedback control of mevalonate synthesis by dietary cholesterol. *J Biol Chem.* (1966) 241:602–9.
12. Linn TC. The effect of cholesterol feeding and fasting upon beta-hydroxy-beta-methylglutaryl coenzyme A reductase. *J Biol Chem.* (1967) 242:990–3.
13. Swann A, Wiley MH, Siperstein MD. Tissue distribution of cholesterol feedback control in the guinea pig. *J Lipid Res.* (1975) 16:360–6.
14. Wong WW, Dimitroulakos J, Minden MD, Penn LZ. HMG-CoA reductase inhibitors and the malignant cell: the statin family of drugs as triggers of tumor-specific apoptosis. *Leukemia* (2002) 16:508–19. doi: 10.1038/sj.leu.2402476
15. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. *Cancer Treatment Rev.* (2015) 41:554–67. doi: 10.1016/j.ctrv.2015.04.005
16. Yu O, Eberg M, Benayoun S, Aprikian A, Batist G, Suissa S, et al. Use of statins and the risk of death in patients with prostate cancer. *J Clin Oncol.* (2014) 32:5–11. doi: 10.1200/JCO.2013.49.4757

17. Krens LL, Simkens LH, Baas JM, Koomen ER, Gelderblom H, Punt CJ, et al. Statin use is not associated with improved progression free survival in cetuximab treated KRAS mutant metastatic colorectal cancer patients: results from the CAIRO2 study. *PLoS ONE* (2014) 9:e112201. doi: 10.1371/journal.pone.0112201
18. Shao YY, Hsu CH, Yeh KH, Chen HM, Yeh YC, Lai CL, et al. Statin use is associated with improved prognosis of colorectal cancer in Taiwan. *Clin Colorectal Cancer* (2015) 14:177–184.e4. doi: 10.1016/j.clcc.2015.02.003
19. Viers BR, Houston Thompson R, Psutka SP, Lohse CM, Cheville JC, Leibovich BC, et al. The association of statin therapy with clinicopathologic outcomes and survival among patients with localized renal cell carcinoma undergoing nephrectomy. *Urologic Oncol.* (2015) 33:388e11–8. doi: 10.1016/j.urolonc.2015.01.009
20. Cardwell CR, Hicks BM, Hughes C, Murray LJ. Statin use after diagnosis of breast cancer and survival: a population-based cohort study. *Epidemiology* (2015) 26:68–78. doi: 10.1097/EDE.0000000000000189
21. Habis M, Wroblewski K, Bradaric M, Ismail N, Yamada SD, Litchfield L, et al. Statin therapy is associated with improved survival in patients with non-serous-papillary epithelial ovarian cancer: a retrospective cohort analysis. *PLoS ONE* (2014) 9:e104521. doi: 10.1371/journal.pone.0104521
22. Nowakowski GS, Maurer MJ, Habermann TM, Ansell SM, Macon WR, Ristow KM, et al. Statin use and prognosis in patients with diffuse large B-cell lymphoma and follicular lymphoma in the rituximab era. *J Clin Oncol.* (2010) 28:412–7. doi: 10.1200/JCO.2009.23.4245
23. Vuorio A, Kuoppala J, Kovanen PT, Humphries SE, Tonstad S, Wiegman A, et al. Statins for children with familial hypercholesterolemia. *Cochr Database Syst Rev.* (2017) 7:CD006401. doi: 10.1002/14651858.CD006401.pub4
24. Clendening JW, Penn LZ. Targeting tumor cell metabolism with statins. *Oncogene* (2012) 31:4967–78. doi: 10.1038/onc.2012.6
25. Livingstone E, Hollestein LM, van Herk-Sukel MP, van de Poll-Franse L, Joosse A, Schilling B, et al. Statin use and its effect on all-cause mortality of melanoma patients: a population-based Dutch cohort study. *Cancer Med.* (2014) 3:1284–93. doi: 10.1002/cam4.285
26. Nam DH, Lee H, Park JC, Shin SK, Lee SK, Hyung WJ, et al. Long-term statin therapy improves oncological outcome after radical gastrectomy for stage II and III gastric cancer. *Anticancer Res.* (2014) 34:355–61.
27. Wang Z, Li Y, Banerjee S, Kong D, Ahmad A, Nogueira V, et al. Down-regulation of Notch-1 and Jagged-1 inhibits prostate cancer cell growth, migration and invasion, and induces apoptosis via inactivation of Akt, mTOR, and NF-kappaB signaling pathways. *J Cell Biochem.* (2010) 109:726–36. doi: 10.1002/jcb.22451
28. Sezgin E, Levental I, Mayor S, Eggeling C. The mystery of membrane organization: composition, regulation and roles of lipid rafts. *Nat Rev Mol Cell Biol.* (2017) 18:361–74. doi: 10.1038/nrm.2017.16
29. Ikonen E. Roles of lipid rafts in membrane transport. *Curr Opin Cell Biol.* (2001) 13:470–7. doi: 10.1016/S0955-0674(00)00238-6
30. Lisanti MP, Scherer PE, Vidugiriene J, Tang Z, Hermanowski-Vosatka A, Tu YH, et al. Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: implications for human disease. *J Cell Biol.* (1994) 126:111–26. doi: 10.1083/jcb.126.1.111
31. Jeong J, McMahon AP. Cholesterol modification of Hedgehog family proteins. *J Clin Investig.* (2002) 110:591–6. doi: 10.1172/JCI0216506
32. Gao X, Lowry PR, Zhou X, Depry C, Wei Z, Wong GW, et al. PI3K/Akt signaling requires spatial compartmentalization in plasma membrane microdomains. *Proc Natl Acad Sci USA.* (2011) 108:14509–14. doi: 10.1073/pnas.1019386108
33. Shukla S, Gupta S. Apigenin-induced cell cycle arrest is mediated by modulation of MAPK, PI3K-Akt, and loss of cyclin D1 associated retinoblastoma dephosphorylation in human prostate cancer cells. *Cell Cycle* (2007) 6:1102–14. doi: 10.4161/cc.6.9.4146
34. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol.* (2010) 28:1075–83. doi: 10.1200/JCO.2009.25.3641
35. Chen X, Resh MD. Cholesterol depletion from the plasma membrane triggers ligand-independent activation of the epidermal growth factor receptor. *J Biol Chem.* (2002) 277:49631–7. doi: 10.1074/jbc.M208327200
36. Adam RM, Mukhopadhyay NK, Kim J, Di Vizio D, Cinar B, Boucher K, et al. Cholesterol sensitivity of endogenous and myristoylated Akt. *Cancer Res.* (2007) 67:6238–46. doi: 10.1158/0008-5472.CAN-07-0288
37. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol.* (2007) 35:495–516. doi: 10.1080/01926230701320337
38. Oliferenko S, Paiha K, Harder T, Gerke V, Schwarzler C, Schwarz H, et al. Analysis of CD44-containing lipid rafts: Recruitment of annexin II and stabilization by the actin cytoskeleton. *J Cell Biol.* (1999) 146:843–54. doi: 10.1083/jcb.146.4.843
39. Murai T. The role of lipid rafts in cancer cell adhesion and migration. *Int J Cell Biol.* (2012) 2012:763283. doi: 10.1155/2012/763283
40. Murai T, Sato C, Sato M, Nishiyama H, Suga M, Mio K, et al. Membrane cholesterol modulates the hyaluronan-binding ability of CD44 in T lymphocytes and controls rolling under shear flow. *J Cell Sci.* (2013) 126:3284–94. doi: 10.1242/jcs.120014
41. Li YC, Park MJ, Ye SK, Kim CW, Kim YN. Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents. *Am J Pathol.* (2006) 168:1107–18; quiz 1404–5. doi: 10.2353/ajpath.2006.050959
42. Coogan PF, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Shapiro S. Statin use and the risk of breast and prostate cancer. *Epidemiology* (2002) 13:262–7. doi: 10.1097/00001648-200205000-00005
43. Galbiati F, Razani B, Lisanti MP. Emerging themes in lipid rafts and caveolae. *Cell* (2001) 106:403–11. doi: 10.1016/S0092-8674(01)00472-X
44. Gajate C, Gonzalez-Camacho F, Mollinedo F. Lipid raft connection between extrinsic and intrinsic apoptotic pathways. *Biochem Biophys Res Commun.* (2009) 380:780–4. doi: 10.1016/j.bbrc.2009.01.147
45. Li AJ, Elmore RG, Pavelka JC, Karlan BY. Hyperandrogenism, mediated by obesity and receptor polymorphisms, promotes aggressive epithelial ovarian cancer biology. *Gynecol Oncol.* (2007) 107:420–3. doi: 10.1016/j.ygyno.2007.07.059
46. Song JH, Tse MC, Bellail A, Phuphanich S, Khuri F, Kneteman NM, et al. Lipid rafts and nonrafts mediate tumor necrosis factor related apoptosis-inducing ligand induced apoptotic and nonapoptotic signals in non small cell lung carcinoma cells. *Cancer Res.* (2007) 67:6946–55. doi: 10.1158/0008-5472.CAN-06-3896
47. Badana AK, Chintala M, Gavara MM, Naik S, Kumari S, Kappala VR, et al. Lipid rafts disruption induces apoptosis by attenuating expression of LRP6 and survivin in triple negative breast cancer. *Biomed Pharmacother.* (2018) 97:359–68. doi: 10.1016/j.biopha.2017.10.045
48. Raghu H, Sodadasu PK, Malla RR, Gondi CS, Estes N, Rao JS. Localization of uPAR and MMP-9 in lipid rafts is critical for migration, invasion and angiogenesis in human breast cancer cells. *BMC Cancer* (2010) 10:647. doi: 10.1186/1471-2407-10-647
49. Staubach S, Hanisch FG. Lipid rafts: signaling and sorting platforms of cells and their roles in cancer. *Expert Rev Proteomics* (2011) 8:263–77. doi: 10.1586/epr.11.2
50. Waugh MG, Minogue S, Anderson JS, dos Santos M, Hsuan JJ. Signalling and non-caveolar rafts. *Biochem Soc Transact.* (2001) 29:509–11. doi: 10.1042/bst0290509
51. Menter DG, Ramsauer VP, Harirforoosh S, Chakraborty K, Yang P, Hsi L, et al. Differential effects of pravastatin and simvastatin on the growth of tumor cells from different organ sites. *PLoS ONE* (2011) 6:e28813. doi: 10.1371/journal.pone.0028813
52. Zhuang L, Kim J, Adam RM, Solomon KR, Freeman MR. Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. *J Clin Investig.* (2005) 115:959–68. doi: 10.1172/JCI200519935
53. Dimitroulakos J, Lorimer IA, Goss G. Strategies to enhance epidermal growth factor inhibition: targeting the mevalonate pathway. *Clin Cancer Res.* (2006) 12:4426s−31s. doi: 10.1158/1078-0432.CCR-06-0089
54. Sekine Y, Furuya Y, Nishii M, Koike H, Matsui H, Suzuki K. Simvastatin inhibits the proliferation of human prostate cancer PC-3 cells via down-regulation of the insulin-like growth factor 1 receptor. *Biochem Biophys Res Commun.* (2008) 372:356–61. doi: 10.1016/j.bbrc.2008.05.043
55. Sekine Y, Nakayama H, Miyazawa Y, Kato H, Furuya Y, Arai S, et al. Simvastatin in combination with meclofenamic acid inhibits the proliferation and migration of human prostate cancer PC-3

cells via an AKR1C3 mechanism. *Oncol Lett.* (2018) 15:3167–72. doi: 10.3892/ol.2017.7721
56. Garnett DJ, Greenhough TJ. Statins cause profound effects on gene expression in human cancer cells *in vitro*: the role of membrane microdomains. *Gene Expression* (2012) 15:225–34. doi: 10.3727/105221613X13571653093240
57. Walz C, Crowley BJ, Hudon HE, Gramlich JL, Neuberg DS, Podar K, et al. Activated Jak2 with the V617F point mutation promotes G1/S phase transition. *J Biol Chem.* (2006) 281:18177–83. doi: 10.1074/jbc.M600064200
58. Jedidi A, Marty C, Oligo C, Jeanson-Leh L, Ribeil JA, Casadevall N, et al. Selective reduction of JAK2V617F-dependent cell growth by siRNA/shRNA and its reversal by cytokines. *Blood* (2009) 114:1842–51. doi: 10.1182/blood-2008-09-176875
59. Griner LN, McGraw KL, Johnson JO, List AF, Reuther GW. JAK2-V617F-mediated signalling is dependent on lipid rafts and statins inhibit JAK2-V617F-dependent cell growth. *Br J Haematol.* (2013) 160:177–87. doi: 10.1111/bjh.12103
60. von Tresckow B, Kallen KJ, von Strandmann EP, Borchmann P, Lange H, Engert A, et al. Depletion of cellular cholesterol and lipid rafts increases shedding of CD30. *J Immunol.* (2004) 172:4324–31. doi: 10.4049/jimmunol.172.7.4324
61. Mechtersheimer S, Gutwein P, Agmon-Levin N, Stoeck A, Oleszewski M, Riedle S, et al. Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. *J Cell Biol.* (2001) 155:661–73. doi: 10.1083/jcb.200101099
62. Zimina EP, Bruckner-Tuderman L, Franzke CW. Shedding of collagen XVII ectodomain depends on plasma membrane microenvironment. *J Biol Chem.* (2005) 280:34019–24. doi: 10.1074/jbc.M503751200
63. Veit G, Zimina EP, Franzke CW, Kutsch S, Siebolds U, Gordon MK, et al. Shedding of collagen XXIII is mediated by furin and depends on the plasma membrane microenvironment. *J Biol Chem.* (2007) 282:27424–35. doi: 10.1074/jbc.M703425200
64. Miller T, Yang F, Wise CE, Meng F, Priester S, Munshi MK, et al. Simvastatin stimulates apoptosis in cholangiocarcinoma by inhibition of Rac1 activity. *Dig Liver Dis.* (2011) 43:395–403. doi: 10.1016/j.dld.2011.01.010
65. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocr Rev.* (2004) 25:947–70. doi: 10.1210/er.2003-0030
66. Baulieu EE. Neurosteroids: a novel function of the brain. *Psychoneuroendocrinology* (1998) 23:963–87. doi: 10.1016/S0306-4530(98)00071-7
67. Mellon SH, Griffin LD. Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab.* (2002) TEM 13:35–43. doi: 10.1016/S1043-2760(01)00503-3
68. Kayes-Wandover KM, White PC. Steroidogenic enzyme gene expression in the human heart. *J Clin Endocrinol Metab.* (2000) 85:2519–25. doi: 10.1210/jc.85.7.2519
69. Labrie F, Belanger A, Luu-The V, Labrie C, Simard J, Cusan L, et al. DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: its role during aging. *Steroids* (1998) 63:322–8. doi: 10.1016/S0039-128X(98)00007-5
70. Russell DW. Oxysterol biosynthetic enzymes. *Biochim Biophys Acta* (2000) 1529:126–35. doi: 10.1016/S1388-1981(00)00142-6
71. Luoma PV. Cytochrome P450–physiological key factor against cholesterol accumulation and the atherosclerotic vascular process. *Ann Med.* (2007) 39:359–70. doi: 10.1080/07853890701379767
72. Bjorkhem I. Rediscovery of cerebrosterol. *Lipids* (2007) 42:5–14. doi: 10.1007/s11745-006-1003-2
73. Bjorkhem I, Eggertsen G. Genes involved in initial steps of bile acid synthesis. *Curr Opin lipidol.* (2001) 12:97–103. doi: 10.1097/00041433-200104000-00002
74. Babiker A, Andersson O, Lund E, Xiu RJ, Deeb S, Reshef A, et al. Elimination of cholesterol in macrophages and endothelial cells by the sterol 27-hydroxylase mechanism. Comparison with high density lipoprotein-mediated reverse cholesterol transport. *J Biol Chem.* (1997) 272:26253–61. doi: 10.1074/jbc.272.42.26253
75. Lehmann JM, Kliewer SA, Moore LB, Smith-Oliver TA, Oliver BB, Su JL, et al. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J Biol Chem.* (1997) 272:3137–40. doi: 10.1074/jbc.272.6.3137
76. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature* (1996) 383:728–31. doi: 10.1038/383728a0
77. Cummins CL, Volle DH, Zhang Y, McDonald JG, Sion B, Lefrancois-Martinez AM, et al. Liver X receptors regulate adrenal cholesterol balance. *J Clin Investig.* (2006) 116:1902–12. doi: 10.1172/JCI28400
78. Repa JJ, Mangelsdorf DJ. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. *Ann Rev Cell Devel Biol.* (2000) 16:459–81. doi: 10.1146/annurev.cellbio.16.1.459
79. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. *Science* (2001) 294:1866–70. doi: 10.1126/science.294.5548.1866
80. Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, Lustig K, et al. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science* (2000) 289:1524–9. doi: 10.1126/science.289.5484.1524
81. Venkateswaran A, Repa JJ, Lobaccaro JM, Bronson A, Mangelsdorf DJ, Edwards PA. Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols. *J Biol Chem.* (2000) 275:14700–7. doi: 10.1074/jbc.275.19.14700
82. Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA, et al. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. *Proc Natl Acad Sci US A.* (2000) 97:12097–102. doi: 10.1073/pnas.200367697
83. Luo Y, Tall AR. Sterol upregulation of human CETP expression *in vitro* and in transgenic mice by an LXR element. *J Clin Investig.* (2000) 105:513–20. doi: 10.1172/JCI8573
84. Hamilton KJ, Hewitt SC, Arao Y, Korach KS. Estrogen hormone biology. *Curr Topics Devel Biol.* (2017) 125:109–46. doi: 10.1016/bs.ctdb.2016.12.005
85. Filardo EJ. A role for G-protein coupled estrogen receptor (GPER) in estrogen-induced carcinogenesis: dysregulated glandular homeostasis, survival and metastasis. *J Steroid Biochem Mol Biol.* (2018) 176:38–48. doi: 10.1016/j.jsbmb.2017.05.005
86. Sun G, Porter W, Safe S. Estrogen-induced retinoic acid receptor alpha 1 gene expression: role of estrogen receptor-Sp1 complex. *Mol Endocrinol.* (1998) 12:882–90.
87. Liu MM, Albanese C, Anderson CM, Hilty K, Webb P, Uht RM, et al. Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression. *J Biol Chem.* (2002) 277:24353–60. doi: 10.1074/jbc.M201829200
88. Ray A, Prefontaine KE, Ray P. Down-modulation of interleukin-6 gene expression by 17 beta-estradiol in the absence of high affinity DNA binding by the estrogen receptor. *J Biol Chem.* (1994) 269:12940–6.
89. Folkerd EJ, Dowsett M. Influence of sex hormones on cancer progression. *J Clin Oncol.* (2010) 28:4038–44. doi: 10.1200/JCO.2009.27.4290
90. Frasor J, Danes JM, Komm B, Chang KC, Lyttle CR, Katzenellenbogen BS. Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. *Endocrinology* (2003) 144:4562–74. doi: 10.1210/en.2003-0567
91. Hershberger PA, Vasquez AC, Kanterewicz B, Land S, Siegfried JM, Nichols M. Regulation of endogenous gene expression in human non-small cell lung cancer cells by estrogen receptor ligands. *Cancer Res.* (2005) 65:1598–605. doi: 10.1158/0008-5472.CAN-04-2694
92. Sirianni R, Zolea F, Chimento A, Ruggiero C, Cerquetti L, Fallo F, et al. Targeting estrogen receptor-alpha reduces adrenocortical cancer (ACC) cell growth *in vitro* and *in vivo*: potential therapeutic role of selective estrogen receptor modulators (SERMs) for ACC treatment. *J Clin Endocrinol Metab.* (2012) 97:E2238–50. doi: 10.1210/jc.2012-2374
93. Yee D, Lee AV. Crosstalk between the insulin-like growth factors and estrogens in breast cancer. *J Mammary Gland Biol Neoplasia* (2000) 5:107–15. doi: 10.1023/A:1009575518338
94. Osborne CK, Shou J, Massarweh S, Schiff R. Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. *Clin Cancer Res.* (2005) 11:865s—70s.

95. Ignatov A, Ignatov T, Roessner A, Costa SD, Kalinski T. Role of GPR30 in the mechanisms of tamoxifen resistance in breast cancer MCF-7 cells. *Breast Cancer Res Treat.* (2010) 123:87–96. doi: 10.1007/s10549-009-0624-6
96. McNamara KM, Sasano H. Beyond the C18 frontier: androgen and glucocorticoid metabolism in breast cancer tissues: the role of non-typical steroid hormones in breast cancer development and progression. *Steroids* (2015) 103:115–22. doi: 10.1016/j.steroids.2015.05.008
97. McNamara KM, Sasano H. Androgen and breast cancer: an update. *Curr Opin Endocrinol Diab Obes.* (2016) 23:249–56. doi: 10.1097/MED.0000000000000251
98. Hickey TE, Robinson JL, Carroll JS, Tilley WD. Minireview: the androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? *Mol Endocrinol.* (2012) 26:1252–67. doi: 10.1210/me.2012-1107
99. Lim E, Tarulli G, Portman N, Hickey TE, Tilley WD, Palmieri C. Pushing estrogen receptor around in breast cancer. *Endocrine-Related Cancer* (2016) 23:T227–41. doi: 10.1530/ERC-16-0427
100. McNamara KM, Moore NL, Hickey TE, Sasano H, Tilley WD. Complexities of androgen receptor signalling in breast cancer. *Endocr Related Cancer* (2014) 21:T161–81. doi: 10.1530/ERC-14-0243
101. Feng J, Li L, Zhang N, Liu J, Zhang L, Gao H, et al. Androgen and AR contribute to breast cancer development and metastasis: an insight of mechanisms. *Oncogene* (2017) 36:2775–90. doi: 10.1038/onc.2016.432
102. Li Y, Wan X, Wei Y, Liu X, Lai W, Zhang L, et al. LSD1-mediated epigenetic modification contributes to ovarian cancer cell migration and invasion. *Oncol Rep.* (2016) 35:3586–92. doi: 10.3892/or.2016.4729
103. Ferrari-Amorotti G, Fragliasso V, Esteki R, Prudente Z, Soliera AR, Cattelani S, et al. Inhibiting interactions of lysine demethylase LSD1 with snail/slug blocks cancer cell invasion. *Cancer Res.* (2013) 73:235–45. doi: 10.1158/0008-5472.CAN-12-1739
104. Ye Q, Chung LW, Cinar B, Li S, Zhau HE. Identification and characterization of estrogen receptor variants in prostate cancer cell lines. *J Steroid Biochem Mol Biol.* (2000) 75:21–31. doi: 10.1016/S0960-0760(00)00118-7
105. Denmeade SR, Isaacs JT. Activation of programmed (apoptotic) cell death for the treatment of prostate cancer. *Adv Pharmacol.* (1996) 35:281–306. doi: 10.1016/S1054-3589(08)60278-1
106. Takezawa Y, Izumi K, Machioka K, Iwamoto H, Naito R, Makino T, et al. Crosstalk between androgen-sensitive and androgen-insensitive prostate cancer cells. *Anticancer Res.* (2018) 38:2045–55. doi: 10.21873/anticanres.12444
107. Yu T, Liu M, Luo H, Wu C, Tang X, Tang S, et al. GPER mediates enhanced cell viability and motility via non-genomic signaling induced by 17beta-estradiol in triple-negative breast cancer cells. *J Steroid Biochem Mol Biol.* (2014) 143:392–403. doi: 10.1016/j.jsbmb.2014.05.003
108. Steiman J, Peralta EA, Louis S, Kamel O. Biology of the estrogen receptor, GPR30, in triple negative breast cancer. *Am J Surg.* (2013) 206:698–703. doi: 10.1016/j.amjsurg.2013.07.014
109. Shen Y, Yang F, Zhang W, Song W, Liu Y, Guan X. The androgen receptor promotes cellular proliferation by suppression of g-protein coupled estrogen receptor signaling in triple-negative breast cancer. *Cell Physiol Biochem.* (2017) 43:2047–61. doi: 10.1159/000484187
110. Zhu A, Li Y, Song W, Xu Y, Yang F, Zhang W, et al. Antiproliferative effect of androgen receptor inhibition in mesenchymal stem-like triple-negative breast cancer. *Cell Physiol Biochem.* (2016) 38:1003–14. doi: 10.1159/000443052
111. Chen ZJ, Wei W, Jiang GM, Liu H, Wei WD, Yang X, et al. Activation of GPER suppresses epithelial mesenchymal transition of triple negative breast cancer cells via NF-kappaB signals. *Mol Oncol.* (2016) 10:775–88. doi: 10.1016/j.molonc.2016.01.002
112. Bosland MC, Ford H, Horton L. Induction at high incidence of ductal prostate adenocarcinomas in NBL/Cr and Sprague-Dawley Hsd:SD rats treated with a combination of testosterone and estradiol-17 beta or diethylstilbestrol. *Carcinogenesis* (1995) 16:1311–7. doi: 10.1093/carcin/16.6.1311
113. Ricke WA, McPherson SJ, Bianco JJ, Cunha GR, Wang Y, Risbridger GP. Prostatic hormonal carcinogenesis is mediated by in situ estrogen production and estrogen receptor alpha signaling. *FASEB J.* (2008) 22:1512–20. doi: 10.1096/fj.07-9526com
114. Bonkhoff H, Fixemer T, Hunsicker I, Remberger K. Estrogen receptor expression in prostate cancer and premalignant prostatic lesions. *Am J Pathol.* (1999) 155:641–7. doi: 10.1016/S0002-9440(10)65160-7
115. Fixemer T, Remberger K, Bonkhoff H. Differential expression of the estrogen receptor beta (ERbeta) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. *Prostate* (2003) 54:79–87. doi: 10.1002/pros.10171
116. Chan QK, Lam HM, Ng CF, Lee AY, Chan ES, Ng HK, et al. Activation of GPR30 inhibits the growth of prostate cancer cells through sustained activation of Erk1/2, c-jun/c-fos-dependent upregulation of p21, and induction of G(2) cell-cycle arrest. *Cell Death Differentiation* (2010) 17:1511–23. doi: 10.1038/cdd.2010.20
117. Liu J, Heikkila P, Meng QH, Kahri AI, Tikkanen MJ, Voutilainen R. Expression of low and high density lipoprotein receptor genes in human adrenals. *Eur J Endocrinol.* (2000) 142:677–82. doi: 10.1530/eje.0.1420677
118. Bochem AE, Holleboom AG, Romijn JA, Hoekstra M, Dallinga-Thie GM, Motazacker MM, et al. High density lipoprotein as a source of cholesterol for adrenal steroidogenesis: a study in individuals with low plasma HDL-C. *J Lipid Res.* (2013) 54:1698–704. doi: 10.1194/jlr.P033449
119. Chan S, Debono M. Replication of cortisol circadian rhythm: new advances in hydrocortisone replacement therapy. *Ther Adv Endocrinol Metab.* (2010) 1:129–38. doi: 10.1177/2042018810380214
120. Bohm M, Herrmann W, Wassmann S, Laufs U, Nickenig G. Does statin therapy influence steroid hormone synthesis? *Zeitschrift Kardiologie* (2004) 93:43–8. doi: 10.1007/s00392-004-1003-2
121. Clauss SB, Holmes KW, Hopkins P, Stein E, Cho M, Tate A et al. Efficacy and safety of lovastatin therapy in adolescent girls with heterozygous familial hypercholesterolemia. *Pediatrics* (2005) 116:682–8. doi: 10.1542/peds.2004-2090
122. Venkatesh B, Imeson L, Kruger P, Cohen J, Jones M, Bellomo R, et al. Elevated plasma-free cortisol concentrations and ratios are associated with increased mortality even in the presence of statin therapy in patients with severe sepsis. *Crit Care Med.* (2015) 43:630–5. doi: 10.1097/CCM.0000000000000750
123. Sahebkar A, Rathouska J, Simental-Mendia LE, Nachtigal P. Statin therapy and plasma cortisol concentrations: A systematic review and meta-analysis of randomized placebo-controlled trials. *Pharmacol Res.* (2016) 103:17–25. doi: 10.1016/j.phrs.2015.10.013
124. Schooling CM, Au Yeung SL, Freeman G, Cowling BJ The effect of statins on testosterone in men and women, a systematic review and meta-analysis of randomized controlled trials. *BMC Med.* (2013) 11:57. doi: 10.1186/1741-7015-11-57
125. Harshman LC, Wang X, Nakabayashi M, Xie W, Valenca L, Werner L, et al. Statin use at the time of initiation of androgen deprivation therapy and time to progression in patients with hormone-sensitive prostate cancer. *JAMA Oncol.* (2015) 1:495–504. doi: 10.1001/jamaoncol.2015.0829
126. Bennett NC, Hooper JD, Lambie D, Lee CS, Yang T, Vesey DA, et al. Evidence for steroidogenic potential in human prostate cell lines and tissues. *Am J Pathol.* (2012) 181:1078–87. doi: 10.1016/j.ajpath.2012.06.009
127. Hu J, La Vecchia C, de Groh M, Negri E, Morrison H, Mery L, et al. Dietary cholesterol intake and cancer. *Ann Oncol.* (2012) 23:491–500. doi: 10.1093/annonc/mdr155
128. Swan R, Alnabulsi A, Cash B, Alnabulsi A, Murray GI. Characterisation of the oxysterol metabolising enzyme pathway in mismatch repair proficient and deficient colorectal cancer. *Oncotarget* (2016) 7:46509–27. doi: 10.18632/oncotarget.10224
129. Nagano K, Imai S, Zhao X, Yamashita T, Yoshioka Y, Abe Y, et al. Identification and evaluation of metastasis-related proteins, oxysterol binding protein-like 5 and calumenin, in lung tumors. *Int J Oncol.* (2015) 47:195–203. doi: 10.3892/ijo.2015.3000
130. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, et al. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* (2013) 342:1094–8. doi: 10.1126/science.1241908
131. Wu Q, Ishikawa T, Sirianni R, Tang H, McDonald JG, Yuhanna IS, et al. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Rep.* (2013) 5:637–45. doi: 10.1016/j.celrep.2013.10.006
132. Tian W, Pang W, Ge Y, He X, Wang D, Li X, et al. Hepatocyte-generated 27-hydroxycholesterol promotes the growth of melanoma by

activation of estrogen receptor alpha. *J Cell Biochem.* (2018) 119:2929–38. doi: 10.1002/jcb.26498
133. Raza S, Meyer M, Goodyear CK, Hammer DP, Guo B, Ghribi O. The cholesterol metabolite 27-hydroxycholesterol stimulates cell proliferation via ERbeta in prostate cancer cells. *Cancer Cell Int.* (2017) 17:52. doi: 10.1186/s12935-017-0422-x
134. Loilome W, Wechagama P, Namwat N, Jusakul A, Sripa B, Miwa M, et al. Expression of oxysterol binding protein isoforms in opisthorchiasis-associated cholangiocarcinoma: a potential molecular marker for tumor metastasis. *Parasitol Int.* (2012) 61:136–9. doi: 10.1016/j.parint.2011.07.003
135. Umetani M, Shaul PW. 27-Hydroxycholesterol: the first identified endogenous *SERM Trends Endocrinol Metab*: (2011) 22:130–5. doi: 10.1016/j.tem.2011.01.003
136. Lappano R, Recchia AG, De Francesco EM, Angelone T, Cerra MC, Picard D, et al. The cholesterol metabolite 25-hydroxycholesterol activates estrogen receptor alpha-mediated signaling in cancer cells and in cardiomyocytes. *PLoS ONE* (2011) 6:e16631. doi: 10.1371/journal.pone.0016631
137. Simigdala N, Gao Q, Pancholi S, Roberg-Larsen H, Zvelebil M, Ribas R, et al. Cholesterol biosynthesis pathway as a novel mechanism of resistance to estrogen deprivation in estrogen receptor-positive breast cancer. *Breast Cancer Res.* (2016) 18:58. doi: 10.1186/s13058-016-0713-5
138. Dalenc F, Iuliano L, Filleron T, Zerbinati C, Voisin M, Arellano C, et al. Circulating oxysterol metabolites as potential new surrogate markers in patients with hormone receptor-positive breast cancer: results of the OXYTAM study. *J Steroid Biochem Mol Biol.* (2017) 169:210–8. doi: 10.1016/j.jsbmb.2016.06.010
139. Raychaudhuri S, Im YJ, Hurley JH, Prinz WA. Nonvesicular sterol movement from plasma membrane to ER requires oxysterol-binding protein-related proteins and phosphoinositides. *J Cell Biol.* (2006) 173:107–19. doi: 10.1083/jcb.200510084
140. Wang PY, Weng J, Anderson RG. OSBP is a cholesterol-regulated scaffolding protein in control of ERK 1/2 activation. *Science* (2005) 307:1472–6. doi: 10.1126/science.1107710
141. Charman M, Colbourne TR, Pietrangelo A, Kreplak L, Ridgway ND. Oxysterol-binding protein (OSBP)-related protein 4 (ORP4) is essential for cell proliferation and survival. *J Biol Chem.* (2014) 289:15705–17. doi: 10.1074/jbc.M114.571216
142. Li JW, Xiao YL, Lai CF, Lou N, Ma HL, Zhu BY, et al. Oxysterol-binding protein-related protein 4L promotes cell proliferation by sustaining intracellular Ca2+ homeostasis in cervical carcinoma cell lines. *Oncotarget* (2016) 7:65849–61. doi: 10.18632/oncotarget.11671
143. Burgett AW, Poulsen TB, Wangkanont K, Anderson DR, Kikuchi C, Shimada K, et al. Natural products reveal cancer cell dependence on oxysterol-binding proteins. *Nat Chem Biol.* (2011) 7:639–47. doi: 10.1038/nchembio.625
144. Kimbung S, Chang CY, Bendahl PO, Dubois L, Thompson JW, McDonnell DP, et al. Impact of 27-hydroxylase (CYP27A1) and 27-hydroxycholesterol in breast cancer. *Endocr Related Cancer* (2017) 24:339–49. doi: 10.1530/ERC-16-0533
145. Nguyen VT, Barozzi I, Faronato M, Lombardo Y, Steel JH, Patel N, et al. Differential epigenetic reprogramming in response to specific endocrine therapies promotes cholesterol biosynthesis and cellular invasion. *Nat Commun.* (2015) 6:10044. doi: 10.1038/ncomms10044
146. Murtola TJ, Tammela TL, Maattanen L, Hakama M, Auvinen A. Prostate cancer risk among users of finasteride and alpha-blockers - a population based case-control study. *Eur J Cancer* (2007) 43:775–81. doi: 10.1016/j.ejca.2006.12.001
147. Shannon J, Tewoderos S, Garzotto M, Beer TM, Derenick R, Palma A, et al. Statins and prostate cancer risk: a case-control study. *Am J Epidemiol.* (2005) 162:318–25. doi: 10.1093/aje/kwi203
148. Jacobs EJ, Rodriguez C, Bain EB, Wang Y, Thun MJ, Calle EE. Cholesterol-lowering drugs and advanced prostate cancer incidence in a large U.S. cohort. *Cancer Epidemiol Biomarkers Prevent.* (2007) 16:2213–7. doi: 10.1158/1055-9965.EPI-07-0448
149. Brown M, Hart C, Tawadros T, Ramani V, Sangar V, Lau M, et al. The differential effects of statins on the metastatic behaviour of prostate cancer. *Br J Cancer* (2012) 106:1689–96. doi: 10.1038/bjc.2012.138
150. Heaney RP. Vitamin D in health and disease. *Clin J Am Soc Nephrol.* (2008) 3:1535–41. doi: 10.2215/CJN.01160308
151. Japelt RB, Jakobsen J. Vitamin D in plants: a review of occurrence, analysis, and biosynthesis. *Front Plant Sci.* (2013) 4:136. doi: 10.3389/fpls.2013.00136
152. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. *J Lipid Res.* (2014) 55:13–31. doi: 10.1194/jlr.R031534
153. Hobaus J, Thiem U, Hummel DM, Kallay E. Role of calcium, vitamin D, and the extrarenal vitamin D hydroxylases in carcinogenesis. *Anti Cancer Agents Med Chem.* (2013) 13:20–35. doi: 10.2174/187152013804487434
154. Jeon SM, Shin EA. Exploring vitamin D metabolism and function in cancer. *Exp Mol Med.* (2018) 50:20. doi: 10.1038/s12276-018-0038-9
155. Slominski AT, Brozyna AA, Skobowiat C, Zmijewski MA, Kim TK, Janjetovic Z, et al. On the role of classical and novel forms of vitamin D in melanoma progression and management. *J Steroid Biochem Mol Biol.* (2018) 177:159–70. doi: 10.1016/j.jsbmb.2017.06.013
156. Grant WB. A review of the evidence supporting the vitamin D-cancer prevention hypothesis in 2017. *Anticancer Res.* (2018) 38:1121–36. doi: 10.21873/anticanres.12331
157. Castronovo C, Castronovo V, Nikkels A, Peulen O. Vitamin D anti-cancer activities: observations, doubts and certainties. *Revue Medicale Liege* (2015) 70:495–500.
158. Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control* (2000) 11:847–52. doi: 10.1023/A:1008923802001
159. Schwartz GG. Vitamin D and the epidemiology of prostate cancer. *Semin Dialysis* (2005) 18:276–89. doi: 10.1111/j.1525-139X.2005.18403.x
160. Moreno J, Krishnan AV, Feldman D. Molecular mechanisms mediating the anti-proliferative effects of Vitamin D in prostate cancer. *J Steroid Biochem Mol Biol.* (2005) 97:31–6. doi: 10.1016/j.jsbmb.2005.06.012
161. Simboli-Campbell M, Narvaez CJ, van Weelden K, Tenniswood M, Welsh J. Comparative effects of 1,25(OH)2D3 and EB1089 on cell cycle kinetics and apoptosis in MCF-7 breast cancer cells. *Breast Cancer Res Treat.* (1997) 42:31–41. doi: 10.1023/A:1005772432465
162. James SY, Mackay AG, Colston KW. Effects of 1,25 dihydroxyvitamin D3 and its analogues on induction of apoptosis in breast cancer cells. *J Steroid Biochem Mol Biol.* (1996) 58:395–401. doi: 10.1016/0960-0760(96)00048-9
163. James SY, Mackay AG, Binderup L, Colston KW. Effects of a new synthetic vitamin D analogue, EB1089, on the oestrogen-responsive growth of human breast cancer cells. *J Endocrinol.* (1994) 141:555–63. doi: 10.1677/joe.0.1410555
164. Krishnan AV, Swami S, Peng L, Wang J, Moreno J, Feldman D. Tissue-selective regulation of aromatase expression by calcitriol: implications for breast cancer therapy. *Endocrinology* (2010) 151:32–42. doi: 10.1210/en.2009-0855
165. Scaglione-Sewell BA, Bissonnette M, Skarosi S, Abraham C, Brasitus TA. A vitamin D3 analog induces a G1-phase arrest in CaCo-2 cells by inhibiting cdk2 and cdk6: roles of cyclin E, p21Waf1, and p27Kip1. *Endocrinology* (2000) 141:3931–9. doi: 10.1210/endo.141.11.7782
166. Diaz GD, Paraskeva C, Thomas MG, Binderup L, Hague A. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: possible implications for prevention and therapy. *Cancer Res.* (2000) 60:2304–12.
167. Bises G, Kallay E, Weiland T, Wrba F, Wenzl E, Bonner E, et al. 25-hydroxyvitamin D3-1alpha-hydroxylase expression in normal and malignant human colon. *J Histochem Cytochem.* (2004) 52:985–9. doi: 10.1369/jhc.4B6271.2004
168. Tangpricha V, Flanagan JN, Whitlatch LW, Tseng CC, Chen TC, Holt PR, et al. 25-hydroxyvitamin D-1alpha-hydroxylase in normal and malignant colon tissue. *Lancet* (2001) 357:1673–4. doi: 10.1016/S0140-6736(00)04831-5
169. Shabahang M, Buras RR, Davoodi F, Schumaker LM, Nauta RJ, Evans SR. 1,25-Dihydroxyvitamin D3 receptor as a marker of human colon carcinoma cell line differentiation and growth inhibition. *Cancer Res.* (1993) 53:3712–8.
170. Sheinin Y, Kaserer K, Wrba F, Wenzl E, Kriwanek S, Peterlik M, et al. *In situ* mRNA hybridization analysis and immunolocalization of the vitamin D receptor in normal and carcinomatous human colonic mucosa: relation to epidermal growth factor receptor expression. *Virchows Archiv Int J Pathol.* (2000) 437:501–7. doi: 10.1007/s004280000275

171. Cross HS, Bareis P, Hofer H, Bischof MG, Bajna E, Kriwanek S, et al. 25-Hydroxyvitamin D3-1α-hydroxylase and vitamin D receptor gene expression in human colonic mucosa is elevated during early cancerogenesis. *Steroids* (2001) 66:287–92. doi: 10.1016/S0039-128X(00)00153-7
172. Marshall TG. Are statins analogues of vitamin D? *Lancet* (2006) 368:1234; author reply 1235. doi: 10.1016/S0140-6736(06)69509-3
173. Horkko TT, Tuppurainen K, George SM, Jernvall P, Karttunen TJ, Makinen MJ. Thyroid hormone receptor beta1 in normal colon and colorectal cancer-association with differentiation, polypoid growth type and K-ras mutations. *Int J Cancer* (2006) 118:1653–9. doi: 10.1002/ijc.21556
174. Horvath HC, Lakatos P, Kosa JP, Bacsi K, Borka K, Bises G, et al. The candidate oncogene CYP24A1: A potential biomarker for colorectal tumorigenesis. *J Histochem Cytochem.* (2010) 58:277–85. doi: 10.1369/jhc.2009.954339
175. Pilon C, Urbanet R, Williams TA, Maekawa T, Vettore S, Sirianni R, et al. 1alpha,25-Dihydroxyvitamin D(3) inhibits the human H295R cell proliferation by cell cycle arrest: a model for a protective role of vitamin D receptor against adrenocortical cancer. *J Steroid Biochem Mol Biol.* (2014) 140:26–33. doi: 10.1016/j.jsbmb.2013.11.008
176. Sahebkar A, Reiner Z, Simental-Mendia LE, Ferretti G, Della Corte C, Nobili V. Impact of statin therapy on plasma vitamin D levels: a systematic review and meta-analysis. *Curr Pharmaceut Design* (2017) 23:861–9. doi: 10.2174/1381612822666161006150542
177. Mazidi M, Rezaie P, Vatanparast H, Kengne AP. Effect of statins on serum vitamin D concentrations: a systematic review and meta-analysis. *Eur J Clin Investig.* (2017) 47:93–101. doi: 10.1111/eci.12698
178. Wilczek H, Sobra J, Justova V, Ceska R, Juzova Z, Prochazkova R, et al. [Iatropathogenic effect of Mevacor on vitamin D metabolism]. *Casopis Lekaru Ceskych* (1989) 128:1254–6.
179. Wilczek H, Sobra J, Ceska R, Justova V, Juzova Z, Prochazkova R, et al. [Monitoring plasma levels of vitamin D metabolites in simvastatin (Zocor) therapy in patients with familial hypercholesterolemia]. *Casopis Lekaru Ceskych* (1994) 133:727–9.
180. Perez-Castrillon JL, Vega G, Abad L, Sanz A, Chaves J, Hernandez G, et al. Effects of atorvastatin on vitamin D levels in patients with acute ischemic heart disease. *Am J Cardiol.* (2007) 99:903–5. doi: 10.1016/j.amjcard.2006.11.036
181. Yavuz B, Ertugrul DT, Cil H, Ata N, Akin KO, Yalcin AA, et al. Increased levels of 25 hydroxyvitamin D and 1,25-dihydroxyvitamin D after rosuvastatin treatment: a novel pleiotropic effect of statins? *Cardiovasc Drugs Ther.* (2009) 23:295–9. doi: 10.1007/s10557-009-6181-8
182. Ertugrul DT, Yavuz B, Cil H, Ata N, Akin KO, Kucukazman M, et al. STATIN-D study: comparison of the influences of rosuvastatin and fluvastatin treatment on the levels of 25 hydroxyvitamin D. *Cardiovasc Therapeut.* (2011) 29:146–52. doi: 10.1111/j.1755-5922.2010.00141.x
183. Agarwal B, Bhendwal S, Halmos B, Moss SF, Ramey WG, Holt PR. Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. *Clin Cancer Res.* (1999) 5:2223–9.
184. Wachtershauser A, Akoglu B, Stein J. HMG-CoA reductase inhibitor mevastatin enhances the growth inhibitory effect of butyrate in the colorectal carcinoma cell line Caco-2. *Carcinogenesis* (2001) 22:1061–7. doi: 10.1093/carcin/22.7.1061
185. Poynter JN, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, et al. Statins and the risk of colorectal cancer. *New Engl J Med.* (2005) 352:2184–92. doi: 10.1056/NEJMoa043792
186. Giguere V, Yang N, Segui P, Evans RM. Identification of a new class of steroid hormone receptors. *Nature* (1988) 331:91–4. doi: 10.1038/331091a0
187. Wei W, Schwaid AG, Wang X, Wang X, Chen S, Chu Q, et al. Ligand activation of ERRalpha by cholesterol mediates statin and bisphosphonate effects. *Cell Metab.* (2016) 23:479–91. doi: 10.1016/j.cmet.2015.12.010
188. Vanacker JM, Pettersson K, Gustafsson JA, Laudet V. Transcriptional targets shared by estrogen receptor- related receptors (ERRs) and estrogen receptor (ER) alpha, but not by ERbeta. *EMBO J.* (1999) 18:4270–9. doi: 10.1093/emboj/18.15.4270
189. Zhang Z, Teng CT. Estrogen receptor alpha and estrogen receptor-related receptor alpha1 compete for binding and coactivator. *Mol Cell Endocrinol.* (2001) 172:223–33. doi: 10.1016/S0303-7207(00)00372-5
190. Gallet M, Vanacker JM. ERR receptors as potential targets in osteoporosis. *Trends Endocrinol Metab.* (2010) 21:637–41. doi: 10.1016/j.tem.2010.06.008
191. LaBarge S, McDonald M, Smith-Powell L, Auwerx J, Huss JM. Estrogen-related receptor-alpha (ERRalpha) deficiency in skeletal muscle impairs regeneration in response to injury. *FASEB J.* (2014) 28:1082–97. doi: 10.1096/fj.13-229211
192. Wu F, Wang J, Wang Y, Kwok TT, Kong SK, Wong C. Estrogen-related receptor alpha (ERRalpha) inverse agonist XCT-790 induces cell death in chemotherapeutic resistant cancer cells. *Chemico Biol Interact.* (2009) 181:236–42. doi: 10.1016/j.cbi.2009.05.008
193. Casaburi I, Avena P, De Luca A, Chimento A, Sirianni R, Malivindi R, et al. Estrogen related receptor alpha (ERRalpha) a promising target for the therapy of adrenocortical carcinoma (ACC). *Oncotarget* (2015) 6:25135–48. doi: 10.18632/oncotarget.4722
194. Duellman SJ, Calaoagan JM, Sato BG, Fine R, Klebansky B, Chao WR, et al. A novel steroidal inhibitor of estrogen-related receptor alpha (ERR alpha). *Biochem Pharmacol.* (2010) 80:819–26. doi: 10.1016/j.bcp.2010.05.024
195. Patch RJ, Searle LL, Kim AJ, De D, Zhu X, Askari HB, et al. Identification of diaryl ether-based ligands for estrogen-related receptor alpha as potential antidiabetic agents. *J Med Chem.* (2011) 54:788–808. doi: 10.1021/jm101063h

Conflict of Interest Statement: 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 Endocrinology

REVIEW
published: 02 April 2019
doi: 10.3389/fendo.2019.00204

The Roles of Cholesterol and Its Metabolites in Normal and Malignant Hematopoiesis

*Hideyuki Oguro**

Cellular Engineering, The Jackson Laboratory for Genomic Medicine, Farmington, CT, United States

Hematopoiesis is sustained throughout life by hematopoietic stem cells (HSCs) that are capable of self-renewal and differentiation into hematopoietic progenitor cells (HPCs). There is accumulating evidence that cholesterol homeostasis is an important factor in the regulation of hematopoiesis. Increased cholesterol levels are known to promote proliferation and mobilization of HSCs, while hypercholesterolemia is associated with expansion of myeloid cells in the peripheral blood and links hematopoiesis with cardiovascular disease. Cholesterol is a precursor to steroid hormones, oxysterols, and bile acids. Among steroid hormones, 17β-estradiol (E2) induces HSC division and E2-estrogen receptor α (ERα) signaling causes sexual dimorphism of HSC division rate. Oxysterols are oxygenated derivatives of cholesterol and key substrates for bile acid synthesis and are considered to be bioactive lipids, and recent studies have begun to reveal their important roles in the hematopoietic and immune systems. 27-Hydroxycholesterol (27HC) acts as an endogenous selective estrogen receptor modulator and induces ERα-dependent HSC mobilization and extramedullary hematopoiesis. 7α,25-dihydroxycholesterol (7α,25HC) acts as a ligand for Epstein-Barr virus-induced gene 2 (EBI2) and directs migration of B cells in the spleen during the adaptive immune response. Bile acids serve as chemical chaperones and alleviate endoplasmic reticulum stress in HSCs. Cholesterol metabolism is dysregulated in hematologic malignancies, and statins, which inhibit *de novo* cholesterol synthesis, have cytotoxic effects in malignant hematopoietic cells. In this review, recent advances in our understanding of the roles of cholesterol and its metabolites as signaling molecules in the regulation of hematopoiesis and hematologic malignancies are summarized.

Keywords: cholesterol, oxysterols, steroids, hematopoietic stem cells, hematopoiesis, hematologic malignancies

OPEN ACCESS

Edited by:
Rosa Sirianni,
University of Calabria, Italy

Reviewed by:
Luca De Toni,
University of Padova, Italy
Alexander Birbrair,
Federal University of Minas Gerais, Brazil

***Correspondence:**
Hideyuki Oguro
hideyuki.oguro@jax.org

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *08 June 2018*
Accepted: *12 March 2019*
Published: *02 April 2019*

Citation:
Oguro H (2019) The Roles of Cholesterol and Its Metabolites in Normal and Malignant Hematopoiesis. Front. Endocrinol. 10:204. doi: 10.3389/fendo.2019.00204

INTRODUCTION

Hematopoietic stem cells (HSCs) sustain blood production throughout life and are the functional units of bone marrow transplantation. HSCs are capable of self-renewal to maintain their pool while producing all mature blood cells through differentiation into multipotent progenitors (MPPs) and subsequent hematopoietic progenitor cells (HPCs) with limited differentiation potentials (**Figure 1**). In adult mice, all HSCs and MPPs fall within the Lineage marker$^-$Sca-1$^+$c-Kit$^+$ (LSK) fraction (1–3), which is a heterogeneous population that contains a mixture of hematopoietic stem and progenitor cells (HSPCs), including HSCs, MPPs, and HPCs. HSCs can be further purified by selecting the CD150$^+$CD48$^{-/low}$ subset (4, 5) or CD34$^{-/low}$Flt3$^-$ subset (6, 7) of

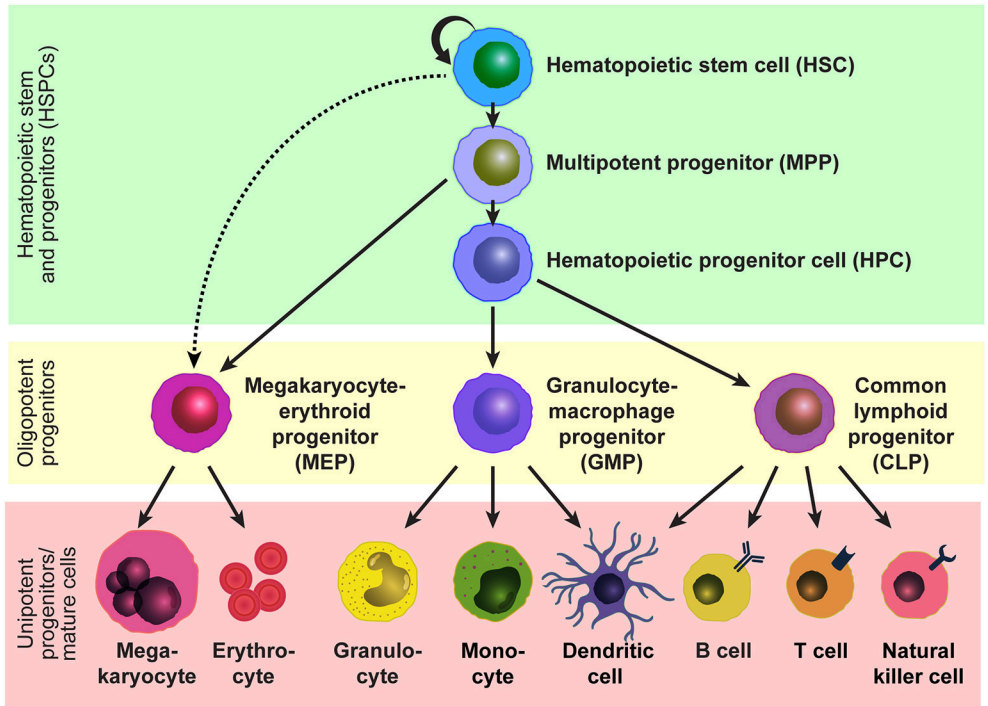

FIGURE 1 | Model of the hematopoietic hierarchy. A hierarchical structure of the adult hematopoietic system based on studies by us and others using mice. HSCs reside at the top of the hierarchy and give rise to MPPs and potentially MEPs. MPPs generate HPCs and MEPs, and HPCs generate GMPs and CLPs. Mature cells are developed from these oligopotent progenitors, MEPs, GMPs, and CMPs, through intermediate progenitors. Some lineage relationships are under debate.

LSK cells. The HSC population is functionally heterogenous in terms of cell-cycle kinetics, self-renewal capacity, and differentiation potential, and the heterogeneity can be distinguished by additional markers such as CD229 (5) and von Willebrand factor (8). Human HSPCs can be marked by Lineage marker^{-}CD38^{-}CD34^{+} and the HSC population can be further refined by marking the CD45RA^{-}CD49f^{+} subset of Lineage marker^{-}CD38^{-}CD34^{+} cells (9). HSCs reside in specialized niches which are local tissue microenvironments that support HSC behavior and regulate their function, such as self-renewal, differentiation, and localization, by producing factors that act directly on HSCs (10). In adults, HSCs are quiescent and localize primarily in the bone marrow, and their number is tightly regulated under steady-state conditions, comprising <0.01% of bone marrow cells in mice. In response to acute hematopoietic demands such as blood loss, myeloablation, infection, or pregnancy, HSCs change two aspects of their steady-state behaviors in order to increase production of necessary hematopoietic cells (11–14). First, quiescent HSCs re-enter the cell cycle to proliferate or differentiate through symmetric or asymmetric cell divisions, and second, they mobilize from the bone marrow to extramedullary tissues, such as the spleen, to expand the physical space for hematopoiesis.

In addition to the regulation of HSC behaviors by short-range factors, such as cytokines, cell-surface proteins, extracellular matrix components, oxygen tension, and ion levels, that are generated in their niches, HSCs are also regulated by long-range systemic signals, such as circulating cytokines, hormones, lipids, and vitamins. Cholesterol is found in the bloodstream and within cells, and is an essential structural component of mammalian plasma membranes and is essential to maintain both membrane structural integrity and to modulate membrane fluidity (15). Cholesterol also serves as a precursor for the biosynthesis of steroid hormones, oxysterols, and bile acids (**Figure 2**) (16). These cholesterol metabolites have important biological roles as signal transducers and chemical chaperones, and there is accumulating evidence that these metabolites act as systemic signals that regulate normal and malignant hematopoiesis. This review discusses recent advances in understanding the roles of cholesterol and its metabolites in the regulation of hematopoiesis and hematologic malignancies.

CHOLESTEROL LEVELS AND HEMATOPOIESIS

In the bloodstream, cholesterol is transported within lipoprotein particles, which are organized by apolipoproteins that can be recognized and bound by specific receptors on cell membranes. There are several types of lipoproteins, such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), very-low-density lipoprotein (VLDL), and chylomicrons, in order of higher density to lower density. LDL is the major cholesterol carrier in the blood and is

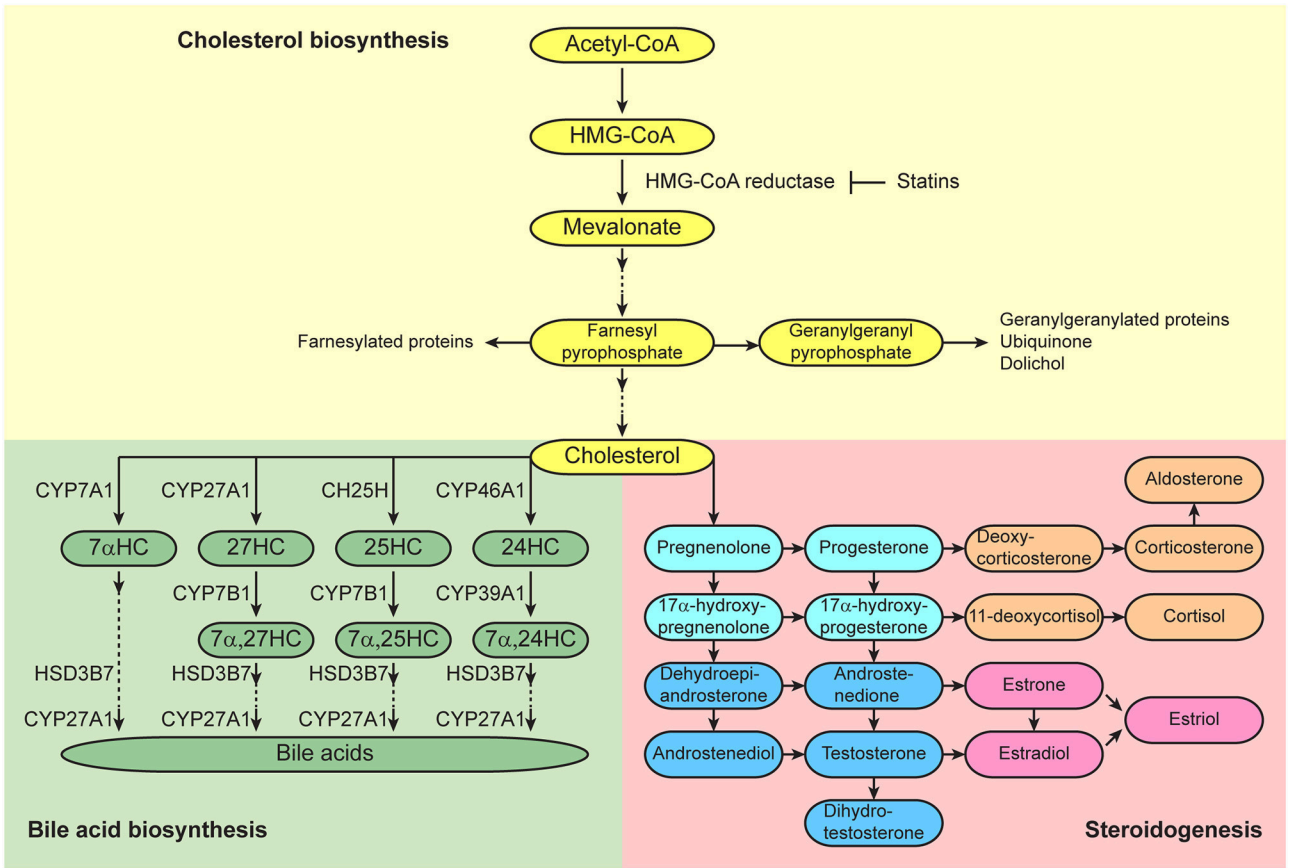

FIGURE 2 | Cholesterol synthetic/metabolic pathways**.** Pathways of cholesterol biosynthesis (top), bile acid biosynthesis (bottom left), and steroidogenesis (bottom right) are presented. Enzymes that catalyze the conversions are shown adjacent to the arrows.

recognized by the LDL receptor (LDLR) in peripheral tissues. LDL is the atherogenic lipoprotein, and increased LDL levels promote cholesterol accumulation. Macrophages accumulate oxidized LDL and give rise to foam cells, and contribute to atherosclerotic plaque formation. HDL opposes this process and reduces inflammation. HDL is involved in reverse cholesterol transport, in which HDL serves to shuttle cholesterol from peripheral tissues to the liver where cholesterol is eventually converted into bile acids.

Hypercholesterolemia Induces Proliferation and Mobilization of Mouse Hematopoietic Stem and Progenitor Cells

Several studies have shown a strong correlation between plasma cholesterol levels and the mobilized HSC number in the peripheral blood using mouse models. Gomes et al. showed that mice fed a high-fat/high-cholesterol (HFHC) diet for 30 days displayed thrombocytosis, lymphocytosis, and an increase in the number of HSPCs mobilized into the peripheral blood, while the HSPC number in the bone marrow decreased (17). They also found that the HFHC diet induced increased plasma levels of C-X-C motif chemokine ligand 12/ stromal cell-derived factor 1 (CXCL12/SDF1), a chemokine which is chemotactic for HSCs that express its receptor C-X-C motif chemokine receptor 4 (CXCR4) (18). Apolipoprotein E (APOE) is a key component in cholesterol metabolism and *Apoe*-deficient mice cause hypercholesterolemia (19, 20). *Apoe*-deficient mice fed an HFHC diet developed monocytosis (21–23), and Murphy et al. also reported neutrophilia associated with the proliferation and expansion of HSPCs in the bone marrow (23). Interestingly, APOE was expressed on the surface of HSPCs and acted cell autonomously to control HSPC proliferation, monocytosis, neutrophilia, and monocyte accumulation in atherosclerotic lesions, as revealed by transplantation of *Apoe*-deficient bone marrow cells. LDLR deficiency causes impaired LDL clearance, resulting in high plasma LDL-cholesterol levels and causing familial hypercholesterolemia. LDL receptor-deficient ($Ldlr^{-/-}$) mice fed an HFHC diet displayed hypercholesterolemia associated with increased HSPCs in both bone marrow and peripheral blood, and increased monocytes and granulocytes in the peripheral blood (24, 25). In addition to their mobilization, more HSPCs in the bone marrow incorporated the DNA synthesis marker 5-bromo-2′-deoxyuridine (BrdU) in $Ldlr^{-/-}$ mice on an HFHC diet as compared to in $Ldlr^{-/-}$ mice on a normal diet, indicating that hypercholesterolemia promoted

HSPC proliferation. In contrast, infusion with reconstituted HDL reduced the frequency and proliferation rate of HSPCs in the bone marrow, highlighting the opposing effects of LDL and HDL on HSPC proliferation. Scavenger receptor type BI (SR-BI, encoded by *Scarb1* gene) is a HDL receptor, and *Scarb1*$^{-/-}$ mice showed increased plasma total cholesterol levels with unchanged plasma concentration of apoA-I, the major protein in HDL (26). Gao et al. reported that *Scarb1*$^{-/-}$ mice fed an HFHC diet showed significantly increased the number of HSPCs in the bone marrow, spleen, and peripheral blood, as well as the proliferation of HSPCs as compared to wild-type mice fed an HFHC diet (27). Interestingly, HSPCs in *Scarb1*$^{-/-}$ mice fed an HFHC diet displayed increased levels of reactive oxygen species (ROS). Elevation of ROS levels hinders HSC quiescence and self-renewal, and accelerates HSC exhaustion (28). Injection of ROS inhibitor N-acetylcysteine attenuated HSPC expansion and leukocytosis in *Scarb1*$^{-/-}$ mice fed an HFHC diet, suggesting a correlation between ROS levels and HSPC proliferation. Tie et al. also reported that *Apoe*-deficiency increased the number and ROS levels of HSPCs, and these were further increased by the HFHC diet (29). They also observed shorter telomere length in HSPCs in *Apoe*$^{-/-}$ mice as compared to wild-type mice, suggesting accelerated aging of HSPCs, and this phenotype was reversed by treating *Apoe*$^{-/-}$ mice with N-acetylcysteine. These studies clearly indicate increased systemic cholesterol levels promote proliferation and mobilization of HSPCs.

Cholesterol Efflux Pathways Regulate Proliferation and Mobilization of Mouse Hematopoietic Stem and Progenitor Cells

ABCA1 and ABCG1, adenosine triphosphate-binding cassette transporters, play a key role in promoting active cellular cholesterol efflux (30). Yvan-Charvet et al. reported that *Ldlr*$^{-/-}$ mice on an HFHC diet that were transplanted with *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ bone marrow cells showed accelerated atherosclerosis and extensive infiltration of myocardium and spleen with macrophage foam cells as compared to transplantation with wild-type bone marrow cells (31). The same group subsequently reported that *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice on a normal diet displayed five-fold increase of HSPCs (including the CD34^{+}CD150^{+}Flt3^{-} highly-pure HSC population) in the bone marrow, as well as the increase in the S/G2/M fraction in HSPCs (32). The overall BrdU incorporation of *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ bone marrow cells was increased *in vitro*, whereas when wild-type bone marrow cells were mixed with *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ bone marrow cells, the overall BrdU incorporation of wild-type bone marrow cells was not increased, suggesting that HSPC proliferation in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice was caused by cell autonomous effects. Interestingly, in their next report, they showed that *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice also displayed an increase in HSPCs in the peripheral blood, spleen, and liver, indicating HSPC mobilization and extramedullary hematopoiesis (33). In this study, they performed a competitive bone marrow transplantation experiment by transplanting a mixture of equal numbers of bone marrow cells from wild-type and *Abca1*$^{-/-}$ *Abcg1*$^{-/-}$ mice into wild-type recipient mice, and found that HSPC mobilization of both the *Abca1*$^{-/-}$ *Abcg1*$^{-/-}$ and wild-type donor cells was induced, suggesting that there is a cell-extrinsic factor that induces HSPC mobilization of wild-type donor cells from *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ donor cells. Plasma levels of granulocyte colony-stimulating factor (G-CSF) were significantly increased in recipients of *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ bone marrow cells and the mobilization of *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ HSPCs was reduced by injection of G-CSF-neutralizing antibody. Interleukin-17 (IL-17) is a potent inducer of G-CSF (34), and the production of IL-17 can be mediated by the secretion of interleukin-23 (IL-23) from splenic phagocytic macrophages and dendritic cells (35). In the recipients of *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ bone marrow cells, plasma G-CSF levels and colony-forming HSPC numbers in the blood were normalized by an IL-17-blocking antibody, and plasma levels of IL-17 and G-CSF, as well as colony-forming HSPCs in the blood, were reduced by administration of IL-23 receptor-neutralizing antibody. Both myeloid cell (including macrophages) -specific and dendritic-cell specific deletion of *Abca1* and *Abcg1* using *lysM-cre*; *Abca1*$^{fl/fl}$; *Abcg1*$^{fl/fl}$ mice, and *CD11c-cre*; *Abca1*$^{fl/fl}$; *Abcg1*$^{fl/fl}$ mice, respectively, exhibited increased levels of splenic IL-23, plasma IL-17 and G-CSF, and colony-forming HSPCs in the blood, suggesting that IL-23/IL-17/G-CSF signaling is associated with enhanced HSPC mobilization in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice. They further reported that CXCL12 levels and the number of N-Cadherin^{+} osteoblasts, one of the CXCL12-expressing cell populations in the bone marrow (36), were decreased in the bone marrow of *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice, an effect that might be caused by depletion of bone marrow macrophage populations due to an enhanced IL-23/IL-17/G-CSF signaling axis. Thus, this study supports a step-wise mechanism by which increased intracellular cholesterol levels lead to mobilization of HSCs: (1) increased cholesterol initially promotes secretion of pro-inflammatory cytokines from immune cells, (2) this increases production of G-CSF by bone marrow stromal cells, (3) reducing osteoblast number and osteoblast production of CXCL12, a chemokine which attracts HSCs, and (4) leads to HSC mobilization into the bloodstream. They subsequently reported that *Ldlr*$^{-/-}$ recipient mice that received *lysM-cre*; *Abca1*$^{fl/fl}$; *Abcg1*$^{fl/fl}$ bone marrow cells and were fed an HFHC diet developed atherosclerosis associated with monocytosis and neutrophilia (37). The authors demonstrated a cell-extrinsic mechanism in which the expression of macrophage colony-stimulating factor (M-CSF) and G-CSF were increased in the spleen, and this might cause monocyte and neutrophil production in the bone marrow.

Cholesterol Levels and Human Hematopoiesis

Cholesterol homeostasis also affects human hematopoiesis. Crysandt et al. performed a retrospective analysis of a variety of clinical parameters in 83 patients following high-dose cyclophosphamide and G-CSF treatment and found that patients with hypercholesterolemia showed a substantially higher number of harvested CD34^{+} HSPCs in the peripheral blood

as compared to normocholesterolemic patients (38). 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a rate-limiting enzyme of *de novo* cholesterol synthesis, and statins, as inhibitors of HMG-CoA reductase, prevent the conversion of HMG-CoA to L-mevalonate and inhibit downstream cholesterol biosynthesis (**Figure 2**). Cimato et al. treated human subjects with different statins, atorvastatin, pravastatin, and rosuvastatin, to vary cholesterol levels and analyzed the number of mobilized $CD34^+$ HSPCs in the peripheral blood (39). They found a positive correlation between $CD34^+$ HSPC number and both total and LDL-cholesterol levels. In addition, G-CSF and its upstream regulator IL-17 both correlated positively with LDL-cholesterol levels. Gao et al. studied the correlation between HDL and white blood cell levels in patients with coronary heart disease (27). They found negative correlations between HDL levels and both total white blood cell and neutrophil counts in the peripheral blood, and patients with low HDL-cholesterol had more mobilized $Lineage^-CD34^+CD38^-CD45RA^{-/low}$ HSCs in the peripheral blood as compared to the patients with normal HDL-cholesterol. Tolani et al. analyzed data from a clinical trial of rosuvastatin in children with heterozygous familial hypercholesterolemia and found that the children with the lowest HDL-cholesterol levels had higher monocyte counts in the peripheral blood, and there was an inverse correlation between HDL levels and monocyte percentage (40). Thus, increased cholesterol levels induce mobilization of not only mouse HSCs but human HSCs, which suggests that cholesterol level is a factor that should be considered when mobilizing HSCs for clinical transplantation.

ROLES OF CHOLESTEROL METABOLITES IN HEMATOPOIESIS

Sex Steroid Hormones

Estrogens and androgens are classically recognized as sex steroid hormones, and progestogen are recognized as a third class of sex steroid hormones. Each of these sex steroid hormones is synthesized from cholesterol, and the first and rate-limiting step of the steroidogenic pathway is the cleavage of the cholesterol side chain by P450scc (CYP11A1) to convert into pregnenolone (**Figure 2**) (41). Estrogens are produced in gonadal and extra-gonadal tissues. In females, 17β-estradiol (E2), a most potent estrogen, is produced primarily by theca and granulosa cells in the ovaries. Androstenedione is generated from cholesterol and is converted into testosterone by aromatase in theca cells, and they are further converted into E2 by aromatase in granulosa cells. Testosterone is the primary androgen secreted from Leydig cells in the testes, and small amounts are also secreted from theca cells in the ovaries. Progesterone is a critical progestogen to establish and maintain pregnancy. Progesterone is produced from cholesterol in the corpus luteum of the ovary during early pregnancy and the production is sustained by the placenta in humans and rodents. In addition to their well-recognized effects on reproductive tissues, the sex steroid hormones are also being recognized as having broad physiological effects on non-reproductive tissues, such as nervous, cardiovascular, skeletal, immune, and hematopoietic systems. It is known that females and males differ in innate and adaptive immune responses, and these sex-biased differences in the immune system contribute to variations in the prevalence of autoimmune diseases and malignancies, susceptibility to infectious diseases, and responses to vaccines (42).

Androgens and Lympho-Hematopoiesis

Several studies have shown that androgens negatively regulate B lymphopoiesis. Castration of male mice leads to spleen enlargement and expansion of the B-cell population in the bone marrow and spleen (43–45). This effect is reversed by androgen replacement with either testosterone or dihydrotestosterone (DHT) (46). Androgen-resistant "testicular feminization" mutant male mice also show expansion of B-cell populations in the bone marrow and spleen (45, 47). In addition to the regulation of B lymphopoiesis, castrated mice and testicular feminization male mice also show thymic hypertrophy, which can be rescued by DHT administration (43, 45, 48–50). Experiments transplanting wild-type bone marrow cells into testicular feminization male mice suggest that androgen receptors expressed by bone marrow stromal cells or thymic epithelium modulate B-cell development or thymus size, respectively (50, 51). Velardi et al. showed that one mechanism by which androgens influence thymopoiesis is through direct inhibition of the Notch ligand *Dll4* in cortical thymic epithelial cells (52). Immune function progressively declines with age in mice and humans (53). In male mice, castration rejuvenates aged bone marrow and thymus, enhances peripheral T- and B-cell functions, and promotes immune recovery following chemotherapy-induced immunodepletion and HSC transplantation (54–62). These studies indicate that androgens are critical mediators of age-related lymphoid decline. Castration also enhances the recovery of bone marrow-resident HSCs after chemotherapy-induced immunodepletion (62). Khong et al. demonstrated that the number of HSCs marked by $CD34^-Flt3^-LSK$ was significantly increased at 7 days after castration of 9-month-old mice as compared to sham-treated mice, and the repopulation potential during serial bone marrow transplantations was enhanced when using these mice as donors (63). Their gene expression analyses suggest that castration induces qualitative changes in both HSCs and their bone marrow environment.

Estrogens and Lympho-Hematopoiesis

B lymphopoiesis in the bone marrow and T lymphopoiesis in thymus are drastically reduced during pregnancy (64, 65). Ovariectomy stimulates B lymphopoiesis and results in increased numbers of B cells in the bone marrow (66, 67), and it is reversed by administration of E2 (68). Genetically hypogonadal female mice which have a partial deletion of the hypothalamic gonadotropin-releasing hormone gene have a secondary deficiency in gonadal steroidogenesis and show expansion of B cell progenitors. Estrogen replacement with E2 reversed the increased numbers of B cell progenitors in these hypogonadal mice (69). Exogenous E2 treatment suppresses B cell development in both male and female mice (68, 70, 71).

Very early lymphoid precursors marked by Lineage marker^{-}IL-7Rα^{+}c-Kit^{lo}Terminal dexynucleotidyl transferase^{+} in the bone marrow are selectively depleted by exogenous E2 treatment (72). Both stromal-dependent and independent pathways of estrogen action on developing B cells have been postulated. It is proposed that bone marrow stromal cells expressing estrogen receptors mediate negative regulatory effects of E2 on early pre-B development (47, 73). A study of estrogen receptor α (ERα) male knockout mice by Thurmond et al. indicated that ERα is necessary for development of normal numbers of B cells in the bone marrow (74). They investigated the effect of E2 on lymphopoiesis by performing bone marrow transplantation using ERα-deficient mice as donors, recipients, or both, and treated with E2. They demonstrated that exogenous E2-induced alteration of B cell populations was primarily caused by a hematopoietic cell-intrinsic mechanism rather than by their environment.

Estrogens also regulate other hematopoietic lineages. Administration of a high-dose of estrogen induces anemia in rodents and dogs, regardless of their sex (75–77). Schroeder et al. reported that E2 sustained proliferation of erythroid progenitors from chick bone marrow, but E2 also caused erythroid differentiation arrest and blocked erythrocyte gene expression (78). Blobel et al. also showed that E2 added to the culture also reduced the number of erythroid progenitors from human bone marrow (79). They demonstrated that the transcriptional activity of GATA1, an erythroid master transcription factor that is necessary for full maturation of erythrocytes, was strongly repressed by direct binding of ER in an E2-dependent manner in NIH 3T3 and COS cell lines. The same group subsequently reported that inhibition of GATA1 activity by ER induced apoptosis in a murine Friend virus-induced erythroleukemia cell line (80). E2 treatment also stimulates the megakaryocyte colony formation potential of CD34^{+} human cord blood cells *in vitro* (81). E2 promotes megakaryocyte polyploidization and maturation via activation of ERβ accompanied by a significant upregulation of the expression of GATA1, which is also a key regulator of megakaryocyte differentiation (82). Differentiation of dendritic cells with characteristics of Langerhans cells from myeloid progenitors in culture, as induced by granulocyte-macrophage colony-stimulating factor (GM-CSF), is promoted by addition of E2 and inhibited by ER antagonists and ERα-deficiency (83). Interferon regulatory factor 4 (IRF4), a transcription factor induced by GM-CSF and critical for Langerhans cell development, is a target of ERα signaling during this process (84). In contrast, E2 significantly inhibits Flt3-ligand-induced plasmacytoid and conventional dendritic-cell differentiation in culture by decreasing numbers of viable differentiated cells (85). Thus, the effects of E2 are likely to be dependent on the cytokine pathways that might be operative in the steady state or during inflammation and disease.

Estrogens Regulate HSC Division Rate

Estrogens also regulate HSC behavior. In our previous study, we observed that HSCs divide more often in female mice as compared to male mice (14). Ovariectomy in females (which depletes both estrogens and progesterone) significantly reduced HSC division to male levels, while castration of males has no effect on HSC division. Conversely, administration of exogenous E2, but not progesterone or dihydrotestosterone, significantly increased HSC division in both female and male mice. Because E2 treatment also increased HSC division in ovariectomized female mice and castrated male mice, its action is independent of the gonads. Although HSC division rate was increased in untreated female mice as compared to male mice and in E2-treated mice of either sex as compared to vehicle-treated control mice, we did not observe an increase in absolute HSC numbers in those untreated female mice or E2-treated mice. Instead, we observed an increased generation of megakaryocyte-erythroid progenitors (MEPs) in female mice as compared to male mice, and increased splenic erythropoiesis in E2-treated mice of both sexes as compared to vehicle-treated control mice. Given that increased myeloid progenitors including MEPs may arise directly from the asymmetric division of HSCs (**Figure 1**) (86), our observations raise the possibility that the increased MEPs in female mice reflects increased asymmetric division of an HSC to produce one HSC and one MEP in response to E2. ERα, but not ERβ, is highly expressed by HSCs. Conditional deletion of *Esr1*, which encodes ERα, from hematopoietic cells significantly reduced the HSC division rate in female mice, but not in male mice. *Esr1*-deficient HSCs of both sexes were insensitive to exogenous E2 treatment. Moreover, E2 treatment of chimeric recipient mice transplanted with equal numbers of wild-type and *Esr1*-deficient hematopoietic cells revealed that E2 significantly induced division of wild-type HSCs but not *Esr1*-deficient HSCs in the same recipient mice, indicating that E2 acts directly on HSCs, rather than acting indirectly by stimulating secondary signals from other cells. E2 levels increase in females during pregnancy (87), when extramedullary hematopoiesis is induced to increase the production of red blood cells. Notably, pregnant mice exhibit significantly increased HSC division rate relative to non-pregnant female mice, and the deletion of *Esr1* in hematopoietic cells significantly reduced the normal increase in HSC division during pregnancy (**Figure 3**). Increased spleen size is observed during pregnancy in mice and humans (88, 89). In addition to the increased HSC division rate, we found that pregnant mice exhibited significantly increased cellularity, erythropoiesis, myelopoiesis, and HSC number in the spleen, indicating extramedullary hematopoiesis and HSC mobilization, and these processes also depend upon ERα function in HSCs. Pregnant mice also had increased absolute HSC numbers in the bone marrow, but deletion of *Esr1* in hematopoietic cells did not reverse this phenomenon, suggesting the existence of ERα-independent factor(s) that increase(s) HSC numbers in pregnant mice. Nevertheless, ERα signaling is critical for the induction of HSC division and mobilization to the spleen for the expansion of splenic erythropoiesis.

Illing et al. also reported the effects of E2 on HSC function (90). In this study, they treated mice with E2 at a dose of 0.24 mg/kg/day for 30 days, which is a higher dose and longer treatment than our study at a dose of 0.1 mg/kg/day for 7 days. In this condition, they observed a profound reduction

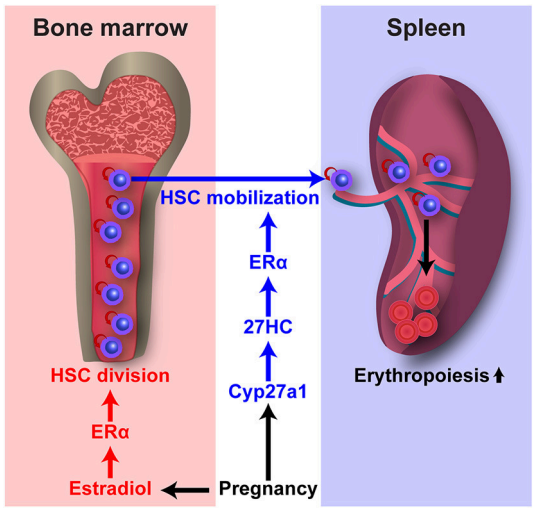

FIGURE 3 | E2 and 27HC differentially regulate HSC division and mobilization during pregnancy. Pregnancy upregulates E2–ERα and 27HC–ERα signaling. E2–ERα signaling induces HSC division in the bone marrow. 27HC–ERα signaling induces HSC mobilization into the circulation and spleen, and augments splenic erythropoiesis.

in bone marrow cellularity by E2 treatment. E2 treatment caused more HSPCs to enter the S phase. They also observed an increased frequency of long-term reconstituting HSCs in E2-treated mice by performing bone marrow transplantation with a limiting dilution assay. However, donor-derived HSPCs of the bone marrow of the recipient mice after tertiary transplantation was decreased in the recipients that received bone marrow cells from E2-treated mice, suggesting exhaustion of reconstituting cells during serial transplantation. Deletion of *Esr1* reversed the reduction of bone marrow cellularity by E2 treatment, however it did not reverse the increased frequency of long-term reconstituting HSCs by E2 treatment. Sanchez-Aguilera et al. reported the effect of tamoxifen, a selective estrogen receptor modulator (SERM), on HSC function (91). Tamoxifen induced HSC division as well as apoptosis in MPPs, and these effects are mediated by ERα but not by ERβ. Tamoxifen treatment significantly reduced MPP number, but not HSC number, and compromised activation of hematopoiesis after chemotherapy. They observed increased expression of *Myc* in HSCs after tamoxifen treatment, and *Myc*-deficient MPPs did not undergo apoptosis upon tamoxifen treatment. Interestingly, female immunodeficient recipient mice support reconstitution of the blood system by transplanted human HSCs more efficiently than male immunodeficient recipient mice (92, 93). Since female mice have higher E2 levels than male mice and E2 induces HSC division, it is anticipated that higher E2 levels in female recipient mice will promote proliferation and differentiation of transplanted HSCs as compared to male recipient mice with very low E2 levels. The molecular mechanisms by which E2 regulates HSCs are not fully understood. Chapple et al. proposed that HSCs from mice treated with E2 had increased regenerative capacity after transplantation or irradiation (94). They demonstrated that E2–ERα signaling induced expression of *Ern1*, which encodes Ire1α, to activate the Ire1α-Xbp1 pathway of the unfolded protein response, and promoted resistance of HSCs against proteotoxic stress.

27-Hydroxycholesterol Regulates HSC Mobilization

Oxysterols are oxygenated derivatives of cholesterol and key substrates for bile acid synthesis (**Figure 2**) (16). In the classic pathway of bile acid synthesis, cholesterol is converted into 7α-hydroxycholesterol (7αHC) by cholesterol 7α-hydroxylase CYP7A1, a rate limiting step that occurs in the liver. In alternative pathways that occur primarily in extrahepatic tissues, cholesterol is converted into 24-hydroxycholesterol (24HC), 25-hydroxycholesterol (25HC), and 27-hydroxycholesterol (27HC) by cholesterol 24-hydroxylase CYP46A1, cholesterol 25-hydroxylase CH25H, and sterol 27-hydroxylase CYP27A1, respectively. Oxysterols are considered to be bioactive lipids and recent studies have started to reveal their important roles in both the hematopoietic and immune systems. 27HC is the most abundant circulating oxysterol and acts as an endogenous SERM which can bind to ERs and regulate their function (95). Plasma 27HC levels strongly correlate with total cholesterol levels (96), as 27HC is generated directly from cholesterol by the sterol 27-hydroxylase CYP27A1. Plasma 27HC levels are greatly reduced in *Cyp27a1*-deficient mice (97). CYP27A1 is abundant in the liver, but it is also expressed in extrahepatic tissues (16). Dietary or genetic changes that elevate 27HC levels modulate ER activity, thereby inhibiting vascular repair in cardiovascular disease (95), promoting ER-positive breast cancer growth (98, 99), and increasing the severity of atherosclerosis (100).

We have demonstrated that the increases in HSC division, HSC mobilization, and extramedullary hematopoiesis during pregnancy require ERα in HSCs, and E2 treatment induces HSC division as described above (14). Interestingly, our recent study revealed that treatment of mice with E2 did not increase HSC number in the spleen, indicating that E2 treatment does not induce HSC mobilization (101). In contrast, treatment with 27HC, another endogenous ER ligand, increased HSC number in the spleen but not HSC division in the bone marrow, indicating a role in inducing HSC mobilization. We demonstrated that the effect of 27HC on HSC mobilization is nullified by deletion of *Esr1* in hematopoietic cells, indicating that 27HC-induced HSC mobilization is dependent on ERα. Plasma cholesterol levels increase in humans during pregnancy (102). During pregnancy in mice, we observed significant increases in 27HC levels in HSPCs. *Cyp27a1*-deficient mice had significantly reduced HSC mobilization and extramedullary hematopoiesis during pregnancy, while the increased rate of HSC division in the bone marrow during pregnancy was not affected. These findings indicate that 27HC acts in concert with E2 to promote hematopoiesis during pregnancy by regulating ERα signaling in HSCs (**Figure 3**). As described above, increased cholesterol

levels promote HSC mobilization in mice and humans, and increased HSC mobilization in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice is associated with elevated serum G-CSF levels (33). In our study, we observed that 27HC treatment significantly induced HSC mobilization in mice deficient for *Csf3*, which encodes G-CSF (101). Together, 27HC and G-CSF additively increased the numbers of colony-forming HSPCs in the peripheral blood. Therefore, 27HC and G-CSF likely act through distinct mechanisms to induce HSC mobilization. These findings suggest an alternative model that the ability of elevated cholesterol levels to promote HSC mobilization is mediated by increases in 27HC production, because treating mice with 27HC induces HSC mobilization and 27HC levels increase as cholesterol levels increase.

E2, 27HC, and ERα

ERα is a nuclear receptor transcription factor and E2 is the most potent endogenous estrogen. Different ER ligands are known to have distinct effects on ERα-mediated regulation of gene expression, and ER ligands differ in their structures and their effects on ER conformation (103–106). For example, Wardell et al. used breast cancer cell lines to test E2 and five different synthetic ER ligands and observed different gene expression patterns regulated by different ER–ligand complexes (106). 27HC induces a unique conformational change in the ER that is different from that mediated by E2 and other ER ligands (107). Different ER–ligand complexes also engage functionally distinct coregulators by selective recruitment of coactivators and corepressors to activate and repress expression of target genes, respectively (108). Thus, as different ER ligands can act through the ERα to differentially regulate gene expression, this may explain why E2 and 27HC have distinct effects on HSCs even though both act through ERα (**Figure 3**). It will be interesting to explore the nature of the ERα target genes as well as the mechanism by which ERα function is differentially regulated by E2 and 27HC, two major endogenous ER ligands.

Bile Acids

Bile acids are synthesized from cholesterol in the liver (**Figure 2**), secreted into the bile, and delivered to the lumen of the small intestine where they act as emulsifiers of dietary lipids, cholesterol, and fat-soluble vitamins (16). However, bile acids have additional roles (109). Tauroursodeoxycholic acid (TUDCA) serves as a chemical chaperone and reduces endoplasmic reticulum stress (110). HSCs are predisposed to apoptosis through misfolded protein accumulation in the endoplasmic reticulum caused by cellular stress that subsequently activates the unfolded protein response (111). Addition of TUDCA to cultured mouse HSCs alleviates endoplasmic reticulum stress and increases their capacity to reconstitute the hematopoietic system in recipient mice upon transplantation (112). In contrast to the quiescent state of adult HSCs in the bone marrow, HSCs undergo a rapid expansion in the fetal liver during development (113). Adult HSCs in the bone marrow have a lower rate of protein synthesis as compared to most other hematopoietic cells (114). Sigurdsson et al. reported that although fetal-liver HSCs had a higher rate of protein synthesis as compared to adult HSCs in the bone marrow, fetal-liver HSCs had lower expression of endoplasmic reticulum stress response genes (115). In addition to the role of CYP27A1 for side chain oxidation of cholesterol for the generation of 27HC to initiate the alternative acidic bile acid biosynthetic pathway, CYP27A1 also catalyzes another side chain oxidation after the ring modification step of both classic and alternative acidic bile acid synthesis pathways (**Figure 2**) (16). *Cyp27a1*-deficient mice have significantly decreased bile acids (97). Sigurdsson et al. also reported that fetuses in *Cyp27a1*-deficient mothers displayed significantly reduced levels of total bile acids as wells as secondary bile acids in the fetal liver, suggesting that maternal bile acids are transferred to the fetus during pregnancy (115). Interestingly, the livers of *Cyp27a1*-deficient fetuses in *Cyp27a1*-deficient mothers contained a significantly lower number of long-term reconstituting HSCs, as assessed by limiting dilution transplantation assay, and these fetal HSCs showed significantly higher levels of protein aggregation. These findings imply that bile acids enable fetal-liver HSCs to have a higher level of protein synthesis without activating a stress response, allowing expansion of the HSC pool during fetal development.

25-Hydroxycholesterol

25HC is generated directly from cholesterol by CH25H, and 25HC is further converted into 7α,25-dihydroxycholesterol (7α,25HC) by the oxysterol 7α-hydroxylase CYP7B1-mediated hydroxylation (**Figure 2**) (116). Expression of *Ch25h* is upregulated in macrophages and dendritic cells when they are exposed to various inflammatory mediators (117–120). 25HC augments the production of inflammatory cytokines in macrophages, and mediates feedback inhibition of *IL1b* expression and inflammasome activation in activated macrophages in the DNA sensor protein absent in melanoma 2 (AIM2)-dependent manner (121–124). 25HC is strongly induced following viral infection and by interferon, and it inhibits the replication of a wide range of enveloped viruses (125, 126). 25HC also promotes macrophage foam cell formation (127). Epstein-Barr virus-induced gene 2 (EBI2), a G protein-coupled receptor also known as GPR183, controls follicular B-cell migration and T-cell-dependent antibody production (128, 129). 7α,25HC acts as a ligand for EBI2 and directs migration of B cells in the spleen during the adaptive immune response (130, 131). EBI2 and 7α,25HC also regulate splenic CD4^{+} dendritic cells for positioning in marginal zone bridging channels to maintain their homeostasis and mount a response against certain antigens, and positioning of activated CD4 T cells at the interface of the follicle and T zone to interact with activated dendritic cells (132–134). Thus, 25HC broadly regulates innate and adaptive immune cell behavior. The 7α,25HC/EBI2 axis also regulates bone mass homeostasis (135). EBI2 is expressed in monocyte/osteoclast precursors, and 7α,25HC is secreted by osteoblasts. EBI2 guides osteoclast precursors toward bone surfaces by promoting their movement and positioning, which facilitates fusion of osteoclasts and enhances the development of large osteoclasts to maintain bone mass homeostasis.

CHOLESTEROL SYNTHESIS/METABOLISM AND HEMATOLOGIC MALIGNANCIES

Cholesterol metabolism is dysregulated in hematologic malignancies. The rate of cholesterol synthesis is higher in cells from acute myeloid leukemia (AML) patients as compared to healthy subjects (136). Hypocholesterolemia is frequently observed due to elevated LDL uptake by leukemia cells (137–139), but elevated cholesterol levels in leukemia cells have also been reported (140–143). Yvan-Charvet et al. reported that HFHC diet administered *Ldlr*$^{+/-}$ mice transplanted with cellular cholesterol efflux pathway-deficient *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ bone marrow cells displayed a myeloproliferative neoplasm (MPN)-like phenotype, and expression of an *APOA1* transgene that elevates HDL levels suppressed this phenotype (32). Thus, changes in intracellular cholesterol levels are associated with the development and maintenance of hematologic malignancies. Statins have cytotoxic effects in various types of malignant hematopoietic cells including AML (144–158), chronic myeloid leukemia (CML) (153, 159–161), MPNs (162), acute lymphocytic leukemia (ALL) (163, 164), chronic lymphocytic leukemia (CLL) (165–167), adult T-cell leukemia (ATL) (168), lymphoma (169, 170), and multiple myeloma (171–176). To identify compounds that can inhibit the stem cell activity of leukemia-initiating cells (LICs), Hartwell et al. performed a high-throughput screen in a bone marrow-mimicking culture system in which LICs expressing the *MLL-AF9* fusion oncogene were co-cultured with a bone marrow stromal cell line (177). Among the compounds that selectively inhibited LICs but not normal HSPCs, lovastatin also inhibited LIC stem cell activity in an *in vivo* bone marrow transplantation model. Although these reports demonstrate the effectiveness of statins, the mechanisms of their anticancer effects are not fully understood. Griner et al. reported that MPN-associated JAK2^{V617F} localized to lipid rafts, subdomains of the plasma membrane that contain protein receptors and a high concentration of cholesterol, and simvastatin inhibited this localization and JAK2^{V617F}-dependent cell growth in MPN model cell lines (162). Simvastatin also inhibited erythroid colony formation of primary cells from MPN patients, but had no effect on cells from healthy individuals. Other than the cholesterol-lowering effect, inhibition of the mevalonate pathway by statins also reduces the levels of farnesyl pyrophosphate and geranylgeranyl pyrophosphate and thereby inhibits protein farnesylation and geranylgeranylation, modifications that are important for a variety of cellular processes including cell proliferation, survival, and migration (**Figure 2**). Thus, the anticancer effects of statins could also be rendered through changes in these other cellular processes (157, 158, 170, 176).

Among the metabolites of cholesterol, oxysterols such as 7βHC, 25HC, 7β,25HC, 7-ketocholestanol, and 7-ketocholestanol have cytotoxic effects on leukemia and lymphoma cells (178–183). Tsujioka et al. reported that DNA methyltransferase inhibitors induced *CH25H* expression with enhanced 25HC production and promoted apoptosis in leukemia and myelodysplastic syndrome (MDS) cell lines, while exogenous 25HC treatment suppressed cell growth of leukemia and MDS cell lines (184). Other than oxysterol, Sanchez-Aguilera et al. reported that tamoxifen treatment blocked development of *JAK2*^{V617F}-induced MPN in mice and induced apoptosis of human MPN cells from patients with *JAK2*^{V617F} mutation in a xenograft model (91). They also demonstrated that tamoxifen treatment reduced leukemic burden in a mouse model of AML using mice transplanted with bone marrow cells expressing the *MLL-AF9* oncogene. Their findings have uncovered the potential role of estrogen signaling in leukemia and suggest the potential use of SERMs as a treatment for leukemia. The roles of cholesterol metabolites in hematologic malignancies are not yet fully explored, and further studies of cholesterol metabolites are expected to elucidate their roles in hematologic malignancies and their potential in preventing and treating hematologic malignancies.

CONCLUSION AND FUTURE DIRECTIONS

Cholesterol and its metabolites are now being recognized to have important roles in broad biological processes by regulating a wide variety of molecular machinery. Advances in understanding these molecular mechanisms will benefit human health. One potential clinical application of molecules that regulate or are regulated by cholesterol metabolism is to enhance mobilization of HSCs for transplantation. To enable efficient collection of mobilized HSCs from the peripheral blood for HSC transplantation, donors are treated with HSC-mobilizing agents such as G-CSF (185). However, a significant proportion of donors fail to reach the minimum HSC collection threshold required for transplantation using traditional strategies (186). The failure of mobilization can increase patient morbidity because patients cannot proceed to transplantation. Thus, advances in mobilization strategies that could increase the success of HSC collection without introducing additional side effects are needed to improve patient outcomes. For example, administration of 27HC enhances the mobilization of HSPCs by G-CSF (101). Identification of the genes downstream of 27HC-ERα signaling that mediate HSC mobilization may contribute to the development of new methods that improve the yield of mobilized HSCs for transplantation, while also offering an explanation for the long-standing observation that increased cholesterol levels are associated with increased HSC mobilization in mice and humans.

High blood cholesterol levels are associated with the development of atherosclerosis. Atherosclerosis is a progressive disease in which the inside of the artery become thick and stiff due to the buildup of the atheromatous plaque which consists of cholesterol, fat and other substances, and restricts blood flow and causes complications including myocardial infarction, peripheral artery disease, and stroke. In addition to lipids, various types of leukocytes also accumulate in the atheromatous plaque. Hypercholesterolemia causes monocytosis, and these monocytes give rise to macrophages which eventually turn into foam cells by ingesting LDL in the plaque, and promotes plaque growth and inflammation. Other than monocytes, diverse immune-cell subsets, such as neutrophils, mast cells,

B and T lymphocytes, are associated with atherosclerosis [reviewed in (187, 188)]. Oxysterols are formed and accumulate in the plaque as a result of LDL oxidation due to the inflammatory response. Although atherosclerotic properties of oxysterols have been tested, it is still unclear whether oxysterols have pro- or anti-atherosclerotic properties [reviewed in (189)]. Estrogens also affect atherogenesis. Despite of reports that support the atheroprotective effects of estrogens, it is also controversial whether they have pro- or anti-atherosclerotic properties [reviewed in (190)]. After myocardial infarction, monocyte recruitment is increased, and sustained and accelerated atherosclerosis is observed in a mouse model. Interestingly, myocardial infarction causes HSPC mobilization into the spleen and sustains augmented monocytepoiesis, providing a possibility of novel therapy to mitigate progression of atherosclerosis (191, 192). Thus, cholesterol and its metabolites link hematopoiesis with cardiovascular health, and deciphering this link is critical for developing new targeted therapies.

The molecular mechanisms underlying the regulation of normal and malignant hematopoiesis by cholesterol and its metabolites are not yet fully understood. There are many drugs that target cholesterol synthetic and metabolic pathways, and further studies are expected to generate novel strategies for enhancing hematopoiesis, augmenting hematopoietic recovery after hematopoietic injuries, improving collection of mobilized HSCs for transplantation, and treating hematologic malignancies.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

The study was supported by The Jackson Laboratory and the National Blood Foundation Scientific Research Grants Program.

ACKNOWLEDGMENTS

The author gives appreciation to colleagues and collaborators, and apologizes to researchers whose work could not be cited and this review owing to space limitations. The author thanks Carmen Robinett for thoughtful editing of the manuscript.

REFERENCES

1. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science*. (1988) 241:58–62. doi: 10.1126/science.2898810
2. Okada S, Nakauchi H, Nagayoshi K, Nishikawa S, Miura Y, Suda T. Enrichment and characterization of murine hematopoietic stem cells that express c-kit molecule. *Blood*. (1991) 78:1706–12.
3. Ikuta K, Weissman IL. Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proc Natl Acad Sci USA*. (1992) 89:1502–6. doi: 10.1073/pnas.89.4.1502
4. Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. (2005) 121:1109–21. doi: 10.1016/j.cell.2005.05.026
5. Oguro H, Ding L, Morrison SJ. SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. *Cell Stem Cell*. (2013) 13:102–16. doi: 10.1016/j.stem.2013.05.014
6. Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science*. (1996) 273:242–5. doi: 10.1126/science.273.5272.242
7. Christensen JL, Weissman IL. Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. *Proc Natl Acad Sci USA*. (2001) 98:14541–6. doi: 10.1073/pnas.261562798
8. Sanjuan-Pla A, Macaulay IC, Jensen CT, Woll PS, Luis TC, Mead A, et al. Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. *Nature*. (2013) 502:232–6. doi: 10.1038/nature12495
9. Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science*. (2011) 333:218–21. doi: 10.1126/science.1201219
10. Crane GM, Jeffery E, Morrison SJ. Adult haematopoietic stem cell niches. *Nat Rev Immunol*. (2017) 17:573–90. doi: 10.1038/nri.2017.53
11. Cheshier SH, Prohaska SS, Weissman IL. The effect of bleeding on hematopoietic stem cell cycling and self-renewal. *Stem Cells Dev*. (2007) 16:707–17. doi: 10.1089/scd.2007.0017
12. Randall TD, Weissman IL. Phenotypic and functional changes induced at the clonal level in hematopoietic stem cells after 5-fluorouracil treatment. *Blood*. (1997) 89:3596–606.
13. Baldridge MT, King KY, Boles NC, Weksberg DC, Goodell MA. Quiescent haematopoietic stem cells are activated by IFN-gamma in response to chronic infection. *Nature*. (2010) 465:793–7. doi: 10.1038/nature09135
14. Nakada D, Oguro H, Levi BP, Ryan N, Kitano A, Saitoh Y, Takeichi M, Wendt GR, Morrison SJ. Oestrogen increases haematopoietic stem-cell self-renewal in females and during pregnancy. *Nature*. (2014) 505:555–8. doi: 10.1038/nature12932
15. Ikonen E. Cellular cholesterol trafficking and compartmentalization. *Nat Rev Mol Cell Biol*. (2008) 9:125–38. doi: 10.1038/nrm2336
16. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem*. (2003) 72:137–74. doi: 10.1146/annurev.biochem.72.121801.161712
17. Gomes AL, Carvalho T, Serpa J, Torre C, Dias S. Hypercholesterolemia promotes bone marrow cell mobilization by perturbing the SDF-1:CXCR4 axis. *Blood*. (2010) 115:3886–94. doi: 10.1182/blood-2009-08-240580
18. Karpova D, Bonig H. Concise review: CXCR4/CXCL12 signaling in immature hematopoiesis–lessons from pharmacological and genetic models. *Stem Cells*. (2015) 33:2391–9. doi: 10.1002/stem.2054
19. Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, et al. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell*. (1992) 71:343–53. doi: 10.1016/0092-8674(92)90362-G
20. Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science*. (1992) 258:468–71. doi: 10.1126/science.1411543
21. Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R, Pittet MJ. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J Clin Invest*. (2007) 117:195–205. doi: 10.1172/JCI29950
22. Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, et al. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest*. (2007) 117:185–94. doi: 10.1172/JCI28549
23. Murphy AJ, Akhtari M, Tolani S, Pagler T, Bijl N, Kuo CL, et al. ApoE regulates hematopoietic stem cell proliferation, monocytosis, and monocyte

accumulation in atherosclerotic lesions in mice. *J Clin Invest*. (2011) 121:4138–49. doi: 10.1172/JCI57559
24. Feng Y, Schouteden S, Geenens R, Van Duppen V, Herijgers P, Holvoet P, et al. Hematopoietic stem/progenitor cell proliferation and differentiation is differentially regulated by high-density and low-density lipoproteins in mice. *PLoS ONE*. (2012) 7:e47286. doi: 10.1371/journal.pone.0047286
25. Seijkens T, Hoeksema MA, Beckers L, Smeets E, Meiler S, Levels J, et al. Hypercholesterolemia-induced priming of hematopoietic stem and progenitor cells aggravates atherosclerosis. *FASEB J*. (2014) 28:2202–13. doi: 10.1096/fj.13-243105
26. Rigotti A, Trigatti BL, Penman M, Rayburn H, Herz J, Krieger M. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci USA*. (1997) 94:12610–5. doi: 10.1073/pnas.94.23.12610
27. Gao M, Zhao D, Schouteden S, Sorci-Thomas MG, Van Veldhoven PP, Eggermont K, et al. Regulation of high-density lipoprotein on hematopoietic stem/progenitor cells in atherosclerosis requires scavenger receptor type BI expression. *Arterioscler Thromb Vasc Biol*. (2014) 34:1900–9. doi: 10.1161/ATVBAHA.114.304006
28. Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, et al. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med*. (2006) 12:446–51. doi: 10.1038/nm1388
29. Tie G, Messina KE, Yan J, Messina JA, Messina LM. Hypercholesterolemia induces oxidant stress that accelerates the ageing of hematopoietic stem cells. *J Am Heart Assoc*. (2014) 3:e000241. doi: 10.1161/JAHA.113.000241
30. Westerterp M, Bochem AE, Yvan-Charvet L, Murphy AJ, Wang N, Tall AR. ATP-binding cassette transporters, atherosclerosis, and inflammation. Circ Res. (2014) 114:157–70. doi: 10.1161/CIRCRESAHA.114.300738
31. Yvan-Charvet L, Ranalletta M, Wang N, Han S, Terasaka N, Li R, Welch C, Tall AR. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. *J Clin Invest*. (2007) 117:3900–8. doi: 10.1172/JCI33372
32. Yvan-Charvet L, Pagler T, Gautier EL, Avagyan S, Siry RL, Han S, et al. ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. *Science*. (2010) 328:1689–93. doi: 10.1126/science.1189731
33. Westerterp M, Gourion-Arsiquaud S, Murphy AJ, Shih A, Cremers S, Levine RL, et al. Regulation of hematopoietic stem and progenitor cell mobilization by cholesterol efflux pathways. *Cell Stem Cell*. (2012) 11:195–206. doi: 10.1016/j.stem.2012.04.024
34. Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J Exp Med*. (1996) 183:2593–603. doi: 10.1084/jem.183.6.2593
35. Stark MA, Huo Y, Burcin TL, Morris MA, Olson TS, Ley K. Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. *Immunity*. (2005) 22:285–94. doi: 10.1016/j.immuni.2005.01.011
36. Semerad CL, Christopher MJ, Liu F, Short B, Simmons PJ, Winkler I, et al. G-CSF potently inhibits osteoblast activity and CXCL12 mRNA expression in the bone marrow. *Blood*. (2005) 106:3020–7. doi: 10.1182/blood-2004-01-0272
37. Westerterp M, Murphy AJ, Wang M, Pagler TA, Vengrenyuk Y, Kappus MS, et al. Deficiency of ATP-binding cassette transporters A1 and G1 in macrophages increases inflammation and accelerates atherosclerosis in mice. *Circ Res*. (2013) 112:1456–65. doi: 10.1161/CIRCRESAHA.113.301086
38. Crysandt M, Hilgers RD, von Hobe S, Eisert A, Jost E, Panse J, et al. Hypercholesterolemia and its association with enhanced stem cell mobilization and harvest after high-dose cyclophosphamide+G-CSF. *Bone Marrow Transplant*. (2011) 46:1426–9. doi: 10.1038/bmt.2010.327
39. Cimato TR, Palka BA, Lang JK, Young RF. LDL cholesterol modulates human CD34+ HSPCs through effects on proliferation and the IL-17 G-CSF axis. *PLoS ONE*. (2013) 8:e73861. doi: 10.1371/journal.pone.0073861
40. Tolani S, Pagler TA, Murphy AJ, Bochem AE, Abramowicz S, Welch C, et al. Hypercholesterolemia and reduced HDL-C promote hematopoietic stem cell proliferation and monocytosis: studies in mice and FH children. *Atherosclerosis*. (2013) 229:79–85. doi: 10.1016/j.atherosclerosis.2013.03.031
41. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*. (2011) 32:81–151. doi: 10.1210/er.2010-0013
42. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. (2016) 16:626–38. doi: 10.1038/nri.2016.90
43. Viselli SM, Stanziale S, Shults K, Kovacs WJ, Olsen NJ. Castration alters peripheral immune function in normal male mice. *Immunology*. (1995) 84:337–42.
44. Wilson CA, Mrose SA, Thomas DW. Enhanced production of B lymphocytes after castration. *Blood*. (1995) 85:1535–9.
45. Ellis TM, Moser MT, Le PT, Flanigan RC, Kwon ED. Alterations in peripheral B cells and B cell progenitors following androgen ablation in mice. *Int Immunol*. (2001) 13:553–8. doi: 10.1093/intimm/13.4.553
46. Viselli SM, Reese KR, Fan J, Kovacs WJ, Olsen NJ. Androgens alter B cell development in normal male mice. *Cell Immunol*. (1997) 182:99–104. doi: 10.1006/cimm.1997.1227
47. Smithson G, Couse JF, Lubahn DB, Korach KS, Kincade PW. The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. *J Immunol*. (1998) 161:27–34.
48. Olsen NJ, Watson MB, Kovacs WJ. Studies of immunological function in mice with defective androgen action. Distinction between alterations in immune function due to hormonal insensitivity and alterations due to other genetic factors. *Immunology*. (1991) 73:52–7.
49. Olsen NJ, Watson MB, Henderson GS, Kovacs WJ. Androgen deprivation induces phenotypic and functional changes in the thymus of adult male mice. *Endocrinology*. (1991) 129:2471–6. doi: 10.1210/endo-129-5-2471
50. Olsen NJ, Olson G, Viselli SM, Gu X, Kovacs WJ. Androgen receptors in thymic epithelium modulate thymus size and thymocyte development. *Endocrinology*. (2001) 142:1278–83. doi: 10.1210/endo.142.3.8032
51. Olsen NJ, Gu X, Kovacs WJ. Bone marrow stromal cells mediate androgenic suppression of B lymphocyte development. *J Clin Invest*. (2001) 108:1697–704. doi: 10.1172/JCI200113183
52. Velardi E, Tsai JJ, Holland AM, Wertheimer T, Yu VW, Zakrzewski JL, et al. Sex steroid blockade enhances thymopoiesis by modulating Notch signaling. *J Exp Med*. (2014) 211:2341–9. doi: 10.1084/jem.20131289
53. Dorshkind K, Montecino-Rodriguez E, Signer RA. The ageing immune system: is it ever too old to become young again? *Nat Rev Immunol*. (2009) 9:57–62. doi: 10.1038/nri2471
54. Dudakov JA, Goldberg GL, Reiseger JJ, Chidgey AP, Boyd RL. Withdrawal of sex steroids reverses age- and chemotherapy-related defects in bone marrow lymphopoiesis. *J Immunol*. (2009) 182:6247–60. doi: 10.4049/jimmunol.0802446
55. Fitzpatrick FT, Kendall MD, Wheeler MJ, Adcock IM, Greenstein BD. Reappearance of thymus of ageing rats after orchidectomy. *J Endocrinol*. (1985) 106:R17–9. doi: 10.1677/joe.0.106R017
56. Goldberg GL, Sutherland JS, Hammet MV, Milton MK, Heng TS, Chidgey AP, Boyd RL. Sex steroid ablation enhances lymphoid recovery following autologous hematopoietic stem cell transplantation. *Transplantation*. (2005) 80:1604–13. doi: 10.1097/01.tp.0000183962.64777.da
57. Goldberg GL, Alpdogan O, Muriglan SJ, Hammett MV, Milton MK, Eng JM, et al. Enhanced immune reconstitution by sex steroid ablation following allogeneic hemopoietic stem cell transplantation. *J Immunol*. (2007) 178:7473–84. doi: 10.4049/jimmunol.178.11.7473
58. Goldberg GL, King CG, Nejat RA, Suh DY, Smith OM, Bretz JC, et al. Luteinizing hormone-releasing hormone enhances T cell recovery following allogeneic bone marrow transplantation. *J Immunol*. (2009) 182:5846–54. doi: 10.4049/jimmunol.0801458
59. Greenstein BD, Fitzpatrick FT, Adcock IM, Kendall MD, Wheeler MJ. Reappearance of the thymus in old rats after orchidectomy: inhibition of regeneration by testosterone. *J Endocrinol*. (1986) 110:417–22. doi: 10.1677/joe.0.1100417
60. Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, et al. Activation of thymic regeneration in mice and humans following androgen blockade. *J Immunol*. (2005) 175:2741–53. doi: 10.4049/jimmunol.175.4.2741
61. Goldberg GL, Dudakov JA, Reiseger JJ, Seach N, Ueno T, Vlahos K, et al. Sex steroid ablation enhances immune reconstitution following cytotoxic

antineoplastic therapy in young mice. *J Immunol*. (2010) 184:6014–24. doi: 10.4049/jimmunol.0802445
62. Dudakov JA, Goldberg GL, Reiseger JJ, Vlahos K, Chidgey AP, Boyd RL. Sex steroid ablation enhances hematopoietic recovery following cytotoxic antineoplastic therapy in aged mice. *J Immunol*. (2009) 183:7084–94. doi: 10.4049/jimmunol.0900196
63. Khong DM, Dudakov JA, Hammett MV, Jurblum MI, Khong SM, Goldberg GL, et al. Enhanced hematopoietic stem cell function mediates immune regeneration following sex steroid blockade. *Stem Cell Reports*. (2015) 4:445–58. doi: 10.1016/j.stemcr.2015.01.018
64. Rijhsinghani AG, Bhatia SK, Tygrett LT, Waldschmidt TJ. Effect of pregnancy on thymic T cell development. *Am J Reprod Immunol*. (1996) 35:523–8. doi: 10.1111/j.1600-0897.1996.tb00052.x
65. Medina KL, Smithson G, Kincade PW. Suppression of B lymphopoiesis during normal pregnancy. *J Exp Med*. (1993) 178:1507–15. doi: 10.1084/jem.178.5.1507
66. Erben RG, Raith S, Eberle J, Stangassinger M. Ovariectomy augments B lymphopoiesis and generation of monocyte-macrophage precursors in rat bone marrow. *Am J Physiol*. (1998) 274(3 Pt 1):E476–83. doi: 10.1152/ajpendo.1998.274.3.E476
67. Miyaura C, Onoe Y, Inada M, Maki K, Ikuta K, Ito M, Suda T. Increased B-lymphopoiesis by interleukin 7 induces bone loss in mice with intact ovarian function: similarity to estrogen deficiency. *Proc Natl Acad Sci USA*. (1997) 94:9360–5. doi: 10.1073/pnas.94.17.9360
68. Masuzawa T, Miyaura C, Onoe Y, Kusano K, Ohta H, Nozawa S, et al. Estrogen deficiency stimulates B lymphopoiesis in mouse bone marrow. *J Clin Invest*. (1994) 94:1090–7. doi: 10.1172/JCI117424
69. Smithson G, Beamer WG, Shultz KL, Christianson SW, Shultz LD, Kincade PW. Increased B lymphopoiesis in genetically sex steroid-deficient hypogonadal (hpg) mice. *J Exp Med*. (1994) 180:717–20. doi: 10.1084/jem.180.2.717
70. Medina KL, Kincade PW. Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. *Proc Natl Acad Sci USA*. (1994) 91:5382–6. doi: 10.1073/pnas.91.12.5382
71. Medina KL, Strasser A, Kincade PW. Estrogen influences the differentiation, proliferation, and survival of early B-lineage precursors. *Blood*. (2000) 95:2059–67.
72. Medina KL, Garrett KP, Thompson LF, Rossi MI, Payne KJ, Kincade PW. Identification of very early lymphoid precursors in bone marrow and their regulation by estrogen. *Nat Immunol*. (2001) 2:718–24. doi: 10.1038/90659
73. Smithson G, Medina K, Ponting I, Kincade PW. Estrogen suppresses stromal cell-dependent lymphopoiesis in culture. *J Immunol*. (1995) 155:3409–17.
74. Thurmond TS, Murante FG, Staples JE, Silverstone AE, Korach KS, Gasiewicz TA. Role of estrogen receptor alpha in hematopoietic stem cell development and B lymphocyte maturation in the male mouse. *Endocrinology*. (2000) 141:2309–18. doi: 10.1210/endo.141.7.7560
75. Dukes PP, Goldwasser E. Inhibition of erythropoiesis by estrogens. *Endocrinology*. (1961) 69:21–9. doi: 10.1210/endo-69-1-21
76. Mirand EA, Gordon AS. Mechanism of estrogen action in erythropoiesis. *Endocrinology*. (1966) 78:325–32. doi: 10.1210/endo-78-2-325
77. Crafts RG. The effects of estrogens on the bone marrow of adult female dogs. *Blood*. (1948) 3:276–85.
78. Schroeder C, Gibson L, Nordstrom C, Beug H. The estrogen receptor cooperates with the TGF alpha receptor (c-erbB) in regulation of chicken erythroid progenitor self-renewal. *EMBO J*. (1993) 12:951–60. doi: 10.1002/j.1460-2075.1993.tb05736.x
79. Blobel GA, Sieff CA, Orkin SH. Ligand-dependent repression of the erythroid transcription factor GATA-1 by the estrogen receptor. *Mol Cell Biol*. (1995) 15:3147–53. doi: 10.1128/MCB.15.6.3147
80. Blobel GA, Orkin SH. Estrogen-induced apoptosis by inhibition of the erythroid transcription factor GATA-1. *Mol Cell Biol*. (1996) 16:1687–94. doi: 10.1128/MCB.16.4.1687
81. Bord S, Frith E, Ireland DC, Scott MA, Craig JI, Compston JE. Estrogen stimulates differentiation of megakaryocytes and modulates their expression of estrogen receptors alpha and beta. *J Cell Biochem*. (2004) 92:249–57. doi: 10.1002/jcb.20035
82. Du C, Xu Y, Yang K, Chen S, Wang X, Wang S, et al. Estrogen promotes megakaryocyte polyploidization via estrogen receptor beta-mediated transcription of GATA1. *Leukemia*. (2017) 31:945–56. doi: 10.1038/leu.2016.285
83. Paharkova-Vatchkova V, Maldonado R, Kovats S. Estrogen preferentially promotes the differentiation of CD11c+ CD11b(intermediate) dendritic cells from bone marrow precursors. *J Immunol*. (2004) 172:1426–36. doi: 10.4049/jimmunol.172.3.1426
84. Carreras E, Turner S, Frank MB, Knowlton N, Osban J, Centola M, et al. Estrogen receptor signaling promotes dendritic cell differentiation by increasing expression of the transcription factor IRF4. *Blood*. (2010) 115:238–46. doi: 10.1182/blood-2009-08-236935
85. Carreras E, Turner S, Paharkova-Vatchkova V, Mao A, Dascher C, Kovats S. Estradiol acts directly on bone marrow myeloid progenitors to differentially regulate GM-CSF or Flt3 ligand-mediated dendritic cell differentiation. *J Immunol*. (2008) 180:727–38. doi: 10.4049/jimmunol.180.2.727
86. Yamamoto R, Morita Y, Ooehara J, Hamanaka S, Onodera M, Rudolph KL, et al. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. *Cell*. (2013) 154:1112–26. doi: 10.1016/j.cell.2013.08.007
87. Mahendroo MS, Cala KM, Landrum DP, Russell DW. Fetal death in mice lacking 5alpha-reductase type 1 caused by estrogen excess. *Mol Endocrinol*. (1997) 11:917–27.
88. Sheehan HL, Falkiner NM. Splenic aneurysm and splenic enlargement in pregnancy. Br *Med J*. (1948) 2:1105. doi: 10.1136/bmj.2.4590.1105
89. Fowler JH, Nash DJ. Erythropoiesis in the spleen and bone marrow of the pregnant mouse. *Dev Biol*. (1968) 18:331–53. doi: 10.1016/0012-1606(68)90045-6
90. Illing A, Liu P, Ostermay S, Schilling A, de Haan G, Krust A, et al. Estradiol increases hematopoietic stem and progenitor cells independent of its actions on bone. *Haematologica*. (2012) 97:1131–5. doi: 10.3324/haematol.2011.052456
91. Sanchez-Aguilera A, Arranz L, Martin-Perez D, Garcia-Garcia A, Stavropoulou V, et al. Estrogen signaling selectively induces apoptosis of hematopoietic progenitors and myeloid neoplasms without harming steady-state hematopoiesis. *Cell Stem Cell*. (2014) 15:791–804. doi: 10.1016/j.stem.2014.11.002
92. Notta F, Doulatov S, Dick JE. Engraftment of human hematopoietic stem cells is more efficient in female NOD/SCID/IL-2Rgc-null recipients. *Blood*. (2010) 115:3704–7. doi: 10.1182/blood-2009-10-249326
93. Miller PH, Rabu G, MacAldaz M, Knapp DJ, Cheung AM, Dhillon K, et al. Analysis of parameters that affect human hematopoietic cell outputs in mutant c-kit-immunodeficient mice. *Exp Hematol*. (2017) 48:41–9. doi: 10.1016/j.exphem.2016.12.012
94. Chapple RH, Hu T, Tseng YJ, Liu L, Kitano A, Luu V, et al. ERalpha promotes murine hematopoietic regeneration through the Ire1alpha-mediated unfolded protein response. *Elife*. (2018) 7. doi: 10.7554/eLife.31159
95. Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, et al. Mangelsdorf DJ. 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nat Med*. (2007) 13:1185–92. doi: 10.1038/nm1641
96. Karuna R, Holleboom AG, Motazacker MM, Kuivenhoven JA, Frikke-Schmidt R, Tybjaerg-Hansen A, et al. Plasma levels of 27-hydroxycholesterol in humans and mice with monogenic disturbances of high density lipoprotein metabolism. *Atherosclerosis*. (2011) 214:448–55. doi: 10.1016/j.atherosclerosis.2010.10.042
97. Rosen H, Reshef A, Maeda N, Lippoldt A, Shpizen S, Triger L, et al. Markedly reduced bile acid synthesis but maintained levels of cholesterol and vitamin D metabolites in mice with disrupted sterol 27-hydroxylase gene. *J Biol Chem*. (1998) 273:14805–12. doi: 10.1074/jbc.273.24.14805
98. Wu Q, Ishikawa T, Sirianni R, Tang H, McDonald JG, Yuhanna IS, et al. Shaul PW. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Rep*. (2013) 5:637–45. doi: 10.1016/j.celrep.2013.10.006
99. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, et al. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science*. (2013) 342:1094–8. doi: 10.1126/science.1241908
100. Umetani M, Ghosh P, Ishikawa T, Umetani J, Ahmed M, Mineo C, et al. The cholesterol metabolite 27-hydroxycholesterol promotes atherosclerosis via proinflammatory processes mediated by estrogen receptor alpha. *Cell Metab*. (2014) 20:172–82. doi: 10.1016/j.cmet.2014.05.013

101. Oguro H, McDonald JG, Zhao Z, Umetani M, Shaul PW, Morrison SJ. 27-Hydroxycholesterol induces hematopoietic stem cell mobilization and extramedullary hematopoiesis during pregnancy. *J Clin Invest*. (2017) 127:3392–401. doi: 10.1172/JCI94027
102. Ordovas JM, Pocovi M, Grande F. Plasma lipids and cholesterol esterification rate during pregnancy. *Obstet Gynecol*. (1984) 63:20–5.
103. Schwartz JA, Skafar DF. Ligand-mediated modulation of estrogen receptor conformation by estradiol analogs. *Biochemistry*. (1993) 32:10109–15. doi: 10.1021/bi00089a029
104. Paige LA, Christensen DJ, Gron H, Norris JD, Gottlin EB, Padilla KM, et al. Estrogen receptor (ER) modulators each induce distinct conformational changes in ER alpha and ER beta. *Proc Natl Acad Sci USA*. (1999) 96:3999–4004. doi: 10.1073/pnas.96.7.3999
105. Bruning JB, Parent AA, Gil G, Zhao M, Nowak J, Pace MC, et al. Coupling of receptor conformation and ligand orientation determine graded activity. *Nat Chem Biol*. (2010) 6:837–43. doi: 10.1038/nchembio.451
106. Wardell SE, Kazmin D, McDonnell DP. Research resource: transcriptional profiling in a cellular model of breast cancer reveals functional and mechanistic differences between clinically relevant SERM and between SERM/estrogen complexes. *Mol Endocrinol*. (2012) 26:1235–48. doi: 10.1210/me.2012-1031
107. DuSell CD, Umetani M, Shaul PW, Mangelsdorf DJ, McDonnell DP. 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator. *Mol Endocrinol*. (2008) 22:65–77. doi: 10.1210/me.2007-0383
108. Feng Q, O'Malley BW. Nuclear receptor modulation–role of coregulators in selective estrogen receptor modulator (SERM) actions. *Steroids*. (2014) 90:39–43. doi: 10.1016/j.steroids.2014.06.008
109. de Aguiar Vallim TQ, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. *Cell Metab*. (2013) 17:657–69. doi: 10.1016/j.cmet.2013.03.013
110. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS. chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science*. (2006) 313:1137–40. doi: 10.1126/science.1128294
111. van Galen P, Kreso A, Wienholds E, Laurenti E, Eppert K, Lechman ER, et al. Reduced lymphoid lineage priming promotes human hematopoietic stem cell expansion. *Cell Stem Cell*. (2014) 14:94–106. doi: 10.1016/j.stem.2013.11.021
112. Miharada K, Sigurdsson V, Karlsson S. Dppa5 improves hematopoietic stem cell activity by reducing endoplasmic reticulum stress. *Cell Rep*. (2014) 7:1381–92. doi: 10.1016/j.celrep.2014.04.056
113. Ema H, Nakauchi H. Expansion of hematopoietic stem cells in the developing liver of a mouse embryo. *Blood*. (2000) 95:2284–8.
114. Signer RA, Magee JA, Salic A, Morrison SJ. Haematopoietic stem cells require a highly regulated protein synthesis rate. *Nature*. (2014) 509:49–54. doi: 10.1038/nature13035
115. Sigurdsson V, Takei H, Soboleva S, Radulovic V, Galeev R, Siva K, et al. Bile acids protect expanding hematopoietic stem cells from unfolded protein stress in fetal liver. *Cell Stem Cell*. (2016) 18:522–32. doi: 10.1016/j.stem.2016.01.002
116. Cyster JG, Dang EV, Reboldi A, Yi T. 25-Hydroxycholesterols in innate and adaptive immunity. *Nat Rev Immunol*. (2014) 14:731–43. doi: 10.1038/nri3755
117. Bauman DR, Bitmansour AD, McDonald JG, Thompson BM, Liang G, Russell DW. 25-Hydroxycholesterol secreted by macrophages in response to Toll-like receptor activation suppresses immunoglobulin A production. *Proc Natl Acad Sci USA*. (2009) 106:16764–9. doi: 10.1073/pnas.0909142106
118. Park K, Scott AL. Cholesterol 25-hydroxylase production by dendritic cells and macrophages is regulated by type I interferons. *J Leukoc Biol*. (2010) 88:1081–7. doi: 10.1189/jlb.0610318
119. Diczfalusy U, Olofsson KE, Carlsson AM, Gong M, Golenbock DT, Rooyackers O, et al. Marked upregulation of cholesterol 25-hydroxylase expression by lipopolysaccharide. *J Lipid Res*. (2009) 50:2258–64. doi: 10.1194/jlr.M900107-JLR200
120. Zou T, Garifulin O, Berland R, Boyartchuk VL. Listeria monocytogenes infection induces prosurvival metabolic signaling in macrophages. *Infect Immun*. (2011) 79:1526–35. doi: 10.1128/IAI.01195-10
121. Reboldi A, Dang EV, McDonald JG, Liang G, Russell DW, Cyster JG. Inflammation. 25-Hydroxycholesterol suppresses interleukin-1-driven inflammation downstream of type I interferon. *Science*. (2014) 345:679–84. doi: 10.1126/science.1254790
122. Gold ES, Diercks AH, Podolsky I, Podyminogin RL, Askovich PS, Treuting PM, Aderem A. 25-Hydroxycholesterol acts as an amplifier of inflammatory signaling. *Proc Natl Acad Sci USA*. (2014) 111:10666–71. doi: 10.1073/pnas.1404271111
123. Koarai A, Yanagisawa S, Sugiura H, Ichikawa T, Kikuchi T, Furukawa K, et al. 25-Hydroxycholesterol enhances cytokine release and Toll-like receptor 3 response in airway epithelial cells. *Respir Res*. (2012) 13:63. doi: 10.1186/1465-9921-13-63
124. Dang EV, McDonald JG, Russell DW, Cyster JG. Oxysterol restraint of cholesterol synthesis prevents AIM2 inflammasome activation. *Cell*. (2017) 171:1057–71 e11. doi: 10.1016/j.cell.2017.09.029
125. Blanc M, Hsieh WY, Robertson KA, Kropp KA, Forster T, Shui G, et al. The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. *Immunity*. (2013) 38:106–18. doi: 10.1016/j.immuni.2012.11.004
126. Liu SY, Aliyari R, Chikere K, Li G, Marsden MD, Smith JK, et al. Interferon-inducible cholesterol-25-hydroxylase broadly inhibits viral entry by production of 25-hydroxycholesterol. *Immunity*. (2013) 38:92–105. doi: 10.1016/j.immuni.2012.11.005
127. Gold ES, Ramsey SA, Sartain MJ, Selinummi J, Podolsky I, Rodriguez DJ, et al. ATF3 protects against atherosclerosis by suppressing 25-hydroxycholesterol-induced lipid body formation. *J Exp Med*. (2012) 209:807–17. doi: 10.1084/jem.20111202
128. Gatto D, Paus D, Basten A, Mackay CR, Brink R. Guidance of B cells by the orphan G protein-coupled receptor EBI2 shapes humoral immune responses. *Immunity*. (2009) 31:259–69. doi: 10.1016/j.immuni.2009.06.016
129. Pereira JP, Kelly LM, Xu Y, Cyster JG. EBI2 mediates B cell segregation between the outer and centre follicle. *Nature*. (2009) 460:1122–6. doi: 10.1038/nature08226
130. Liu C, Yang XV, Wu J, Kuei C, Mani NS, Zhang L, et al. Oxysterols direct B-cell migration through EBI2. *Nature*. (2011) 475:519–23. doi: 10.1038/nature10226
131. Hannedouche S, Zhang J, Yi T, Shen W, Nguyen D, Pereira JP, et al. Oxysterols direct immune cell migration via EBI2. *Nature*. (2011) 475:524–7. doi: 10.1038/nature10280
132. Gatto D, Wood K, Caminschi I, Murphy-Durland D, Schofield P, Christ D, et al. The chemotactic receptor EBI2 regulates the homeostasis, localization and immunological function of splenic dendritic cells. *Nat Immunol*. (2013) 14:446–53. doi: 10.1038/ni.2555
133. Yi T, Cyster JG. EBI2-mediated bridging channel positioning supports splenic dendritic cell homeostasis and particulate antigen capture. *Elife*. (2013) 2:e00757. doi: 10.7554/eLife.00757
134. Li J, Lu E, Yi T, Cyster JG. EBI2 augments Tfh cell fate by promoting interaction with IL-2-quenching dendritic cells. *Nature*. (2016) 533:110–4. doi: 10.1038/nature17947
135. Nevius E, Pinho F, Dhodapkar M, Jin H, Nadrah K, Horowitz MC, et al. Oxysterols and EBI2 promote osteoclast precursor migration to bone surfaces and regulate bone mass homeostasis. *J Exp Med*. (2015) 212:1931–46. doi: 10.1084/jem.20150088
136. Ho YK, Smith RG, Brown MS, Goldstein JL. Low-density lipoprotein (LDL) receptor activity in human acute myelogenous leukemia cells. *Blood*. (1978) 52:1099–114.
137. Vitols S, Angelin B, Ericsson S, Gahrton G, Juliusson G, Masquelier M, et al. Uptake of low density lipoproteins by human leukemic cells *in vivo*: relation to plasma lipoprotein levels and possible relevance for selective chemotherapy. *Proc Natl Acad Sci USA*. (1990) 87:2598–602. doi: 10.1073/pnas.87.7.2598
138. Vitols S, Gahrton G, Bjorkholm M, Peterson C. Hypocholesterolaemia in malignancy due to elevated low-density-lipoprotein-receptor activity in tumour cells: evidence from studies in patients with leukaemia. *Lancet*. (1985) 2:1150–4. doi: 10.1016/S0140-6736(85)92679-0
139. Scribano D, Baroni S, Pagano L, Zuppi C, Leone G, Giardina B. Return to normal values of lipid pattern after effective chemotherapy in acute lymphoblastic leukemia. *Haematologica*. (1996) 81:343–45.
140. Moschovi M, Trimis G, Apostolakou F, Papassotiriou I, Tzortzatou-Stathopoulou F. Serum lipid alterations in acute lymphoblastic

leukemia of childhood. *J Pediatr Hematol Oncol*. (2004) 26:289–93. doi: 10.1097/00043426-200405000-00006
141. Liebes LF, Pelle E, Zucker-Franklin D, Silber R. Comparison of lipid composition and 1,6-diphenyl-1,3,5-hexatriene fluorescence polarization measurements of hairy cells with monocytes and lymphocytes from normal subjects and patients with chronic lymphocytic leukemia. *Cancer Res*. (1981) 41:4050–6.
142. Yachnin S, Golomb HM, West EJ, Saffold C. Increased cholesterol biosynthesis in leukemic cells from patients with hairy cell leukemia. *Blood*. (1983) 61:50–60.
143. Golomb HM, Saffold CW, Nathans AH, Dawson G. Phospholipid and cholesterol differences amongst leukemic cell types with special reference to hairy cell leukemia: a preliminary report. *Clin Chim Acta*. (1981) 116:311–8. doi: 10.1016/0009-8981(81)90050-4
144. Clutterbuck RD, Millar BC, Powles RL, Newman A, Catovsky D, Jarman M, et al. Inhibitory effect of simvastatin on the proliferation of human myeloid leukaemia cells in severe combined immunodeficient (SCID) mice. *Br J Haematol*. (1998) 102:522–7. doi: 10.1046/j.1365-2141.1998.00783.x
145. Dimitroulakos J, Nohynek D, Backway KL, Hedley DW, Yeger H, Freedman MH, et al. Increased sensitivity of acute myeloid leukemias to lovastatin-induced apoptosis: a potential therapeutic approach. *Blood*. (1999) 93:1308–18.
146. Dimitroulakos J, Thai S, Wasfy GH, Hedley DW, Minden MD, Penn LZ. Lovastatin induces a pronounced differentiation response in acute myeloid leukemias. *Leuk Lymphoma*. (2000) 40:167–78. doi: 10.3109/10428190009054894
147. Perez-Sala D, Mollinedo F. Inhibition of isoprenoid biosynthesis induces apoptosis in human promyelocytic HL-60 cells. *Biochem Biophys Res Commun*. (1994) 199:1209–15. doi: 10.1006/bbrc.1994.1359
148. Wang IK, Lin-Shiau SY, Lin JK. Induction of apoptosis by lovastatin through activation of caspase-3 and DNase II in leukaemia HL-60 cells. *Pharmacol Toxicol*. (2000) 86:83–91. doi: 10.1034/j.1600-0773.2000.d01-16.x
149. Lishner M, Bar-Sef A, Elis A, Fabian I. Effect of simvastatin alone and in combination with cytosine arabinoside on the proliferation of myeloid leukemia cell lines. *J Investig Med*. (2001) 49:319–24. doi: 10.2310/6650.2001.33896
150. Wong WW, Tan MM, Xia Z, Dimitroulakos J, Minden MD, Penn LZ. Cerivastatin triggers tumor-specific apoptosis with higher efficacy than lovastatin. *Clin Cancer Res*. (2001) 7:2067–75.
151. Holstein SA, Hohl RJ. Synergistic interaction of lovastatin and paclitaxel in human cancer cells. *Mol Cancer Ther*. (2001) 1:141–9.
152. Li HY, Appelbaum FR, Willman CL, Zager RA, Banker DE. Cholesterol-modulating agents kill acute myeloid leukemia cells and sensitize them to therapeutics by blocking adaptive cholesterol responses. *Blood*. (2003) 101:3628–34. doi: 10.1182/blood-2002-07-2283
153. Maksumova L, Ohnishi K, Muratkhodjaev F, Zhang W, Pan L, Takeshita A, et al. Increased sensitivity of multidrug-resistant myeloid leukemia cell lines to lovastatin. *Leukemia*. (2000) 14:1444–50. doi: 10.1038/sj.leu.2401856
154. Newman A, Clutterbuck RD, Powles RL, Millar JL. Selective inhibition of primary acute myeloid leukaemia cell growth by simvastatin. *Leukemia*. (1994) 8:2023–9.
155. Sassano A, Katsoulidis E, Antico G, Altman JK, Redig AJ, Minucci S, et al. Suppressive effects of statins on acute promyelocytic leukemia cells. *Cancer Res*. (2007) 67:4524–32. doi: 10.1158/0008-5472.CAN-06-3686
156. Stirewalt DL, Appelbaum FR, Willman CL, Zager RA, Banker DE. Mevastatin can increase toxicity in primary AMLs exposed to standard therapeutic agents, but statin efficacy is not simply associated with ras hotspot mutations or overexpression. *Leuk Res*. (2003) 27:133–45. doi: 10.1016/S0145-2126(02)00085-1
157. Xia Z, Tan MM, Wong WW, Dimitroulakos J, Minden MD, Penn LZ. Blocking protein geranylgeranylation is essential for lovastatin-induced apoptosis of human acute myeloid leukemia cells. *Leukemia*. (2001) 15:1398–407. doi: 10.1038/sj.leu.2402196
158. Wu J, Wong WW, Khosravi F, Minden MD, Penn LZ. Blocking the Raf/MEK/ERK pathway sensitizes acute myelogenous leukemia cells to lovastatin-induced apoptosis. *Cancer Res*. (2004) 64:6461–8. doi: 10.1158/0008-5472.CAN-04-0866
159. Chen R, Xiao W, Li D, Mu S. Combination of simvastatin and imatinib sensitizes the CD34+ cells in K562 to cell death. *Med Oncol*. (2011) 28:528–31. doi: 10.1007/s12032-010-9472-9
160. Yang YC, Xiao DW, Liu H, Chuan LM, Zeng YL, Zhou DA, Liu W, Xu GQ, Huang WF. Mechanism of simvastatin-induced K562 cell apoptosis. *Pharmacology*. (2009) 84:191–5. doi: 10.1159/000235907
161. Oh B, Kim TY, Min HJ, Kim M, Kang MS, Huh JY, Kim Y, Lee DS. Synergistic killing effect of imatinib and simvastatin on imatinib-resistant chronic myelogenous leukemia cells. *Anticancer Drugs*. (2013) 24:20–31. doi: 10.1097/CAD.0b013e32835a0fbd
162. Griner LN, McGraw KL, Johnson JO, List AF, Reuther GW. JAK2-V617F-mediated signalling is dependent on lipid rafts and statins inhibit JAK2-V617F-dependent cell growth. *Br J Haematol*. (2013) 160:177–87. doi: 10.1111/bjh.12103
163. Yasuda N, Matzno S, Iwano C, Nishikata M, Matsuyama K. Evaluation of apoptosis and necrosis induced by statins using fluorescence-enhanced flow cytometry. *J Pharm Biomed Anal*. (2005) 39:712–7. doi: 10.1016/j.jpba.2005.04.022
164. Sheen C, Vincent T, Barrett D, Horwitz EM, Hulitt J, Strong E, Grupp SA, Teachey DT. Statins are active in acute lymphoblastic leukaemia (ALL): a therapy that may treat ALL and prevent avascular necrosis. *Br J Haematol*. (2011) 155:403–7. doi: 10.1111/j.1365-2141.2011.08696.x
165. Chapman-Shimshoni D, Yuklea M, Radnay J, Shapiro H, Lishner M. Simvastatin induces apoptosis of B-CLL cells by activation of mitochondrial caspase 9. *Exp Hematol*. (2003) 31:779–83. doi: 10.1016/S0301-472X(03)00192-9
166. Podhorecka M, Halicka D, Klimek P, Kowal M, Chocholska S, Dmoszynska A. Simvastatin and purine analogs have a synergic effect on apoptosis of chronic lymphocytic leukemia cells. *Ann Hematol*. (2010) 89:1115–24. doi: 10.1007/s00277-010-0988-z
167. Vitols S, Angelin B, Juliusson G. Simvastatin impairs mitogen-induced proliferation of malignant B-lymphocytes from humans–*in vitro* and *in vivo* studies. *Lipids*. (1997) 32:255–62. doi: 10.1007/s11745-997-0032-1
168. Nonaka M, Uota S, Saitoh Y, Takahashi M, Sugimoto H, Amet T, et al. Role for protein geranylgeranylation in adult T-cell leukemia cell survival. *Exp Cell Res*. (2009) 315:141–50. doi: 10.1016/j.yexcr.2008.10.010
169. Rozados VR, Hinrichsen LI, Binda MM, Gervasoni SI, Matar P, Bonfil RD, et al. Lovastatin enhances the antitumoral and apoptotic activity of doxorubicin in murine tumor models. *Oncol Rep*. (2008) 19:1205–11. doi: 10.3892/or.19.5.1205
170. van de Donk NW, Schotte D, Kamphuis MM, van Marion AM, van Kessel B, Bloem AC, et al. Protein geranylgeranylation is critical for the regulation of survival and proliferation of lymphoma tumor cells. *Clin Cancer Res*. (2003) 9:5735–48.
171. Caffario P, Dammacco F, Gernone A, Silvestris F. Statins activate the mitochondrial pathway of apoptosis in human lymphoblasts and myeloma cells. *Carcinogenesis*. (2005) 26:883–91. doi: 10.1093/carcin/bgi036
172. Dmoszynska A, Podhorecka M, Klimek P, Grzasko N. Lovastatin and thalidomide have a combined effect on the rate of multiple myeloma cell apoptosis in short term cell cultures. *Eur J Clin Pharmacol*. (2006) 62:325–9. doi: 10.1007/s00228-006-0106-2
173. Gronich N, Drucker L, Shapiro H, Radnay J, Yarkoni S, Lishner M. Simvastatin induces death of multiple myeloma cell lines. *J Investig Med*. (2004) 52:335–44. doi: 10.1136/jim-52-05-34
174. Hus M, Grzasko N, Szostek M, Pluta A, Helbig G, Woszczyk D, et al. Thalidomide, dexamethasone and lovastatin with autologous stem cell transplantation as a salvage immunomodulatory therapy in patients with relapsed and refractory multiple myeloma. *Ann Hematol*. (2011) 90:1161–6. doi: 10.1007/s00277-011-1276-2
175. van der Spek E, Bloem AC, Lokhorst HM, van Kessel B, Bogers-Boer L, van de Donk NW. Inhibition of the mevalonate pathway potentiates the effects of lenalidomide in myeloma. *Leuk Res*. (2009) 33:100–8. doi: 10.1016/j.leukres.2008.06.001
176. van de Donk NW, Kamphuis MM, van Kessel B, Lokhorst HM, Bloem AC. Inhibition of protein geranylgeranylation induces apoptosis in myeloma plasma cells by reducing Mcl-1 protein levels. *Blood*. (2003) 102:3354–62. doi: 10.1182/blood-2003-03-0970

177. Hartwell KA, Miller PG, Mukherjee S, Kahn AR, Stewart AL, Logan DJ, et al. Niche-based screening identifies small-molecule inhibitors of leukemia stem cells. *Nat Chem Biol*. (2013) 9:840–8. doi: 10.1038/nchembio.1367
178. Christ M, Luu B, Mejia JE, Moosbrugger I, Bischoff P. Apoptosis induced by oxysterols in murine lymphoma cells and in normal thymocytes. *Immunology*. (1993) 78:455–60.
179. Hwang PL. Inhibitors of protein and RNA synthesis block the cytotoxic effects of oxygenated sterols. *Biochim Biophys Acta*. (1992) 1136:5–11. doi: 10.1016/0167-4889(92)90077-O
180. Hietter H, Bischoff P, Beck JP, Ourisson G, Luu B. Comparative effects of 7 beta-hydroxycholesterol towards murine lymphomas, lymphoblasts and lymphocytes: selective cytotoxicity and blastogenesis inhibition. *Cancer Biochem Biophys*. (1986) 9:75–83.
181. Rosa Fernandes L, Stern AC, Cavaglieri RC, Nogueira FC, Domont G, et al. 7-Ketocholesterol overcomes drug resistance in chronic myeloid leukemia cell lines beyond MDR1 mechanism. *J Proteomics*. (2017) 151:12–23. doi: 10.1016/j.jprot.2016.06.011
182. Aupeix K, Weltin D, Mejia JE, Christ M, Marchal J, Freyssinet JM, et al. Oxysterol-induced apoptosis in human monocytic cell lines. *Immunobiology*. (1995) 194:415–28. doi: 10.1016/S0171-2985(11)80108-7
183. Lim HK, Kang HK, Yoo ES, Kim BJ, Kim YW, Cho M, et al. Oxysterols induce apoptosis and accumulation of cell cycle at G/M phase in the human monocytic THP-1 cell line. *Life Sci*. (2003) 72:1389–99. doi: 10.1016/S0024-3205(02)02377-9
184. Tsujioka T, Yokoi A, Itano Y, Takahashi K, Ouchida M, Okamoto S, et al. Five-aza-2′-deoxycytidine-induced hypomethylation of cholesterol 25-hydroxylase gene is responsible for cell death of myelodysplasia/leukemia cells. *Sci Rep*. (2015) 5:16709. doi: 10.1038/srep16709
185. Bonig H, Papayannopoulou T. Hematopoietic stem cell mobilization: updated conceptual renditions. *Leukemia*. (2013) 27:24–31. doi: 10.1038/leu.2012.254
186. Giralt S, Costa L, Schriber J, Dipersio J, Maziarz R, McCarty J, et al. Optimizing autologous stem cell mobilization strategies to improve patient outcomes: consensus guidelines and recommendations. *Biol Blood Marrow Transplant*. (2014) 20:295–308. doi: 10.1016/j.bbmt.2013.10.013
187. Tabas I, Lichtman AH. Monocyte-macrophages and T cells in atherosclerosis. *Immunity*. (2017) 47:621–34. doi: 10.1016/j.immuni.2017.09.008
188. Nahrendorf M. Myeloid cell contributions to cardiovascular health and disease. *Nat Med*. (2018) 24:711–20. doi: 10.1038/s41591-018-0064-0
189. Zmyslowski A, Szterk A. Current knowledge on the mechanism of atherosclerosis and pro-atherosclerotic properties of oxysterols. *Lipids Health Dis*. (2017) 16:188. doi: 10.1186/s12944-017-0579-2
190. Nofer JR. Estrogens and atherosclerosis: insights from animal models and cell systems. *J Mol Endocrinol*. (2012) 48:R13–29. doi: 10.1530/JME-11-0145
191. Dutta P, Courties G, Wei Y, Leuschner F, Gorbatov R, Robbins CS, et al. Myocardial infarction accelerates atherosclerosis. *Nature*. (2012) 487:325–9. doi: 10.1038/nature11260
192. Dutta P, Sager HB, Stengel KR, Naxerova K, Courties G, Saez B, et al. Myocardial infarction activates CCR2(+) hematopoietic stem and progenitor cells. *Cell Stem Cell*. (2015) 16:477–87. doi: 10.1016/j.stem.2015.04.008

Conflict of Interest Statement: The author declares 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 Endocrinology

REVIEW
published: 17 July 2019
doi: 10.3389/fendo.2019.00483

Oxysterols and Gastrointestinal Cancers Around the Clock

*Urša Kovač†, Cene Skubic†, Laura Bohinc, Damjana Rozman and Tadeja Režen**

Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

This review focuses on the role of oxidized sterols in three major gastrointestinal cancers (hepatocellular carcinoma, pancreatic, and colon cancer) and how the circadian clock affects the carcinogenesis by regulating the lipid metabolism and beyond. While each field of research (cancer, oxysterols, and circadian clock) is well-studied within their specialty, little is known about the intertwining mechanisms and how these influence the disease etiology in each cancer type. Oxysterols are involved in pathology of these cancers, but final conclusions about their protective or damaging effects are elusive, since the effect depends on the type of oxysterol, concentration, and the cell type. Oxysterol concentrations, the expression of key regulators liver X receptors (LXR), farnesoid X receptor (FXR), and oxysterol-binding proteins (OSBP) family are modulated in tumors and plasma of cancer patients, exposing these proteins and selected oxysterols as new potential biomarkers and drug targets. Evidence about how cholesterol/oxysterol pathways are intertwined with circadian clock is building. Identified key contact points are different forms of retinoic acid receptor related orphan receptors (ROR) and LXRs. RORs and LXRs are both regulated by sterols/oxysterols and the circadian clock and in return also regulate the same pathways, representing a complex interplay between sterol metabolism and the clock. With this in mind, in addition to classical therapies to modulate cholesterol in gastrointestinal cancers, such as the statin therapy, the time is ripe also for therapies where time and duration of the drug application is taken as an important factor for successful therapies. The final goal is the personalized approach with chronotherapy for disease management and treatment in order to increase the positive drug effects.

Keywords: oxysterols, circadian rhythm, hepatocellular carcinoma, pancreatic cancer, colorectal cancer, ROR, LXR, FXR

OPEN ACCESS

Edited by:
Vincenzo Pezzi,
University of Calabria, Italy

Reviewed by:
Carsten Carlberg,
University of Eastern Finland, Finland
Thomas Burris,
Washington University in St. Louis, United States
Jean-Marc A. Lobaccaro,
Université Clermont Auvergne, France

***Correspondence:**
Tadeja Režen
tadeja.rezen@mf.uni-lj.si

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *08 January 2019*
Accepted: *03 July 2019*
Published: *17 July 2019*

Citation:
Kovač U, Skubic C, Bohinc L, Rozman D and Režen T (2019) Oxysterols and Gastrointestinal Cancers Around the Clock. Front. Endocrinol. 10:483. doi: 10.3389/fendo.2019.00483

INTRODUCTION

Cholesterol and Oxysterols

Cholesterol is an essential molecule that participates in many cellular processes. It enables proper functioning of cellular membrane, is a precursor for synthesis of steroid hormones, oxysterols and bile acid, and functions as a signaling molecule regulating cell cycle, modifying proteins, and affecting its own synthesis (1–3). Most of the cholesterol in cells resides in the cellular membranes, where it plays a crucial role in stabilization of membranes, affects its fluidity and has an important role in lipid rafts (3). Oxysterols also have multiple functions, such as affecting membrane fluidity,

regulating SREBP (sterol regulatory element binding transcription protein) signaling pathway through regulation of INSIGs (insulin induced genes) and by this sterol synthesis, interacting with NPC1 (NPC intracellular cholesterol transporter 1) and OSBP/OSBPL [oxysterol-binding proteins (like)] and, most importantly, are ligands and activators of several nuclear receptors, such as RORs (NR1F1-3, retinoic acid receptor related orphan receptors A, B, C), FXR (NR1H4, farnesoid X receptor alpha), PXR (NR1I2, pregnane X receptor), ESR1/2 (NR3A1/2, estrogen receptor alpha/beta), and LXR (NR1H3, liver X receptor alpha) (4, 5). The term oxysterol usually means oxidized sterols that are produced from cholesterol enzymatically or by auto oxidation (**Figure 1**). However, also other sterols, including intermediates of cholesterol synthesis can be oxidized at least enzymatically by cytochrome P450 (CYP) enzymes (6). Auto oxidation of cholesterol usually happens in the presence of reactive oxygen species and oxidation occurs on the B ring of sterol nucleus, mainly at positions C7 or C6. With this process 7α/β-hydroxycholesterol, 7-ketocholesterol and 6-hydroxycholesterol are formed (7, 8). Enzymatic synthesis includes the side chain oxidation by CYP or non-CYP. For example, 24- hydroxycholesterol is synthesized by CYP46A1, 25-hydroxycholesterol by 25-hydroxylase (non-heme iron-containing protein) and 27-hydroxycholesterol by CYP27A1 (7, 9). Other oxysterols are formed by oxidation of the sterol nucleus, like 4β-hydroxycholesterol synthesized by CYP3A4 or 7α-hydroxycholesterol by CYP7A1 (10). The concentration of oxysterols in normal healthy tissue and blood is 10^4- to 10^6-fold lower compared to cholesterol (11). In addition to endogenous synthesis, oxysterols can also derive from the diet. The cholesterol-rich food contains 10 to 100 μM concentration of oxysterols. Most common are 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, cholestane-3ß,5α,6ß-triol, 5α,6α-epoxycholesterol, and 5β,6β-epoxycholesterol (12). When oxysterols were fed to humans, they were found in chylomicrons and lipoproteins (13).

SREBPs are transcription factors that activate transcription of genes necessary for cholesterol synthesis and uptake. Mammalian cells express three SREBP isoforms, SREBP-1a, SREBP-1c, and SREBP-2, which are responsible for expression of different lipid associated genes. SREBPs are located in endoplasmic reticulum (ER) together with SCAP (SREBP cleavage-activating protein) the escort protein, and INSIGs, the inhibitors of translocation. When cholesterol level declines, SREBPs are translocated to Golgi apparatus together with SCAP where they are proteolytic cleaved. A smaller SREBP is translocated to nucleus where it induces transcription of target genes (14). The major regulator of cholesterol homeostasis is SREBP-2 (15) as shown by the early mouse knockout experiments as well as in follow-up studies (16, 17). When SCAP is bound to INSIG, the vesicular transport of SREBP from ER is disabled. The cholesterol and oxysterols level regulate the INSIG inhibition of SCAP/SREBP transport (18, 19). Interestingly, cholesterol and oxysterols both induce the SCAP-INSIG interaction but by different mechanisms. Cholesterol acts by binding to SCAP and causing its conformational change that promotes binding to INSIG. Oxysterols, on the other hand, act directly on INSIG. For INSIG-2 it was shown that hydroxyl group on the side chain of sterols (22, 24, 25, or 27 position) is necessary for successful binding. A study confirmed these results, when 27-hydroxycholesterol levels were upregulated by CYP27A1 overexpression in mice or when primary hepatocytes were treated with 27-hydroxycholesterol, *Insig-2* expression was induced and SREBP-1 translocation was prevented (20). In this way, oxysterols regulate cholesterol synthesis and uptake through regulation of SREBP signaling pathway (18).

All these pathways are regulated by cholesterol and cholesterol synthesis intermediates but also by oxysterols themselves. Oxysterol synthesis itself is regulated in a similar manner as cholesterol and bile acid synthesis since many enzymes, transporters, and transcription factors are common. The excess of oxysterols is toxic for cells; therefore, oxysterols are transported to the liver, where they are metabolized to bile acid products and excreted from the body (21). FXR is a nuclear receptor of bile acids and is also the major regulator of bile acid synthesis. Hence, FXR regulates directly or indirectly expression and activity of oxysterol producing enzymes (CYP7A1, CYP27A1, CYP3A) (22). Also 22(R)-hydroxycholesterol has been shown to regulate expression of ABCB11 (ATP binding cassette subfamily B member 11, alternatively also BSEP- bile salt export protein) through FXR in hepatocytes (23). Since bile acids can act through different signaling pathways not connected to oxysterols, we will focus only on FXR direct involvement in selected cancers.

Circadian Rhythm

Circadian clock as the inner rhythm in mammals is recognized as a cell-autonomous and self-sustaining mechanism, which controls almost every aspect of our life. The period of these rhythms is ∼24 h long, thus circadian (from latin *circa*—approximately; *diem*—day) (24). The circadian rhythmicity is known to be a crucial endogenous process of organisms, described in almost every live species from cyanobacteria to human, and is capable to adapt to the environmental rhythm of the day. Circadian homeostasis in mammals is maintained by the central clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus which orchestrates numerous clocks in peripheral tissues. The peripheral clocks have been observed in cells and tissues all over the mammalian body. Light, however, is not the only signal for entrainment of internal clocks. Systemic cues including hormones, body temperature, feeding/fasting cycles also influence the circadian rhythm in tissues throughout the body (25). The molecular basis of clock is constituted of periodical expression of clock genes driven by the autoregulatory transcription-translation feedback loops involving *cis*-regulatory elements such as E-boxes, D-boxes, and ROREs (ROR-elements) (26). The positive transcriptional loop is formed by transcriptional activators ARNTL/BMAL1 (Aryl Hydrocarbon Receptor Nuclear Translocator Like) and CLOCK (Clock Circadian Regulator, NPAS2 in neuronal tissue, Neuronal PAS Domain Protein). The CLOCK:BMAL1 heterodimer binds to conserved E-box sequences in target gene promotors of *PER1,2,3* (Period Circadian Regulator 1, 2, 3), *CRY1,2* (Cryptochrome 1, 2), and *DEC1,2* (Deleted In Esophageal Cancer 1, 2) genes contributing to the activation of their expression (27). PER and CRY proteins dimerize in the

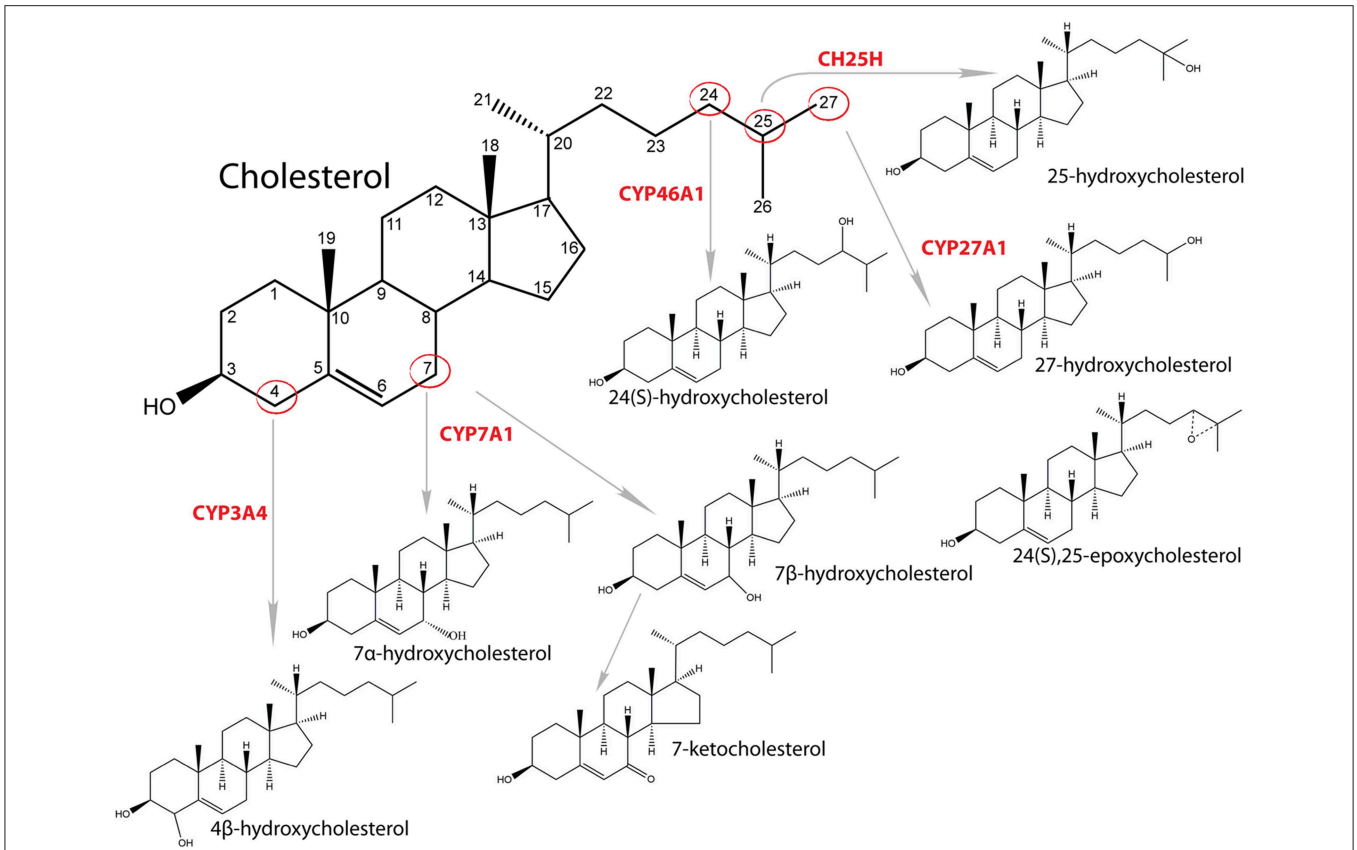

FIGURE 1 | Synthesis of oxysterols and catalyzing enzymes.

cytoplasm and after translocation to the nucleus inhibit further *CLOCK:BMAL1* transcription, forming the negative feedback loop. Also DEC1 and DEC2 can interact with the component of the SCN core clock genes. DEC1 and DEC2 proteins form dimers and translocate to the nucleus where they inhibit the activity of CLOCK:BMAL1 heterodimer (28). The degradation of PER, CRY and DEC proteins is essential for the restart of a new cycle of the transcription with ∼24 h periodicity. An additional regulatory loop of BMAL1 and CLOCK dimer activity is through interaction with RORs and REVERBA (Nuclear Receptor Subfamily 1 Group D Member 1, NR1D1). RORs and REVERBA compete for ROR response element in the promotor region of *BMAL1*, where REVERBA acts as inhibitor and RORs as activators of *BMAL1* transcription. In turn, CLOCK:BMAL1 heterodimers activate *REVERBA* transcription. Besides E-boxes and ROREs, D-boxes play an important role as well. Transcription regulation through D-boxes goes via different transcription factors, such as DBP (albumin gene D-site binding protein) expressed in the SCN with a clear rhythm in the light-dark or constant dark conditions. The role of TIMELESS (timeless circadian regulator gene), which is mammalian ortholog of *Drosophila* TIM, remains ambiguous (29). In *Drosphila* TIM protein plays an important role and entrains the internal clock through light. After heterodimerization with PER, translocation to the nucleus occurs and transcription of core clock cycle genes is inhibited.

Regulation of circadian rhythm, however does not stop with transcription-translation regulatory loops. Post-translational changes such as phosphorylation, ubiquitination and acetylation as well as epigenetic changes have been proven to play an important role in regulation of circadian rhythms (30–32). PER1-3 proteins are subjects to temporal changes in phosphorylation by CKIε (casein kinase Iε) and CKIδ, reaching a peak right before its destruction. Their reversible phosphorylation is a dynamic process with kinases and phosphatases as counterparts, their net effect leading to altered PER2 protein stability and cellular location, ultimately affecting the length of circadian period (33). Additionally, CLOCK has been found to have HAT (histone acetyltransferase) activity (34). While HAT activity results in transcription chromatin states, its counterpart, histone deacetylase, would condense chromatin and silence gene expression. Indeed, SIRT1 (sirtuin 1), a NAD+ (nicotinamide adenine dinucleotide) dependent protein deacetylase, is a representative of HDAC (histone deacetylase) family, whose deacetylation is under circadian control. In the liver, SIRT1 deacetylates BMAL1 and PER2 as well as histone H3 in promotors of clock controlled genes (31, 35). With its reaction depending on NAD+, SIRT1 scopes far beyond its circadian

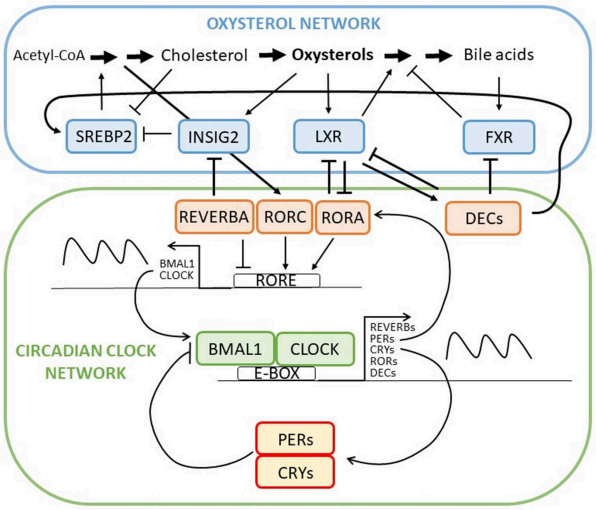

FIGURE 2 | Intertwined oxysterol and circadian clock networks with multiple negative feedback loops and several contact points between oxysterol network and circadian clock regulation through RORA-LXR, REVERBA-INSIG, and DEC-FXR/LXR/SREBP interactions. Through contact points circadian network regulates circadian expression of oxysterol network genes and metabolites and *vice versa*.

regulation. Primarily known physiological effects of SIRT1 deacetylation were regulation of metabolism and response to oxidative stress. It therefore represents a functional link between cellular metabolic activity and stress response, cell proliferation and genome stability, standing at crossroads of the processes we are tackling (31, 36–38).

Cholesterol and Oxysterols Around the Clock

Disorders of lipid metabolism are responsible for many adverse pathologies, including tumor development. It is already well-established that circadian clock controls every aspect of our life, also the lipid metabolism (**Figure 2**). The core circadian machinery is placed in the hypothalamus and orchestrates other peripheral clocks in tissues like liver, colon, and pancreas. Peripheral clocks can be synchronized trough different channels. They can be directly synchronized by SCN (neural and hormones signals), trough food entrainment or body temperature (39). Lipid metabolism focusing on cholesterol metabolism is under a daytime specific regulation. Recently it was shown that adropin, identified as a protein that has a role in maintaining the energy homeostasis, could represent a link between circadian clock and cholesterol metabolism. Expression of adropin is diminished by cholesterol and 7-oxygenated sterols that can modulate the RORA/C signaling. The RORA/C and REVERB receptors might thus link adropin and its synthesis to circadian rhythm of lipid metabolism which is a novel and important connection (40).

Liver X receptors whose natural ligands are oxysterols have been found to regulate *Dec1*, a transcription factor involved in hepatic clock system and metabolism (41). *In vivo* observation showed reciprocal suppression between RORA and LXR (42). Research on *Reverbα* mutant mice showed a circadian controlled expression of *Cyp7a1*. Furthermore, Martelot et al. also demonstrated that REVERBA cooperates in modulation of cholesterol and bile acid synthesis through control of SREBP. Thus, REVERBA control the rhythmic abundance of *Insig2* and is further responsible for diurnal translocation of SREBP to the nucleus. The circadian activation of SREBP drives the circadian transcription of cholesterol biosynthesis genes and hence the oxysterols, which activate LXR. The cyclically activated LXR is responsible for cyclic expression of CYP7A1 (43). Both, *Cyp7a1* and *Cyp27a1* showed circadian expression where sex differences in circadian variation were observed, indicting the importance of sex when planning the therapy (44). In the *Clock* mutant mice on a diet containing cholesterol and cholic acid showed reduced and disrupted *Cyp7a1* expression and high liver cholesterol accumulation (45). Timed high fat diet in mice, on the other hand, resulted in changed *Cyp7a1* expression via PPARα (peroxisome proliferator activated receptor alpha) as well as increased hepatic cholesterol (46). *Cyp7a1* anomalies were found in *Reverbα* deficient mice as well as $Per1^{-/-}$ and $Per2^{-/-}$ mice, where bile acid homeostasis was disrupted (47, 48). Other CYP enzymes involved in cholesterol biosynthesis have been reported in association with circadian clock metabolism. CYP3A has a circadian pattern, but researched did not find it to be important for drug therapy due to small changes observed (49). mRNA levels and metabolic activity of CYP3A4 in serum-shocked HepG2 cells showed a 24 h rhythmicity. DBP was responsible for CYP3A4 activation (50). CYP8B1, an important player in bile acid and liver metabolism, is also under the RORA regulation resulting in its diurnal rhythm and fasting induction (51). Furthermore, DEC2 was shown as an important regulator that convey the circadian signal to the liver that has role in *CYP7A*, *CYP8B*, and *CYP51A1* expression (52).

HEPATOCELLULAR CARCINOMA (HCC)

Hepatocellular carcinoma (HCC) is one of the most common causes of death worldwide and represents the most common primary malignancy in the liver. It is commonly associated with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, aflatoxins and alcoholic cirrhosis (53). It is a global health problem and over 80% of HCC cases are seen in developing countries with the highest incidence in areas endemic for HCV and HBV (54). Most patients with HCC have liver cirrhosis but there is also a number of cases where patients with non-alcoholic fatty liver disease (NAFLD) and without cirrhosis develop HCC, demonstrating the role of NAFLD in its pathogenesis (55, 56). The incidence is increasing rapidly and is higher in men compared to women (57). Other types of liver cancers like intrahepatic cholangiocarcinoma, hepatoblastoma, and angiosarcoma are not so common in comparison to HCC. The molecular pathogenesis of HCC is extremely complex where circadian clock and lipid metabolism play an important role (58). Genetic and epigenetic alterations have been shown to also drive hepatocarcinogenesis. Knowing the crucial signaling pathways in HCC will enable us to provide better therapies to treat this cancer

TABLE 1 | Oxysterol-circadian factors influencing HCC development.

Factor	Notes	Proposed function	References
Oxysterols	Increased in serum of NAFLD, HCV infected patients	Potential biomarkers	(60, 61)
	Treatment of HepG2	Cytotoxicity	(62)
25-hydrohycholesterol	Treatment of rat hepatoma cells	Pro-apoptotic	(62, 63)
25-hydroxycholesterol and OSBPL8	Treatment and overexpression in HepG2	Pro-apoptotic	
OSBPL8	Downregulation in Huh7 and HepG2	Pro-proliferative	(62)
	Overexpression in HCC cell line	Pro-apoptotic	(64)
LXR	Lower expression in HCC tumors	Lower post-operative survival rate	(65)
	Activity	Anti-proliferative via SOCS3	(66)
FXR	Lower expression and activity in HCC tumors	Association with multiple malignant characteristics	(67–72)
	Overexpression in HCC cell lines	Anti-proliferative, suppressed tumor growth in nude mice	(69)
	Downregulation in HCC cell lines	Pro-proliferative, increased migration and invasion, accelerated tumor growth in nude mice	(70)
SREBP1	Increased expression in HCC cell line	Required for tumor proliferation	(73)
CLOCK (NPAS2)	Increased expression in HCC tumors		(74)
	SNPs	Associated with increased risk for HCC, survival of TACE treated patients	(75)
PER3	SNP	Association with survival	(76)
PER2	Knockout mice	Higher diethylnitrosamine induced carcinogenesis	(77)
Chronic circadian disruption	Spontaneous hepatocarcinogenesis in mice	Metabolic disruption, CAR activation	(77–79)
TIMELESS	HepG2	Oncogenic	(80)

(59). The overview of factors associated with HCC are presented in **Table 1**.

In patients infected with HCV, the cholesterol transport in the liver is modified and serum concentration of cholesterol was lower compared to healthy individuals. Interestingly, the serum levels of oxysterols 4β-hydroxycholesterol, 25-hydroxycholesterol, and 7α-hydroxycholesterol were elevated despite lower cholesterol concentration. When patients with HCV were treated with anti-viral therapy the oxysterols where reduced back to normal values. Further analyses indicated that these oxysterols were probably not elevated because of the activity of liver enzymes CYP3A4 and CYP7A1. *CYP7A1* expression was unchanged in patients with HCV (61) and CYP3A4 activity was even downregulated (81). Direct effect of higher oxysterol concentrations on the development of HCC remains unknown, but oxysterols may be potential biomarkers and even potential novel targets for inhibiting disease progression (61). One of the major primary risk factors for HCC is NAFLD. With increasing control over hepatitis infections, it is predicted that NAFLD will soon become the major risk factor for HCC development. NAFLD is a complex multi systemic disease, caused by many factors such as genetics, dietary, environmental, and others (82, 83). In NAFLD patients, serum cholesterol is elevated and consequently some oxysterols (4β-, 25-, and 27-hydroxycholesterol) are also elevated. These oxysterols can affect the resorption of cholesterol in some tissues, through LXR activation, which regulates ATP-binding cassette transporters. In this way oxysterols affect the cholesterol concentration in NAFLD, which can evolve in HCC (60). In rat model with induced hepatoma tumor in the liver, the measurements showed elevated levels of following oxysterols: 24S-, 25- and 27-hydroxycholesterol, and 24S,25-epoxycholesterol, which are all known to bind to LXR. In these tumors, the ABCA1 (ATP binding cassette subfamily A member 1) and ABCG1 (ATP binding cassette subfamily G member 1) cholesterol transporters were elevated on mRNA and protein levels, most likely through LXR activation. There is some data indicating that 25-hydroxycholesterol has a pro-apoptotic function in rat hepatoma cells and is able to induce sub-Gl apoptosis (63). Authors suggested that local addition of 25-hydroxycholesterol in combination with other drugs could be potentially used in treatment of hepatoma patients. HCC cells have changed metabolism to sustain the fast proliferation. Because of this, many cellular functions are altered, including cholesterol metabolism and transport. It was shown that oxysterols are cytotoxic, can induce cell death in HepG2 cells, and can suppress the growth of HCC cells by inhibiting ACAT2 (Acyl-coenzyme A:cholesterol acyltransferase) enzyme, which results in intracellular unesterified oxysterol accumulation (62). When OSBPL8 (also known as ORP8) is downregulated, cell proliferation is promoted. When ORP8 was overexpressed in HepG2 cells and 25-hydroxycholesterol was added, the ER stress and apoptosis were promoted (62). ORP8 overexpression was already enough to trigger apoptosis in primary HCC cells isolated from human liver and cell lines (64). Lower LXR expression was associated with lower post-operative survival rate (65). LXR regulated HCC response to TGFβ1 (transforming growth factor beta 1), which acts as cytostatic and pro-apoptotic (84). LXR suppressed HCC proliferation through activation of SOCS3 (suppressor of cytokine signaling 3) (66).

FXR's role in HCC development was indicated when whole body *Fxr* knockout mice developed spontaneous HCC by the age of 14 months (85, 86). These mice had abnormal level of bile acids in the serum and administration of cholestyramine decreased HCC incidence (85). Many studies confirmed a significant decrease in FXR expression and activity in human HCC tumors and this was associated with multiple malignant characteristics (67–72). However, intestine-specific *Fxr* reactivation in *Fxr* knockout mice restored bile acid enterohepatic circulation, limiting hepatic inflammation and proliferation while preventing spontaneous hepatocarcinogenesis (87). Also only 20 and 5% incidence of hepatic tumors was observed in hepatocyte specific and enterocyte specific *Fxr* knockout mice, respectively. No serum or hepatic increase in bile acid level was observed in either of cell specific knockout models and no change in expression of cell-cycle regulators (88). These results lead to a hypothesis that high bile acid levels and FXR deregulation are needed for HCC development. There is, however, a mounting data pointing to a direct involvement of FXR in tumor suppression.

Overexpression of FXR decreased proliferation of HCC cell line and suppressed tumor growth in nude mice (69). Inhibition of FXR expression resulted in enhanced cell proliferation, migration and invasion in HCC cell lines and accelerated tumor growth in nude mice. FXR was shown to directly interact with β-catenin and repress its transcriptional activity (70). FXR also directly upregulates NDRG2 (N-myc downregulated gene family member 2) (72). NDRG2 is a known tumor suppressor, whose expression is reduced in HCC samples and correlates with aggressive tumor characteristics (72, 89). FXR also directly inhibited gankyrin expression via C/EBPβ-HDAC1 (CCAAT enhancer binding protein beta/ histone deacetylase 1) complex (90). Gankyrin is a known oncogene and is increased in HCC (91). In this study, they also showed that long-lived Little mice, which have high FXR liver expression, do not develop liver cancer after diethylnitrosoamine injection in comparison to wild type mice (90). FXR upregulated mir-122 which suppressed proliferation of HCC cells and the growth of HCC xenografts *in vivo* (71). In HCC human tumors miR-122 was significantly downregulated and this correlated with FXR expression (71). FXR and its agonist waltonitone repressed HCC cell proliferation by activating mir-22 repression of CCNA2 (Cyclin A2) (92). In HCC tumors FXR and miR-22 were downregulated while CCNA2 expression was opposite (92, 93). FXR also repressed inflammation via SOCS3 and via inhibiting NFκB (nuclear factor Kappa B) signaling (94, 95). FXR is a direct regulator of SOCS3, which enabled FXR-mediated cell growth repression, and their expression is correlated in HCC human samples (96). In summary, a direct role of FXR in protection against hepatocarcinogenesis could also be *via* defense against bile acid-induced injury, prevention of liver injury and apoptosis, prevention of ROS generation, promoting liver repair and generation after injury, suppressing cancer cell proliferation, etc. (97). There are some potential explanations available for the mechanisms behind FXR downregulation in HCC. In HCC cell lines miR-421 downregulated FXR and by this promoted cell proliferation, migration and invasion (98). Increased expression of miR-421 and its correlation with patient's survival was confirmed in HCC patients (99). Another factor decreasing the expression and activity of FXR is inflammation and inflammatory cytokines were shown to inhibit HNF1A (HNF1 homeobox A) binding to *FXR* promotor (68). SIRT1 overexpression leads to deacetylation of FXR in the mouse liver, and inverse expression of SIRT1 and FXR was confirmed in human HCC samples (100). In spite of many tumor suppressor actions discovered, there is also some caution needed. FXR also upregulates FGF19 (fibroblast growth factor 19) which is anti-cholestatic and anti-fibrotic factor in the liver and was proposed for the treatment of NASH. However, FGF19, but not its rodent counterpart FGF15, is potentially pro-tumorigenic as shown by ectopic expression in mice (101). FGF19 expression is upregulated in HCC samples and correlates with poor prognosis (102). This is an important factor to consider when translating results from studies in rodent models to humans. However, new engineered FGF19 variants have been developed excluding pro-tumorigenic activity and are currently in evaluation for the treatment of NASH (103).

More and more results show the importance of SREBPs, especially SREBP-1, as a link between oncogenic signaling and tumor metabolism. It was shown that cancerous cells have higher *de novo* lipogenesis, which is required for rapid tumor proliferation. In the cancer cells, SREBP-1 is highly activated and it was shown, that pharmacological targeting of SREBP-1 greatly inhibits tumor growth. This rises hope for SREBP-1 to be potential anti-cancerous target (73). Higher expression of SREBP-1 was shown in different cancer cell lines (104) and higher expression of SREBP-1 is associated with many metabolic diseases; including NAFLD and NASH. One of the factors activating SREBP-1 in HCC is hepatoma-derived growth factor (HDGF). Co-expression of HDGF and SREBP-1 is an indicator of poor HCC prognosis. HDGR knockdown or its mutation in HepG2 cells resulted in decreased expression of SREBP-1 targeted genes (105). A study on a mouse model of HCC showed that lipid biosynthesis (fatty acids and cholesterol) is crucial for HCC development and that targeting SREBP pathway can be used as an anti-tumor strategy (106). Study on hepatic stellate cells, which have an important role in liver fibrosis, showed that in these cell line INSIG-1 and INSIG-2 expression is downregulated. By downregulation of INSIGs, SREPB signaling is promoted, which is the characteristic of HCC and crucial for cancer proliferation (107). Bioinformatics analysis showed that two miRNA (has-miR-221 and has-miR-29c) are potential HCC diagnostic markers and that they have one common target, which is *INSIG1* (108). All these data show the importance of SREBP signaling and its potential as drug target.

Data on human and mice reported a connection between circadian rhythms and liver disease where alterations in rhythmicity had a profound effect on metabolic pathways resulting also in adverse outcomes, like HCC. NPAS2 or CLOCK was shown to have a critical role in HCC and expression of the protein was significantly upregulated in HCC patients (74). In other study, single nucleotide polymorphisms (SNPs) in *NPAS2* were associated with the increased risk for HCC or with overall survival in HCC patients treated with transcatheter arterial chemoembolization (TACE) (75). The rs2640908 polymorphism within *PER3* was also associated

with overall survival in HCC patients treated with TACE (76). SNPs from circadian negative feedback loop genes were further studied and were suggested to have an independent role as prognostic biomarkers for prediction of HCC clinical outcome (109). Study on mice showed that circadian disruption due to *Per2* mutation or jet lag has a profound role in liver carcinogenesis induced by diethhylnitrosamine (77). Other research addressed the importance of the circadian clock metabolism in hepatocarcinogenesis where the changes in expression of several core clock genes were downregulated (*BMAL1, CLOCK, PER1, PER2, CRY1, CRY2*), while upregulation of clock related genes (*NR1D1* and *DBP*) was observed (110). Circadian metabolism and carcinogenesis are highly interconnected and the connection is complicated. Kettner et al. demonstrated that chronic circadian disruption induces NAFLD and spontaneous hepatocarcinogenesis, promotes genome-wide deregulation and metabolic disruption, and activates CAR (NR1I3, constitutive androstane receptor) which drives NAFLD to NASH, fibrosis and HCC progression (78). Chronic circadian disruption in mice had a profound effect on metabolism, what was also observed in humans and circadian disruption with or without steroid receptor Coactivator-2 (SRC-2) ablation showed association with human HCC gene signature (79). The TIMELESS protein has oncogenic function in human HCC as well (80). Studies on HepG2 cell line demonstrated the importance of *RORA* in different steps in liver carcinogenesis (111).

PANCREATIC CANCER (PC)

Pancreatic cancer is one of the most malignant cancers, estimated to cause 432 242 deaths in 2018 and ranking seventh among estimated cancer deaths in the world (112). Estimated 5-year survival rate is extremely low and only 4% of patients will live 5 years after diagnosis. An important risk factor is smoking, since around 20% of tumors are attributable to smoking. Other risk factors include family history of chronic pancreatitis or pancreatic cancer, male sex, advancing age, diabetes mellitus, obesity and occupational exposure (113, 114). Novel epidemiological studies propose that dietary factors also impact pancreatic cancer risk. In a large population saturated fatty acid intake was linked to higher pancreatic cancer risk, while omega-3 fatty acids, increased vitamin C and vitamin E intake lowered cancer risk (115). Pancreatic cancer patients have unspecific signs, making the diagnosis difficult, often discovering already advanced stages of the disease. The overview of factors associated with PC are presented in **Table 2**.

Very little is known about the oxysterol role in the development of pancreatic cancer. Serum cholesterol, 5α,6α-epoxide, and lanosterol were identified as highly discriminating between pancreatic cancer patients and healthy subjects (116). Two meta-analyses showed that dietary cholesterol may be associated with pancreatic cancer in worldwide populations (128, 129). OSBPL have been shown to be involved in pathology of PC. Increased expression of OSBPL5 was associated with higher invasion and poor prognosis of pancreatic cancer patients (117, 118). In pancreatic ductal adenocarcinoma (PDAC) patients, OSBPL3 was found as differentially expressed in four studies and was correlated with poor prognosis of these patients (119). The role of nuclear receptors also remains unknown. Activation of LXR by synthetic ligands had anti-proliferative effects on PDAC cells (120). Very few studies measured the expression FXR and its role in pancreatic cancer. Moreover, there are conflicting results available. The increase in FXR mRNA and protein expression was confirmed in pancreatic tumors in comparison to adjacent tissue, and high FXR expression was correlated with the poor prognosis. FXR inhibition reduced cell proliferation, migration and invasion in pancreatic cancer cell lines (121, 122). It was proposed that FXR induced phosphorylation of SP1 (Sp1 transcription factor) and by this promoted cancer progression (122). While another study found correlation between high FXR expression with less aggressive phenotype, smaller tumors, absence of metastases and better prognosis (123). The difference in FXR expression and correlation between studies can simply be attributed to a low sample number, different groups of patients, different length of time in survival analyses, correlation with other factors etc. However, bile acids were increased in plasma and pancreatic juice in PC patients, and the mouse model confirmed the correlation between the increase in serum bile acids level with the severity of the disease (124). FXR was significantly increased in pancreatic cancer cell lines in comparison to normal pancreatic cells and FXR was found as the regulator of FAK (PTK2, protein tyrosine kinase 2)/c-Jun (JUN, Jun proto-oncogene, AP-1 transcription factor subunit) / MUC4 (mucin 4) signaling pathway (124). Similar to HCC, SREBP1 pathway and *de novo* lipogenesis is upregulated in PC. Study on 60 PC patients showed high correlation between elevated SREBP1 expression and poor disease prognosis. Inhibition of SREBP1 in a mouse model lead to decreased *in vivo* weight of tumor, indication the importance of SREBP1 upregulation for cancer cell growth (130). Study on PC cell model showed that inhibitors of SREBP1 decrease PC cell viability and proliferation. This indicate that targeting SREBP1 pathway is potential target for PC disease management and should be further explored (131). One of the potential molecules for targeting SREBP1 is resveratrol. Recent study on mouse model of PC and human cell lines treated with gemcitabine (chemotherapy medication) showed that resveratrol suppressed stemness induced by gemcitabine. Resveratrol should be considered as an additive for chemotherapeutic drugs (132).

Circadian activity in pancreas is also controlled by SCN (133, 134). The clock genes expression (*BMAL1, PER1, PER2, PER3, CRY2, TIMELESS, and CK1ε*) is altered in PDAC which is the most common type of pancreatic malignant tumors (135, 136). In humans, lower *BMAL1* expression was associated with poorer survival rate, and correlated with higher tumor stage, poorer histological differentiation, and increased vascular invasion in PDAC (125, 126). Since knockdown of *Bmal1* resulted in an anti-apoptotic and pro-proliferative profile, while its overexpression had the opposite effect, *Bmal1* can act as an anti-oncogene by binding directly to *p53* promoter region (126). Since circadian rhythms and metabolic pathways go both ways, and pancreatic

TABLE 2 | Oxysterol-circadian factors influencing PC development.

Factor	Notes	Proposed function	References
Cholesterol, 5α,6α-epoxide, and lanosterol	Increased in serum of PC patients	Potential biomarkers	(116)
OSBPL5	Increased expression in PC tumors	Higher invasion and poor prognosis	(117, 118)
OSBPL3	Increased expression in PDAC tumors	Poor prognosis	(119)
LXR	Activation by ligands in PDAC cell lines	Anti-proliferative	(120)
FXR	Increased expression in PC tumors	Poor prognosis	(121, 122)
	Increased expression in PC tumors	Better prognosis	(123)
	Inhibition in PC cell lines	Anti-proliferative, lower migration and invasion	(121, 122)
	Increased expression in PC cell lines	Regulator of FAK/JUN/MUC4 pathway	(124)
BMAL1	Lower expression in PC tumors	Poor prognosis	(125, 126)
	Knockdown in PC cell lines	Anti-apoptotic, pro-proliferative	(126)
SIRT1	Significant down regulation in PC tumors	Lower mortality rate	(127)

cancer risk is increased in people with metabolic syndrome (137), the role of metabolism and its disruption plays an important role in pancreatic cancerogenesis. A recent study evaluating dietary influence on cancer risk in women confirmed the role of obesity (138). *Bmal1* and its downstream regulation seems to play a crucial role in pancreatic regulation of metabolic processes, as *Bmal1* knock out in mouse β-cells led to glucose intolerance and development of diabetes (139). Since glucose metabolism is also under the circadian control, it is not surprising that alterations in clock genes (*Clock, Bmal1*) lead to impaired glucose tolerance (140), with a profound role in pathogenesis of pancreatic cancer (141). Deregulation of SIRT1, which regulates the central and peripheral clock, has an important role in PDAC as well (127).

COLORECTAL CANCER (CRC)

Colorectal cancer is the third most commonly diagnosed cancer, the second most common cause of oncological death in the world estimated in 2018 (112) and as such represents a major public health issue worldwide. About 6% of CRCs are predetermined by a defined hereditary syndrome, while around 25% of CRCs are familial, the latter being tightly intertwined with many of the modifiable risk factors as well as genetic factors. Non-modifiable risk factors for CRCs include increasing age, male gender, African American race and residence in high-income countries (142), while modifiable factors include obesity, moderate to heavy alcohol consumption, smoking, high consumption of red or processed meat and a diet with low fiber, fruit, and vegetables intake (112, 142). Also, genetics plays an important role in its pathogenesis (142). In CRC one of the most commonly dysregulated pathway is the Wnt/β-catenin signaling, leading to β-catenin accumulation, ultimately resulting in activation of several gene targets, including oncogenes that contribute to the development of cancerous phenotype (143). Its component APC (APC regulator of WNT signaling pathway) mutations are present in majority of sporadic CRCs (144). Since onset and progression of CRC are tightly intertwined with its mutational pathways, detailed knowledge of their regulation is crucial for obtaining new markers in order to better understand risk factors as well as treatment and prognosis. The overview of factors associated with CRC are presented in **Table 3**.

High total cholesterol level is associated with a higher risk for colon cancer in men (145). Another study taking into an account also genetic factors again confirmed a link between hypercholesterolemia and colorectal cancer risk (146). A *CYP7A1* haplotype and SNPs were significantly associated with colorectal cancer and it was proposed to be the effect of fecal bile acids (147–150). Whole grain food significantly reduces colon cancer risk and phytochemicals that are involved in this protection are especially interesting (182).

Studies in colon cancer cell lines indicate that different oxysterols are cytotoxic and induce apoptosis. A study of wheat bran showed that it contains several different oxyphytosterols with anti-proliferative effect on colon cancer cell lines, some could also induce apoptosis (151). Dietary-representative oxysterol mixture induced apoptosis in differentiated colonic CaCo-2 cells (152). A study in major cell line models for CRC, Caco2 and SW620 cells, showed that 27-hydroxycholesterol decreased proliferation of these cells (153). This effect was not due to cellular cytotoxicity or induction of apoptosis and it was independent of nuclear receptors LXR and ESRs. 7α- and 7β-hydroxycholesterol, 5α,6α-epoxycholesterol and 7β-hydroxysitosterol were all able to induce apoptosis in human colon cancer cell line Caco-2 (154, 155). 7β- hydroxycholesterol was cytotoxic to colon cancer cell lines in concentrations from 3 to 10 μM (156). 7-ketocholesterol and 25-hydroxycholesterol reduced barrier functions, apoptosis and induced viability of Caco-2 cells (158). 7-ketocholesterol besides decreasing epithelial barrier also induced inappropriate development of inflammatory response to food (159). 7-ketocholesterol induced ER stress in HT-29 colon cancer cell line (160) and affected mitochondrial functionality in Caco-2 cells, while co-treatment with 7-ketostigmasterol reduced the toxic effect (161). Only 25-hydroxycholesterol, but not the

TABLE 3 | Oxysterol-circadian factors influencing CRC development.

Factor	Notes	Proposed function	References
Total serum cholesterol	Increased in humans	Higher risk for CRC	(145, 146)
CYP7A1	SNP and haplotype	Associated with CRC	(147–150)
Oxyphytosterols	Treatment of CRC cell lines	Anti-proliferative, pro-apoptotic	(151)
Dietary oxysterols	Treatment of Caco-2	Pro-apoptotic	(152)
27-hydroxy cholesterol	Treatment of CRC cell lines	Anti-proliferative	(153)
7α- and 7β-hydroxycholesterol, 5α,6α-epoxycholesterol, and 7β-hydroxysitosterol	Treatment of Caco-2	Pro-apoptotic	(154–156)
7β-hydroxycholesterol	Treatment of Caco-2	Induced expression of inflammatory and chemotactic cytokines	(157)
7-ketocholesterol	Treatment of Caco-2	Reduced barrier functions, anti-apoptotic, induced viability, lower inflammatory response	(158, 159)
	Treatment of HT-29	Induction of ER stress	(160)
	Treatment of Caco-2	Mitochondrial functionality	(161)
25-hydroxycholesterol	Treatment of Caco-2	Reduced barrier functions, anti-apoptotic, and induced viability	(158)
	Treatment of DLD-1	Induction of cell death, anoikis	(162)
	Treatment of Caco-2	Enhanced IL1B induction of IL8	(163)
LXR	Induction by agonist in CRC cell lines	Anti-proliferative	(164)
CYP8B1, CYP46A1, CYP2R1	Higher expression in CRC	Poor prognosis	(165)
CYP7B1	Higher expression in CRC	Good prognosis	(165)
OSBPL9	Downregulation in CRC tumors	Poor prognosis	(166)
LXR	Increased expression	Good prognosis	(167)
	Activation in HT-92	Anti-proliferative	(168, 169)
FXR	Decreased expression in CRC tumors	Poor prognosis	(170–175)
	Knockout in mice	Increased susceptibility to chemically-induced	(171, 176)
	Overexpression in CRC cell lines	Reduced tumor growth, anti-proliferative, pro-apoptotic	(92, 171, 175–177)
PER1, PER3	Decreased expression in CRC tumors	Poor prognosis	(178)
PER1, BMAL1	Decreased PER1 and increased BMAL1 in CRC tumors	Poor prognosis	(179)
PER2	Increased in CRC tumors	Good prognosis	(178)
RORA	SNPs	Risk of development of CRC	(180)
	Lower expression in CRC tumors	Good prognosis	(180)
SIRT1	Transgenic mice and human tumor specimens	Suppression of *in vivo* tumor formation	(181)

22-(R)-hydroxycholesterol and other oxysterols (7 beta-hydroxycholesterol and 5-cholesten-3beta-ol-7-one) induced anoikis, a type of programmed cell death, in DLD-1 cells (162). Some of these effects could be through activation of LXRs, since LXR induction by GW3965 had anti-proliferative effects on colon cancer cells (164).

On the other hand, oxysterols also affect expression of inflammatory molecules in colon cancer cells. Inflammatory bowel disease is an important risk factor for development of CRC and chronic inflammation and oxidative stress are part of pathogenesis (183). 7β-hydroxycholesterol induced expression of key inflammatory and chemotactic cytokines in CaCo-2 cell line (157). 25-hydroxycholesterol pre-treatment enhanced IL1B (interleukin 1 beta) induced IL8 (interleukin 8) production in Caco-2 cells (184). Representative mixture of oxysterols increased oxidative stress in differentiated Caco-2 cells and was followed by the production of cytokines IL6 (interleukin 6) and IL8 (163). In CRC patients, serum level of IL8 was increasing with the progression of cancer (185). Expression of enzymes and oxysterol receptors is modulated in CRC. Human tissue microarray analyses revealed significantly higher protein expression of CYP2R1, CYP7B1, CYP8B1, CYP46A1, CYP51A1, while CYP27A1 and CYP39A1 had no significant change, in primary colorectal tumor compared to normal colonic mucosa (165). In this study, they also showed a significant association between patient prognosis and survival with CYP expression. For example, higher expression of CYP8B1, CYP2R1, CYP27A1, and CYP46A1 in tumor was associated with poor prognosis, while high CYP7B1 expression correlated with good prognosis. OSBPL1A short transcript variant was down-regulated in colon cancer tumors, but the long variant was unchanged in comparison to normal samples (186). OSBPL9 was

included in a gene expression signature as a predictor of survival in colon cancer and was downregulated in poor prognosis patients (166).

LXR expression was proposed to be a prognostic indicator for CRC and its expression was associated with favorable clinical outcome. Positive LXR expression was associated with better survival rate and there was a significant negative association between LXR expression and vascular invasion, but no association was found between LXR expression and the patient's age, sex, tumor size, grade or TNM (tumor/node/metastasis) stage (167). A genome wide analyses revealed regulatory programs of LXR activation which lead to inhibition of HT29, a colorectal cancer cell line, proliferation (168). LXR was shown to directly bind to β-catenin and suppress its activity and cellular proliferation (169). A recent review has already summarized FXR and bile acids role in the development of colon cancer (187). Several studies showed that FXR expression is reduced in intestinal tumors in humans and that it is inversely correlated with the degree of malignancy and clinical outcome (170–175). The FXR knockout mice have increased susceptibility to chemically-induced colorectal carcinogenesis, while FXR overexpression in gut cells reduced tumor development and growth (171, 176). The activation of FXR was shown to suppress proliferation and induce apoptosis in colon cancer cell lines (171, 177). The observed downregulation of FXR expression is due to increased methylation of *FXR* promotor by the loss of APC function, which was confirmed in CRC cell lines, animal models and colonic tumors from patients (174, 188, 189). However, *FXR* is also downregulated by intestinal inflammation, western diet, and microRNA (187, 190). The current hypothesis is that the decreased FXR regulation in combination with Western diet and hence higher levels of secondary bile acids results in pro-tumorigenic colon environment leading to the development of colon cancer. Moreover, recent studies showed that FXR exhibits anti-cancerogenesis effects beyond regulation of bile acid level, by also affecting other cellular signaling pathways in colon cells. A study in mouse and organoid models, showed that FXR regulated proliferation of intestinal cancer stem cells (191). FXR repressed proliferation of colon cells by inhibiting the *MMP7* (matrix metallopeptidase 7), a known intestinal tumor promotor expression; by activating mir-22 repression of CCNA2 (Cyclin A2); and by activating the EGFR/SRC (epidermal growth factor receptor/SRC proto-oncogene) pathway in colon cells (92, 175, 177). Upregulation of SREBP1 pathway is also present in CRC (192). Inhibition of SREBP1 in CRC shows promising result for cancer management. It was shown that ordonin (diterpenoid isolated from *Rabdosia rubescens*) reduced expression of *SREBP1* and induce apoptosis in CRC cells cultures (193).

Epidemiological studies have shed light on connection between circadian disruption and elevated risk of cancer, including colorectal cancer (128, 194, 195). Meta-analysis showed increased incidence of colorectal cancer in people with long-term exposure to night light (shift work). The proposed mechanism for circadian disruption on oncogenesis is melatonin suppression and loss of its protective effect against cancer via apoptosis, anti-angiogenesis, anti-oxidation and regulation of the immune system. One of the proposed additional factors is low level of 25-hydroxyvitamin D due to lower sun exposure (128). In mouse models, physical destruction of the SCN as well as functional disturbance of circadian rhythms (chronic jet lag) resulted in accelerated tumor growth in transplantable tumor models (Glasgow osteosarcoma and Pancreatic adenocarcinoma), indicating a role of circadian system in controlling malignant growth (196). In human CRC, there are multiple studies reporting abnormal expression of circadian genes including altered expressions of *CLOCK, BMAL1, PER1, PER2, PER3,* and *CK1ε* (179, 197–200). Whether the disruption originates in core clock genes and drives tumorous transformation or measured disruption is a consequence of cancer, remains unclear. However, studies suggest that clock genes have an important role in tumor suppression (201). Although clinical correlations between specific mechanisms of clock gene disruption and colorectal cancer phenotype and prognosis have not been conclusive, some pathways show typical clinical and pathological features. Decreased expression of *PER1* and *PER3* in tumor tissue as such indicate poorer survival rate (178), decreased *PER1* and high *BMAL1* expression correlate with poorer outcome and liver metastasis (179), and high *PER2* expression correlate with significantly better disease outcome (178). In recent systematic evaluation of genetic variants in the circadian pathways connected with CRC, examining 119 SNPs in *RORA* was proposed as potentially important marker for CRC risk and prognosis. While people carrying SNP in *RORA* were much more inclined to developing CRC, lower RORA correlated with better differentiated tumors and better disease outcome (180). On the other hand, changes in cellular metabolism may cause circadian disruption, further influencing colorectal cancer phenotype (202). It has been shown that SIRT1 suppresses colorectal tumor formation *in vivo* by β-catenin deacetylation (181). A recent study showed that both metabolic and circadian dysregulation progressed during cancer progression. Their findings suggested that clock-related glycolysis genes alterations might add to a clock-driven rewiring of metabolism, connected to cancer progression and altering response to cancer therapy (203).

NEW THERAPEUTIC STRATEGIES

Targeting Oxysterol-Cholesterol Network

Statins are widely used lipid-lowering drugs which inhibit HMGCR, a rate limiting enzyme from cholesterol synthesis. Statins have been proposed in several studies as potential drugs used for reducing the risk of development and mortality in gastrointestinal cancers. Statin use was associated with a decreased risk of mortality in several cancers also colorectal cancer (204). However, another study showed no association with colorectal cancer incidence in United States cohort (205). Longer statin use was connected to a reduction in all-cause mortality in patients after colorectal diagnosis (206). However, overall conclusion of meta-analyses of 42 studies was that statin use was associated with a modest reduction in risk of CRC (207). This association was confirmed for lipophilic

statins but not for hydrophilic statins. In addition, long-term statin use (>5 years) did not significantly affect the CRC risk.

HCC occurs mainly in cirrhotic liver and statins may affect the risk of HCC by their anti-fibrotic effect. Three meta-analyses confirmed that statin use is associated with lower risk of HCC in different populations (58, 208, 209). Fluvastatin was pointed out in one of these studies. A registry-based study also confirmed an association between statin use and the risk of HCC and additionally showed a dose response relationship (210). Stain use also significantly decreased the risk of HCC in patients with HBV (211, 212). Studies suggest that statins lower the risk of cancer in general in chronic hepatitis patients (213). Overview of studies on statin effect on mean survival rate revealed a link to extended survival, but the length of survival was variable among studies (214). Perioperative statins have been associated with improved recurrence-free survival in HCC patients (215, 216). Meta-analysis confirmed an association between statin use and decreased risk in mortality in pancreatic patients, but other studies found no association (217, 218). A review of studies revealed that observational studies found the association but randomized controlled trials did not. Statins have anti-neoplastic properties through anti-proliferative, pro-apoptotic, anti-angiogenic and immunomodulatory effects and can affect multiple signaling pathways in cells (219). Most important is the inhibition of HMGCR and by this synthesis of mevalonic acid. *HMGCR* genetic variant significantly modified the protective association between statins and CRC risk (220). Mevalonate pathway is upregulated in several cancers, also pancreatic and hepatic, and is responsible for activation of small G proteins (221). All these data make statins compelling therapy for decreasing the risk of cancer and lowering mortality of patients, but due to conflicting results, it is difficult to form final recommendations. An important issue in prescribing statins are the side effects of statins such as hepatotoxicity and drug-induced myopathy.

Since SREBPs target genes are upregulated in many cancers, SREBPs could have therapeutic potential (106). Several studies showed a successful targeting of SREBP pathway and suppression of lipid metabolism with substances like TAK1, Emodin and using different miRNA (222–225). Since oxysterols bind to INSIG and affect SREBP signaling pathway, oxysterols also have a potential to be used in cancer proliferation inhibition, but more studies on oxysterol-INSIG interaction are needed.

Oxysterols activate several nuclear receptors most importantly LXR, which is hypothesized to exert their anti-cancerogenic effects. Targeting LXR for prevention and therapy of cancers is already evaluated in clinical studies. LXR is an interesting target, because it is activated by phytosterols, which can reduce the incidence of colon cancer (226). LXR is connected to TGFB1 actions and was also proposed as a potential target for treatment of HCC (84). Bergapten, a LXR agonist, was already evaluated for HCC treatment (227). LXR agonists were proposed also for treatment of CRC since LXR activation reduced intestinal tumor formation in a mouse ($APC^{min/+}$) CRC model and also blocked proliferation of human colorectal cells (228). LXR is a promising target, but majority of evidence was gathered through studying the effects of LXR activation in cell lines (4).

FXR is also a promising target for cancer treatment. Activation of FXR would prevent toxic bile acids build up, but also repress other tumorigenic proteins. In preclinical and clinical trials FXR agonists show a potential for treatment of different liver disease among them also HCC (175, 229, 230). In the mouse ($APC^{min/+}$) CRC model on high fat diet, treatment with FexD, a deuterated analog of fexaramine with the gut-restricted activity, resulted in slower tumor progression, improved bile acid homeostasis and improved survival (191). However, FXR's role in pancreatic cancer is conflicting and the fact that in humans it activates a potentially pro-tumorigenic FGF19 emphasizes the needed for more studies that will confirm the positive role of FXR agonists.

Chronotherapy as a New Therapeutic Strategy

Cancer represents the second leading cause of dead worldwide (231). The available treatment is not always the most efficient thus new therapeutic strategies are needed to be developed. In addition to standard therapy (chemotherapy, radiation, and surgery), additional factors need to be taken into consideration, such as lifestyle and biology, when providing integrative treatment for different cancer types (232). Since the circadian clock metabolism has a profound role in pathogenesis of cancers, chronotherapy might be a better therapeutic strategy. We need to adjust the pattern of drug delivery to improve the treatment efficiency, by reducing the drug at the time point where tissue is most susceptible to toxicity, and increasing the dose at times with most susceptibility to the positive drug effect (233).

Therapeutic strategy for HCC is well-established. Since the incidence is rising drastically, early diagnosis and definitive treatment is currently the only way to increase the survival rate of HCC patients. Great research breakthroughs in chronobiology led to the development of this field. Recently it was demonstrated that isoform of the HNF4A (nuclear factor 4 alpha) plays a crucial role in HCC progression. Forced expression of *BMAL1* in HCC that is positive for HNF4A stops the growth of tumors *in vivo* (234). Furthermore, SULT1A1 (sulfotransferase 1a1) that has a circadian pattern at the mRNA and protein level and is responsible for detoxifications of various drugs in the liver is regulated trough BMAL1. Knockdown of *Bmal1* resulted in changed rhythmicity in Hepa-1c1c7 cells (235). The role of IFNA (interferon alpha) was assessed in circadian manner in HepG2 cells as well. It was proposed that IFNA could have pharmacological role, since its continuous administration resulted in significantly decreased levels of CLOCK and BMAL1 protein (236).

Pancreatic cancer is one of the most aggressive tumors, responding poorly to therapy; therefore, new therapeutic strategies are in high demand. Circadian gene *Per2* overexpression increases the sensitivity to cisplatin, possibly by inducing a reduction in of the BCL (B-cell lymphoma) proteins

(237). In search of cancer cell growth inhibitors downstream circadian controlled pathways, ligands of PPARG (peroxisome proliferator activated receptor gamma) showed to be reducing tumor aggressiveness and enhancing cytotoxic action of anti-cancer agents (238, 239). Another mechanism showing promise in pancreatic cancer treatment might be TIMELESS, involved in DNA damage response, whose expression was found to be altered in pancreatic cancer (135).

Chronotherapy has also proven to be effective in CRC. In metastatic CRC patients chemotherapy induced circadian disruption correlated with poorer disease outcome, suggesting its prevention could improve treatment results (240). Furthermore, a meta-analysis of five randomized controlled trials showed a significant improvement in overall survival in metastatic CRC patients treated with chronobiologically timed chemotherapy compared to conventional chemotherapy (241). Chronomodulated hepatic arterial infusion also showed promise as a possible drug administration strategy in heavily pretreated patients with CRC liver metastases (242). Recent study examining the role of PER3 in CRC found that its overexpression enhanced fluorouracil sensitivity in CRC cells, proposing it as a potential target in CRC treatment (243). Moreover, another study showed sex-specific expression and sex-specific prognostic value of clock and clock-controlled genes, shedding light on colorectal cancer and patient characteristics that have to be taken into consideration in order to provide optimal treatment (244).

CIRCADIAN-OXYSTEROL NETWORK IN CANCEROGENESIS

Members of the oxysterol and circadian clock networks were exposed as the new promising prognostic biomarkers, genetic risk factors and potential therapeutics in gastrointestinal cancers. The roles of oxysterols in various cancers have been reviewed previously and mechanisms by which oxysterol can affect cancer pathogenesis and disease development were pointed out (8). Oxysterols activate different signaling pathways in cells which can either promote or inhibit cancer development. For example, 22(R)-, 24-, 25-, and 27-hydroxycholesterol are *in vivo* ligands of LXR and act as tumor suppressor in selected cancers. Increased LXR activity was shown to be beneficial in all three presented cancers exposing LXR as a promising drug target in oncology. However, one oxysterol can have a different role depending on the tissue. Current hypothesis is that the dual role of oxysterols is due to activation of different signaling pathways resulting in tumor suppressor LXR-dependent or oncogenic LXR-independent actions (245). Oxysterols also interact with OSBP/L proteins and these have been connected to many human diseases, such as dyslipidemia and cancers (246). Studies indicate that OSBPL family members could also have a dual role. In HCC and CRC their expression is downregulated and this is correlated with a poor prognosis. While in PC their expression is increased and correlates with a poor prognosis. The opposite roles could be explained by the fact that members of OSBPL family have tissue specific expression, which is disturbed in tumors, and also have different roles in cell physiology. Enzymes involved in oxysterol synthesis are also potential new prognostic biomarkers and drug targets in gastrointestinal cancers. The expression and the SNPs in cytochromes P450 enzymes are associated with the risk for development and prognosis of selected cancers. The level of serum cholesterol itself is a prognostic biomarker associated with a higher risk for development of numerous cancers. The association is consolidated by the fact that statins are emerging novel therapies in gastrointestinal cancers. Cholesterol is the key player not only due to being the oxysterol progenitor molecule but also one of the key cellular ingredients needed in growing cancerous cells. The oxysterol metabolites, the bile acids, and their receptor FXR are emerging new biomarkers and therapeutic targets in gastrointestinal cancers. The role of FXR in these cancers is two faced. FXR downregulation is observed with progression of cancerogenesis and FXR role in suppression of proliferation, migration and invasion in colon cells and hepatocytes was confirmed. However, FXR role in pancreatic cancer seems to be the opposite. These data indicate that FXR activity is essential in tissues with high FXR expression such as liver and colon, while FXR activity in tissues where FXR and bile acids are not common is potentially tumorigenic.

Disruptions of circadian rhythm are clearly tumorigenic in gastrointestinal cancers as indicated by rodent models. The circadian molecular clock genes are also new emerging prognostic biomarkers and therapeutic targets. Several genes involved in regulation of cellular circadian expression have direct oncogenic or tumor suppressor roles. Not only their expression correlates with prognosis in patients, knockouts in rodent models lead to spontaneous cancerogenesis and SNPs are associated with the risk of cancer development and prognosis. They are interesting therapeutic targets, moreover, the time of the therapy must also be considered and chronotherapy has already been shown to be effective in gastrointestinal cancers.

SUMMARY

Summary of reviewed data revealed that circadian regulatory network, cytochromes P450, nuclear receptors, cholesterol biosynthesis and oxysterols have overlapping roles in gastrointestinal cancers. Several factors from sterol homeostasis and circadian rhythm have been identified as potential novel prognostic biomarkers, genetic risk factors and drug targets. However, there are still several issues that remain open. The role of oxysterols in carcinogenesis is far from being conclusive. The cells are dealing with a variety of oxysterol molecules that promote their actions through modulation of transcription factors, such as LXR, RORs and others. Depending on the type of cancer, an oxysterol modulated pathway can have beneficial or damaging effect on carcinogenesis. This is one of the obstacles in developing new therapeutic strategies, besides statins, where it is clear that a more personalized approach is essential to increase the positive drug effects. The link between cholesterol, oxysterol synthesis and circadian rhythm was until recently

mostly unidirectional: the clock controlled the expression of lipogenic genes. It now becomes evident that both pathways are interconnected by energy metabolism where RORA/C and LXR are at the crossroad. The ROR receptors are circadian regulators, being co-responsible for the rhythmic expression of output metabolic genes. However, their own transcriptional activity depends on sterols and oxysterols whose endogenous synthesis is regulated by the clock. This is again a new field of translational research termed chronotherapy which was proven to be successful particularly in treatments of colorectal cancer.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by Slovenian Research Agency program grant P1-0390 and research project J1-9176. UK was supported by young researcher scholarship. LB is a masters' student of medicine at the Faculty of Medicine University of Ljubljana.

REFERENCES

1. Ačimovič J, Rozman D. Steroidal triterpenes of cholesterol synthesis. *Molecules*. (2013) 18:4002–17. doi: 10.3390/molecules18044002
2. Singh P, Saxena R, Srinivas G, Pande G, Chattopadhyay A. Cholesterol biosynthesis and homeostasis in regulation of the cell cycle. *PloS ONE. (*2013) 8:e58833. doi: 10.1371/journal.pone.0058833
3. Simons K, Ikonen E. How cells handle cholesterol. *Science*. (2000) 290:1721–6. doi: 10.1126/science.290.5497.1721
4. De Boussac H, Alioui A, Viennois E, Dufour J, Trousson A, Vega A, et al. Oxysterol receptors and their therapeutic applications in cancer conditions. *Exp Opin Ther Targets*. (2013) 17:1029–38. doi: 10.1517/14728222.2013.820708
5. Björkhem I, Diczfalusy U. Oxysterols: friends, foes, or just fellow passengers? *Arterioscler Thromb Vasc Biol*. (2002) 22:734–42. doi: 10.1161/01.ATV.0000013312.32196.49
6. Ačimovič J, Goyal S, Košir R, Goličnik M, Perše M, Belič A, et al. Cytochrome P450 metabolism of the post-lanosterol intermediates explains enigmas of cholesterol synthesis. *Sci Rep*. (2016) 6:28462. doi: 10.1038/srep28462
7. Brown AJ, Jessup W. Oxysterols: sources, cellular storage and metabolism, and new insights into their roles in cholesterol homeostasis. *Mol Aspects Med*. (2009) 30:111–22. doi: 10.1016/j.mam.2009.02.005
8. Kloudova A, Peter Guengerich F, Soucek P. The role of oxysterols in human cancer. *Trends Endocrinol Metab*. (2017) 28:485–96. doi: 10.1016/j.tem.2017.03.002
9. Russell, DW. Oxysterol biosynthetic enzymes. *Biochim Biophys Acta*. (2000) 1529:126–35. doi: 10.1016/S1388-1981(00)00142-6
10. Gill S, Chow R, Brown AJ. Sterol regulators of cholesterol homeostasis and beyond: the oxysterol hypothesis revisited and revised. *Prog Lipid Res*. (2008) 47:391–404. doi: 10.1016/j.plipres.2008.04.002
11. DeBose-Boyd, RA. Feedback regulation of cholesterol synthesis. *Cell Res*. (2008) 18:609–21. doi: 10.1038/cr.2008.61
12. Kanner J. Dietary advanced lipid oxidation endproducts are risk factors to human health. *Mol Nutr Food Res*. (2007) 51:1094–101. doi: 10.1002/mnfr.200600303
13. Staprans I, Pan X-M, Rapp JH, Feingold KR. Oxidized cholesterol in the diet is a source of oxidized lipoproteins in human serum. *J Lipid Res*. (2003) 44:705–15. doi: 10.1194/jlr.M200266-JLR200
14. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell*. (2006) 124:35–46. doi: 10.1016/j.cell.2005.12.022
15. Shimano, H. Sterol Regulatory Element-Binding Proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog Lipid Res*. (2001) 40:439–52. doi: 10.1016/S0163-7827(01)00010-8
16. Shimano H, Shimomura I, Hammer RE, Herz J, Goldstein JL, Brown MS, et al. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *J Clin Investig*. (1997) 100:2115–24. doi: 10.1172/JCI119746
17. Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, Brown MS. Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem*. (2002) 277:9520–8. doi: 10.1074/jbc.M111421200
18. Radhakrishnan A, Ikeda Y, Kwon HJ, Brown MS, Goldstein JL. Sterol-regulated transport of srebps from endoplasmic reticulum to golgi : oxysterols block transport by binding to insights. *PNAS*. (2007) 104:6511–18. doi: 10.1073/pnas.0700899104
19. Dong XY, Tang SQ, Chen JD. Dual functions of insig proteins in cholesterol homeostasis. *Lipids Health Dis*. (2012) 11:173. doi: 10.1186/1476-511X-11-173
20. Li D, Long W, Huang R, Chen Y, Xia M. 27-Hydroxycholesterol inhibits sterol regulatory element-binding protein 1 activation and hepatic lipid accumulation in mice. *Obesity*. (2018) 26:713–22. doi: 10.1002/oby.22130
21. Lu M, Hu XH, Li Q, Xiong Y, Hu GJ, Xu JJ, et al. A specific cholesterol metabolic pathway is established in a subset of hccs for tumor growth. *J Mol Cell Biol*. (2013) 5:404–15. doi: 10.1093/jmcb/mjt039
22. Rezen T, Rozman D, Pascussi JM, Monostory K. Interplay between cholesterol and drug metabolism. *Biochim Biophys Acta*. (2011) 1814:146–60. doi: 10.1016/j.bbapap.2010.05.014
23. Deng R, Yang D, Yang J, Yan B. Oxysterol 22 (R) -hydroxycholesterol induces the expression of the bile salt export pump through nuclear receptor farsenoid X receptor but not liver X receptor. *J Pharmacol Exp Ther*. (2006) 317:317–25. doi: 10.1124/jpet.105.097758
24. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature*. (2002) 418:935–41. doi: 10.1038/nature00965
25. Zmrzljak UP, Rozman D. Circadian regulation of the hepatic endobiotic and xenobitoic detoxification pathways: the time matters. *Chem Res Toxicol*. (2012) 25:811–24. doi: 10.1021/tx200538r
26. Korencic A, Bordyugov G, Kosir R, Rozman D, Golicnik M, Herzel H. The interplay of cis-regulatory elements rules circadian rhythms in mouse liver. *PloS ONE. (*2012) 7:e46835. doi: 10.1371/journal.pone.0046835
27. Dumbell R, Matveeva O, Oster H. Circadian clocks, stress, and immunity. *Front Endocrinol*. (2016) 7:37. doi: 10.3389/fendo.2016.00037
28. Honma S, Kawamoto T, Takagi Y, Fujimoto K, Sato F, Noshiro M, et al. Dec1 and Dec2 are regulators of the mammalian molecular clock. *Nature*. (2002) 419:841–4. doi: 10.1038/nature01123
29. Lowrey PL, Takahashi JS. Mammalian circadian biology: elucidating genome-wide levels of temporal organization. *Ann Rev Genomics Hum Genet*. (2004) 5:407–41. doi: 10.1146/annurev.genom.5.061903.175925
30. Ripperger JA, Merrow M. Perfect timing: epigenetic regulation of the circadian clock. *FEBS Lett*. (2011) 585:1406–11. doi: 10.1016/j.febslet.2011.04.047
31. Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, et al. SIRT1 Regulates circadian clock gene expression through PER2 deacetylation. *Cell*. (2008) 134:317–28. doi: 10.1016/j.cell.2008.06.050
32. Hirano A, Fu YH, Ptáek LJ. The intricate dance of post-translational modifications in the rhythm of life. *Nat Struct Mol Biol*. (2016) 23:1053–60. doi: 10.1038/nsmb.3326
33. Eide EJ, Woolf MF, Kang H, Woolf P, Hurst W, Camacho F, et al. Control of mammalian circadian rhythm by CKI -regulated proteasome-mediated PER2 degradation. *Mol Cell Biol*. (2005) 25:2795–807. doi: 10.1128/MCB.25.7.2795-2807.2005
34. Doi M, Hirayama J, Sassone-Corsi P. Circadian regulator CLOCK is a histone acetyltransferase. *Cell*. (2006) 125:497–508. doi: 10.1016/j.cell.2006.03.033

35. Bellet MM, Masri S, Astarita G, Sassone-Corsi P, Della Fazia MA, Servillo G. Histone deacetylase SIRT1 controls proliferation, circadian rhythm, and lipid metabolism during liver regeneration in mice. *J Biol Chem*. (2016) 291:23318–29. doi: 10.1074/jbc.M116.737114
36. Grimaldi B, Nakahata Y, Kaluzova M, Masubuchi S, Sassone-Corsi P. Chromatin remodeling, metabolism and circadian clocks: the interplay of CLOCK and SIRT1. *Int J Biochem Cell Biol*. (2009) 41:81–6. doi: 10.1016/j.biocel.2008.08.035
37. Utani K, Fu H, Jang SM, Marks AB, Smith OK, Zhang Y, et al. Phosphorylated SIRT1 associates with replication origins to prevent excess replication initiation and preserve genomic stability. *Nucleic Acids Res.* (2017) 45:7807–24. doi: 10.1093/nar/gkx468
38. Guarente L. Sirtuins, aging, and metabolism. *Cold Spring Harb Symp Quant Biol*. (2011) 76:81–90. doi: 10.1101/sqb.2011.76.010629
39. Gnocchi D, Pedrelli M, Hurt-Camejo E, Parini P, Gnocchi D, Pedrelli M, et al. Lipids around the Clock: focus on circadian rhythms and lipid metabolism. *Biology*. (2015) 4:104–32. doi: 10.3390/biology4010104
40. Ghoshal S, Stevens JR, Billon C, Girardet C, Sitaula S, Leon AS, et al. Adropin: an endocrine link between the biological clock and cholesterol homeostasis. *Mol Metab*. (2018) 8:51–64. doi: 10.1016/j.molmet.2017.12.002
41. Cho Y, Noshiro M, Choi M, Morita K, Kawamoto T, Fujimoto K, et al. The basic helix-loop-helix proteins Differentiated Embryo Chondrocyte (DEC) 1 and DEC2 function as corepressors of retinoid X receptors. *Mol Pharmacol*. (2009) 76:1360–9. doi: 10.1124/mol.109.057000
42. Wada T, Kang HS, Angers M, Gong H, Bhatia S, Khadem S, et al. Identification of oxysterol 7 -hydroxylase (Cyp7b1) as a novel retinoid-Related Orphan Receptor (ROR) (NR1F1) target gene and a functional cross-talk between ROR and liver X receptor (NR1H3). *Mol Pharmacol*. (2007) 73:891–9. doi: 10.1124/mol.107.040741
43. Martelot GL, Claudel T, Gatfield D, Schaad O, Kornmann B, Sasso GL, et al. REV-ERBα participates in circadian SREBP signaling and bile acid homeostasis. *PLoS Biol*. (2009) 7:1–12. doi: 10.1371/journal.pbio.1000181
44. Lu YF, Jin T, Xu Y, Zhang D, Wu Q, Zhang Y-K, et al. Sex differences in the circadian variation of cytochrome P450 genes and corresponding nuclear receptors in mouse liver. *Chronobiol Int*. (2013) 30:1135–43. doi: 10.3109/07420528.2013.805762
45. Kudo T, Kawashima M, Tamagawa T, Shibata S. Clock mutation facilitates accumulation of cholesterol in the liver of mice fed a cholesterol and/or cholic acid diet. *Am J Physiol Endocrinol Metab*. (2008) 294:E120–30. doi: 10.1152/ajpendo.00061.2007
46. Wang X, Xue J, Yang J, Xie M. Timed high-fat diet in the evening affects the hepatic circadian clock and PPARα-mediated lipogenic gene expressions in mice. *Genes Nutr*. (2013) 8:457–63. doi: 10.1007/s12263-013-0333-y
47. Duez HJ, van der Veen N, Duhem C, Pourcet B, Touvier T, Fontaine C, et al. Regulation of bile acid synthesis by the nuclear receptor rev-Erbα. *Gastroenterology*. (2008) 135:689–98. doi: 10.1053/j.gastro.2008.05.035
48. Ma K, Xiao R, Tseng HT, Shan L, Fu L, Moore DD. Circadian dysregulation disrupts bile acid homeostasis. *PLoS ONE*. (2009) 4:e6843. doi: 10.1371/journal.pone.0006843
49. Tomalik-Scharte D, Suleiman AA, Frechen S, Kraus D, Kerkweg U, Rokitta D, et al. Population pharmacokinetic analysis of circadian rhythms in hepatic CYP3A activity using midazolam. *J Clin Pharmacol*. (2014) 54:1162–9. doi: 10.1002/jcph.318
50. Takako T, Tomita M, Matsunaga N, Nakagawa H, Koyanagi S, Ohdo S. Molecular basis for rhythmic expression of Cyp3a4 in serum-shocked Hepg2 cells. *Pharmacogenet Genomics*. (2007) 17:1047–56. doi: 10.1097/FPC.0b013e3282f12a61
51. Pathak P, Li T, Chiang JYL. Retinoic acid-related orphan receptor α regulates diurnal rhythm and fasting induction of sterol 12α-hydroxylase in bile acid synthesis. *J Biol Chem*. (2013) 288:37154–65. doi: 10.1074/jbc.M113.485987
52. Noshiro M, Kawamoto T, Furukawa M, Fujimoto K, Yoshida Y, Sasabe E, et al. Rhythmic expression of DEC1 and DEC2 in peripheral tissues: DEC2 is a potent suppressor for hepatic cytochrome P450s opposing DBP. *Genes Cells*. (2004) 9:317–29. doi: 10.1111/j.1356-9597.2004.00722.x
53. Bartosch, B. Hepatitis B and C viruses and hepatocellular carcinoma. *Viruses*. (2010) 2:1504–9. doi: 10.3390/v2081504
54. El-Serag, HB. Hepatocellular carcinoma. *N Eng J Med*. (2011) 365:1118–27. doi: 10.1056/NEJMra1001683
55. Kovac U, Rozman D. Genetics of non-alcoholic fatty liver disease. In: *eLS*. American Cancer Society (2015). p. 1–8. doi: 10.1002/9780470015902.a0025335
56. Naik A, Košir R, Rozman D. Genomic aspects of NAFLD pathogenesis. *Genomics*. (2013) 102:84–95. doi: 10.1016/j.ygeno.2013.03.007
57. Petrick JL, Kelly SP, Altekruse SF, McGlynn KA, Rosenberg PS. Future of hepatocellular carcinoma incidence in the united states forecast through 2030. *J Clin Oncol*. (2016) 34:1787–94. doi: 10.1200/JCO.2015.64.7412
58. Zhou YY, Zhu GQ, Wang Y, Zheng JN, Ruan LY, Cheng Z, et al. Systematic review with network meta-analysis: statins and risk of hepatocellular carcinoma. *Oncotarget*. (2016) 7:21753–62. doi: 10.18632/oncotarget.7832
59. Blagotinšek K, Rozman D. Targeting signalling pathways in hepatocellular carcinoma. *Curr Pharmaceut Design*. (2016) 22:1–1. doi: 10.2174/1381612822666161006160005
60. Ikegami T, Hyogo H, Honda A, Miyazaki T, Tokushige K, Hashimoto E, et al. Increased serum liver X receptor ligand oxysterols in patients with non-alcoholic fatty liver disease. *J Gastroenterol*. (2012) 47:1257–66. doi: 10.1007/s00535-012-0585-0
61. Ikegami T, Honda A, Miyazaki T, Kohjima M, Nakamuta M, Matsuzaki Y. Increased serum oxysterol concentrations in patients with chronic hepatitis C virus infection. *Biochem Biophys Res Commun*. (2014) 446:736–40. doi: 10.1016/j.bbrc.2014.01.176
62. Li J, Zheng X, Lou N, Zhong W, Yan D. Oxysterol binding protein-related protein 8 mediates the cytotoxicity of 25-hydroxycholesterol. *J Lipid Res*. (2016) 57:1845–53. doi: 10.1194/jlr.M069906
63. Yokoyama S, Nozawa F, Tanaka S, Muneyuki S, Ogawa M. Anti-tumor effects of 25-hydroxycholesterol and low-dose recombinant tumor necrosis factor-alpha on rat liver tumorigenesis: modulated differentiation therapy for hepatocellular carcinoma. *Int J Oncol*. (2000) 16:1029–33. doi: 10.3892/ijo.16.5.1029
64. Zhong W, Qin S, Zhu B, Pu M, Liu F, Wang L, et al. Oxysterol-binding Protein-Related Protein 8 (ORP8) increases sensitivity of hepatocellular carcinoma cells to fas-mediated apoptosis. *J Biol Chem*. (2015) 290:8876–87. doi: 10.1074/jbc.M114.610188
65. Long H, Guo X, Qiao S, Huang Q. Tumor LXR expression is a prognostic marker for patients with hepatocellular carcinoma. *Pathol Oncol Res*. (2018) 24:339–44. doi: 10.1007/s12253-017-0249-8
66. Xiong H, Zhang Y, Chen S, Ni Z, He J, Li X, et al. Induction of SOCS3 by liver X receptor suppresses the proliferation of hepatocellular carcinoma cells. *Oncotarget*. (2017) 8:64083–94. doi: 10.18632/oncotarget.19321
67. Wolfe A, Thomas A, Edwards G, Jaseja R, Guo GL. Increased activation of the wnt-/- $N_L$catenin pathway in spontaneous hepatocellular carcinoma observed in farnesoid X receptor knockout mice. *J Pharmacol Exp Ther*. (2011) 2011:jpet.111.179390. doi: 10.1124/jpet.111.179390
68. Liu N, Meng Z, Lou G, Zhou W, Wang X, Zhang Y et al. Hepatocarcinogenesis in FXR-/- mice mimics human hcc progression that operates through hnf1α regulation of fxr expression. *Mol Endocrinol*. (2012) 26:775–85. doi: 10.1210/me.2011-1383
69. Su H, Ma C, Liu J, Li N, Gao M, Huang A, et al. Downregulation of nuclear receptor FXR Is associated with multiple malignant clinicopathological characteristics in human hepatocellular carcinoma. *Am J Physiol Gastrointest Liver Physiol*. (2012) 303:G1245–53. doi: 10.1152/ajpgi.00439.2011
70. Liu X, Zhang X, Ji L, Gu J, Zhou M, Chen S. Farnesoid X receptor associates with β-catenin and inhibits its activity in hepatocellular carcinoma. *Oncotarget*. (2015) 6:4226–38. doi: 10.18632/oncotarget.2899
71. He J, Zhao K, Zheng L, Xu Z, Gong W, Chen S, et al. Upregulation of microRNA-122 by farnesoid X receptor suppresses the growth of hepatocellular carcinoma cells. *Mol Cancer*. (2015) 4:163. doi: 10.1186/s12943-015-0427-9
72. Schulz A, Schlu T, Kinzel O, Abel U, Deuschle U, Schu J, et al. FXR controls the tumor suppressor NDRG2 and FXR agonists reduce liver tumor growth and metastasis in an orthotopic mouse xenograft model. *PLoS ONE*. (2012) 7:e43044. doi: 10.1371/journal.pone.0043044
73. Guo D, Bell EH, Mischel P, Chakravarti A. Targeting SREBP-1-driven lipid metabolism to treat cancer. *Curr Pharma*

Design. (2014) 20:2619–26. doi: 10.2174/13816128113199990486
74. Yuan P, Li J, Zhou F, Huang Q, Zhang J, Guo X, et al. NPAS2 promotes cell survival of hepatocellular carcinoma by transactivating CDC25A. Cell Death Dis. (2017) 8:e2704. doi: 10.1038/cddis.2017.131
75. Yuan P, Wang S, Zhou F, Wan S, Yang Y, Huang X, et al. Functional polymorphisms in the NPAS2Gene are associated with overall survival in transcatheter arterial chemoembolization-treated hepatocellular carcinoma patients. *Cancer Sci*. (2014) 105:825–32. doi: 10.1111/cas.12428
76. Zhao B, Lu J, Yin J, Liu H, Guo X, Yang Y, et al. a functional polymorphism in per3 gene is associated with prognosis in hepatocellular carcinoma. *Liver Int*. (2012) 32:1451–9. doi: 10.1111/j.1478-3231.2012.02849.x
77. Filipski E, Subramanian P, Carrière J, Guettier C, Barbason H, Lévi F. Circadian disruption accelerates liver carcinogenesis in mice. *Mutat Res Genet Toxicol Environ Mutagenesis*. (2009) 680:95–105. doi: 10.1016/j.mrgentox.2009.10.002
78. Kettner NM, Voicu H, Finegold MJ, Coarfa C, Sreekumar A, Putluri N, et al. Circadian homeostasis of liver metabolism suppresses hepatocarcinogenesis. *Cancer Cell*. (2016) 30:909–24. doi: 10.1016/j.ccell.2016.10.007
79. Fleet T, Stashi E, Zhu B, Rajapakshe K, Marcelo KL, Kettner NM, et al. Genetic and Environmental models of circadian disruption link src-2 function to hepatic pathology. *Journal of Biol Rhythms*. (2016) 31:443–60. doi: 10.1177/0748730416657921
80. Elgohary N, Pellegrino R, Neumann O, Elzawahry HM, Saber MM, Zeeneldin AA, et al. Protumorigenic role of timeless in hepatocellular carcinoma. *Int J Oncol*. (2015) 46:597–606. doi: 10.3892/ijo.2014.2751
81. Morcos PN, Moreira SA, Brennan BJ, Blotner S, Shulman NS, Smith PF. Influence of chronic hepatitis C infection on cytochrome P450 3A4 activity using midazolam as an *in vivo* probe substrate. *Eur J Clin Pharmacol*. (2013) 69:1777–84. doi: 10.1007/s00228-013-1525-5
82. Jepsen P, Turati F, La Vecchia C. NAFLD and cancer: more cause for concern? *J Hepatol*. (2018) 68:10–2. doi: 10.1016/j.jhep.2017.10.008
83. Skubic C, Drakulić Ž, Rozman D. Pharmacogenetic considerations for personalized therapy when tackling nonalcoholic fatty liver disease: the perspective of genes, drugs, and the sex. *Expert Opin Drug Metab Toxicol*. (2018) 14:831–41. doi: 10.1080/17425255.2018.1492552
84. Bellomo C, Caja L, Fabregat I, Mikulits W, Kardassis D, Heldin CH, et al. Snail mediates crosstalk between TGFβ and LXRα in hepatocellular carcinoma. *Cell Death Differ*. (2018) 25:885–903. doi: 10.1038/s41418-017-0021-3
85. Yang F, Huang X, Yi T, Yen Y, Moore DD, Huang W. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. *Cancer Res*. (2007) 67:863–7. doi: 10.1158/0008-5472.CAN-06-1078
86. Kim I, Morimura K, Shah Y, Yang Q, Ward JM, Gonzalez FJ. Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice. *Carcinogenesis*. (2007) 28:940–6. doi: 10.1093/carcin/bgl249
87. Degirolamo C, Modica S, Vacca M, Di Tullio G, Morgano A, Orazio AD, et al. Prevention of spontaneous hepatocarcinogenesis n farnesoid X receptor-null mice by intestinal-specific farnesoid X receptor reactivation. *Hepatology*. (2014) 61:161–70. doi: 10.1002/hep.27274
88. Takahashi S, Tanaka N, Fukami T, Xie C, Yagai T, Kim D, et al. Role of farnesoid X receptor and bile acids in hepatic tumor development. *Hepatol Commun*. (2018) 2:1567–82. doi: 10.1002/hep4.1263
89. Lee DC, Kang YK, Kim WH, Jang YJ, Kim DJ, Park IY, et al. Functional and Clinical evidence for NDRG2 as a candidate suppressor of liver cancer metastasis. *Cancer Res*. (2008) 11:4210–21. doi: 10.1158/0008-5472.CAN-07-5040
90. Jiang Y, Iakova P, Jin J, Sullivan E, Sharin VI, Hong H, et al. Farnesoid X receptor inhibits gankyrin in mouse livers and prevents development of liver cancer. *Hepatology*. (2013) 57:1098–106. doi: 10.1002/hep.26146
91. Dawson SP. Hepatocellular carcinoma and the ubiquitin - proteasome system. *Biochim Biophys Acta*. (2008) 1782:775–84. doi: 10.1016/j.bbadis.2008.08.003
92. Yang F, Hu Y, Liu HX, Wan YJY. MiR-22-silenced cyclin a expression in colon and liver cancer cells is regulated by bile acid receptor. *J Biol Chem*. (2015) 290:6507–15. doi: 10.1074/jbc.M114.620369
93. Yang F, Gong J, Wang G, Chen P, Yang L. Waltonitone inhibits proliferation of hepatoma cells and tumorigenesis via FXR-MiR-22-CCNA2 signaling pathway. *Oncotarget*. (2016) 7:75165–75. doi: 10.18632/oncotarget.12614
94. Xu Z, Huang G, Gong W, Zhou P, Zhao Y, Zhang Y, et al. FXR ligands protect against hepatocellular in Fl ammation via SOCS3 induction. *Cell Signal*. (2012) 24:1658–64. doi: 10.1016/j.cellsig.2012.04.015
95. Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor κB in hepatic inflammatory response. *Hepatology*. (2008) 48:1632–43. doi: 10.1002/hep.22519
96. Guo F, Xu Z, Zhang Y, Jiang P, Huang G, Chen S. FXR induces SOCS3 and suppresses hepatocellular carcinoma. *Oncotarget*. (2015) 6:34606–16. doi: 10.18632/oncotarget.5314
97. Wang X, Fu X, Van Ness C, Meng Z, Ma X, Huang W. Bile acid receptors and liver cancer. *Curr Pathobiol Rep*. (2013) 1:29–35. doi: 10.1007/s40139-012-0003-6
98. Zhang Y, Gong W, Dai S, Huang G, Shen X, Gao M. Downregulation of human farnesoid X receptor by MiR-421 promotes proliferation and migration of hepatocellular carcinoma cells. *Mol Cancer Res*. (2012) 10:516–23. doi: 10.1158/1541-7786.MCR-11-0473
99. Lu M, Kong X, Wang H, Huang G, Ye C, He Z. A novel MicroRNAs expression signature for hepatocellular carcinoma diagnosis and prognosis. *Oncotarget*. (2017) 8:8775–84. doi: 10.18632/oncotarget.14452
100. Garcia-Rodriguez JL, Barbier-Torres L, Fernandez-Alvarez S, Gutierrez-de Juan V, Monte MJ, Halilbasic E, et al. SIRT1 controls liver regeneration by regulating bile acid metabolism through farnesoid X receptor and mammalian target of rapamycin signaling. *Hepatology*. (2014) 59:1972–83. doi: 10.1002/hep.26971
101. Zhou M, Luo J, Chen M, Yang H, Learned RM, Depaoli AM, et al. Mouse species-specific control of hepatocarcinogenesis and metabolism by FGF19/FGF15. *J Hepatol*. (2017) 66:1182–92. doi: 10.1016/j.jhep.2017.01.027
102. Miura S, Mitsuhashi N, Shimizu H, Kimura F, Yoshidome H, Otsuka M. Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma. *BMC Cancer*. (2012) 12:56. doi: 10.1186/1471-2407-12-56
103. Piglionica M, Cariello M, Moschetta A. The gut-liver axis in hepatocarcinoma: a focus on the nuclear receptor fxr and the enterokine fgf19. *Curr Opin Pharmacol*. (2018) 43:93–8. doi: 10.1016/j.coph.2018.08.005
104. Li C, Yang W, Zhang J, Zheng X, Yao Y, Tu K, et al. SREBP-1 has a prognostic role and contributes to invasion and metastasis in human hepatocellular carcinoma. *Int J Mol Sci*. (2014) 15:7124–38. doi: 10.3390/ijms15057124
105. Min X, Wen J, Zhao L, Wang K, Li Q, Huang G, et al. Role of hepatoma-derived growth factor in promoting *de novo* lipogenesis and tumorigenesis in hepatocellular carcinoma. *Mol Oncol*. (2018) 12:1480–97. doi: 10.1002/1878-0261.12357
106. Li NZ, Zhou S, Shen Y, Xu J,Miao HH, Xiong Y, et al. Inhibition of the sterol regulatory element-binding protein pathway suppresses hepatocellular carcinoma by repressing inflammation in mice. *Hepatology*. (2017) 65:1936–47. doi: 10.1002/hep.29018
107. Tomita K, Toshiaki T, Suzuki T, Shimizu M, Sato H, Narimatsu K, et al. Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice. *Hepatology*. (2014) 59:154–69. doi: 10.1002/hep.26604
108. Ji J, Chen H, Liu XP, Wang YH, Luo CL, Zhang W, Xie, W, et al. A MiRNA combination as promising biomarker for hepatocellular carcinoma diagnosis: a study based on bioinformatics analysis. *J Cancer*. (2018) 9:3435–46. doi: 10.7150/jca.26101
109. Zhang Y, Qiu Z, Wei L, Tang R, Lian B, Zhao Y, et al. Integrated analysis of mutation data from various sources identifies key genes and signaling pathways in hepatocellular carcinoma. *PloS ONE*. (2014) 9:e100854. doi: 10.1371/journal.pone.0100854
110. Li H, Lu YF, Chen H, Liu J. Dysregulation of metallothionein and circadian genes in human hepatocellular carcinoma. *Chronobiol Int*. (2017) 34:192–202. doi: 10.1080/07420528.2016.1256300
111. Chauvet C, Vanhoutteghem A, Duhem C, Saint-Auret G, Bois-Joyeux B, Djian P, et al. Control of gene expression by the retinoic acid-related orphan

receptor alpha in HepG2 Human hepatoma cells. *PloS ONE.* (2011) 6:e22545. doi: 10.1371/journal.pone.0022545
112. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J Clin*. (2018) 68:394–424. doi: 10.3322/caac.21492
113. Wolfgang CL, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, et al. Recent progress in pancreatic cancer. *Cancer J Clin*. (2013) 63:318–48. doi: 10.3322/caac.21190
114. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet*. (2011) 378:607–20. doi: 10.1016/S0140-6736(10)62307-0
115. Gong Z, Holly EA, Wang F, Chan JM, Bracci PM. Intake of fatty acids and antioxidants and pancreatic cancer in a large population-based case-control study in the San Francisco Bay Area. *Int J Cancer*. (2010) 127:1893–904. doi: 10.1002/ijc.25208
116. Di Gangi IM, Mazza T, Fontana A, Copetti M, Fusilli C, Ippolito A, et al. Metabolomic Profile in pancreatic cancer patients: a consensus-based approach to identify highly discriminating metabolites. *Oncotarget*. (2016) 7:5815–29. doi: 10.18632/oncotarget.6808
117. Ishikawa S, Nagai Y, Masuda T, Koga Y, Nakamura T, Imamura Y, et al. The role of oxysterol binding protein-related protein 5 in pancreatic cancer. *Cancer Sci*. (2010) 101:898–905. doi: 10.1111/j.1349-7006.2009.01475.x
118. Koga Y, Ishikawa S, Nakamura T, Masuda T, Nagai Y, Takamori H, et al. Oxysterol Binding protein-related protein-5 is related to invasion and poor prognosis in pancreatic cancer. *Cancer Sci*. (2008) 99:2387–94. doi: 10.1111/j.1349-7006.2008.00987.x
119. Li H, Wang X, Fang Y, Huo Z, Lu X, Zhan X, et al. Integrated expression profiles analysis reveals novel predictive biomarker in pancreatic ductal adenocarcinoma. *Oncotarget*. (2017) 8:52571–83. doi: 10.18632/oncotarget.16732
120. Candelaria NR, Addanki S, Zheng J, Nguyen-Vu T, Karaboga H, Dey P, et al. Antiproliferative effects and mechanisms of liver X receptor ligands in pancreatic ductal adenocarcinoma cells. *PLoS ONE*. (2014) 9:e106289. doi: 10.1371/journal.pone.0106289
121. Lee JY, Lee KT, Lee JK, Lee KH, Jang KT, Heo JS, et al. Farnesoid X receptor, overexpressed in pancreatic cancer with lymph node metastasis promotes cell migration and invasion. *Br J Cancer*. (2011) 104:1027–37. doi: 10.1038/bjc.2011.37
122. Hu H, Wu L, Han T, Zhuo M, Lei W, Cui J. Correlated high expression of FXR and Sp1 in cancer cells confers a poor prognosis for pancreatic cancer : a study based on TCGA and tissue microarray. *Oncotarget*. (2017) 8:33265–75. doi: 10.18632/oncotarget.16633
123. Giaginis C, Koutsounas I, Alexandrou P, Patsouris E, Kouraklis G, Theocharis S. Elevated Farnesoid X Receptor (FXR) and Retinoid X Receptors (RXRs) expression is associated with less tumor aggressiveness and favourable prognosis in patients with pancreatic adenocarcinoma. *Neoplasma*. (2015) 62:332–41. doi: 10.4149/neo_2015_040
124. Joshi S, Cruz E, Rachagani S, Guha S, Brand RE, Ponnusamy MP, et al. Bile acids-mediated overexpression of MUC4 via FAK-dependent c-Jun activation in pancreatic cancer. *Mol Oncol*. (2016) 10:1063–77. doi: 10.1016/j.molonc.2016.04.007
125. Li W, Liu L, Liu D, Jin S, Yang Y, Tang W, et al. Decreased circadian component Bmal1 predicts tumor progression and poor prognosis in human pancreatic ductal adenocarcinoma. *Biochem Biophys Res Commun*. (2016) 472:156–62. doi: 10.1016/j.bbrc.2016.02.087
126. Jiang W, Zhao S, Jiang X, Zhang E, Hu G, Hu B, et al. The circadian clock gene Bmal1 acts as a potential anti-oncogene in pancreatic cancer by activating the P53 tumor suppressor pathway. *Cancer Lett*. (2016) 371:314–25. doi: 10.1016/j.canlet.2015.12.002
127. Tavano F, Pazienza V, Fontana A, Burbaci FP, Panebianco C, Saracino C, et al. SIRT1 and circadian gene expression in pancreatic ductal adenocarcinoma: effect of starvation. *Chronobiol Int*. (2015) 32:497–512. doi: 10.3109/07420528.2014.1003351
128. Wang X, Ji A, Zhu Y, Liang Z, Wu J, Li S, et al. A meta-analysis including dose-response relationship between night shift work and the risk of colorectal cancer. *Oncotarget*. (2015) 6:25046–60. doi: 10.18632/oncotarget.4502
129. Chen H, Qin S, Wang M, Zhang T, Zhang S. Association between cholesterol intake and pancreatic cancer risk: evidence from a meta-analysis. *Sci Rep*. (2015) 5:8243. doi: 10.1038/srep08243
130. Sun Y, He W, Luo M, Zhou Y, Chang G, Ren W, et al. SREBP1 regulates tumorigenesis and prognosis of pancreatic cancer through targeting lipid metabolism. *Tumour Biol*. (2015) 36:4133–41. doi: 10.1007/s13277-015-3047-5
131. Siqingaowa SS, Gopalakrishnan V, Taghibiglou C. Sterol regulatory element-binding protein 1 inhibitors decrease pancreatic cancer cell viability and proliferation. *Biochem Biophys Res Commun*. (2017) 488:136–40. doi: 10.1016/j.bbrc.2017.05.023
132. Zhou C, Qian W, Ma J, Cheng L, Jiang Z, Yan B, et al. Resveratrol enhances the chemotherapeutic response and reverses the stemness induced by gemcitabine in pancreatic cancer cells via targeting SREBP1. *Cell Proliferat*. (2019) 52:e12514. doi: 10.1111/cpr.12514
133. Rong W, Jiang W, Wan R. It is about time: circadian clock in the pancreas. *Pancreatic Disord Ther*. (2015) 5:2–5. doi: 10.4172/2165-7092.1000155
134. Mühlbauer E, Wolgast S, Finckh U, Peschke D, Peschke E. Indication of circadian oscillations in the rat pancreas. *FEBS Lett*. (2004) 564:91–6. doi: 10.1016/S0014-5793(04)00322-9
135. Relles D, Sendecki J, Chipitsyna G, Hyslop T, Yeo CJ, Arafat HA. Circadian gene expression and clinicopathologic correlates in pancreatic cancer. *J Gastrointest Surg*. (2013) 17:443–50. doi: 10.1007/s11605-012-2112-2
136. Luchini C, Capelli P, Scarpa A. Pancreatic ductal adenocarcinoma and its variants. *Surg Pathol Clin*. (2016) 9:547–60. doi: 10.1016/j.path.2016.05.003
137. Esposito K, Chiodini P, Colao A, Lenzi A, Giugliano D. Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. *Diabetes Care*. (2012) 35:2402–11. doi: 10.2337/dc12-0336
138. Jiao L, Chen L, White DL, Tinker L, Chlebowski RT, Van Horn LV, et al. Low-fat dietary pattern and pancreatic cancer risk in the women's health initiative dietary modification randomized controlled trial. *J Natl Cancer Inst*. (2018) 110:49–56. doi: 10.1093/jnci/djx117
139. Lee J, Moulik M, Fang Z, Saha P, Zou F, Xu Y, et al. Bmal1 and -cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced -cell failure in mice. *Mol Cell Biol*. (2013) 33:2327–38. doi: 10.1128/MCB.01421-12
140. Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature*. (2010) 466:627–31. doi: 10.1038/nature09253
141. Rosato V, Tavani A, Bosetti C, Pelucchi C, Talamini R, Polesel J, et al. Metabolic syndrome and pancreatic cancer risk: a case-control study in italy and meta-analysis. *Metabolism*. (2011) 60:1372–8. doi: 10.1016/j.metabol.2011.03.005
142. Obuch JC, Ahnen DJ. Colorectal cancer: genetics is changing everything. *Gastroenterol Clin N Am*. (2016) 45:459–76. doi: 10.1016/j.gtc.2016.04.005
143. Karantanos T, Theodoropoulos G, Pektasides D, Gazouli M. Clock genes: their role in colorectal cancer. *World J Gastroenterol*. (2014) 20:1986–92. doi: 10.3748/wjg.v20.i8.1986
144. Schneikert J, Behrens J. The canonical Wnt signalling pathway and its APC partner in colon cancer development. *Gut*. (2007) 56:417–25. doi: 10.1136/gut.2006.093310
145. Kitahara CM, Berrington de González A, Freedman ND, Huxley R, Mok Y, Jee SH, et al. Total cholesterol and cancer risk in a large prospective study in korea. *J Clin Oncol*. (2011) 29:1592–8. doi: 10.1200/JCO.2010.31.5200
146. Rodriguez-Broadbent H, Law PJ, Sud A, Palin K, Tuupanen S, Gylfe A, et al. Mendelian randomisation implicates hyperlipidaemia as a risk factor for colorectal cancer. *Int J Cancer*. (2017) 140:2701–8. doi: 10.1002/ijc.30709
147. Ito H, Matsuo K, Hosono S, Watanabe M, Kawase T, Suzuki T, et al. Association between CYP7A1 and the risk of proximal colon cancer in japanese. *Int J Mol Epidemiol Genet*. (2010) 1:35–46. doi: 10.1158/1940-6207.PREV-09-B116
148. Wertheim BC, Smith JW, Fang C, Alberts DS, Lance P, Thompson PA. Risk modification of colorectal adenoma by CYP7A1 polymorphisms and the role of bile acid metabolism in carcinogenesis. *Cancer Prev Res*. (2012) 5:197–204. doi: 10.1158/1940-6207.CAPR-11-0320

149. Tabata S, Yin G, Ogawa S, Yamaguchi K, Mineshita M, Kono S. Genetic polymorphism of cholesterol 7α-hydroxylase (CYP7A1) and colorectal adenomas: self defense forces health study. *Cancer Sci*. (2006) 97:406–10. doi: 10.1111/j.1349-7006.2006.00182.x
150. Hagiwara T, Kono S, Yin G, Toyomura K, Nagano J, Mizoue T, et al. Genetic polymorphism in cytochrome P450 7A1 and risk of colorectal cancer: the fukuoka colorectal cancer study. *Cancer Res*. (2005) 65:2979–82. doi: 10.1158/0008-5472.CAN-04-3872
151. Zhu Y, Soroka D, Sang S. Oxyphytosterols as active ingredients in wheat bran suppress human colon cancer cell growth: identification, chemical synthesis, and biological evaluation. *J Agric Food Chem*. (2015) 63:2267–76. doi: 10.1021/jf506361r
152. Biasi F, Mascia C, Astegiano M, Chiarpotto E, Nano M, Vizio B, et al. Pro-oxidant and proapoptotic effects of cholesterol oxidation products on human colonic epithelial cells: a potential mechanism of inflammatory bowel disease progression. *Free Radic Biol Med*. (2009) 47:1731–41. doi: 10.1016/j.freeradbiomed.2009.09.020
153. Warns J, Marwarha G, Freking N, Ghribi O. 27-Hydroxycholesterol decreases cell proliferation in colon cancer cell lines. *Biochimie*. (2018) 153:171–80. doi: 10.1016/j.biochi.2018.07.006
154. Biasi F, Chiarpotto E, Sottero B, Maina M, Mascia C, Guina T, et al. Evidence of cell damage induced by major components of a diet-compatible mixture of oxysterols in human colon cancer CaCo-2 cell line. *Biochimie*. (2013) 95:632–40. doi: 10.1016/j.biochi.2012.10.011
155. Roussi S, Winter A, Gosse F, Werner D, Zhang X, Marchioni E, et al. Different apoptotic mechanisms are involved in the antiproliferative effects of 7β-hydroxysitosterol and 7β-hydroxycholesterol in human colon cancer cells. *Cell Death Differ*. (2005) 12:128–35. doi: 10.1038/sj.cdd.4401530
156. Maier G, Bing G, Wagner E, Unger C. Antitumor activity and induction of apoptosis by water-soluble derivatives of 7β-hydroxycholesterol in human colon carcinoma cell lines. *Anticancer Res*. (1999) 19:4251–56.
157. Mascia C, Maina M, Chiarpotto E, Leonarduzzi G, Poli G, Biasi F. Proinflammatory effect of cholesterol and its oxidation products on CaCo-2 human enterocyte-like cells: effective protection by epigallocatechin-3-gallate. *Free Radic Biol Med*. (2010) 49:2049–57. doi: 10.1016/j.freeradbiomed.2010.09.033
158. Chalubinski M, Zemanek K, Skowron W, Wojdan K, Gorzelak P, Broncel M. The effect of 7-ketocholesterol and 25-hydroxycholesterol on the integrity of the human aortic endothelial and intestinal epithelial barriers. *Inflamm Res*. (2013) 62:1015–23. doi: 10.1007/s00011-013-0660-x
159. Chalubinski M, Wojdan K, Gorzelak P, Borowiec M, Broncel M. The effect of oxidized cholesterol on barrier functions and IL-10 MRNA expression in human intestinal epithelium co-cultured with dendritic cells in the transwell system. *Food Chem Toxicol*. (2014) 69:289–93. doi: 10.1016/j.fct.2014.04.005
160. Lee WH, Lee CS, Kwon K, Kwon YS, Kim SW, Goo TW, et al. 7-Ketocholesterol induces endoplasmic reticulum stress in HT-29 cells. *Z Naturforsch C*. (2009) 64:307–10. doi: 10.1515/znc-2009-3-425
161. Alemany L, Laparra JM, Barberá R, Alegría A. Evaluation of the cytotoxic effect of 7keto-stigmasterol and 7keto-cholesterol in human intestinal (Caco-2) cells. *Food Chem Toxicol*. (2012) 50:3106–13. doi: 10.1016/j.fct.2012.06.036
162. Tanaka AR, Noguchi K, Fukazawa H, Igarashi Y, Arai H, Uehara Y. P38MAPK and Rho-dependent kinase are involved in anoikis induced by anicequol or 25-hydroxycholesterol in DLD-1 colon cancer cells. *Biochem Biophys Res Commun*. (2013) 430:1240–5. doi: 10.1016/j.bbrc.2012.12.067
163. Serra G, Incani A, Serreli G, Porru L, Melis MP, Tuberoso CIG, et al. Olive oil polyphenols reduce oxysterols -induced redox imbalance and pro-inflammatory response in intestinal cells. *Redox Biol*. (2018) 17:348–54. doi: 10.1016/j.redox.2018.05.006
164. Vedin LL, Gustafsson JA, Steffensen KR. The oxysterol receptors LXRα and LXRβ suppress proliferation in the colon. *Mol Carcinogenesis*. (2013) 52:835–44. doi: 10.1002/mc.21924
165. Swan R, Alnabulsi A, Cash B, Alnabulsi A, Murray GI. Characterisation of the oxysterol metabolising enzyme pathway in mismatch repair proficient and deficient colorectal cancer. *Oncotarget*. (2016) 7:46509–27. doi: 10.18632/oncotarget.10224
166. Abdul Aziz NA, Mokhtar NM, Harun R, Mollah MMH, Mohamed Rose I, Sagap I, et al. A 19-gene expression signature as a predictor of survival in colorectal cancer. *BMC Med Genomics*. (2016) 9:58. doi: 10.1186/s12920-016-0218-1
167. Yun SH, Park MG, Kim YM, Roh MS, Park JI. Expression of chicken ovalbumin upstream promoter-transcription factor II and liver X receptor as prognostic indicators for human colorectal cancer. *Oncol Lett*. (2017) 14:4011–20. doi: 10.3892/ol.2017.6659
168. Savic D, Ramaker RC, Roberts SB, Dean EC, Burwell TC, Meadows SK, et al. Distinct gene regulatory programs define the inhibitory effects of liver X receptors and PPARG on cancer cell proliferation. *Genome Med*. (2016) 8:74. doi: 10.1186/s13073-016-0328-6
169. Uno S, Endo K, Jeong Y, Kawana K, Miyachi H, Hashimoto Y, et al. Suppression of β-catenin signaling by liver X receptor ligands. *Biochem Pharmacol*. (2009) 77:186–95. doi: 10.1016/j.bcp.2008.10.007
170. De Gottardi ADE, Touri F, Maurer CA, Perez A, Maurhofer O, Ventre G, et al. The bile acid nuclear receptor FXR and the bile acid binding protein IBABP are differently expressed in colon cancer. *Dig Dis Sci*. (2004) 49:982–9. doi: 10.1023/B:DDAS.0000034558.78747.98
171. Modica S, Murzilli S, Salvatore L, Schmidt DR, Moschetta A. Nuclear bile acid receptor FXR protects against intestinal tumorigenesis. *Cancer Res*. (2008) 23:9589–95. doi: 10.1158/0008-5472.CAN-08-1791
172. Modica S, Gofflot F, Murzilli S, Orazio AD, Salvatore L, Pellegrini F, et al. The intestinal nuclear receptor signature with epithelial localization patterns and expression modulation in tumors. *YGAST*. (2010) 138:636–48.e12. doi: 10.1053/j.gastro.2009.09.060
173. Lax S, Schauer G, Prein K, Kapitan M, Silbert D, Berghold A, et al. Expression of the nuclear bile acid receptor / farnesoid x receptor is reduced in human colon carcinoma compared to nonneoplastic mucosa independent from site and may be associated with adverse prognosis. *Int J Cancer*. (2012) 2239:2232–9. doi: 10.1002/ijc.26293
174. Bailey AM, Zhan L, Maru D, Shureiqi I, Pickering CR, Kiriakova G, et al. FXR silencing in human colon cancer by DNA methylation and KRAS signaling. *Am J Physiol Gastrointest Liver Physiol*. (2014) 306:G48–58. doi: 10.1152/ajpgi.00234.2013
175. Peng Z, Chen J, Drachenberg CB, Raufman JP, Xie G. Farnesoid X receptor represses matrix metalloproteinase 7 expression, revealing this regulatory axis as a promising therapeutic target in colon cancer. *J Biol Chem*. (2019) 294:8529–42. doi: 10.1074/jbc.RA118.004361
176. Maran RRM, Thomas A, Roth M, Sheng Z, Esterly N, Pinson D, et al. Farnesoid X receptor deficiency in mice leads to increased intestinal epithelial cell proliferation and tumor development. *J Pharmacol Exp Ther*. (2009) 328:469–77. doi: 10.1124/jpet.108.145409
177. Peng Z, Raufman JP, Xie G. Src-mediated cross-talk between farnesoid X and epidermal growth factor receptors inhibits human intestinal cell proliferation and tumorigenesis. *PLoS ONE*. (2012) 7:e48461. doi: 10.1371/journal.pone.0048461
178. Mazzoccoli G, Panza A, Valvano MR, Palumbo O, Carella M, Pazienza V, et al. Clock gene expression levels and relationship with clinical and pathological features in colorectal cancer patients. *Chronobiol Int*. (2011) 28:841–51. doi: 10.3109/07420528.2011.615182
179. Oshima T, Takenoshita S, Akaike M, Kunisaki C, Fujii S. Expression of circadian genes correlates with liver metastasis and outcomes in colorectal cancer. *Oncol Rep*. (2011) 25:1439–46. doi: 10.3892/or.2011.1207
180. Gu D, Li S, Ben S, Du M, Chu H, Zhang Z, et al. Circadian clock pathway genes associated with colorectal cancer risk and prognosis. *Arch Toxicol*. (2018) 92:2681–9. doi: 10.1007/s00204-018-2251-7
181. Firestein R, Blander G, Michan S, Oberdoerffer P, Ogino S, Campbell J, et al. The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. *PLoS ONE*. (2008) 3:e2020. doi: 10.1371/journal.pone.0002020
182. Aune D, Chan DSM, Lau R, Vieira R, Greenwood DC, Kampman E, et al. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *BMJ*. (2011) 343:d6617. doi: 10.1136/bmj.d6617
183. Kim ER, Chang DK. Colorectal cancer in inflammatory bowel disease: the risk, pathogenesis, prevention and diagnosis. *World J Gastroenterol*. (2014) 20:9872. doi: 10.3748/wjg.v20.i29.9872
184. Bai B, Yamamoto K, Sato H, Sugiura H, Tanaka T. Combined effect of 25-hydroxycholesterol and IL-1β on IL-8 production in human

colon carcinoma cell line (Caco-2). *Inflammation*. (2005) 29:141–6. doi: 10.1007/s10753-006-9009-8
185. Biasi F, Guina T, Maina M, Nano M, Falcone A, Aroasio E, et al. Progressive increase of matrix metalloprotease-9 and interleukin-8 serum levels during carcinogenic process in human colorectal tract. *PLoS ONE*. (2012) 7:e41839. doi: 10.1371/journal.pone.0041839
186. Thorsen K, Schepeler T, Øster B, Rasmussen MH, Vang S, Wang K, et al. Tumor-specific usage of alternative transcription start sites in colorectal cancer identified by genome-wide exon array analysis. *BMC Genomics*. (2011) 12:505. doi: 10.1186/1471-2164-12-505
187. Gadaleta RM, Oldenburg B, Willemsen ECL, Spit M, Murzilli S, Salvatore L, et al. Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating nf- κ b signaling in the intestine. *BBA Mol Basis Dis*. (2011) 1812:851–8. doi: 10.1016/j.bbadis.2011.04.005
188. Selmin OI, Fang C, Lyon AM, Doetschman TC, Thompson PA, Martinez JD, et al. Inactivation of adenomatous polyposis coli reduces bile acid / farnesoid X receptor expression through fxr gene cpg methylation in mouse colon tumors and human colon cancer. *J Nutr*. (2016) 146:236–42. doi: 10.3945/jn.115.216580
189. Romagnolo DF, Donovan MG, Doetschman TC, Selmin OI. n-6 linoleic acid induces epigenetics alterations associated with colonic inflammation and cancer. *Nutrients*. (2019) 11:171. doi: 10.3390/nu11010171
190. Krattinger R, Boström A, Schiöth HB, Thasler WE, Mwinyi J, Kullak-ublick GA. MicroRNA-192 suppresses the expression of the farnesoid X receptor. *Am J Physiol Gastrointest Liver Physiol*. (2019) 310:1044–51. doi: 10.1152/ajpgi.00297.2015
191. Fu T, Coulter S, Yoshihara E, Oh TG, Fang S, Cayabyab F, et al. FXR regulates intestinal cancer stem cell proliferation. *Cell. (*2019) 176:1098–112.e18. doi: 10.1016/j.cell.2019.01.036
192. Li JN, Mahmoud MA, Han WF, Ripple M, Pizer ES. Sterol regulatory element-binding protein-1 participates in the regulation of fatty acid synthase expression in colorectal neoplasia. *Exp Cell Res*. (2000) 261:159–65. doi: 10.1006/excr.2000.5054
193. Kwan HY, Yang Z, Fong WF, Hu YM, Yu ZL, Hsiao WL. The anticancer effect of oridonin is mediated by fatty acid synthase suppression in human colorectal cancer cells. *J Gastroenterol*. (2013) 48:182–92. doi: 10.1007/s00535-012-0612-1
194. Erren TC, Morfeld P, Foster RG, Reiter RJ, GroßJV, Westermann IK. Sleep and cancer: synthesis of experimental data and meta-analyses of cancer incidence among some 1,500,000 study individuals in 13 countries. *Chronobiol Int*. (2016) 33:325–50. doi: 10.3109/07420528.2016.1149486
195. Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, et al. Night-shift work and risk of colorectal cancer in the nurses' health study. *J Natl Cancer Inst*. (2003) 95:825–8. doi: 10.1093/jnci/95.11.825
196. Filipski E, Li XM, Lévi F. Disruption of circadian coordination and malignant growth. *Cancer Causes Control*. (2006) 17:509–14. doi: 10.1007/s10552-005-9007-4
197. Mazzoccoli G, Colangelo T, Panza A, Rubino R, De Cata A, Tiberio C, et al. Deregulated expression of cryptochrome genes in human colorectal cancer. *Mol Cancer*. (2016) 15:1–20. doi: 10.1186/s12943-016-0492-8
198. Karantanos T, Theodoropoulos G, Gazouli M, Vaiopoulou A, Karantanou C, Lymberi M, et al. Expression of clock genes in patients with colorectal cancer. *Int J Biol Mark*. (2013) 28:280–5. doi: 10.5301/JBM.5000033
199. Yang X, Wood PA, Ansell CM, Ohmori M, Hrushesky WJM. B -catenin induces b -trcp-mediated per2 degradation altering circadian clock gene expression in intestinal mucosa of apc min / + mice. *J Biol Chem*. (2009) 145:289–97. doi: 10.1093/jb/mvn167
200. Momma T, Okayama H, Saitou M, Sugeno H, Yoshimoto N, Takebayashi Y, et al. Expression of circadian clock genes in human colorectal adenoma and carcinoma. *Oncol Lett*. (2017) 14:5319–25. doi: 10.3892/ol.2017.6876
201. Chen-Goodspeed M, Lee CC. Tumor suppression and circadian function. *J Biol Rhythms*. (2007) 22:291–8. doi: 10.1177/0748730407303387
202. Ishida N. Circadian clock, cancer and lipid metabolism. *Neurosci Res*. (2007) 57:483–90. doi: 10.1016/j.neures.2006.12.012
203. Fuhr L, El-Athman R, Scrima R, Cela O, Carbone A, Knoop H, et al. The circadian clock regulates metabolic phenotype rewiring via HKDC1 and modulates tumor progression and drug response in colorectal cancer. *EBiomedicine*. (2018) 33:105–21. doi: 10.1016/j.ebiom.2018.07.002
204. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. *Cancer Treat Rev*. (2015) 41:554–67. doi: 10.1016/j.ctrv.2015.04.005
205. Jacobs EJ, Newton CC, Thun MJ, Gapstur SM. Long-term use of cholesterol-lowering drugs and cancer incidence in a large united states cohort. *Cancer Res*. (2011) 71:1763–71. doi: 10.1158/0008-5472.CAN-10-2953
206. Cardwell CR, Hicks BM, Hughes C, Murray LJ. Statin use after colorectal cancer diagnosis and survival: a population-based cohort study. *J Clin Oncol*. (2014) 32:3177–83. doi: 10.1200/JCO.2013.54.4569
207. Liu Y, Tang W, Wang J, Xie L, Li T, He Y, et al. Association between statin use and colorectal cancer risk: a meta-analysis of 42 studies. *Cancer Causes Control*. (2014) 25:237–49. doi: 10.1007/s10552-013-0326-6
208. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. *Gastroenterology*. (2013) 144:323–32. doi: 10.1053/j.gastro.2012.10.005
209. Shi M, Zheng H, Nie B, Gong W, Cui X. Statin use and risk of liver cancer: an update meta-analysis. *BMJ Open*. (2014) 4:e005399. doi: 10.1136/bmjopen-2014-005399
210. Tsan YT, Lee CH, Ho WC, Lin MH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. *J Clin Oncol*. (2013) 31:1514–21. doi: 10.1200/JCO.2012.44.6831
211. Tsan YT, Lee CH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis b virus infection. *J Clin Oncol*. (2012) 30:623–30. doi: 10.1200/JCO.2011.36.0917
212. Hsiang JC, Wong GL, Tse YK, Wong VW, Yip TC, Chan HL. Statin and the risk of hepatocellular carcinoma and death in a hospital-based hepatitis B-infected population: a propensity score landmark analysis. *J Hepatol*. (2015) 63:1190–7. doi: 10.1016/j.jhep.2015.07.009
213. Chen CI, Kuan CF, Fang YA, Liu SH, Liu JC, Wu LL, et al. Cancer risk in HBV patients with statin and metformin use: a population-based cohort study. *Medicine*. (2015) 94:e462. doi: 10.1097/MD.0000000000000462
214. Janicko M, Drazilova S, Pella D, Fedacko J, Jarcuska P. Pleiotropic effects of statins in the diseases of the liver. *World J Gastroenterol*. (2016) 22:6201–13. doi: 10.3748/wjg.v22.i27.6201
215. Nishio T, Taura K, Nakamura N, Seo S, Yasuchika K, Kaido T, et al. Impact of statin use on the prognosis of patients with hepatocellular carcinoma undergoing liver resection: a subgroup analysis of patients without chronic hepatitis viral infection. *Surgery*. (2018) 163:264–9. doi: 10.1016/j.surg.2017.09.026
216. Kawaguchi Y, Sakamoto Y, Ito D, Ito K, Arita J, Akamatsu N, et al. Statin use is associated with a reduced risk of hepatocellular carcinoma recurrence after initial liver resection. *BioSci Trends*. (2017) 11:574–80. doi: 10.5582/bst.2017.01191
217. Archibugi L, Arcidiacono PG, Capurso G. Statin use is associated to a reduced risk of pancreatic cancer: a meta-analysis. *Digest Liver Dis*. (2018) 51:28–37. doi: 10.1016/j.dld.2018.09.007
218. Hamada T, Khalaf N, Yuan C, Babic A, Morales-Oyarvide V, Qian ZR, et al. Statin use and pancreatic cancer risk in two prospective cohort studies. *J Gastroenterol*. (2018) 53:959–66. doi: 10.1007/s00535-018-1430-x
219. Singh S, Singh PP, Roberts LR, Sanchez W. chemopreventive strategies in hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol*. (2014) 11:45–54. doi: 10.1038/nrgastro.2013.143
220. Lipkin SM, Chao EC, Moreno V, Rozek LS, Rennert H, Pinchev M, et al. Genetic variation in 3-hydroxy-3-methylglutaryl coa reductase modifies the chemopreventive activity of statins for colorectal cancer. *Cancer Prev Res*. (2010) 3:597–603. doi: 10.1158/1940-6207.CAPR-10-0007
221. Amin S, Boffetta P, Lucas AL. The role of common pharmaceutical agents on the prevention and treatment of pancreatic cancer. *Gut Liver*. (2016) 10:665–71. doi: 10.5009/gnl15451
222. Kim YS,Lee YM, Oh TI, Shin DHG, Kim HS, Kan Y, et al. Emodin sensitizes hepatocellular carcinoma cells to the anti-cancer effect of sorafenib through suppression of cholesterol metabolism. *Int J Mol Sci*. (2018) 19:E3127. doi: 10.3390/ijms19103127

223. Morioka S, Sai K, Omori E, Ikeda Y, Matsumoto K, Ninomiya-Tsuji J. TAK1 Regulates hepatic lipid homeostasis through SREBP. *Oncogene*. (2016) 35:3829–38. doi: 10.1038/onc.2015.453
224. Zhang H, Feng Z, Huang R, Xia Z, Xiang G, Zhang J. MicroRNA-449 suppresses proliferation of hepatoma cell lines through blockade lipid metabolic pathway related to SIRT1. *Int J Oncol*. (2014) 45:2143–52. doi: 10.3892/ijo.2014.2596
225. Gabitova L, Gorin A, Astsaturov I. Molecular pathways: sterols and receptor signaling in cancer. *Clin Cancer Res*. (2014) 20:28–34. doi: 10.1158/1078-0432.CCR-13-0122
226. Chuu, C-P. Modulation of liver X receptor signaling as a prevention and therapy for colon cancer. *MedHypotheses*. (2011) 76:697–9. doi: 10.1016/j.mehy.2011.01.037
227. Pattanayak SP, Bose P, Sunita P, Siddique MUM, Lapenna A. Bergapten inhibits liver carcinogenesis by modulating LXR/PI3K/Akt and IDOL/LDLR pathways. *Biomed Pharmacother*. (2018) 108:297–308. doi: 10.1016/j.biopha.2018.08.145
228. Sasso GLO, Bovenga F, Murzilli S, Salvatore L, Tullio GDI, Martelli N, et al. Liver X receptors inhibit proliferation of human colorectal cancer cells and growth of intestinal tumors in mice. *YGAST*. (2013) 144:1497–1507.e13. doi: 10.1053/j.gastro.2013.02.005
229. Alawad AS, Levy C. FXR agonists: from bench to bedside, a guide for clinicians. *Digest Dis Sci*. (2016) 61:3395–404. doi: 10.1007/s10620-016-4334-8
230. Kowdley KV, Luketic V, Chapman R, Hirschfield GM, Poupon R, Schramm C, et al. A randomized trial of obeticholic acid biliary cholangitis. *Hepatology*. (2018) 67:1890–902. doi: 10.1002/hep.29569
231. Ortiz R, Melguizo C, Prados J, Alvarez PJ, Caba O, Rodriguez-Serrano F, et al. New gene therapy strategies for cancer treatment: a review of recent patents. *Recent Patents Anti Cancer Drug Discov*. (2012) 7:297–312. doi: 10.2174/157489212801820093
232. Block KI, Block PB, Gyllenhaal C. Integrative treatment for colorectal cancer: a comprehensive approach. *J Altern Complement Med*. (2018) 24:890–901. doi: 10.1089/acm.2018.0125
233. Davidson AJ, Straume M, Block GD, Menaker M. Daily timed meals dissociate circadian rhythms in hepatoma and healthy host liver. *Int J Cancer*. (2006) 118:1623–7. doi: 10.1002/ijc.21591
234. Fekry B, Ribas-Latre A, Baumgartner C, Deans JR, Kwok C, Patel P, et al. Incompatibility of the circadian protein BMAL1 and HNF4α in hepatocellular carcinoma. *Nat Commun*. (2018) 9:4349. doi: 10.1038/s41467-018-06648-6
235. Guo L, Yu F, Zhang T, Wu B. The clock protein BMAL1 regulates circadian expression and activity of sulfotransferase 1A1 in mice. *Drug Metab Disposit*. (2018) 46:1403–10. doi: 10.1124/dmd.118.082503
236. Koyanagi S, Ohdo S. Alteration of intrinsic biological rhythms during interferon treatment and its possible mechanism. *Mol Pharmacol*. (2002) 62:1393–9. doi: 10.1124/mol.62.6.1393
237. Akira ODA, Katayose YU, Yabuuchi S, Yamamoto K, Mizuma M, Shirasou S, et al. Clock gene mouse period2 overexpression inhibits growth of human pancreatic cancer cells and has synergistic effect with cisplatin. *Anticancer Res*. (2009) 29:1201–10.
238. Pazienza V, Tavano F, Francavilla M, Fontana A, Pellegrini F, Benegiamo G, et al. Time-qualified patterns of variation of ppar γ, dnmt1, and dnmt3b expression in pancreatic cancer cell lines. *PPAR Res*. (2012) 2012:1–8. doi: 10.1155/2012/890875
239. Hashimoto K, Ethridge RT, Evers BM. Peroxisome proliferator-activated receptor γ ligand inhibits cell growth and invasion of human pancreatic cancer cells. *Int J Gastrointest Cancer*. (2002) 32:7–22. doi: 10.1385/IJGC:32:1:7
240. Innominato PF, Giacchetti S, Moreau T, Bjarnason GA, Smaaland R, Focan C, et al. Fatigue and weight loss predict survival on circadian chemotherapy for metastatic colorectal cancer. *Cancer*. (2013) 119:2564–73. doi: 10.1002/cncr.28072
241. Liao C, Li J, Bin Q, Cao Y, Gao F. Chronomodulated chemotherapy versus conventional chemotherapy for advanced colorectal cancer: a meta-analysis of five randomized controlled trials. *Int J Colorectal Dis*. (2010) 25:343–50. doi: 10.1007/s00384-009-0838-4
242. Bouchahda M, Adam R, Giacchetti S, Castaing D, Brezault-Bonnet C, Hauteville D, et al. Rescue chemotherapy using multidrug chronomodulated hepatic arterial infusion for patients with heavily pretreated metastatic colorectal cancer. *Cancer*. (2009) 115:4990–9. doi: 10.1002/cncr.24549
243. Zhang F, Sun H, Zhang S, Yang X, Zhang G, Su T. Overexpression of PER3 inhibits self-renewal capability and chemoresistance of colorectal cancer stem-like cells via inhibition of notch and β-catenin signaling. *Oncol Res Featuring Preclin Clin Cancer Ther*. (2017) 25:709–19. doi: 10.3727/096504016X14772331883976
244. Hasakova K, Vician M, Reis R, Zeman M, Herichova I. The expression of clock genes cry1 and cry2 in human colorectal cancer and tumor adjacent tissues correlates differently dependent on tumor location. *Neoplasma*. (2018) 65:986–92. doi: 10.4149/neo_2018_180122N47
245. Rossin D, Calfapietra S, Sottero B, Poli G, Biasi F. HNE and cholesterol oxidation products in colorectal inflammation and carcinogenesis. *Free Radic Biol Med*. (2017) 111:186–95. doi: 10.1016/j.freeradbiomed.2017.01.017
246. Pietrangelo A, Ridgway ND. Bridging the molecular and biological functions of the oxysterol-binding protein family. *Cell Mol Life Sci*. (2018) 75:3079–98. doi: 10.1007/s00018-018-2795-y

Conflict of Interest Statement: 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: info@frontiersin.org | +41 21 510 17 00

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