frontiers | Research Topics

Metabolic reprogramming in cancer

Edited by
Valentina Audrito and Elisa Giovannetti

Published in
Frontiers in Pharmacology
Frontiers in Oncology

ISSN 1664-8714
ISBN 978-2-8325-6162-1
DOI 10.3389/978-2-8325-6162-1

About Frontiers

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

Frontiers journal series

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

Dedication to quality

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

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Metabolic reprogramming in cancer

Topic editors
Valentina Audrito — Università del Piemonte Orientale, Italy
Elisa Giovannetti — VU Medical Center, Netherlands

Citation
Audrito, V., Giovannetti, E., eds. (2025). *Metabolic reprogramming in cancer*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6162-1

Table of contents

frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 11 March 2025
DOI 10.3389/fphar.2025.1583986

OPEN ACCESS

EDITED AND REVIEWED BY
Qingbin Cui,
University of Toledo College of Medicine and Life Sciences, United States

*CORRESPONDENCE
Valentina Audrito,
valentina.audrito@uniupo.it
Elisa Giovannetti,
elisa.giovannetti@gmail.com

RECEIVED 26 February 2025
ACCEPTED 28 February 2025
PUBLISHED 11 March 2025

CITATION
Audrito V and Giovannetti E (2025) Editorial: Metabolic reprogramming in cancer.
Front. Pharmacol. 16:1583986.
doi: 10.3389/fphar.2025.1583986

Editorial: Metabolic reprogramming in cancer

Valentina Audrito[1]* and Elisa Giovannetti[2,3]*

[1]Department of Science and Technological Innovation, University of Eastern Piedmont, Alessandria, Italy, [2]Department of Medical Oncology, Lab of Medical Oncology, Cancer Center Amsterdam, Imaging and Biomarkers, Amsterdam UMC, Vrije Universiteit, Amsterdam, Netherlands, [3]Cancer Pharmacology Lab, Fondazione Pisana Per La Scienza, Pisa, Italy

KEYWORDS

metabolic reprogramming, cancer, tumor microenvironment, metabolic pathways, metabolites, tumorigenesis, metabolic plasticity

Editorial on the Research Topic
Metabolic reprogramming in cancer

Technological advancements over the past few decades have unraveled the diversity and adaptability of tumors, shedding light on key genetic aberrations and metabolic pathways that support tumor growth. Specifically, cancer cells alter their metabolic pathways to fulfill the augmented energy and building block requirements while managing oxidative stress crucial for their proliferation and survival (Nong et al., 2023). The flux through these metabolic pathways, underlying of cancer metabolic plasticity, is controlled by cancer driver mutations and environmental nutrient availability.

The tumor microenvironment (TME), often deficient in specific nutrients, compels cancer cells to adapt by inducing mechanisms to scavenge nutrients and sustain their proliferation. Moreover, it is increasingly recognized that the metabolism of non-cancerous cell types within the TME, such as endothelial cells, fibroblasts, and immune cells, can influence tumor progression (Xia et al., 2021). Specifically, metabolic reprogramming is also essential for maintaining self and body homeostasis of various types of immune cells. Recent studies have highlighted that immune cells undergo metabolic reprogramming during proliferation, differentiation, and execution of effector functions, which are crucial for regulating the antitumor immune response (Hu et al., 2024). This impact is achieved by the release of metabolites and its effects on the expression of immune molecules. Considering that metastases are a significant cause of cancer-related deaths, ongoing efforts focus on comprehending how metabolism is employed by metastatic cells, especially in aggressive tumor types such as lung and pancreatic cancers (Comandatore et al., 2022). Furthermore, there is a newfound interest in utilizing cancer genetic analysis to stratify patients and design dietary interventions along with metabolism-targeting therapies.

This Research Topic included 12 original and review papers addressing different features of metabolic reprogramming in tumors, offering novel knowledge on this topic, also at a translational point of view.

In their review article Chen et al. summarized the main features of metabolic reprogramming in tumors, addressing different aspects including increased glycolytic metabolism, lipid synthesis, alteration in amino acids production, and the relationship between altered metabolism and immune response. Then, they focused the paper on the roles played by metabolic adaptation mechanisms in the prognosis and progression of kidney cancer, discussing recent advancements in the diagnosis and treatment of renal

cancer targeting metabolic vulnerabilities. The role of metabolic reprogramming was also emphasized in the systematic review by Li et al. analyzing hepatocellular carcinoma (HCC), a cancer with high morbidity and mortality. The authors selected from 2011 to 2023 a total of 575 publications on this field to identify the hotspots and frontiers of metabolic reprogramming research in HCC and to provide future directions for novel scientific research and decision-making in HCC therapeutic strategies.

In the context of metabolic rewiring serine hydroxymethyltransferases (SHMTs) and methylenetetrahydrofolate dehydrogenases (MTHFDs) are recognized as important one-carbon metabolic enzymes for regulating tumor initiation and development, representing potential therapeutic targets for anti-tumor strategies, as illustrated in Zhang et al. MTHFD1/2 have been identified as oncogenic enzymes upregulated in various tumors, involved in metastasis formation and chemoresistance. Cytoplasmic SHMT1 and mitochondrial SHMT2 provide one-carbon units for nucleotide biosynthesis, regulating DNA methylation and NADPH generation, altered during cancer development. Wang et al. discuss how esophageal squamous cell carcinoma (ESCC) cells adapt to a hypoxic, nutrient-deprived microenvironment by rewiring glucose, lipid, and amino acid metabolism. This metabolic shift ensures survival and proliferation despite adverse conditions, highlighting new avenues for therapeutic intervention. The study identifies metabolic vulnerabilities in ESCC, suggesting that disrupting these adaptive pathways could improve treatment efficacy.

Xie et al. further explore how hypoxia-related genes influence prognosis and immunotherapeutic outcomes in ESCC. They establish an HPRscore based on hypoxia phenotype-related genes, demonstrating its predictive power for patient survival and response to treatment. Notably, the study identifies PKP1 as a potential therapeutic target, showing that its knockdown reduces tumor proliferation and migration. These findings provide valuable insight into how hypoxia-driven metabolic changes affect tumor behavior and immune evasion. Peppicelli et al. focused their research on melanoma cells resistant to *anoikis*, to investigate the metabolic reprogramming within circulating tumor cells (CTCs), with the aim of identifying new metabolic targets of CTCs. They discovered that *anoikis*-resistant melanoma cells in suspension show a metabolic rewiring from a characteristic glycolytic pathway toward a more oxidative metabolism based on the use of glutamine and fatty acids, while re-adhesion of CTCs on the dishes reversed the metabolism to glycolysis. The inhibition of the metabolic switch of CTCs led to a reduction of cell viability and colony formation ability of cells capable of surviving in suspension, offering novel and future strategies of treatment of CTCs and melanoma metastases. Similar metabolic adaptations are evident in colorectal cancer (CRC), where immune evasion is closely tied to metabolic shifts in the tumor microenvironment. Nicolini et al. examine how CRC cells undergo metabolic reprogramming—from enhanced glycolysis to increased lipid synthesis—to create an immunosuppressive microenvironment. They discuss how lactate acidification, driven by the Warburg effect, impairs anti-tumor immune cells and promotes tumor-associated macrophages (TAMs) and regulatory T cells (Tregs). The study also explores the role of genetic mutations (e.g., RAS, EGFR) and microbiota in shaping CRC metabolism, emphasizing the potential for metabolic-targeted therapies in combination with immune checkpoint inhibitors (ICIs). Gao et al. provide a broader perspective on metabolic reprogramming as a key tool for predictive and precision medicine. They highlight how different cancer types rely on distinct metabolic adaptations, necessitating tailored therapeutic approaches. The study underscores the growing importance of metabolic profiling in developing personalized treatments, optimizing immune responses, and overcoming drug resistance.

Ferroptosis, a non-apoptotic form of cell death driven by iron-dependent lipid peroxidation, plays a paradoxical role in cancer. Complex metabolic changes within tumor cells and in the tumor microenvironment further influence the response of tumor cells to ferroptosis. As explored by Zhao et al. ferroptosis can both suppress and promote tumor growth depending on cellular context and regulatory mechanisms. The study highlights potential therapeutic strategies for enhancing ferroptosis sensitivity in cancer cells while also addressing resistance mechanisms. Understanding ferroptosis is critical for optimizing cancer treatments, particularly in combination with immunotherapies and metabolic interventions. Of note, one driver of ferroptosis is lipid metabolism, which also plays a vital role in cancer stem cell (CSC) maintenance. Du et al. examine how CSCs manipulate lipid metabolism to sustain their stemness, resist therapy, and adapt to environmental stress. They describe how CSCs increase fatty acid content for energy, engage in β-oxidation to optimize utilization, and enhance cholesterol synthesis through the mevalonate pathway. Additionally, lipid droplets serve as alternative energy reservoirs, protecting CSCs from oxidative stress. This study underscores the need to target lipid metabolism to weaken CSC resilience and improve treatment outcomes. Lastly, Ping et al. in their original article using a retrospective analysis described the predictive value of altered plasma omega-3 polyunsaturated fatty acids (omega-3 PUFAs) levels for early treatment response, progression free survival, and overall survival in patients with cervical squamous cell carcinoma (CSCC) who underwent concurrent chemoradiotherapy (CCRT). Interestingly, the authors demonstrated that pretreatment plasma omega-3 PUFAs level, may be a promising biomarker for predicting recent response in CSCC, increasing the prognostic significance of serum squamous cell carcinoma antigen (SCC)-Ag level alone, opening to create new prognostic tools for clinicians in CSCC.

Together, these studies reveal how metabolic reprogramming drives tumor growth and progression, contributes to metastasis formation and chemoresistance, and is interconnected with ferroptosis and lipid metabolism. Targeting these metabolic vulnerabilities holds great promise for improving cancer treatment, particularly in combination with immunotherapy and precision medicine strategies.

Author contributions

VA: Conceptualization, Writing–original draft, Writing–review and editing. EG: Conceptualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

References

Comandatore, A., Franczak, M., Smolenski, R. T., Morelli, L., Peters, G. J., and Giovannetti, E. (2022). Lactate Dehydrogenase and its clinical significance in pancreatic and thoracic cancers. *Semin. Cancer Biol.* 86 (Pt 2), 93–100. doi:10.1016/j.semcancer.2022.09.001

Hu, T., Liu, C.-H., Lei, M., Zeng, Q., Li, L., Tang, H., et al. (2024). Metabolic regulation of the immune system in health and diseases: mechanisms and interventions. *Signal Transduct. Target. Ther.* 9, 268. doi:10.1038/s41392-024-01954-6

Nong, S., Han, X., Xiang, Y., Qian, Y., Wei, Y., Zhang, T., et al. (2023). Metabolic reprogramming in cancer: mechanisms and therapeutics. *MedComm (2020)* 4 (2), e218. doi:10.1002/mco2.218

Xia, L., Oyang, L., Lin, J., Tan, S., Han, Y., Wu, N., et al. (2021). The cancer metabolic reprogramming and immune response. *Mol. Cancer* 20 (1), 28. doi:10.1186/s12943-021-01316-8

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 11 January 2024
DOI 10.3389/fphar.2023.1330910

OPEN ACCESS

EDITED BY
Elisa Giovannetti,
VU Medical Center, Netherlands

REVIEWED BY
Chunming Cheng,
University of Oklahoma, United States
Mahrou Vahabi,
VU Medical Center, Netherlands

*CORRESPONDENCE
Yinghui Xu,
xuyinghui@jlu.edu.cn

RECEIVED 31 October 2023
ACCEPTED 27 December 2023
PUBLISHED 11 January 2024

CITATION
Zhao X, Li X and Xu Y (2024), Ferroptosis: a dual-edged sword in tumour growth.
Front. Pharmacol. 14:1330910.
doi: 10.3389/fphar.2023.1330910

Ferroptosis: a dual-edged sword in tumour growth

Xiangye Zhao, Xiaoning Li and Yinghui Xu*

Cancer Center, The First Hospital of Jilin University, Changchun, Jilin, China

Ferroptosis, a recently identified form of non-apoptotic cell death, is distinguished by its dependence on iron-triggered lipid peroxidation and accumulation of iron. It has been linked to various disorders, including the development of tumours. Interestingly, ferroptosis appears to exhibit a dual role in the context of tumour growth. This article provides a thorough exploration of the inherent ambivalence within ferroptosis, encompassing both its facilitation and inhibition of tumorous proliferation. It examines potential therapeutic targets associated with ferroptosis, the susceptibility of cancerous cells to ferroptosis, strategies to enhance the efficacy of existing cancer treatments, the interaction between ferroptosis and the immune response to tumours, and the fundamental mechanisms governing ferroptosis-induced tumour progression. A comprehensive understanding of how ferroptosis contributes to tumour biology and the strategic management of its dual nature are crucial for maximizing its therapeutic potential.

KEYWORDS

ferroptosis, tumour, iron, metabolism, antitumour therapy

1 Introduction

Cell death plays a crucial role in maintaining tissue balance and controlling the unregulated growth of tumour cells (Fuchs and Steller, 2011). However, tumour cells have evolved mechanisms to evade cell death regulation, promoting unchecked cell replication. Ferroptosis, a unique form of non-apoptotic cell death characterised by lipid peroxidation and unstable iron buildup, differs in morphology, physiology, and biochemistry from classical programmed cell death (Dixon et al., 2012; Friedmann Angeli et al., 2014; Stockwell et al., 2017; Hassannia et al., 2019). An increasing body of evidence implicates ferroptosis in the development of various diseases, including the onset and progression of tumours (Tang et al., 2021).

Currently, ferroptosis has emerged as a significant focus in oncology research. Most studies suggest a beneficial role in restraining tumour growth through interactions between ferroptosis and tumours (Table 1), highlighting its potential as a therapeutic target in oncology. Tumour cells can bypass ferroptosis to promote their own growth by employing defense mechanisms, such as activating System Xc-, boosting glutathione peroxidase 4 (GPX4) activity, and altering glutathione (GSH) metabolism (Dixon et al., 2012). Disrupting or eliminating these mechanisms can trigger ferroptosis and hinder tumour expansion. Additionally, regulating lipid metabolism and iron metabolism pathways can induce ferroptosis, thereby inhibiting tumour growth (Martinez-Outschoorn et al., 2017; Wolpaw and Dang, 2018; Sang et al., 2019; Zou et al., 2020; Lei et al., 2022). Ferroptosis inducers presents a promising approach to curbing tumour growth. Furthermore, combining ferroptosis with chemotherapy, radiotherapy, targeted therapy, or immunotherapy shows potential to enhance antitumour effectiveness and overcome drug resistance (Yamaguchi et al., 2013; Yu et al., 2015; Wang et al., 2019a; Ye et al., 2020) (Table 3).

Consequently, ferroptosis holds the potential to reshape tumour treatment strategies and improve clinical outcomes.

However, it's important to note that ferroptosis can also have a negative impact on promoting tumour growth (Table 2). Through various pathways, such as ferroptosis metabolic pathways (Dai et al., 2020), inflammation-related pathways (Tang et al., 2021; Li and Li, 2020), antigen presentation process (Legrand et al., 2019), and the modulation of immune cell function (Wang et al., 2020; Luo et al., 2021), ferroptosis has been identified as a promoter of tumour growth. This article offers a comprehensive review of ferroptosis's dual role in both promoting and inhibiting tumours, laying a theoretical foundation for further research into ferroptosis in tumour treatment. A thorough understanding of this duality allows for maximizing the clinical effectiveness of ferroptosis-based treatments while minimizing potential adverse effects.

2 The mechanism of ferroptosis

Ferroptosis is an iron-dependent type of programmed cell death caused by excessive polyunsaturated fatty acids (PUFAs). PUFAs are essential components of cell membrane phospholipid layers, significantly influencing membrane structure, fluidity, and permeability (Luo et al., 2021). The main mechanism behind ferroptosis is that when the balance between cellular oxidation and the antioxidant system is disrupted (Kuang et al., 2020), PUFAs in the cell membrane undergo oxidation, forming hydroxyl radicals catalyzed by Fe2+ or ester oxygenase (Figure 1). This process creates lipid peroxides, leading to cellular ferroptosis (Yang and Stockwell, 2016; Yang et al., 2016; Stockwell et al., 2017). The products of lipid peroxidation in cell membranes act as a source of reactive oxygen species (ROS), triggering increased cellular oxidative stress that damages DNA, proteins, or lipids, ultimately resulting in cellular ferroptosis (Trachootham et al., 2009; Reczek and Chandel, 2018).

3 Inhibitory effect of ferroptosis on tumour growth

Ferroptosis can inhibit tumour growth. The inhibitory effect of ferroptosis on tumour growth is discussed in terms of regulating lipid metabolism, amino acid metabolism, and iron metabolism. It has been reported that inhibition of ferroptosis can also promote tumour growth.

3.1 Inhibition of ferroptosis through regulating lipid metabolism thereby promoting tumour growth

Lipid metabolism is closely related to ferroptosis. Lipid peroxidation is a free radical-driven reaction that primarily affects unsaturated fatty acids in cell membranes (Tang et al., 2021). Acyl-coenzyme A synthetase long chain family member 4 (ACSL4) and Lysophosphatidylcholine acyltransferase 3 (LPCAT3) are key regulators of PUFA-PLs synthesis. Phospholipase A2 (PLA2) cleaves PUFAs into free PUFAs and lysophospholipids (Tang et al., 2021). ACSL4 catalyzes the attachment of free PUFAs to coenzyme A to generate PUFA-CoAs, which are re-esterified and incorporated into phospholipids (PLs) by LPCAT3 to form PUFA-containing phospholipids (PUFA-PLs) (Lei et al., 2022; Doll et al., 2017; Dixon et al., 2015). Due to the presence of a bis-allylic moieties of PUFAs, PUFA-PLs are especially susceptible to peroxidation (Conrad and Pratt, 2019).

The downregulation of PUFAs in tumour cells is associated with ferroptosis evasion and the promotion of tumour growth (Lei et al., 2022) (Figure 1; Table 1). For instance, in renal cell carcinoma (RCC), reducing peroxidized PUFAs through the adipokine chemerin allows tumour cells to avoid ferroptosis and supports RCC growth (Tan et al., 2021). KRAS mutations in lung cancer also increase the expression of Acyl-coenzyme A synthetase long chain family member 3 (ACSL3) to reprogram lipid metabolism, promote Monounsaturated fatty acids-phospholipids (MUFA-PL) biosynthesis and ferroptosis resistance, and facilitate lung cancer progression (Friedmann Angeli et al., 2014; Padanad et al., 2016).

In human tumour cell lines, cells in a mesenchymal-like state show selective susceptibility to ferroptosis (Sang et al., 2019). Research indicates that mesenchymal tumour cells exhibit higher enzyme activity, promoting PUFAs synthesis and lipid peroxide production, ultimately leading to ferroptosis occurrence (Viswanathan et al., 2017; Xu et al., 2019) (Figure 1). Specific overexpression of elongation of very long-chain fatty acid protein 5 (ELOVL5) and fatty acid desaturase 1 (FADS1) in mesenchymal gastric cancer cells, both involved in PUFAs synthesis, makes cancer cells particularly susceptible to ferroptosis (Lee et al., 2020) (Table 1).

3.2 Escaping ferroptosis by interfering with the antioxidant system and affecting amino acid metabolism contributes to tumour growth

Ferroptosis is associated with disruption of the antioxidant system and amino acid metabolism (Figure 1). GSH-GPX4 is involved in the intracellular antioxidant system and is a key factor influencing the onset of ferroptosis. GPX4, the only member of the GPX protein family capable of converting phospholipid hydroperoxides into phosphatidyl alcohols, prevents lipid peroxidation, thus restraining ferroptosis and supporting tumour growth (Ursini et al., 1982; Brigelius-Flohé and Maiorino, 2013; Seibt et al., 2019; Brigelius-Flohé and Flohé, 2020). GSH, a co-factor for GPX4, is synthesized from glycine, glutamate, and cysteine, with cysteine being the rate-limiting precursor (Forman et al., 2009; Koppula et al., 2018; Friedmann Angeli et al., 2019).

Cysteine/glutathione antiporter, also known as System Xc-, is an important intracellular antioxidant element. System Xc-is a transmembrane protein, consisting of SLC7A11 and SLC3A2, responsible for the exchange of extracellular cystine with intracellular glutamate (Bannai, 1986; Conrad and Sato, 2012). SLC7A11 mediates cystine/glutamate antotransporter protein activity and SLC3A2 maintains SLC7A11 protein stability (Bannai, 1986; Sato et al., 1999; Conrad and Sato, 2012; Koppula

TABLE 1 Inhibitory effect of ferroptosis on tumour growth by regulating metabolisms.

Mechanism category		Mode of functioning	Function	*In vitro*/vivo	Model	Tumour type	References
Regulate lipid metabolism		Chemerin	Downregulate peroxidized PUFAs, evade ferroptosis, support RCC growth	*In vitro*	ccRCC model systems	RCC	Tan et al. (2021)
		KRAS mutation	Increase the expression of ACSL3, promote MUFA-PL biosynthesis, ferroptosis resistance, facilitate lung cancer progression	*In vitro* and vivo	A549 and H460 NSCLC cells; *KrasG12D* (*tet-op-KrasG12D*) mice	Lung cancer	Friedmann Angeli et al. (2014), Padanad et al. (2016)
		Mesenchymal tumour cells	Overexpression of ELOVL5 and FADS1, involve PUFAs synthesis, render cancer cells susceptible to ferroptosis	*In vitro*	Mesenchymal-type GCs (including Hs746T, SNU-484, SNU-668, YCC-16, and SNU-216 cells)	Mesenchymal GC cell	Lee et al. (2020)
Regulate amino acid metabolism	GPX4	RSL3	Inactivate GPX4, induce ferroptosis, inhibit tumour growth	*In vivo*	Xenograft mouse model of BJeLR cell origin	Fibroblastic tumour	Fuchs and Steller (2011)
		FIN56	Deplete GPX4 protein, block coenzyme Q10 production, induce ferroptosis	*In vitro*	HT-1080 fibrosarcoma cells and BJeLR cells	Fibroblastic tumour	Badgley et al. (2020)
	GSH	Kras/TP53 mutation	Deplete cystine or cysteine, induce ferroptosis, inhibit tumour growth	*In vitro* and vivo	Human PDAC cell lines and KPC mice	Kras/TP53-driven PDACs	Badgley et al. (2020)
		Sulfasalazine	Inhibits System Xc- and diminish cellular glutathione, induce ferroptosis, inhibit pancreatic cancer growth	*In vitro* and vivo	Human pancreatic cancer cell lines MIA PaCa-2 and PANC-1; mice bearing actively growing MIA PaCa-2 and PANC-1 subcutaneous xenografts	Pancreatic cancer	Lo et al. (2010)
	SLC7A11	Overexpression of SLC7A11	Augment cystine uptake and GSH synthesis, inhibit ferroptosis, promote tumour growth	*In vitro*	Human PDAC cell lines	PDACs	Badgley et al. (2020)
		SLC7A11, KRAS-mutant	Mediate cystine uptake, decrease ROS production, inhibit ferroptosis, promote lung adenocarcinoma proliferation and migration	*In vitro* and vivo	NSCLC A549 cells; lung cell lines (HPNE and HPNE/KRAS; H522 and H522/KRAS); LSL-KrasG12D mouse	KRAS-mutant LUAD	Hu et al. (2020)
		siRNA	Downregulation of SLC7A11, induce ROS accumulation, promoted ferroptosis, inhibit lung cancer cell proliferation	*In vitro*	A549 cell	Lung cancer	Huang et al. (2018)
		XAV939	Downregulate SLC7A11 through lncRNA, induce ferroptosis, inhibit NSCLC	*In vitro*	NCI-H1299 NSCLC cell line	NSCLC	Yu et al. (2019)
Regulate iron metabolism		Artemisinin	Absorb and release iron, heighten their susceptibility to ferroptosis, inhibit tumour growth	*In vitro* and vivo	Mouse embryonic fibroblasts and human osteosarcoma HT1080 cells; athymic nude *Foxn1nu/Foxn1* mice of GPX4 knockout in H292 cells	Lung cancer	Chen et al. (2020)
		Iron metabolism	Increase iron uptake, decrease iron efflux pump FPN, promote the onset of ferroptosis, inhibit tumour growth	*In vitro* and vivo	HGSOC tumour initiating cells; mice inoculated IP with FPN-tet-on FTt cells	HGSOC	Basuli et al. (2017)
		Iron oxide nanoparticles	Release intracellular iron, increase iron and ROS production, induce ferroptosis and hinder tumour growth	*In vitro* and vivo	A2780 and ACP cells; mice of H22 liver cancer model	Ovarian cancer; liver cancer	Ma et al. (2017)

PUFAs, polyunsaturated fatty acids; RCC, renal cell carcinoma; ACSL3, Acyl-coenzyme A synthetase long chain family member 3; MUFA-PL, Monounsaturated fatty acids-phospholipids; NSCLC, non-small cell lung cancer; ELOVL5, elongation of very long-chain fatty acid protein 5; FADS1, fatty acid desaturase 1; GC, gastric cancer; GPX4, glutathione Peroxidase 4; PDAC, pancreatic ductal adenocarcinomas; GSH, glutathione; LUAD, lung adenocarcinoma; HGSOC, high-grade serous ovarian cancer; EGFR, epidermal growth factor receptor; FPN, ferroportin; ROS, reactive oxygen species.

TABLE 2 Promotional effect of ferroptosis on tumour growth.

Mechanism category	Pathway	Mechanisms	Effect	References
Regulate ferroptosis metabolic pathways	Lipid metabolism pathway	High expression of ACSL4, induce ferroptosis, promote fibrosis and hepatocellular cell formation	Promote tumour growth in hepatocellular carcinoma	Tsurusaki et al. (2019), Li et al. (2020a), Luo et al. (2020), Ndiaye et al. (2020), Qi et al. (2020)
	8-OHdG-TMEM173 pathway	GPX4 deletion or an iron-rich diet; induce ferroptosis, activate and migrate macrophages	Promote the development of Kras-driven pancreatic cancer	Cooke et al. (2003), Dai et al. (2020)
Trigger inflammation-related pathways	COX-2/PGE2 pathway	LPS induce ferroptosis, impact on NK cell activity, impede cDC1s and Immunosuppression	Evade the immune system, tumour immunotherapy resistance	Yang et al. (2014), Li and Li (2020), Tang et al. (2021)
	IFN-γ secretion by T cells	Upregulate PD-L1 expression, trigger IFN-γ-related adaptive immune resistance, evade the immune response	Promote tumour progression	Dorand et al. (2016), Tang et al. (2019), Zhang et al. (2019), Li et al. (2021)
	CD36-mediated ferroptosis	T cells take up fatty acids through CD36, induce ferroptosis, diminish the production of cytotoxic cytokines	Impair the antitumour activity of CD8 (+) T cells, promote tumour growth	Su et al. (2020), Wang et al. (2020)
Cancer cells dying from ferroptosis compromise antitumour immune responses	Tumour-associated antigen presentation process	Hinder DCs maturation, phagocytosis, influence neighbouring tumour cells to immunogenic death	Reduce antitumour immunity	Wiernicki et al. (2022)
	PMN-MDSCs	PMN-MDSCs undergo ferroptosis, promote the secretion of immunosuppressive molecules, Inhibit T cell activity	Promote tumour growth	Kim et al. (2022)

LPS, lipopolysaccharide; DCs, dendritic cells; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; IFN-γ, interferon-γ; PD-L1, programmed death ligand-1; PMN-MDSCs, Polymorphonuclear myeloid derived suppressor cells; COX-2, cyclooxygenase-2; PGE2, Prostaglandin E2.

et al., 2018). Therefore, inhibition of System Xc—leads to an imbalance of the antioxidant system thereby causing ferroptosis.

The SLC7A11-GSH-GPX4 system plays a crucial role as the main defense against ferroptosis in tumours (Dixon et al., 2012; Friedmann Angeli et al., 2014; Stockwell et al., 2017; Hassannia et al., 2019). GPX4 is a central control factor of ferroptosis, and intracellular GSH content directly affects GPX4 activity (Maiorino et al., 2018). Ferroptosis inducers have demonstrated efficacy in tumour cells by directly binding to and inhibiting GPX4 (Table 1). The ferroptosis activator RSL3, an inhibitor of the antioxidant system, directly inactivates GPX4 and inhibits tumour growth in a xenograft mouse model of BJeLR cell origin (Fuchs and Steller, 2011). FIN56, induces ferroptosis in HT1080 cells by depleting GPX4 protein as well as activating farnesyl-diphosphate farnesyltransferase 1 (FDFT1/SQS) to block coenzyme Q10 production (Shimada et al., 2016). Kras/TP53-driven pancreatic tumours induce ferroptosis and inhibit tumour growth by depleting cystine or cysteine through cyst (e) inase (Badgley et al., 2020). Sulfasalazine inhibits System Xc- and diminishes cellular glutathione, leading to the excessive buildup of lipid peroxides in tumour cells, inducing ferroptosis. This demonstrates an antitumour effect in pancreatic cancer (Lo et al., 2010).

In pancreatic ductal adenocarcinomas (PDACs), overexpressing SLC7A11 inhibits ferroptosis by increasing cystine uptake and GSH production, promoting tumour growth (Badgley et al., 2020). In patients with KRAS-mutant lung adenocarcinoma (LUAD), SLC7A11 mediates cystine uptake, decreases ROS production and thus promotes lung adenocarcinoma proliferation and migration (Hu et al., 2020; Liu et al., 2020; Chen et al., 2021; Lou et al., 2021). These suggest that SLC7A11 overexpression is positively associated with tumour progression. In contrast, downregulation of SLC7A11 gene expression by siRNA induced ROS accumulation, promoted ferroptosis and inhibited A549 cell proliferation (Huang et al., 2018). XAV939 induces ferroptosis and inhibits non-small cell lung cancer (NSCLC) by downregulating SLC7A11 through long non-coding RNA (lncRNA) (Yu et al., 2019).

3.3 Inhibiting tumour growth by affecting iron metabolism pathways to induce ferroptosis

Iron metabolism is a necessary process for ferroptosis. Iron overload induces ferroptosis through the Fenton reaction, which generates a large number of hydroxyl radicals and triggers a strong oxidative stress response that produces a large number of ROS (Conrad and Pratt, 2019). Transferrin (TFR) and divalent metal ion transporter protein-1 (DMT1) take up extracellular iron, and ferroportin (FPN) transfers intracellular iron to the outside of the cell (Figure 1). These proteins collaborate to maintain intracellular iron homeostasis (Seiler et al., 2008; Mandal et al., 2010). Iron is also essential for participation in lipid peroxidation, and lipoxygenase (LOXs) and cytochrome P450 oxidoreductase (PORs) require iron for catalysis (Jiang et al., 2021a).

Tumour cells show an increased demand for iron and display heightened oxidative metabolic processes compared to non-malignant cells (Martinez-Outschoorn et al., 2017; Wolpaw and

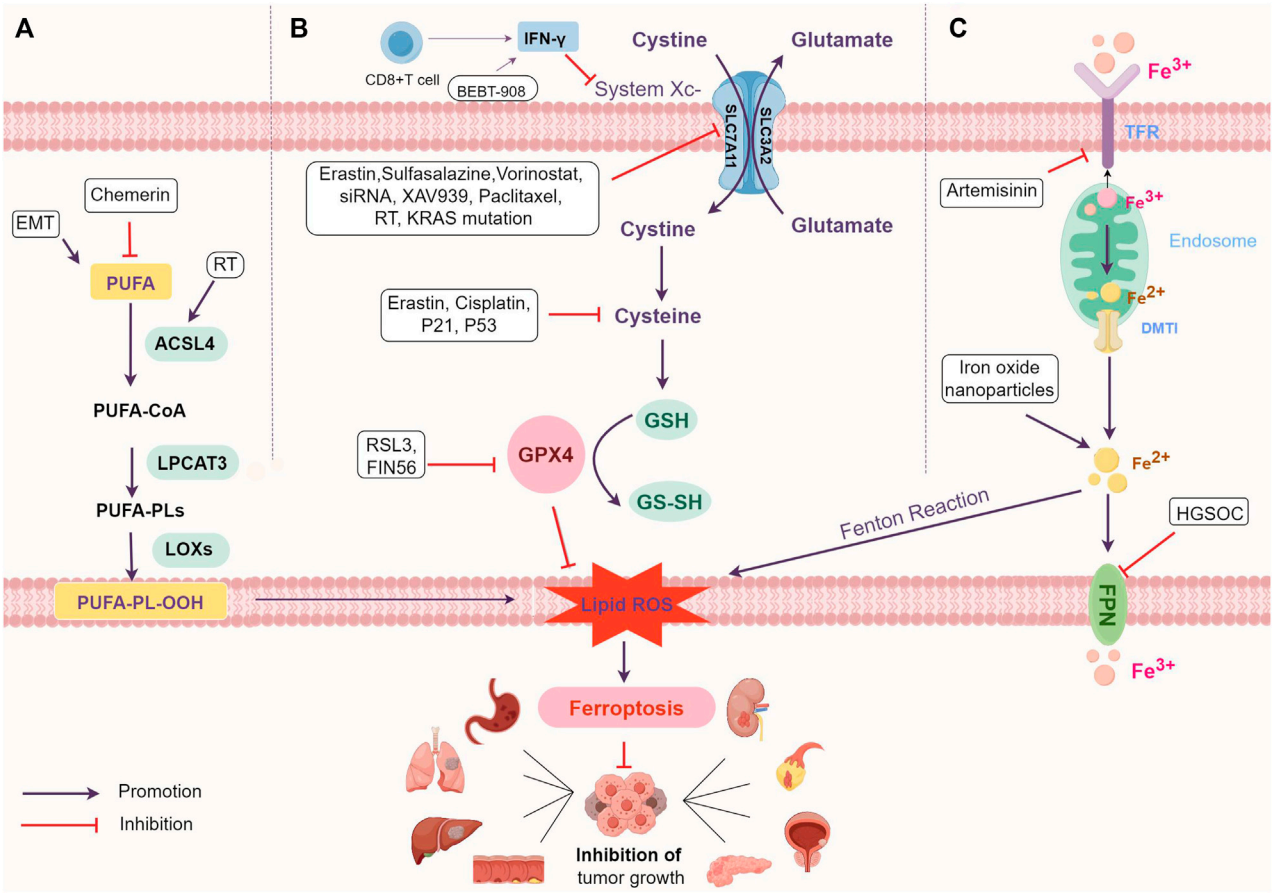


FIGURE 1
The mechanism of ferroptosis and main regulatory mechanism. **(A)** Regulate lipid metabolism pathways. **(B)** Interfere with amino acid metabolism pathways. **(C)** Influence iron metabolism pathways. Abbreviations: PUFAs, polyunsaturated fatty acids; PUFA-PLs, PUFA-containing phospholipids; RT, radiation therapy; ACSL4, Acyl-coenzyme A synthetase long chain family member 4; LPCAT3, Lysophosphatidylcholine acyltransferase 3; LOXs, lipoxygenase; EMT, epithelial-mesenchymal transition; ROS, reactive oxygen species; IFN-γ, interferon-γ; GSH, glutathione; GPX4, glutathione Peroxidase 4; TFR, transferrin; DMT1, divalent metal ion transporter protein-1; FPN, ferroportin; HGSOC, high-grade serous ovarian cancer.

Dang, 2018; Zou et al., 2020). The level of intracellular iron impacts sensitivity to ferroptosis. Elevated intracellular iron in tumour cells leads to higher production of ROS and lipid metabolites, aiding ferroptosis development (Table 1; Figure 1). Tumours abundant in iron, like hepatocellular carcinoma (HCC) and breast cancer, or those rich in ROS like lung cancer, along with tumours with increased iron use and overload, demonstrate heightened sensitivity to ferroptosis (Ma et al., 2016).

Iron oxide nanoparticles, breaking down within the acidic tumour cell environment, release intracellular iron, leading to increased iron and ROS production, ultimately inducing ferroptosis and hindering tumour growth (Ma et al., 2017). Artemisinin prompts lung cancer cells to absorb and release substantial iron amounts, heightening their susceptibility to ferroptosis (Chen et al., 2020). In high-grade serous ovarian cancer (HGSOC), elevated iron intake and reduced expression of the iron efflux pump FPN result in excessive intracellular iron, further promoting ferroptosis onset (Basuli et al., 2017).

Thus, adjusting iron levels—enhancing iron intake, reducing storage, and restricting iron release—holds potential to promote ferroptosis and impede tumour growth.

4 Promotional effect of ferroptosis on tumour growth

Ferroptosis promotes tumour growth and progression by regulating metabolic pathways, triggering inflammation-associated immunosuppression, and cancer cells dying from ferroptosis to compromise antitumour immune responses (Figure 2; Table 2).

4.1 Ferroptosis promotes pancreatic tumour and hepatocellular carcinoma development and progression by regulating metabolic pathways

ACSL4, an enzyme involved in synthesizing phospholipids from PUFAs plays a crucial role in ferroptosis (Dixon et al., 2015; Yuan et al., 2016). In HCC, ACSL4 expression surpasses that in normal liver tissue, and hepatocyte ferroptosis relies on ACSL4, suggesting its involvement in HCC development (Ndiaye et al., 2020) (Figure 2). In a mature toxic injury model, intervention in ACSL4-dependent

ferroptosis notably suppressed HCC progression, likely due to reduced fibrosis in the absence of ACSL4 (Tsurusaki et al., 2019; Li et al., 2020a; Luo et al., 2020; Qi et al., 2020) (Table 2).

Studies suggest that an iron-rich diet or Gpx4 depletion can induce ferroptosis in tumour cells, releasing 8-hydroxy-2′-deoxyguanosine (8-OHdG). This activates the TMEM173/STING-dependent DNA sensor pathway, thereby promoting pancreatic tumour development (Dai et al., 2020; Cooke et al., 2003) (Table 2). TMEM173 regulates inflammation and immune responses, associated with macrophage activation and migration triggered by 8-OHdG (Barber, 2015; Motwani et al., 2019). Tumour-associated macrophages (TAMs) influence early stages of pancreatic tumour formation, activating KRAS-driven PDACs (Mielgo and Schmid, 2013; Zhu et al., 2017). GPX4 deletion or a high-iron diet increases acinar-to-duct metaplasia, ductal lesions, stromal reactions, metastasis, as well as expression of Ki67 and a ferroptosis marker (prostaglandin-endoperoxide synthase 2, PTGS2) in the pancreas (Yang et al., 2014). These studies collectively suggest that ferroptosis contributes to promoting tumour growth.

4.2 Ferroptosis inhibits antitumour immunity by triggering inflammation-related pathways, thereby promoting tumour growth

4.2.1 COX-2/PGE2 pathway

PTGS2, also known as cyclooxygenase-2 (COX-2), is a marker of ferroptosis (Yang et al., 2014). Lipopolysaccharide (LPS) induces lipid peroxidation and PTGS2 expression, which activates ferroptosis (Li et al., 2020b). Ferroptosis detrimentally influences tumour growth by specifically enhancing the COX-2/PGE2 pathway, leading to inflammation-associated immunosuppression (Tang et al., 2021; Li and Li, 2020; Yang et al., 2014) (Figure 2; Table 2). Prostaglandin E2 (PGE2), an inflammatory and immunosuppressive agent, undermines immune control mediated by conventional type 1 dendritic cells (cDC1s) and allows tumour cells to evade the immune system, resulting in immunotherapy resistance (Goodwin and Ceuppens, 1983; Wang and DuBois, 2015). Further studies reveal that PGE2 limits the infiltration of cDC1s into the tumour site by suppressing the chemokines CCL5 and XCL1, secreted by NK cells. Apart from impacting NK cell activity, PGE2 directly hampers cDC1s by reducing levels of tumour-recruited chemokine receptors (Böttcher et al., 2018; Zhang et al., 2022).

4.2.2 IFN-γ-related immune resistance

Ferroptosis can induce immune response through ferroptotic tumour cells, exposing danger-associated molecular patterns (DAMPs) and releasing tumour-associated antigens (TAAs), which stimulate T cells to secrete Interferon-γ (IFN-γ) (Tang et al., 2019; Zhang et al., 2019). Though IFN-γ secretion by T cells induced by ferroptosis effectively eliminates tumour cells, it can also elevate programmed death ligand-1 (PD-L1) levels, triggering IFN-γ-related adaptive immune resistance (Dorand et al., 2016; Li et al., 2021) (Figure 2; Table 2). This phenomenon affects the antitumour efficiency of immune cells, contributing to tumour progression. PD-L1, an immunosuppressive molecule, is overexpressed in various cancers like gastric, kidney, pancreatic, bladder cancers, among others, and is linked to poor clinical prognosis (Ohigashi et al., 2005; Hamanishi et al., 2007; Nakanishi et al., 2007; Hou et al., 2014; Wang et al., 2016). Binding of PD-L1 to PD-1 depletes effector T cells, allowing tumour cells to evade the immune response, ultimately promoting tumour growth.

4.2.3 CD36-mediated ferroptosis

The study noted notably elevated fatty acid levels in tumour tissues compared to normal skin or spleen tissues (Zhang et al., 2018). CD36, involved in DCs' antigen presentation function, emerged as a T cell function regulator. The study showed that within the tumour microenvironment, CD8 (+) T cells uptake fatty acids via CD36, impairing their antitumour functionality by inducing ferroptosis and reducing cytotoxic cytokine production (Wang et al., 2020; Su et al., 2020) (Table 2). Effective blocking of CD36-mediated ferroptosis restored CD8 (+) T cells' antitumour activity. Moreover, inhibiting CD36-mediated ferroptosis alongside immunotherapy notably enhanced the antitumour effects of CD8 (+) T cells (Wang et al., 2020).

4.3 Cancer cells dying from ferroptosis affects the antigen presentation process and produces immunosuppressive effects, thus compromising antitumour immune responses

Immunogenicity refers to antigens' ability to provoke an immune response, involving immune effector molecule production, activation, proliferation, and differentiation (Aaes and Vandenabeele, 2021). Immunogenic cell death occurs when tumour-associated antigens (TAAs) are processed and presented on the surfaces of tumour cells and dendritic cells (DCs) (Legrand et al., 2019; Yatim et al., 2017). Research suggests that ferroptosis may possess immunomodulatory traits influencing neighbouring tumour cells' response to immunogenic death (Blüml et al., 2005; Blüml et al., 2009). Observations indicate that cancer cells undergoing ferroptosis impede TAA processing and presentation. Co-culturing ferroptotic cancer cells with DCs revealed that these "initial" iron-depleted cells hinder DCs maturation, phagocytosis, and antigen-cross-presentation (Wiernicki et al., 2022) (Figure 2; Table 2). Consequently, ferroptosis negatively impacts antigen-presenting cells, influencing adaptive immune responses and antitumour immunity.

Pathologically activated neutrophils, termed polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), negatively impact the regulation of antitumour immunity (Condamine et al., 2015; Wang et al., 2019b; Ostrand-Rosenberg et al., 2020). Their presence in cancer patients correlates with poor prognoses in immunotherapy (Zhou et al., 2018). Studies indicate that genes linked to PMN-MDSCs are enriched in the ferroptosis pathway, suggesting their susceptibility to ferroptosis (Zhang et al., 2020). Observations reveal that PMN-MDSCs can undergo ferroptosis within the tumour microenvironment. While ferroptosis reduces PMN-MDSC numbers, cancer cells undergoing ferroptosis release immunosuppressive molecules hindering T cells, reducing the effectiveness of antitumour therapy (Kim et al., 2022) (Table 2). Conversely, inhibiting ferroptosis decreases immunosuppressive activity, significantly impeding tumour growth. In immune-active mice, ferroptosis inhibition abolishes PMN-MDSCs'

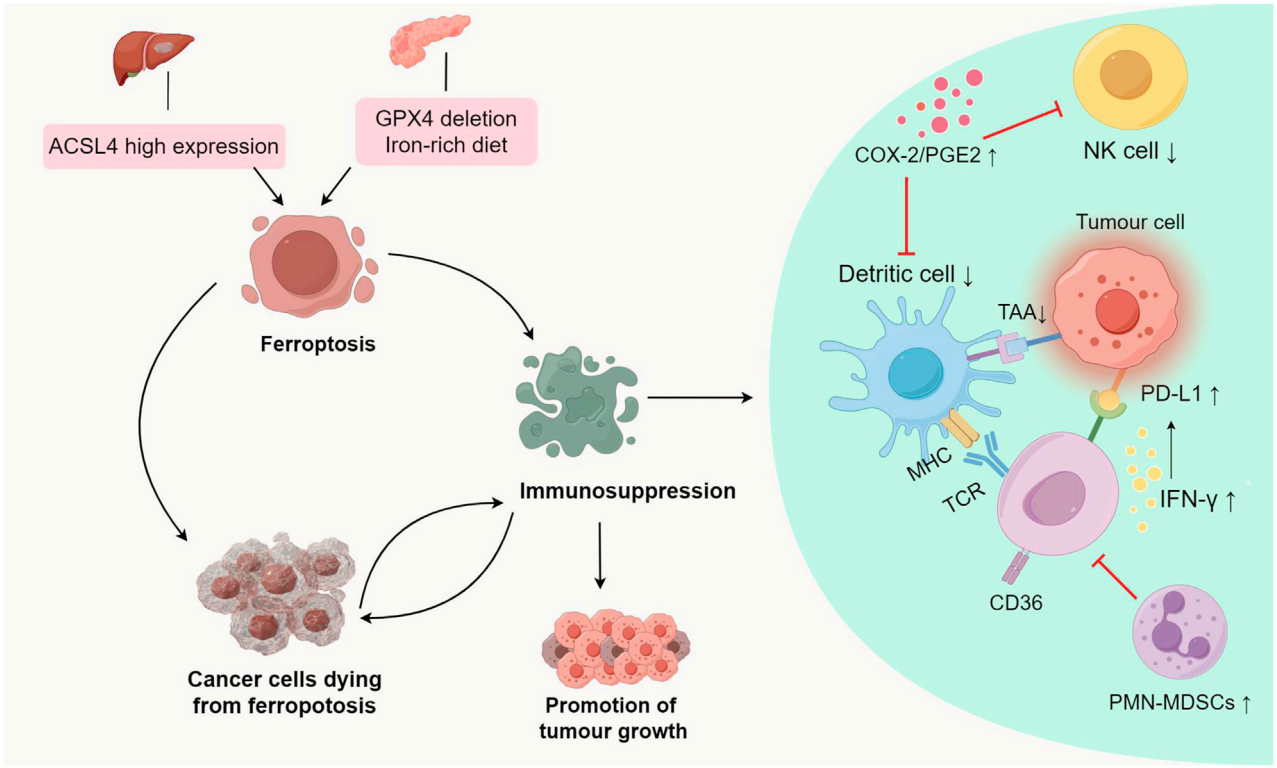


FIGURE 2
Promotional effect of ferroptosis on tumour growth.ACSL4-dependent ferroptosis promotes hepatocellular carcinoma development. A diet rich in iron or GPX4 depletion can induce ferroptosis, thereby promoting the development of pancreatic tumours. Ferroptosis inhibits antitumour immunity by triggering inflammation-related pathways, and influencing antigen presentation processes and the production of immunosuppressive molecules, thereby promoting tumour growth. Abbreviations: ACSL4, Acyl-coenzyme A synthetase long chain family member 4; GPX4, glutathione Peroxidase 4; PGE2, Prostaglandin E2; COX-2, cyclooxygenase-2; TAAs, tumour-associated antigens; IFN-γ, interferon-γ; PD-L1, Programmed cell death ligand 1; MHC, Major Histocompatibility Complex; PMN-MDSCs, polymorphonuclear myeloid derived suppressor cells.

suppressive activity on T cells and synergizes with immune checkpoint inhibitors to halt tumour progression (Kim et al., 2022). Hence, targeting ferroptosis in PMN-MDSCs holds promise as a future therapeutic avenue.

5 The role of ferroptosis in different cancer therapies, such as chemotherapy, immunotherapy, radiotherapy and reversal of tumour resistance

Currently, numerous studies corroborate the synergistic effect of ferroptosis combination therapy in bolstering the efficacy of antitumour treatment. However, evidence supporting the inhibitory effect of combination regimens remains relatively scarce. We've compiled studies showcasing the potential of combined ferroptosis therapy to heighten antitumour efficacy and surmount drug resistance (Table 3). Further pertinent research is warranted to build upon this foundation.

5.1 Ferroptosis combination with chemotherapy

Combining chemotherapeutic agents with ferroptosis inducers amplifies anticancer effects, as these drugs themselves can induce ferroptosis in tumour cells. For instance, the ferroptosis inducer erastin notably enhances cisplatin's efficacy across various tumour types by antagonizing system Xc- or GPX4 functions (Yamaguchi et al., 2013; Yu et al., 2015). Studies illustrate that erastin combined with cisplatin impedes ovarian cancer progression via a ROS-mediated mechanism, augmenting cisplatin's antitumour impact (Cheng et al., 2021) (Table 3). Moreover, cisplatin lowers GSH levels in tumour cells, compromising GPX4 function and triggering ferroptosis in NSCLC cells (Guo et al., 2018). Paclitaxel (PTX) reduces SLC7A11 expression, promoting ferroptosis and retarding colorectal carcinoma cell growth (Lv et al., 2017). The synergy of PTX with RSL3 induces ferroptosis in mutant p53 hypopharyngeal squamous carcinoma (Ye et al., 2019) (Figure 1; Table 3).

5.2 Ferroptosis combination with radiation therapy

Radiation therapy (RT) has been linked to inducing ferroptosis in tumours through diverse pathways (Lei et al., 2020), such as ROS production, GSH depletion, ACSL4 upregulation, and SLC7A11 inhibition (Azzam et al., 2012; Lei et al., 2020) (Figure 1). RT generates surplus ROS and triggers ACSL4 expression by breaking down cellular water, leading to PUFA peroxidation and ferroptosis (Ye et al., 2020). Additionally, RT can promote ferroptosis by suppressing SLC7A11 expression, thereby enhancing radiosensitivity (Lang et al., 2019). Ferroptosis inducers synergize with RT in tumour treatment. For instance, erastin and salazopyridine enhance NSCLC sensitivity to RT (Lei et al., 2020) (Figure 1; Table 3).

5.3 Ferroptosis combination with immunotherapy

The immune system wields significant influence over both tumour development and treatment. Recent research highlights ferroptosis as a factor impeding tumour growth by modulating the immune response (Wang et al., 2019a). Combining immune checkpoint inhibitors with ferroptosis inducers enhances immunotherapy efficacy (Jiang et al., 2021b). Ferroptosis, by recruiting and activating immune cells within the tumour environment, serves as a foundation for using ferroptosis inducers to augment immunotherapy. Ferroptotic tumour cells release DAMPs and trigger Major Histocompatibility Complex (MHC) class I molecule expression, activating T cells and macrophages (Wen et al., 2019). In the tumour microenvironment, CD8 (+) T cells produce IFN-γ, downregulating SLC7A11 expression, reducing cystine uptake, fostering lipid peroxide accumulation, and inducing ferroptosis in Melanoma and ovarian cancer cells (Wang et al., 2019a). The ferroptosis inducer BEBT-908 triggers ferroptosis, elevates MHC class I molecule expression, and activates the IFN-γ signalling pathway in Colorectal cancer (CRC), human diffuse large B-cell lymphoma, and lung cancer, bolstering the body's immune response and exerting antitumour effects (Fan et al., 2021) (Figure 1; Table 3).

5.4 Ferroptosis reverses resistance to tumour therapy

Tumour drug resistance presents a formidable treatment challenge, often reinforced by tumour cells suppressing ferroptosis (Lu et al., 2017). Survival of drug-resistant cells often hinges on GPX4 (Hangauer et al., 2017). Inducing ferroptosis can reverse resistance to conventional chemotherapy, targeted therapy, and immunotherapy. In colon cancer, Lipocalin 2 (LCN2) overexpression leads to resistance to 5-fluorouracil. Targeted inhibition of LCN2 by anti-LCN2 monoclonal antibody (3D12B2) increases intracellular iron levels, decreases GPX4 expression, and induces ferroptosis in tumor cells, thereby overcoming resistance (Chaudhary et al., 2021) (Table 3). EGFR-mutated lung cancer cells, facing acquired drug resistance, exhibit heightened sensitivity to ferroptosis inducers. Vorinostat triggers ferroptosis in resistant cells by inhibiting System Xc- and SLC7A11 expression (Zhang et al., 2021) (Table 3). These findings underscore ferroptosis' therapeutic potential in combatting drug resistance.

6 Future perspective and conclusion

We have summarized multiple studies delving into the interplay between ferroptosis and tumour growth, aiming to grasp their relationship comprehensively and provide insights for targeted therapeutic strategies. It's vital to decipher how to counteract ferroptosis' role in promoting tumour growth while harnessing its therapeutic potential. One study proposed RCH NPs, a self-amplifying nanomedicine, aiming to optimize therapeutic efficacy in tumours by addressing ferroptosis' dual nature. RCH NPs displayed robust ferroptotic damage and bolstered the immune response, enhancing ferroptosis' positive effects in inhibiting tumour growth. They also mitigated inflammation-linked immunosuppression and IFN-γ-associated adaptive immune resistance, countering ferroptosis' negative impact on immunotherapy (Zhang et al., 2022). More research is anticipated to design effective therapies that balance ferroptosis' dual effects on tumour growth.

Yet, ongoing research on ferroptosis remains in its early stages, leaving unanswered queries. Most studies investigating ferroptosis and its tumour association have relied on cellular and animal models, lacking validated clinical evidence. For instance, though targeting GPX4, a crucial component of the ferroptosis defense system, might theoretically restrain tumour growth, GPX4 is essential for life, with studies suggesting its loss heightens mortality rates in mice (Yant et al., 2003). Hence, it's crucial to ascertain the potential harm to normal tissue due to GPX4 inhibitors. Despite numerous potential targets linked to tumours and ferroptosis, it's unclear which of these findings can transition into clinical investigations.

Furthermore, there's a lack of research examining the effectiveness and safety of drugs intended to target ferroptosis. For instance, inhibiting SLC7A11 has shown potential in triggering tumour ferroptosis and reversing tumour resistance without observable impact on the development and survival of mice (Sato et al., 2005). However, this treatment may not be effective for tumours not reliant on the System Xc-. Ferroptosis is linked to the onset and progression of various diseases, extending beyond cancer to include degenerative conditions (Stockwell et al., 2017). Therefore, it's crucial to develop tailored therapies inducing ferroptosis in tumours while avoiding systemic adverse reactions. Combination therapies involving ferroptosis inducers and RT have shown safety in preclinical studies, but there are indications that ferroptosis might also contribute to radiation-induced damage in normal tissues (Su et al., 2022). Hence, further research is necessary to understand the impact of ferroptosis inducers on normal tissues and identify the patient population most likely to benefit from these treatments.

Finally, there's a notable absence of biomarkers available for assessing ferroptosis within the human body. Identifying suitable biomarkers would significantly aid in in vivo studies and clinical monitoring. Discovering predictive biomarkers capable of

TABLE 3 Synergistic effect of ferroptosis combination therapy.

Category		Mode of functioning	Mechanism	*In vitro*/vivo	Model	Tumour type	References
Ferroptosis combination therapy	Chemotherapy	Erastin and cisplatin	ROS-mediated mechanism, diminish GSH, compromise GPX4 function, promote ferroptosis, augment the antitumour impact	*In vitro* and vivo	Human ovarian cancer cell lines (A2780, SKOV3, OVCA433, OVCAR5, OVCAR8 and HEY) and the human ovarian surface epithelial cell line (HOSEpiC); athymic BALB/c female nude mice; NSCLC cell lines (A549, NCIH358, NCIH460)	Ovarian cancer; NSCLC	Guo et al. (2018), Cheng et al. (2021)
		RSL3 and Paclitaxel	Downregulate SLC7A11, promote ferroptosis, retard tumour growth	*In vitro*	Human colorectal carcinoma cell line HCT116; HPSCC cells harboring mutant p53 (mtp53)	CRC, mutant P53 HPSCC	Lv et al. (2017), Ye et al. (2019)
	Radiation therapy	Radiation therapy	Generate ROS production, GSH depletion, ACSL4 upregulation, suppress SLC7A11 expression, enhance radiosensitivity	*In vitro*	H460, A549, H1299 cell lines	NSCLC	Azzam et al. (2012), Lang et al. (2019), Lei et al. (2020)
	Immunotherapy	CD8 (+) T cells release IFN-γ	Downregulate SLC7A11, reduce cystine uptake, induce ferroptosis	*In vitro* and vivo	B16 subcutaneous melanoma model; mice of HT-1080 cells	Melanoma; ovarian cancer	Wang et al. (2019a)
		BEBT-908	Increase MHC class I molecule expression, activate IFN-γ signaling pathway	*In vitro* and *in vivo*	NSCLC H2122 cells, CRC HCT116 cells; MC38 mouse colon adenocarcinoma cell line; female SCID mice with Daudi xenografts	CRC, human diffuse large B-cell lymphoma; lung cancer	Fan et al. (2021)
Reverse resistance		Anti-LCN2 monoclonal antibody (3D12B2)	Increase intracellular iron levels, decrease GPX4 expression, induce ferroptosis, overcome resistance	*In vitro* and *in vivo*	Colon cancer cell line HCT116; CD1 Nude mice	Colon cancer	Chaudhary et al. (2021)
		Vorinostat	Inhibit System Xc- and SLC7A11, stimulate ferroptosis in resistant cells	*In vitro*	EGFR mutant LUAD cell lines, HCC827, HCC4006, H1975, H1650, PC9, HCC4011 and H1993	EGFR-TKI resistant lung adenocarcinoma	Zhang et al. (2021)

ROS, reactive oxygen species; GPX4, glutathione Peroxidase 4; GSH, glutathione; NSCLC, non-small cell lung cancer; HPSCC, hypopharyngeal Squamous Carcinoma; ACSL4, Acyl-coenzyme A synthetase long chain family member 4; IFN-γ, Interferon-γ; LCN2, Lipocalin 2; CRC, colorectal cancer; LUAD, lung adenocarcinoma; EGFR, epidermal growth factor receptor; MHC, Major Histocompatibility Complex.

forecasting a tumour's response to ferroptosis-inducing therapies is crucial for categorizing tumour patients and guiding subsequent antitumour interventions involving ferroptosis induction.

Author contributions

XZ: Conceptualization, Visualization, Writing–original draft, Writing–review and editing. XL: Conceptualization, Writing–original draft. YX: Conceptualization, Supervision, Writing–review and editing.

Funding

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

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.

Publisher's note

References

Aaes, T. L., and Vandenabeele, P. (2021). The intrinsic immunogenic properties of cancer cell lines, immunogenic cell death, and how these influence host antitumor immune responses. *Cell Death Differ.* 28 (3), 843–860. doi:10.1038/s41418-020-00658-y

Azzam, E. I., Jay-Gerin, J.-P., and Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Lett.* 327 (1-2), 48–60. doi:10.1016/j.canlet.2011.12.012

Badgley, M. A., Kremer, D. M., Maurer, H. C., DelGiorno, K. E., Lee, H.-J., Purohit, V., et al. (2020). Cysteine depletion induces pancreatic tumor ferroptosis in mice. *Sci. (New York, N.Y.)* 368 (6486), 85–89. doi:10.1126/science.aaw9872

Bannai, S. (1986). Exchange of cystine and glutamate across plasma membrane of human fibroblasts. *J. Biol. Chem.* 261 (5), 2256–2263. doi:10.1016/s0021-9258(17)35926-4

Barber, G. N. (2015). STING: infection, inflammation and cancer. *Nat. Rev. Immunol.* 15 (12), 760–770. doi:10.1038/nri3921

Basuli, D., Tesfay, L., Deng, Z., Paul, B., Yamamoto, Y., Ning, G., et al. (2017). Iron addiction: a novel therapeutic target in ovarian cancer. *Oncogene* 36 (29), 4089–4099. doi:10.1038/onc.2017.11

Blüml, S., Kirchberger, S., Bochkov, V. N., Krönke, G., Stuhlmeier, K., Majdic, O., et al. (2005). Oxidized phospholipids negatively regulate dendritic cell maturation induced by TLRs and CD40. *J. Immunol.* 175 (1), 501–508. doi:10.4049/jimmunol.175.1.501

Blüml, S., Zupkovitz, G., Kirchberger, S., Seyerl, M., Bochkov, V. N., Stuhlmeier, K., et al. (2009). Epigenetic regulation of dendritic cell differentiation and function by oxidized phospholipids. *Blood* 114 (27), 5481–5489. doi:10.1182/blood-2008-11-191429

Böttcher, J. P., Bonavita, E., Chakravarty, P., Blees, H., Cabeza-Cabrerizo, M., Sammicheli, S., et al. (2018). NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell* 172 (5), 1022–1037. doi:10.1016/j.cell.2018.01.004

Brigelius-Flohé, R., and Flohé, L. (2020). Regulatory phenomena in the glutathione peroxidase superfamily. *Antioxidants redox Signal.* 33 (7), 498–516. doi:10.1089/ars.2019.7905

Brigelius-Flohé, R., and Maiorino, M. (2013). Glutathione peroxidases. *Biochim. Biophys. Acta* 1830 (5), 3289–3303. doi:10.1016/j.bbagen.2012.11.020

Chaudhary, N., Choudhary, B. S., Shah, S. G., Khapare, N., Dwivedi, N., Gaikwad, A., et al. (2021). Lipocalin 2 expression promotes tumor progression and therapy resistance by inhibiting ferroptosis in colorectal cancer. *Int. J. Cancer* 149 (7), 1495–1511. doi:10.1002/ijc.33711

Chen, G. Q., Benthani, F. A., Wu, J., Liang, D., Bian, Z. X., and Jiang, X. (2020). Artemisinin compounds sensitize cancer cells to ferroptosis by regulating iron homeostasis. *Cell Death Differ.* 27 (1), 242–254. doi:10.1038/s41418-019-0352-3

Chen, M., Jiang, Y., and Sun, Y. (2021). KDM4A-mediated histone demethylation of SLC7A11 inhibits cell ferroptosis in osteosarcoma. *Biochem. Biophys. Res. Commun.* 550, 77–83. doi:10.1016/j.bbrc.2021.02.137

Cheng, Q., Bao, L., Li, M., Chang, K., and Yi, X. (2021). Erastin synergizes with cisplatin via ferroptosis to inhibit ovarian cancer growth *in vitro* and *in vivo*. *J. Obstetrics Gynaecol. Res.* 47 (7), 2481–2491. doi:10.1111/jog.14779

Chu, B., Kon, N., Chen, D., Li, T., Liu, T., Jiang, L., et al. (2019). ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. *Nat. Cell Biol.* 21 (5), 579–591. doi:10.1038/s41556-019-0305-6

Condamine, T., Mastio, J., and Gabrilovich, D. I. (2015). Transcriptional regulation of myeloid-derived suppressor cells. *J. Leukoc. Biol.* 98 (6), 913–922. doi:10.1189/jlb.4RI0515-204R

Conrad, M., and Pratt, D. A. (2019). The chemical basis of ferroptosis. *Nat. Chem. Biol.* 15 (12), 1137–1147. doi:10.1038/s41589-019-0408-1

Conrad, M., and Sato, H. (2012). The oxidative stress-inducible cystine/glutamate antiporter, system x (c) (-): cystine supplier and beyond. *Amino Acids* 42 (1), 231–246. doi:10.1007/s00726-011-0867-5

Cooke, M. S., Evans, M. D., Dizdaroglu, M., and Lunec, J. (2003). Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J. Official Publ. Fed. Am. Soc. Exp. Biol.* 17 (10), 1195–1214. doi:10.1096/fj.02-0752rev

Dai, E., Han, L., Liu, J., Xie, Y., Zeh, H. J., Kang, R., et al. (2020). Ferroptotic damage promotes pancreatic tumorigenesis through a TMEM173/STING-dependent DNA sensor pathway. *Nat. Commun.* 11 (1), 6339. doi:10.1038/s41467-020-20154-8

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149 (5), 1060–1072. doi:10.1016/j.cell.2012.03.042

Dixon, S. J., Winter, G. E., Musavi, L. S., Lee, E. D., Snijder, B., Rebsamen, M., et al. (2015). Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. *ACS Chem. Biol.* 10 (7), 1604–1609. doi:10.1021/acschembio.5b00245

Doll, S., Proneth, B., Tyurina, Y. Y., Panzilius, E., Kobayashi, S., Ingold, I., et al. (2017). ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat. Chem. Biol.* 13 (1), 91–98. doi:10.1038/nchembio.2239

Dorand, R. D., Nthale, J., Myers, J. T., Barkauskas, D. S., Avril, S., Chirieleison, S. M., et al. (2016). Cdk5 disruption attenuates tumor PD-L1 expression and promotes antitumor immunity. *Sci. (New York, N.Y.)* 353 (6297), 399–403. doi:10.1126/science.aae0477

Fan, F., Liu, P., Bao, R., Chen, J., Zhou, M., Mo, Z., et al. (2021). A dual PI3K/HDAC inhibitor induces immunogenic ferroptosis to potentiate cancer immune checkpoint therapy. *Cancer Res.* 81 (24), 6233–6245. doi:10.1158/0008-5472.CAN-21-1547

Forman, H. J., Zhang, H., and Rinna, A. (2009). Glutathione: overview of its protective roles, measurement, and biosynthesis. *Mol. Asp. Med.* 30 (1-2), 1–12. doi:10.1016/j.mam.2008.08.006

Friedmann Angeli, J. P., Krysko, D. V., and Conrad, M. (2019). Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. *Nat. Rev. Cancer* 19 (7), 405–414. doi:10.1038/s41568-019-0149-1

Friedmann Angeli, J. P., Schneider, M., Proneth, B., Tyurina, Y. Y., Tyurin, V. A., Hammond, V. J., et al. (2014). Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat. Cell Biol.* 16 (12), 1180–1191. doi:10.1038/ncb3064

Fuchs, Y., and Steller, H. (2011). Programmed cell death in animal development and disease. *Cell* 147 (4), 742–758. doi:10.1016/j.cell.2011.10.033

Gaschler, M. M., and Stockwell, B. R. (2017). Lipid peroxidation in cell death. *Biochem. Biophys. Res. Commun.* 482 (3), 419–425. doi:10.1016/j.bbrc.2016.10.086

Goodwin, J. S., and Ceuppens, J. (1983). Regulation of the immune response by prostaglandins. *J. Clin. Immunol.* 3 (4), 295–315. doi:10.1007/BF00915791

Guo, J., Xu, B., Han, Q., Zhou, H., Xia, Y., Gong, C., et al. (2018). Ferroptosis: a novel anti-tumor action for cisplatin. *Cancer Res. Treat.* 50 (2), 445–460. doi:10.4143/crt.2016.572

Hamanishi, J., Mandai, M., Iwasaki, M., Okazaki, T., Tanaka, Y., Yamaguchi, K., et al. (2007). Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proc. Natl. Acad. Sci. U. S. A.* 104 (9), 3360–3365. doi:10.1073/pnas.0611533104

Hangauer, M. J., Viswanathan, V. S., Ryan, M. J., Bole, D., Eaton, J. K., Matov, A., et al. (2017). Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* 551 (7679), 247–250. doi:10.1038/nature24297

Hassannia, B., Vandenabeele, P., and Vanden Berghe, T. (2019). Targeting ferroptosis to iron out cancer. *Cancer Cell* 35 (6), 830–849. doi:10.1016/j.ccell.2019.04.002

Hou, J., Yu, Z., Xiang, R., Li, C., Wang, L., Chen, S., et al. (2014). Correlation between infiltration of FOXP3+ regulatory T cells and expression of B7-H1 in the tumor tissues of gastric cancer. *Exp. Mol. Pathol.* 96 (3), 284–291. doi:10.1016/j.yexmp.2014.03.005

Hu, K., Li, K., Lv, J., Feng, J., Chen, J., Wu, H., et al. (2020). Suppression of the SLC7A11/glutathione axis causes synthetic lethality in KRAS-mutant lung adenocarcinoma. *J. Clin. Invest.* 130 (4), 1752–1766. doi:10.1172/jci124049

Huang, C., Yang, M., Deng, J., Li, P., Su, W., and Jiang, R. (2018). Upregulation and activation of p53 by erastin-induced reactive oxygen species contribute to cytotoxic and cytostatic effects in A549 lung cancer cells. *Oncol. Rep.* 40 (4), 2363–2370. doi:10.3892/or.2018.6585

Jiang, X., Stockwell, B. R., and Conrad, M. (2021a). Ferroptosis: mechanisms, biology and role in disease. *Nat. Rev. Mol. Cell Biol.* 22 (4), 266–282. doi:10.1038/s41580-020-00324-8

Jiang, Z., Lim, S.-O., Yan, M., Hsu, J. L., Yao, J., Wei, Y., et al. (2021b). TYRO3 induces anti-PD-1/PD-L1 therapy resistance by limiting innate immunity and tumoral ferroptosis. *J. Clin. Investigation* 131 (8), e139434. doi:10.1172/JCI139434

Kim, R., Hashimoto, A., Markosyan, N., Tyurin, V. A., Tyurina, Y. Y., Kar, G., et al. (2022). Ferroptosis of tumour neutrophils causes immune suppression in cancer. *Nature* 612 (7939), 338–346. doi:10.1038/s41586-022-05443-0

Koppula, P., Zhang, Y., Zhuang, L., and Gan, B. (2018). Amino acid transporter SLC7A11/xCT at the crossroads of regulating redox homeostasis and nutrient dependency of cancer. *Cancer Commun. Lond. Engl.* 38 (1), 12. doi:10.1186/s40880-018-0288-x

Kuang, F., Liu, J., Tang, D., and Kang, R. (2020). Oxidative damage and antioxidant defense in ferroptosis. *Front. Cell Dev. Biol.* 8, 586578. doi:10.3389/fcell.2020.586578

Lang, X., Green, M. D., Wang, W., Yu, J., Choi, J. E., Jiang, L., et al. (2019). Radiotherapy and immunotherapy promote tumoral lipid oxidation and ferroptosis via synergistic repression of SLC7A11. *Cancer Discov.* 9 (12), 1673–1685. doi:10.1158/2159-8290.CD-19-0338

Lee, J. Y., Nam, M., Son, H. Y., Hyun, K., Jang, S. Y., Kim, J. W., et al. (2020). Polyunsaturated fatty acid biosynthesis pathway determines ferroptosis sensitivity in gastric cancer. *Proc. Natl. Acad. Sci. U. S. A.* 117 (51), 32433–32442. doi:10.1073/pnas.2006828117

Legrand, A. J., Konstantinou, M., Goode, E. F., and Meier, P. (2019). The diversification of cell death and immunity: memento mori. *Mol. Cell* 76 (2), 232–242. doi:10.1016/j.molcel.2019.09.006

Lei, G., Zhang, Y., Koppula, P., Liu, X., Zhang, J., Lin, S. H., et al. (2020). The role of ferroptosis in ionizing radiation-induced cell death and tumor suppression. *Cell Res.* 30 (2), 146–162. doi:10.1038/s41422-019-0263-3

Lei, G., Zhuang, L., and Gan, B. (2022). Targeting ferroptosis as a vulnerability in cancer. *Nat. Rev. Cancer* 22 (7), 381–396. doi:10.1038/s41568-022-00459-0

Li, D., and Li, Y. (2020). The interaction between ferroptosis and lipid metabolism in cancer. *Signal Transduct. Target. Ther.* 5 (1), 108. doi:10.1038/s41392-020-00216-5

Li, N., Wang, W., Zhou, H., Wu, Q., Duan, M., Liu, C., et al. (2020b). Ferritinophagy-mediated ferroptosis is involved in sepsis-induced cardiac injury. *Free Radic. Biol. Med.* 160, 303–318. doi:10.1016/j.freeradbiomed.2020.08.009

Li, Q., Zhou, Y., He, W., Ren, X., Zhang, M., Jiang, Y., et al. (2021). Platelet-armored nanoplatform to harmonize janus-faced IFN-γ against tumor recurrence and metastasis. *J. Control. Release Official J. Control. Release Soc.* 338, 33–45. doi:10.1016/j.jconrel.2021.08.020

Li, X., Wang, T.-X., Huang, X., Li, Y., Sun, T., Zang, S., et al. (2020a). Targeting ferroptosis alleviates methionine-choline deficient (MCD)-diet induced NASH by suppressing liver lipotoxicity. *Liver Int. Official J. Int. Assoc. Study Liver* 40 (6), 1378–1394. doi:10.1111/liv.14428

Liu, Y., Fan, X., Zhao, Z., and Shan, X. (2020). LncRNA slc7a11-AS1 contributes to lung cancer progression through facilitating TRAIP expression by inhibiting miR-4775. *Onco Targets Ther.* 13, 6295–6302. doi:10.2147/ott.S253082

Lo, M., Ling, V., Low, C., Wang, Y. Z., and Gout, P. W. (2010). Potential use of the anti-inflammatory drug, sulfasalazine, for targeted therapy of pancreatic cancer. *Curr. Oncol.* 17 (3), 9–16. doi:10.3747/co.v17i3.485

Lou, J. S., Zhao, L. P., Huang, Z. H., Chen, X. Y., Xu, J. T., Tai, W. C., et al. (2021). Ginkgetin derived from Ginkgo biloba leaves enhances the therapeutic effect of cisplatin via ferroptosis-mediated disruption of the Nrf2/HO-1 axis in EGFR wild-type non-small-cell lung cancer. *Phytomedicine* 80, 153370. doi:10.1016/j.phymed.2020.153370

Lu, B., Chen, X. B., Ying, M. D., He, Q. J., Cao, J., and Yang, B. (2017). The role of ferroptosis in cancer development and treatment response. *Front. Pharmacol.* 8, 992. doi:10.3389/fphar.2017.00992

Luo, L., Wang, H., Tian, W., Zeng, J., Huang, Y., and Luo, H. (2021). Targeting ferroptosis for cancer therapy: iron metabolism and anticancer immunity. *Am. J. Cancer Res.* 11 (11), 5508–5525.

Luo, Y., Chen, H., Liu, H., Jia, W., Yan, J., Ding, W., et al. (2020). Protective effects of ferroptosis inhibition on high fat diet-induced liver and renal injury in mice. *Int. J. Clin. Exp. Pathol.* 13 (8), 2041–2049.

Lv, C., Qu, H., Zhu, W., Xu, K., Xu, A., Jia, B., et al. (2017). Low-dose paclitaxel inhibits tumor cell growth by regulating glutaminolysis in colorectal carcinoma cells. *Front. Pharmacol.* 8, 244. doi:10.3389/fphar.2017.00244

Ma, P. a., Xiao, H., Yu, C., Liu, J., Cheng, Z., Song, H., et al. (2017). Enhanced cisplatin chemotherapy by iron oxide nanocarrier-mediated generation of highly toxic reactive oxygen species. *Nano Lett.* 17 (2), 928–937. doi:10.1021/acs.nanolett.6b04269

Ma, S., Henson, E. S., Chen, Y., and Gibson, S. B. (2016). Ferroptosis is induced following siramesine and lapatinib treatment of breast cancer cells. *Cell Death Dis.* 7 (7), e2307. doi:10.1038/cddis.2016.208

Maiorino, M., Conrad, M., and Ursini, F. (2018). GPx4, lipid peroxidation, and cell death: discoveries, rediscoveries, and open issues. *Antioxidants redox Signal.* 29 (1), 61–74. doi:10.1089/ars.2017.7115

Mandal, P. K., Seiler, A., Perisic, T., Kölle, P., Banjac Canak, A., Förster, H., et al. (2010). System x(c)- and thioredoxin reductase 1 cooperatively rescue glutathione deficiency. *J. Biol. Chem.* 285 (29), 22244–22253. doi:10.1074/jbc.M110.121327

Martinez-Outschoorn, U. E., Peiris-Pagés, M., Pestell, R. G., Sotgia, F., and Lisanti, M. P. (2017). Cancer metabolism: a therapeutic perspective. *Nat. Rev. Clin. Oncol.* 14 (1), 11–31. doi:10.1038/nrclinonc.2016.60

Mielgo, A., and Schmid, M. C. (2013). Impact of tumour associated macrophages in pancreatic cancer. *BMB Rep.* 46 (3), 131–138. doi:10.5483/bmbrep.2013.46.3.036

Motwani, M., Pesiridis, S., and Fitzgerald, K. A. (2019). DNA sensing by the cGAS-STING pathway in health and disease. *Nat. Rev. Genet.* 20 (11), 657–674. doi:10.1038/s41576-019-0151-1

Nakamura, E., Sato, M., Yang, H., Miyagawa, F., Harasaki, M., Tomita, K., et al. (1999). 4F2 (CD98) heavy chain is associated covalently with an amino acid transporter and controls intracellular trafficking and membrane topology of 4F2 heterodimer. *J. Biol. Chem.* 274 (5), 3009–3016. doi:10.1074/jbc.274.5.3009

Nakanishi, J., Wada, Y., Matsumoto, K., Azuma, M., Kikuchi, K., and Ueda, S. (2007). Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. *Cancer Immunol. Immunother.* 56 (8), 1173–1182. doi:10.1007/s00262-006-0266-z

Ndiaye, H., Liu, J. Y., Hall, A., Minogue, S., Morgan, M. Y., and Waugh, M. G. (2020). Immunohistochemical staining reveals differential expression of ACSL3 and ACSL4 in hepatocellular carcinoma and hepatic gastrointestinal metastases. *Biosci. Rep.* 40 (4). doi:10.1042/BSR20200219

Ohigashi, Y., Sho, M., Yamada, Y., Tsurui, Y., Hamada, K., Ikeda, N., et al. (2005). Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. *Clin. Cancer Res. Official J. Am. Assoc. Cancer Res.* 11 (8), 2947–2953. doi:10.1158/1078-0432.CCR-04-1469

Ostrand-Rosenberg, S., Beury, D. W., Parker, K. H., and Horn, L. A. (2020). Survival of the fittest: how myeloid-derived suppressor cells survive in the inhospitable tumor microenvironment. *Cancer Immunol. Immunother.* 69 (2), 215–221. doi:10.1007/s00262-019-02388-8

Padanad, M. S., Konstantinidou, G., Venkateswaran, N., Melegari, M., Rindhe, S., Mitsche, M., et al. (2016). Fatty acid oxidation mediated by acyl-CoA synthetase long chain 3 is required for mutant KRAS lung tumorigenesis. *Cell Rep.* 16 (6), 1614–1628. doi:10.1016/j.celrep.2016.07.009

Qi, J., Kim, J.-W., Zhou, Z., Lim, C.-W., and Kim, B. (2020). Ferroptosis affects the progression of nonalcoholic steatohepatitis via the modulation of lipid peroxidation-mediated cell death in mice. *Am. J. Pathology* 190 (1), 68–81. doi:10.1016/j.ajpath.2019.09.011

Reczek, C. R., and Chandel, N. S. (2018). ROS promotes cancer cell survival through calcium signaling. *Cancer Cell* 33 (6), 949–951. doi:10.1016/j.ccell.2018.05.010

Sang, M., Luo, R., Bai, Y., Dou, J., Zhang, Z., Liu, F., et al. (2019). Mitochondrial membrane anchored photosensitive nano-device for lipid hydroperoxides burst and inducing ferroptosis to surmount therapy-resistant cancer. *Theranostics* 9 (21), 6209–6223. doi:10.7150/thno.36283

Sato, H., Shiiya, A., Kimata, M., Maebara, K., Tamba, M., Sakakura, Y., et al. (2005). Redox imbalance in cystine/glutamate transporter-deficient mice. *J. Biol. Chem.* 280 (45), 37423–37429. doi:10.1074/jbc.M506439200

Sato, H., Tamba, M., Ishii, T., and Bannai, S. (1999). Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *J. Biol. Chem.* 274 (17), 11455–11458. doi:10.1074/jbc.274.17.11455

Seibt, T. M., Proneth, B., and Conrad, M. (2019). Role of GPX4 in ferroptosis and its pharmacological implication. *Free Radic. Biol. Med.* 133, 144–152. doi:10.1016/j.freeradbiomed.2018.09.014

Seiler, A., Schneider, M., Förster, H., Roth, S., Wirth, E. K., Culmsee, C., et al. (2008). Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* 8 (3), 237–248. doi:10.1016/j.cmet.2008.07.005

Shimada, K., Skouta, R., Kaplan, A., Yang, W. S., Hayano, M., Dixon, S. J., et al. (2016). Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. *Nat. Chem. Biol.* 12 (7), 497–503. doi:10.1038/nchembio.2079

Stockwell, B. R., Friedmann Angeli, J. P., Bayir, H., Bush, A. I., Conrad, M., Dixon, S. J., et al. (2017). Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* 171 (2), 273–285. doi:10.1016/j.cell.2017.09.021

Su, J., Bian, C., Zheng, Z., Wang, H., Meng, L., Xin, Y., et al. (2022). Cooperation effects of radiation and ferroptosis on tumor suppression and radiation injury. *Front. Cell Dev. Biol.* 10, 951116. doi:10.3389/fcell.2022.951116

Su, P., Wang, Q., Bi, E., Ma, X., Liu, L., Yang, M., et al. (2020). Enhanced lipid accumulation and metabolism are required for the differentiation and activation of tumor-associated macrophages. *Cancer Res.* 80 (7), 1438–1450. doi:10.1158/0008-5472.CAN-19-2994

Tan, S. K., Mahmud, I., Fontanesi, F., Puchowicz, M., Neumann, C. K. A., Griswold, A. J., et al. (2021). Obesity-dependent adipokine chemerin suppresses fatty acid oxidation to confer ferroptosis resistance. *Cancer Discov.* 11 (8), 2072–2093. doi:10.1158/2159-8290.CD-20-1453

Tang, D., Chen, X., Kang, R., and Kroemer, G. (2021). Ferroptosis: molecular mechanisms and health implications. *Cell Res.* 31 (2), 107–125. doi:10.1038/s41422-020-00441-1

Tang, D., Kang, R., Berghe, T. V., Vandenabeele, P., and Kroemer, G. (2019). The molecular machinery of regulated cell death. *Cell Res.* 29 (5), 347–364. doi:10.1038/s41422-019-0164-5

Trachootham, D., Alexandre, J., and Huang, P. (2009). Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov.* 8 (7), 579–591. doi:10.1038/nrd2803

Tsurusaki, S., Tsuchiya, Y., Koumura, T., Nakasone, M., Sakamoto, T., Matsuoka, M., et al. (2019). Hepatic ferroptosis plays an important role as the trigger for initiating inflammation in nonalcoholic steatohepatitis. *Cell Death Dis.* 10 (6), 449. doi:10.1038/s41419-019-1678-y

Ursini, F., Maiorino, M., Valente, M., Ferri, L., and Gregolin, C. (1982). Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. *Biochim. Biophys. Acta* 710 (2), 197–211. doi:10.1016/0005-2760(82)90150-3

Viswanathan, V. S., Ryan, M. J., Dhruv, H. D., Gill, S., Eichhoff, O. M., Seashore-Ludlow, B., et al. (2017). Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* 547 (7664), 453–457. doi:10.1038/nature23007

Wang, D., and DuBois, R. N. (2015). Immunosuppression associated with chronic inflammation in the tumor microenvironment. *Carcinogenesis* 36 (10), 1085–1093. doi:10.1093/carcin/bgv123

Wang, H., Franco, F., Tsui, Y.-C., Xie, X., Trefny, M. P., Zappasodi, R., et al. (2020). CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. *Nat. Immunol.* 21 (3), 298–308. doi:10.1038/s41590-019-0589-5

Wang, W., Green, M., Choi, J. E., Gijón, M., Kennedy, P. D., Johnson, J. K., et al. (2019a). CD8+ T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature* 569 (7755), 270–274. doi:10.1038/s41586-019-1170-y

Wang, W., Xia, X., Mao, L., and Wang, S. (2019b). The CCAAT/Enhancer-Binding protein family: its roles in MDSC expansion and function. *Front. Immunol.* 10, 1804. doi:10.3389/fimmu.2019.01804

Wang, X., Teng, F., Kong, L., and Yu, J. (2016). PD-L1 expression in human cancers and its association with clinical outcomes. *Onco Targets Ther.* 9, 5023–5039. doi:10.2147/OTT.S105862

Wen, Q., Liu, J., Kang, R., Zhou, B., and Tang, D. (2019). The release and activity of HMGB1 in ferroptosis. *Biochem. Biophys. Res. Commun.* 510 (2), 278–283. doi:10.1016/j.bbrc.2019.01.090

Wiernicki, B., Maschalidi, S., Pinney, J., Adjemian, S., Vanden Berghe, T., Ravichandran, K. S., et al. (2022). Cancer cells dying from ferroptosis impede dendritic cell-mediated anti-tumor immunity. *Nat. Commun.* 13 (1), 3676. doi:10.1038/s41467-022-31218-2

Wolpaw, A. J., and Dang, C. V. (2018). Exploiting metabolic vulnerabilities of cancer with precision and accuracy. *Trends Cell Biol.* 28 (3), 201–212. doi:10.1016/j.tcb.2017.11.006

Xu, T., Ding, W., Ji, X., Ao, X., Liu, Y., Yu, W., et al. (2019). Molecular mechanisms of ferroptosis and its role in cancer therapy. *J. Cell. Mol. Med.* 23 (8), 4900–4912. doi:10.1111/jcmm.14511

Yamaguchi, H., Hsu, J. L., Chen, C.-T., Wang, Y.-N., Hsu, M.-C., Chang, S.-S., et al. (2013). Caspase-independent cell death is involved in the negative effect of EGF receptor inhibitors on cisplatin in non-small cell lung cancer cells. *Clin. Cancer Res. Official J. Am. Assoc. Cancer Res.* 19 (4), 845–854. doi:10.1158/1078-0432.CCR-12-2621

Yang, W. S., Kim, K. J., Gaschler, M. M., Patel, M., Shchepinov, M. S., and Stockwell, B. R. (2016). Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proc. Natl. Acad. Sci. U. S. A.* 113 (34), E4966–E4975. doi:10.1073/pnas.1603244113

Yang, W. S., SriRamaratnam, R., Welsch, M. E., Shimada, K., Skouta, R., Viswanathan, V. S., et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell* 156 (1-2), 317–331. doi:10.1016/j.cell.2013.12.010

Yang, W. S., and Stockwell, B. R. (2016). Ferroptosis: death by lipid peroxidation. *Trends Cell Biol.* 26 (3), 165–176. doi:10.1016/j.tcb.2015.10.014

Yant, L. J., Ran, Q., Rao, L., Van Remmen, H., Shibatani, T., Belter, J. G., et al. (2003). The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radic. Biol. Med.* 34 (4), 496–502. doi:10.1016/s0891-5849(02)01360-6

Yatim, N., Cullen, S., and Albert, M. L. (2017). Dying cells actively regulate adaptive immune responses. *Nat. Rev. Immunol.* 17 (4), 262–275. doi:10.1038/nri.2017.9

Ye, J., Jiang, X., Dong, Z., Hu, S., and Xiao, M. (2019). Low-concentration PTX and RSL3 inhibits tumor cell growth synergistically by inducing ferroptosis in mutant p53 hypopharyngeal squamous carcinoma. *Cancer Manag. Res.* 11, 9783–9792. doi:10.2147/CMAR.S217944

Ye, L. F., Chaudhary, K. R., Zandkarimi, F., Harken, A. D., Kinslow, C. J., Upadhyayula, P. S., et al. (2020). Radiation-induced lipid peroxidation triggers ferroptosis and synergizes with ferroptosis inducers. *ACS Chem. Biol.* 15 (2), 469–484. doi:10.1021/acschembio.9b00939

Yu, H., Han, Z., Xu, Z., An, C., Xu, L., and Xin, H. (2019). RNA sequencing uncovers the key long non-coding RNAs and potential molecular mechanism contributing to XAV939-mediated inhibition of non-small cell lung cancer. *Oncol. Lett.* 17 (6), 4994–5004. doi:10.3892/ol.2019.10191

Yu, Y., Xie, Y., Cao, L., Yang, L., Yang, M., Lotze, M. T., et al. (2015). The ferroptosis inducer erastin enhances sensitivity of acute myeloid leukemia cells to chemotherapeutic agents. *Mol. Cell. Oncol.* 2 (4), e1054549. doi:10.1080/23723556.2015.1054549

Yuan, H., Li, X., Zhang, X., Kang, R., and Tang, D. (2016). Identification of ACSL4 as a biomarker and contributor of ferroptosis. *Biochem. Biophys. Res. Commun.* 478 (3), 1338–1343. doi:10.1016/j.bbrc.2016.08.124

Zhang, F., Li, F., Lu, G. H., Nie, W., Zhang, L., Lv, Y., et al. (2019). Engineering magnetosomes for ferroptosis/immunomodulation synergism in cancer. *ACS Nano* 13 (5), 5662–5673. doi:10.1021/acsnano.9b00892

Zhang, M., Di Martino, J. S., Bowman, R. L., Campbell, N. R., Baksh, S. C., Simon-Vermot, T., et al. (2018). Adipocyte-derived lipids mediate melanoma progression via FATP proteins. *Cancer Discov.* 8 (8), 1006–1025. doi:10.1158/2159-8290.CD-17-1371

Zhang, M., Qin, X., Zhao, Z., Du, Q., Li, Q., Jiang, Y., et al. (2022). A self-amplifying nanodrug to manipulate the Janus-faced nature of ferroptosis for tumor therapy. *Nanoscale Horizons* 7 (2), 198–210. doi:10.1039/d1nh00506e

Zhang, T., Sun, B., Zhong, C., Xu, K., Wang, Z., Hofman, P., et al. (2021). Targeting histone deacetylase enhances the therapeutic effect of Erastin-induced ferroptosis in EGFR-activating mutant lung adenocarcinoma. *Transl. Lung Cancer Res.* 10 (4), 1857–1872. doi:10.21037/tlcr-21-303

Zhang, Y., Song, J., Zhao, Z., Yang, M., Chen, M., Liu, C., et al. (2020). Single-cell transcriptome analysis reveals tumor immune microenvironment heterogenicity and granulocytes enrichment in colorectal cancer liver metastases. *Cancer Lett.* 470, 84–94. doi:10.1016/j.canlet.2019.10.016

Zhou, J., Nefedova, Y., Lei, A., and Gabrilovich, D. (2018). Neutrophils and PMN-MDSC: their biological role and interaction with stromal cells. *Semin. Immunol.* 35, 19–28. doi:10.1016/j.smim.2017.12.004

Zhu, Y., Herndon, J. M., Sojka, D. K., Kim, K.-W., Knolhoff, B. L., Zuo, C., et al. (2017). Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. *Immunity* 47 (2), 323–338. doi:10.1016/j.immuni.2017.07.014

Zou, Y., Henry, W. S., Ricq, E. L., Graham, E. T., Phadnis, V. V., Maretich, P., et al. (2020). Plasticity of ether lipids promotes ferroptosis susceptibility and evasion. *Nature* 585 (7826), 603–608. doi:10.1038/s41586-020-2732-8

Glossary

GPX4	glutathione peroxidase 4
GSH	glutathione
PGE2	Prostaglandin E2
COX-2	cyclooxygenase-2
PD-L1	programmed death ligand-1
PUFAs	Polyunsaturated fatty acids
ROS	reactive oxygen species
ACSL4	Acyl-coenzyme A synthetase long chain family member 4
PUFA-PLs	PUFA-containing phospholipids
RT	radiation therapy
LPCAT3	Lysophosphatidylcholine acyltransferase 3
LOXs	lipoxygenase
PORs	cytochrome P450 oxidoreductase
ACSL3	Acyl-coenzyme A synthetase long chain family member 3
MUFA-PL	Monounsaturated fatty acids-phospholipids
FDFT1/SQS	farnesyl-diphosphate farnesyltransferase 1
CRC	Colorectal cancer
ELOVL5	elongation of very long-chain fatty acid protein 5
FADS1	fatty acid desaturase 1
PDACs	pancreatic ductal adenocarcinomas
HNC	head and neck cancer
RCC	renal cell carcinoma
HCC	hepatocellular carcinoma
NSCLC	non-small cell lung cancer
TFR	transferrin
DMT1	divalent metal ion transporter protein-1
HGSOC	high-grade serous ovarian cancer
TAMs	Tumour-associated macrophages
PTGS2	prostaglandin-endoperoxide synthase 2
cDC1s	conventional type 1 dendritic cells
DCs	dendritic cells
DAMPs	damage-related molecular patterns
PMN-MDSCs	polymorphonuclear myeloid derived suppressor cells
IFN-γ	Interferon-γ
lncRNA	long non-coding RNA
LCN2	Lipocalin 2
MHC	Major Histocompatibility Complex
8-OHdG	8-hydroxy-2′-deoxyguanosine
PTX	paclitaxel
TAAs	tumour-associated antigens
PLA2	Phospholipase A2
LUAD	lung adenocarcinoma
FPN	ferroportin
LPS	Lipopolysaccharide

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 20 February 2024
DOI 10.3389/fphar.2024.1335785

OPEN ACCESS

EDITED BY
Valentina Audrito,
Università del Piemonte Orientale, Italy

REVIEWED BY
Chang Seon Ryu,
Korea Institute of Science and Technology, Europe, Germany
Elisa Giovannetti,
VU Medical Center, Netherlands
Marika Franczak,
Medical University of Gdansk, Poland, in collaboration with reviewer EG

*CORRESPONDENCE
Zhenhua Wang,
wzhcmusl@163.com

RECEIVED 09 November 2023
ACCEPTED 08 January 2024
PUBLISHED 20 February 2024

CITATION
Zhang X and Wang Z (2024), Targeting SHMTs and MTHFDs in cancer: attractive opportunity for anti-tumor strategy.
Front. Pharmacol. 15:1335785.
doi: 10.3389/fphar.2024.1335785

Targeting SHMTs and MTHFDs in cancer: attractive opportunity for anti-tumor strategy

Xue Zhang[1] and Zhenhua Wang[2]*

[1]The VIP Department, School and Hospital of Stomatology, China Medical University, Shenyang, China, [2]Department of Physiology, School of Life Sciences, China Medical University, Shenyang, China

One-carbon metabolism is a universal metabolic process that mediates the transfer of one-carbon units for purine and thymidine synthesis. One-carbon metabolism has been found to be dysregulated in various cancer types due to its role in production of purine and pyrimidine nucleotides, epigenetic program, and redox homeostasis. One-carbon metabolism is composed a network of one-carbon metabolic enzymes. Disturbing the expression and enzymatic activity of these one-carbon metabolic enzymes could lead to fluctuations of metabolites in the tumor microenvironment. Serine hydroxymethyltransferases (SHMTs) and methylenetetrahydrofolate dehydrogenases (MTHFDs) are gradually recognized as important one-carbon metabolic enzymes for regulating tumor initiation and development, representing potential therapeutic targets for anti-tumor strategies. In the review, we primarily focused on the role of SHMTs and MTHFDs in cancer. Several inhibitors targeting MTHFDs and SHMTs have exert its potential to decrease tumor burden and inhibit tumor proliferation, highlighting the potential of targeting one-carbon metabolic enzymes for anti-cancer strategies.

KEYWORDS

metabolism, one carbon metabolism, metabolic enzyme, tumorigenesis, MTHFD family

Introduction

One-carbon metabolism, also known as folate metabolism, is a universal metabolic process that participates in the transfer of one-carbon units for purine and thymidine synthesis (Ducker and Rabinowitz, 2017). Altered one-carbon metabolism has been emerged as a critical feature of tumor cells, providing building blocks for nucleotide biosynthesis, epigenetic regulation and redox homeostasis (Newman and Maddocks, 2017). One-carbon units are required for the synthesis of purine and pyrimidine nucleotides, which are necessary for DNA and RNA synthesis. Due to the enhanced requirement of nucleotides for proliferation, tumor cells demand increased one-carbon units for nucleotide synthesis (Yang et al., 2021). The interplay between one-carbon metabolism and epigenomic program is also crucial for tumorigenesis. DNA methylation is an epigenetic mechanism that is essential for regulating gene transcription, and the aberrant DNA methylation changes is commonly observed in tumors (Friso et al., 2017) The epigenetic implications of one-carbon metabolism dysregulation in tumor cells are required further study. NADH and NADPH are important co-factors that provide electrons for redox reactions, which can be generated from one-carbon metabolism (Pan et al., 2021a). Herein, one-carbon metabolism is important for tumor initiation and progression.

Targeting the one-carbon metabolism provokes antifolate chemotherapeutic regimens to treat malignant tumors. Currently, the FDA-approved drugs targeting one-carbon metabolic enzymes includes methotrexate, pemetrexed, pralatrexate, trimetrexate, pyrimethamine and 5-Fluorouracil (Cuthbertson et al., 2021). Pemetrexed and 5-fluoruoracil, these two classic antifolate chemotherapeutics, are active in various solid tumors and hematological tumors. Pemetrexed inhibits several enzymes involved in the folate pathway including Dihydrofolate reductase (DHFR), thymidylate synthase (TYMS) and serine hydroxymethyltransferase (SHMT), while 5-fluoruoracil specifically targets TYMS. However, these drugs act non-selectively on normal tissues and tumor tissues, which can not only kill tumor cells to play anti-tumor role, but also rapidly leads to exhaustion of circulating myeloid and lympho-progenitor cells to impair the immune response. Therefore, future therapeutics should be more specifically targeting one-carbon metabolism in tumor cells by more selectively targeting individual one-carbon pathway enzymes. Some other one-carbon enzymes have been found to be selectively upregulated in multiple tumors, making these enzymes promising targets for the development of novel chemotherapeutic agents (Zhao et al., 2021).

Generally, serine by uptake from extracellular environment or *de novo* biosynthesis is the main supply of the one-carbon units. Other amino acids, such as glycine, can also donate a one-carbon unit via glycine cleavage system. The uptake of extracellular serine is mediated by solute carrier family 1 member 4/5 (SLC1A4/SLC1A5), while serine biosynthesis begins with glycolytic intermediate 3-phosphoglycerate and underwent a series of reactions catalyzed by phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSPH) for *de novo* serine biosynthesis. Folate molecules function as carriers for 1C units, allowing them to be manipulated and assembled in support of metabolic processes. Once dihydrofolate reductase catalyzed the reduction of folate to tetrahydrofolate, SHMT1 could transfers a one-carbon unit from serine to from 5,10-methylene-tetrahydrofolate in cytoplasm. SHMT2, as the mitochondrial isoform of SHMTs, catalyzes the same enzymatic reaction to provide a one-carbon unit to form 5,10-methylene-tetrahydrofolate (Cuthbertson et al., 2021). Uniquely among the mitochondrial folate-related reactions, the oxidation of 5,10-methylene-tetrahydrofolate to 10-formyl-tetrahydrofolate can be catalyzed by two isozymes, MTHFD2 and MTHFD2L (Tibbetts and Appling, 2010). MTHFD1L catalyzes the conversion of 10-formyl-tetrahydrofolate to formate and transport formate out of the mitochondria. The illustration of one-carbon metabolism has been depicted in Figure 1. One-carbon pathways are compartmentalized in the cytosol, nucleus and mitochondria (Dekhne et al., 2020). Cytosol one-carbon metabolism has been exploited therapeutically for anti-tumor strategy, and antifolates targeting cytosolic one-carbon pathways are still primary chemotherapeutic regimens for anti-tumor strategy. In the one-carbon metabolism, glycine and serine could activate the mitochondrial enzymes SHMT2 and MTHFD2, which is critical for tumor cell survival (Cuthbertson et al., 2021). SHMTs and MTHFDs are not getting as much attention as other one carbon metabolic enzymes, like TYMS. Considering the potential of SHMTs and MTHFDs for developing anti-tumor

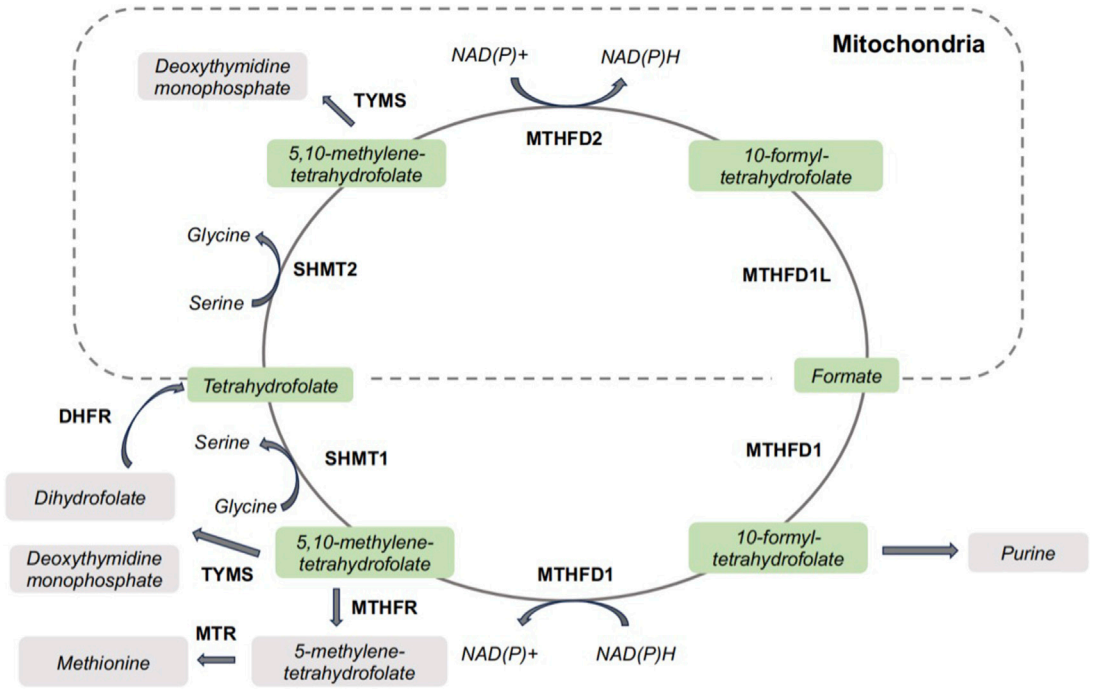


FIGURE 1
One-carbon metabolism in cells. DHFR, dihydrofolate reductase; MTHFD1, methylenetetrahydrofolate dehydrogenase/cyclohydrolase 1; MTHFD2, methylenetetrahydrofolate dehydrogenase/cyclohydrolase 2; MTR, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SHMT1, serine hydroxymethyltransferase 1; SHMT2, serine hydroxymethyltransferase 2; TYMS, thymidylate synthetase.

strategies, we comprehensively summarized the role of SHMTs and MTHFDs in cancer initiation and progression. The clinical implications of SHMTs and MTHFDs are also discussed.

Targeting MTHFDs in cancer

Members of MTHFD family are composed of MTHFD1, MTHFD1L, MTHFD2, and MTHFD2L. Recent studies have revealed that MTHFD1/2 have been identified as oncogenic enzymes upregulated in various tumors, which may highly participate in the initiation and progression of tumors. And it has been demonstrated that MTHFD1L/2L has limited effect in tumor initiation and progression. Herein, we primarily discussed the complex role of MTHFD1/2 in the anti-tumor immunity. The diverse role of MTHFD1/2 in different tumor types has also been summarized. The clinical implications of MTHFD1/2 in cancer have been outlined.

Targeting MTHFD1 in cancer

Arginine methyltransferase 5 (PRMT5) mediates symmetric dimethylation of MTHFD1 on Residue-173. Elevated methylation of MTHFD1 could enhance its metabolic activity for the generation of NADPH, contributing to anoikis resistance and metastasis. Symmetric dimethylation of R173 was correlated with metastasis and prognosis of esophageal squamous cell carcinoma patients (Meng et al., 2022). During the metastatic process, melanoma cells exhibited elevated expression of one-carbon enzymes for the generation of NADPH (Piskounova et al., 2015). Inhibition of one-carbon pathway by MTHFD1 knockdown could attenuate distant organ metastasis of melanoma. In cholangiocarcinoma (CAA), MTHFD1 participated in the regulation of redox status, leading to chemoresistance to gemcitabine. Accordingly, anti-folate compound methotrexate targeting MTHFD1 could elevate ROS level, and the combination of gemcitabine with methotrexate could effectively suppress CAA growth (Pan et al., 2021b).

Targeting MTHFD2 in cancer

The regulation of MTHFD2 is complex and dynamic. p53 could suppress MTHFD2 transcription, and MTHFD2 upregulation by p53 inactivation results in enhanced one-carbon metabolism, purine biosynthesis and tumor proliferation (Li et al, 2021a). MYC transcriptionally acts on MTHFD2, which is regulated by activating transcription factor 4 (ATF4) (Pikman et al., 2016; Pällmann et al., 2021; Gao et al., 2022). MicroRNAs also regulate MTHFD2 expression. miRNA-99a-3p has been found to be an upstream regulator directly inhibiting MTHFD2 expression in lung adenocarcinoma (Mo et al., 2022). In acute myeloid leukemia (AML), miR-92a could suppress cell proliferation by directly inhibiting MTHFD2 expression (Gu et al., 2017). The post-modification of MTHFD2 is complex and dynamic. Under folate stress, SIRT4 mediates the acetylation of MTHFD2 at K50 to regulate its protein stability, therefore driving tumor cell growth (Zhang et al., 2022). Sirtuin 3 (SIRT3), a key mitochondrial deacetylase, mediates the deacetylation of MTHFD2. MTHFD2 is hyperacetylated at lysine 88, which is the common acetylated site (Wan et al., 2020).

One-carbon metabolism is essential for T cell proliferation and function (Ron-Harel et al., 2016). Targeted screening in primary murine T cells has identified MTHFD2 as a key gene in inflammatory disorders. Given that one-carbon metabolism links various energetic sources and is therapeutically targetable, the role of MTHFD2 in $CD4^+$ T effector (Teff), pathogenic T helper-17 (Th17) and regulatory T (Treg) cell subsets has been investigated. It has been demonstrated that MTHFD2 functions as a metabolic checkpoint determining T cell differentiation. In Th17 cells, MTHFD2 deficiency promotes aberrant upregulation of FoxP3 and suppressive capacity. MTHFD2 deficiency also induces Treg cell differentiation. These effects are correlated with impaired *de novo* purine synthesis, 5-aminoimidazole carboxamide ribonucleotide (AICAR) accumulation, guanine depletion, suppressed mTORC1 signaling, enhanced oxidative phosphorylation, and decreased methylation. Herein, MTHFD2 functions as a metabolic checkpoint balancing Th17 and Treg cells, highlighting MTHFD2 as a potential target for inflammatory disease (Sugiura et al., 2022). One-carbon metabolic network is also essential for Teff responses in tumors (Kurniawan et al., 2020). Functional screening has identified MTHFD2 as a driver metabolic gene contribute to resistance against Teff cells. It has been reported that MTHFD2 promotes basal and IFN-γ-induced PD-L1 upregulation, which is important for tumorigenesis. IFN-γ could induce MTHFD2 expression by activating AKT-mTORC1 signaling. MTHFD2 enhances PD-L1-induced resistance to tumor immunity through the one-carbon cycle-HBP pathway and the UDP-GlcNAc-O-GlcNAcylation-MYC-PD-L1 signaling pathway (Shang et al., 2021). These findings indicate the role of MTHFD2 in immune evasion, which may provoke further investigations on tumor immunotherapy.

One-carbon metabolism has been demonstrated to be highly correlated with initiation and development of colorectal cancer (CRC). Various one-carbon metabolic enzymes, including SHMT2 and MTHFD2, are significantly upregulated in CRC tissues than non-tumor tissues. Notably, patients with high expression of SHMT2 and MTHFD2 exhibit lower survival rates than patients with low expression of SHMT2 and MTHFD2 (Miyo et al., 2017). In CRC cells, MTHFD2 has been identified as a new deacetylation substrate of SIRT3. SIRT3 could deacetylate MTHFD2 at lysine 88 to alter its enzymatic activity. Furthermore, deacetylated MTHFD2 could enhance its enzymatic activity and regulate cellular levels of NADPH. Notably, cisplatin could reduce SIRT3 expression and ultimately increase MTHFD2 acetylation, which leads to reduced NADPH levels and increased ROS levels. This cisplatin-SIRT3-MTHFD2 axis in ROS production suggests MTHFD2 K88 acetylation as a potential target for CRC treatment (Wan et al., 2020). It has been found that protein levels of SIRT3 are negatively correlated with K88 MTHFD2 acetylation in CRC tissues, indicating MTHFD2 or SIRT3 inhibition may be promising for CRC treatment. Under oxidative stress, MTHFD2 maintains the level of NADPH. MTHFD2-mediated ROS balance could rescue CRC cells from demands of NADPH production, therefore promoting tumor progression. The folate analog LY345899 as

MTHFD2 inhibitor exerts anti-tumor effect in CRC and provokes further investigation for anti-tumor treatment of CRC (Ju et al., 2019). In colon cancer cells, MTHFD2 promotes non-homologous end joining in response to DNA damage through the formation of complex with PARP3 to enhance its ribosylation. MTHFD2 silencing could restrain p53-deleted cell proliferation and sensitize tumor cells to chemotherapeutic regimens, suggesting potential of MTHFD2 inhibition for the treatment of p53-deleted tumors (Li et al., 2021b). Silencing the expression of MTHFD2 leads to impaired proliferation and migration of CRC cells, arrested in G0/G1-S phase, and increased apoptosis of CRC cells (Wei et al., 2019). In colon cancer cells, MTHFD2 has been confirmed to be the target gene of miR-33a-5p. miR-33a-5p could inhibit cell proliferation and migration of CRC by targeting MTHFD2 (Yan et al., 2019).

Castration-resistant prostate cancer (CRPC) occurs after androgen deprivation is an huge obstacle in developing anti-tumor strategies (Armstrong et al., 2019). Protein tyrosine phosphatase receptor type F polypeptide interacting protein alpha 4 (PPFIA4), localizing to mitochondria, could interact with MTHFD2 for one-carbon metabolism. Androgen deprivation could promote PPFIA4 translocation into mitochondria and enhance the interaction between PPFIA4 and MTHFD2, leading to increased tyrosine phosphorylated MTHFD2. Phosphorylated MTHFD2 increases the levels of NADPH, preventing androgen deprivation-induced mitochondrial dysfunction and promoting tumor growth. DS18561882, an MTHFD2 inhibitor, combined with enzalutamide (a potent androgen-receptor inhibitor) could suppress cell proliferation of CRPC (Zhao et al., 2022). Besides, MYC interacts with ATF4 to activate gene expression of one-carbon cycle in prostate cancer cells, including MTHFD2. MTHFD2 silencing could inhibit prostate cancer cell growth *in vitro* and patient-derived xenografts. Additionally, MTHFD2 inhibition via nanoliposomal siRNA could impair tumor growth *in vivo* models of prostate tumor (Pällmann et al., 2021).

MTHFD2 expression has been found to be highly upregulated in ovarian cancer than nontumoral samples (Cui, et al., 2022a). Ovarian cancer patients with high MTHFD2 expression have been found to be associated with lower survivals. MTHFD2 silencing could induce cell apoptosis and cell cycle arrest, impair ovarian tumor cell proliferation and metastasis. The inhibitory effect of MTHFD2 silencing on ovarian cancer progression may be resulted from inhibited expression and activity of cyclin B1/Cdc2 complex. MTHFD2 could promote tumor progression via STAT3-mediated epithelial-mesenchymal transition (Li et al., 2021a). In UQCR11-deficient ovarian cancer, MTHFD2 functions as a collateral lethal gene. MTHFD2 provides mitochondrial NAD+, and the UQCR11-MTHFD2 collateral lethality has been verified in mousemodels (Achreja et al., 2022).

AML is a heterogenous hematological malignancy of the stem cell precursors of the myeloid lineage (Löwenberg et al., 1999). Enzymatic function of MTHFD2 sustains rapid cell proliferation during early embryogenesis, while transforming to MTHFD2L in mature cells. In tumoral tissues, the re-activation of MTHFD2 indicates an isoform shift from MTHFD2L to MTHFD2 during tumor transformation. MTHFD2 silencing in AML cells could promote cell differentiation in primary AML blasts. In xenografts and mouse models, MTHFD2 silencing could decrease tumor burden and prolong survival. Functional genomic screening identified FLT3-ITD as a biomarker of response to MTHFD2 inhibition. Mechanistically, MYC could regulate the expression of MTHFD2, and MTHFD2 silencing inhibits the TCA cycle (Pikman et al., 2016). MTHFD2 has also implicated in DNA and replication stress. MTHFD2 inhibitors could retard replication fork and induce replication stress in AML cells. Mechanistically, MTHFD2 inhibitors could inhibit thymidine production resulting in DNA and replication stress. The interplay between MTHFD2-related tumor metabolism and replication stress that may be exploited therapeutically for cancer treatment (Bonagas et al., 2022).

Lung cancer is the leading cause of tumor-related deaths worldwide (Sung et al., 2021). MTHFD2 has been found to be upregulated in stage-dependent lung tumor tissues and lung tumor cell lines (Chan et al., 2020). MTHFD2 silencing could impair cell viability, transformation and self-renewal abilities of lung cancer cells. MTHFD2 silencing also reduces NADPH level and induce oxidative stress with increased ROS and cell apoptosis (Chan et al., 2020). The inhibitory effect of MTHFD2 silencing on tumorigenesis and stemness has been found to be correlated with purine depletion, which leads to the accumulation of AICAR-the final intermediate of the purine biosynthesis. Lung tumor cells with acquired resistance to the gefitinib exhibit enhanced stemness and upregulated MTHFD2 expression. MTHFD2 silencing or adding AICAR could reduce stemness and restore sensitivity to gefitinib in the gefitinib-resistant lung tumor cells (Nishimura et al., 2019). MTHFD2 overexpression in gefitinib-responsive lung tumor cells could confer gefitinib resistance. Thus, MTHFD2-induced one-carbon metabolism is important for tumor stemness and gefitinib resistance by reducing intracellular AICAR. Considering cancer stem cells rely on MTHFD2, MTHFD2 may be a target to eradicate stem cells and reduce recurrence.

In glioblastoma, MTHFD2 inhibition could activate the PERK/eIF2α axis, blocking translation and inducing apoptosis, which can be suppressed by a PERK inhibitor. Mechanistical study revealed that MTHFD2 is related to unfolded protein response via the post-transcriptional modification of chaperone protein GRP78. MTHFD2 mediates the progression of glioblastoma via unfolded protein response, indicating a novel link between one-carbon metabolism and stress response. Therefore, MTHFD2 is an attractive target for glioblastoma. (Zhu et al., 2022a). MTHFD2 also participated in cell cycle progression in bladder cancer. Nuclear MTHFD2 could activate CDK2 to promote the growth of bladder cancer by promoting cell cycle progression. (Liu et al., 2021a). In renal cell carcinoma, MTHFD2 get involved in regulating global N6-methyladenosine (m6A) methylation levels. Specifically, MTHFD2 induced m6A methylation of HIF-2α mRNA and enhanced translation of HIF-2α. Increased HIF-2α translation further promoted glycolysis to promote progression of renal cell carcinoma (Green et al., 2019).

Clinical implications of MTHFD1/2 in cancer

An analysis of mRNA profiles of metabolic enzymes across 19 tumor types to explore metabolic enzymes that are differentially expressed indicates mitochondrial one-carbon metabolism as the highest scoring pathway, especially MTHFD2. The analysis of

TABLE 1 Expression patterns and clinical value of one-carbon metabolic enzymes in tumors.

Gene	Tumor type	Expression	Level	Clinical value	Reference
MTHFD1	Hepatocellular carcinoma	Upregulation	Protein	Shorter OS	Yu et al. (2019)
MTHFD2	Colorectal cancer	Upregulation	Protein	Shorter OS and DFS	Ju et al., 2019
					Miyo et al. (2017)
	Ovarian cancer	Upregulation	Protein mRNA	Shorter OS	Cui, et al. (2022b)
		Upregulation			
	Lung cancer	Upregulation	mRNA and protein	N/A	Shi et al. (2021)
	Glioblastoma	Upregulation	Protein	Advanced differentiation grade	Nishimura et al. (2019)
			mRNA	Advanced tumor stage	
	Bladder cancer	Upregulation	mRNA and protein	Poor prognosis	Zhu et al. (2022a)
SHMT1	Hepatocellular carcinoma	Downregulation	Protein	Shorter OS	Dou et al. (2019)
	Renal cell cancer	Downregulation	Protein mRNA	Shorter OS	Yang et al. (2023)
		Downregulation			
SHMT2	Thyroid cancer	Upregulation	Protein	Advanced TNM stage and shorter PFS	Jin et al. (2021)
	Colorectal cancer	Upregulation	Protein	Advanced TNM stage and lymph node metastasis	Cui et al. (2022a)

DFS, disease-free survival; OS, overall survival; PFS, progression-free survival; SHIN1, SHMT, inhibitor; TNM, tumor, lymph node, metastasis.

metabolic enzyme expression indicates an essential role of one-carbon metabolism and MTHFD2 in tumorigenesis and progression (Nilsson et al., 2014). MTHFD2 expression has been found to be dysregulated in multiple tumor types, especially solid and hematological tumors. MTHFD2 expression is correlated to clinicopathological features and clinical outcomes, implying the potential of MTHFD2 expression as a prognostic indicator in a disease-specific manner. MTHFD2 expression has been found to be upregulated in multiple tumor types (illustrated in Table 1).

A limitation of some anti-metabolite drugs is that multiple targeted metabolic enzymes expressed in both tumor cells and healthy proliferative cells, leading to adverse side-effects and therefore limiting the utilization of anti-metabolite regimens in clinical settings. The protein of MTHFD2 is expressed in immature cells and transformed cells, but importantly not in mature and normal cells. Thus, MTHFD2 is a promising therapeutic target to selectively eradicate tumor cells by disturbing one-carbon metabolism while sparing healthy proliferative cells. LY345899 has been characterized as a potent MTHFD2 inhibitor (Gustafsson et al., 2017). In CRC, the anti-tumor effects of LY345899 on tumor growth and metastasis have been verified *in vitro* and *in vivo* (Ju et al., 2019). In models of castration-resistant prostate cancer, another MTHFD2 inhibitor, DS18561882, combined with enzalutamide could suppress tumor growth (Zhao et al., 2022). DS44960156, a new isozyme-selective MTHFD2 inhibitor, has been developed with higher selectivity for MTHFD2 than MTHFD1 and good ligand efficiency (Kawai et al., 2019) (Table 2).

Targeting SHMTs in cancer

The reversible conversion of serine and tetrahydrofolate to glycine and 5,10- methylene tetrahydrofolates is catalyzed by SHMTs, which is a critical step of one-carbon metabolism. SHMTs have two isoforms, namely, the cytoplasmic SHMT1 and mitochondrial SHMT2. This reaction provides one-carbon units for nucleotide biosynthesis, regulating DNA methylation and NADPH generation. Targeting SHMTs may be exploited as potentially promising therapeutic strategy for developing anti-tumor drugs.

Targeting SHMT1 in cancer

In some cancers, SHMT1 acts as a tumor promoter. In CRC, SHMT1 functions as a critical metabolic switch, providing one-carbon units between the thymidylate biosynthesis and methionine biosynthesis. Modifying the expression SHMT1 in $Apc^{min/+}$ mice could affect the contributions of purine, thymidylate and methionine biosynthesis, and ultimately determining genome stability to indue CRC initiation (Macfarlane et al., 2011). In non–small cell lung cancer, glycogen synthase kinase 3 (GSK3) mediated the expression of one-carbon metabolic enzymes, especially SHMT1. Nuclear enrichment of GSK3 could suppress expression of SHMT1 in lung cancer cells. Moreover, pharmaceutical inhibition of GSK3 by CHIR99021 confered a metabolic vulnerability to enhance the efficacy of SHMT1/2 inhibitor SHIN1 in lung cancer cells (He et al., 2022). In addition, SHMT1 inhibition induced p53-dependent apoptosis and cell cycle arrest in lung cancer cells (Paone et al., 2014).

SHMT1 functions as a tumor suppressor in hepatocellular carcinoma (HCC) and renal cell carcinoma. In HCC cells, SHMT1 overexpression could impair the metastatic ability of HCCLM3 cells while SHMT1 inhibition could augment the metastasis of HCC cells (Hep3B). Mechanically, SHMT1 inhibition led to increased reactive oxygen species to promote epithelial and mesenchymal transition in HCC cells

TABLE 2 Targeting one-carbon metabolic enzymes in tumors.

Gene	Methods	Cancer type	Results	Status	Reference
MTHFD2	Inhibitor	Colorectal cancer	Inhibiting growth, lung and peritoneal metastasis	Preclinical	Ju et al. (2019)
	LY345899				
	Inhibitor TH9619	Acute myeloid leukemia	Inhibiting growth	Preclinical	Bonagas et al. (2022)
	Inhibitor DS18561882	Prostate cancer	Inhibiting growth	Preclinical	Zhao et al. (2022)
	siRNA	Non-small-cell lung cancer	Inhibiting growth	Preclinical	Gao et al. (2022)
		Lung adenocarcinoma	Inhibiting growth and metastasis	Preclinical	Shi et al. (2021)
		Lung adenocarcinoma	Inhibiting growth	Preclinical	Mo et al. (2022)
		Ovarian cancer	Inhibiting growth and metastasis	Preclinical	Li et al. (2021b)
		Glioblastoma	Inhibiting growth	Preclinical	Zhu et al. (2022b)
	Administered nanoliposomal siRNA	Prostate cancer	Inhibiting growth	Preclinical	Pällmann et al. (2021)
	shRNA	Acute myeloid leukemia	Inhibiting growth	Preclinical	Pikman et al. (2016)
		Renal cell carcinoma	Inhibiting growth	Preclinical	Green et al. (2019)
		Lung cancer	Inhibiting growth and stemness	Preclinical	Nishimura et al. (2019)
					Chan et al. (2020)
		Pancreatic cancer	Inhibiting growth	Preclinical	Shang et al. (2021)
SHMT1/2	Inhibitor	T-cell lymphoblastic leukemia	Inhibiting growth	Preclinical	Pikman et al. (2022)
	RZ-2994				
	Inhibitor	T-cell lymphoblastic leukemia	Inhibiting growth	Preclinical	Ducker et al. (2017)
	SHIN1				
	Inhibitor	T-cell lymphoblastic leukemia	Inhibiting growth	Preclinical	Garcia-Canaveras et al. (2021)
	SHIN1				

siRNA, small interfering RNA; shRNA, short hairpin RNA; SHIN1, SHMT, inhibitor.

(Hep3B) (Dou et al., 2019). SHMT1 overexpression significantly retarded the growth of renal cell carcinoma. Homeobox D8 (HOXD8) functions as the upstream regulator of SHMT1. HOXD8 upregulated SHMT1 expression to impair the proliferative and migrative ability of renal cell carcinoma, indicating SHMT1 as a tumor suppressor for renal cell carcinoma (Yang et al., 2023).

Targeting SHMT2 in cancer

One-carbon units are required for tRNA methylatatin, which is critical for mitochondrial translation and oxidative phosphorylation. In colon cancer, SHMT2 maintained the expression of mitochondrial respiratory chain proteins to sustain oxidative phosphorylation (Morscher et al., 2018). SHMT2 is required for tumor cells for the adaptation to the tumor microenvironment, rendering tumor cells be more sensitive to glycine cleavage system inhibition. SHMT2 could inhibit PKM2 activity and oxidative phosphorylation, conferring survival advantages for tumor cells in poorly vascularized tumor regions (Kim et al., 2015).

The post-translational modifications in the regulation of SHMT2 in CRC cells are under great investigations. SIRT5 could desuccinylate SHMT2 at lysine 280 to enhance its metabolic activity and promote serine catabolism in CRC cells. Hypersuccinylation of SHMT2 at lysine 280 led to impaired enzymatic activity and CRC proliferation (Yang et al., 2018). Lysine acetylation also participates in the regulation of SHMT2 by disrupting its functional structure and inhibiting its enzymatic activity via TRIM21-mediated K63-ubiquitin-lysosome pathway. SHMT2 K95-Ac impaired CRC proliferation *in vivo* and *in vitro* by reducing serine consumption and NADPH generation (Wei et al., 2018). SHMT2 could interact with β-catenin and inhibit the ubiquitylation-mediated degradation of β-catenin, and ultimately promoting CRC proliferation and metastasis. TCF4 could interact with β-catenin to increase SHMT2 expression and form an SHMT2/β-catenin positive feedback loop (Liu et al., 2021b). This SHMT2/β-catenin loop represents a promising therapeutic target for CRC treatment. Herein, targeting SHMT2 represents a potentially attractive strategy for cancer treatment.

SHMT2 also participates in the regulation of 5-fluorouracil chemoresistance of CRC. Chen et al. found that SHMT2 binds cytosolic p53 and prevents cytosolic p53 degradation, leading to impaired autophagy. Under 5-fluorouracil treatment, SHMT2 abrogation could enhance autophagy and inhibit cell apoptosis in CRC (Chen et al., 2021). The p53-SHMT2

interaction may provide novel targets for overcoming chemoresistance. In another study, Pranzini et al. found that 5-fluorouracil -resistant CRC cells exhibit increased reliance to serine by enhanced serine biosynthesis or exogenous serine uptake. The SHMT2-induced serine metabolism represents a metabolic adaptation of 5-fluorouracil-resistant CRC cells to potentiate DNA damage response (Pranzini et al., 2022).

Clinical implications of SHMT1/2 in cancer

Protein level of SHMT1 has been found to be downregulated in HCC, and reduced SHMT1 expression was associated with decreased overall survival of patients (Dou et al., 2019). SHMT1 protein was reduced in renal cell carcinoma tissues and correlated with poor prognosis of patients. Besides, Shmt1 hemizygosity has been found to be correlated with increased risk for intestinal tumor in $Apc^{min/+}$ mice (Macfarlane et al., 2011).

RZ-2994, a novel inhibitor of SHMT1 and SHMT2, could induce G2 cell cycle arrest in T-cell lymphoblastic leukemia (Pikman et al., 2022). Moreover, RZ-2994 could effectively reduce leukemia burden in the setting of methotrexate resistance. a dual SHMT1/2 inhibitor. Ducker et al. designed a folate-competitive inhibitor of SHMTs, namely, SHIN1. SHIN1 exhibits its potential to specifically target SHMTs and impair cell proliferation in a number of tumor cell lines (Ducker et al., 2017). Another SHMT inhibitor SHIN2 could block the growth of T-cell lymphoblastic leukemia. Moreover, methotrexate-resistant tumor cells exhibited increased sensitivity to SHIN2 (Garcia-Canaveras et al., 2021).

Conclusion

One-carbon metabolism has been found to be dysregulated in various cancer types due to its role in generation of purine and pyrimidine nucleotides, epigenetic program, and redox homeostasis, and is composed a network of one-carbon metabolic enzymes. MTHFDs and SHMTs are participated in the tumor initiation and progression, gradually being recognized as potential therapeutic anti-tumor targets. Several inhibitors targeting MTHFD2, SHMT1/2 has exerted its potential to decrease tumor burden and inhibit tumor proliferation.

Author contributions

XZ: Writing–original draft. ZW: Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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.

Publisher's note

References

Achreja, A., Yu, T., Mittal, A., Choppara, S., Animasahun, O., Nenwani, M., et al. (2022). Metabolic collateral lethal target identification reveals MTHFD2 paralogue dependency in ovarian cancer. *Nat. Metab.* 4 (9), 1119–1137. doi:10.1038/s42255-022-00636-3

Armstrong, A. J., Szmulewitz, R. Z., Petrylak, D. P., Holzbeierlein, J., Villers, A., Azad, A., et al. (2019). ARCHES: a randomized, phase III study of androgen deprivation therapy with enzalutamide or placebo in men with metastatic hormone-sensitive prostate cancer. *J. Clin. Oncol.* 37 (32), 2974–2986. doi:10.1200/JCO.19.00799

Bonagas, N., Gustafsson, N. M. S., Henriksson, M., Marttila, P., Gustafsson, R., Wiita, E., et al. (2022). Pharmacological targeting of MTHFD2 suppresses acute myeloid leukemia by inducing thymidine depletion and replication stress. *Nat. Cancer* 3 (2), 156–172. doi:10.1038/s43018-022-00331-y

Chan, C. H., Wu, C. Y., Dubey, N. K., Wei, H. J., Lu, J. H., Mao, S., et al. (2020). Modulating redox homeostasis and cellular reprogramming through inhibited methylenetetrahydrofolate dehydrogenase 2 enzymatic activities in lung cancer. *Aging (Albany NY)* 12 (18), 17930–17947. doi:10.18632/aging.103471

Chen, J., Na, R., Xiao, C., Wang, X., Wang, Y., Yan, D., et al. (2021). The loss of SHMT2 mediates 5-fluorouracil chemoresistance in colorectal cancer by upregulating autophagy. *Oncogene* 40 (23), 3974–3988. doi:10.1038/s41388-021-01815-4

Cui, X., Cui, Y., Du, T., Jiang, X., Song, C., Zhang, S., et al. (2022b). SHMT2 drives the progression of colorectal cancer by regulating UHRF1 expression. *Can. J. Gastroenterol. Hepatol.* 2022, 3758697. doi:10.1155/2022/3758697

Cui, X., Su, H., Yang, J., Wu, X., Huo, K., Jing, X., et al. (2022a). Up-regulation of MTHFD2 is associated with clinicopathological characteristics and poor survival in ovarian cancer, possibly by regulating MOB1A signaling. *J. Ovarian Res.* 15 (1), 23. doi:10.1186/s13048-022-00954-w

Cuthbertson, C. R., Arabzada, Z., Bankhead, A., 3rd., Kyani, A., and Neamati, N. (2021). A review of small-molecule inhibitors of one-carbon enzymes: SHMT2 and MTHFD2 in the spotlight. *ACS Pharmacol. Transl. Sci.* 4 (2), 624–646. doi:10.1021/acsptsci.0c00223

Dekhne, A. S., Hou, Z., Gangjee, A., and Matherly, L. H. (2020). Therapeutic targeting of mitochondrial one-carbon metabolism in cancer. *Mol. Cancer Ther.* 19 (11), 2245–2255. doi:10.1158/1535-7163.MCT-20-0423

Dou, C., Xu, Q., Liu, J., Wang, Y., Zhou, Z., Yao, W., et al. (2019). SHMT1 inhibits the metastasis of HCC by repressing NOX1-mediated ROS production. *J. Exp. Clin. Cancer Res.* 38 (1), 70. doi:10.1186/s13046-019-1067-5

Ducker, G. S., Ghergurovich, J. M., Mainolfi, N., Suri, V., Jeong, S. K., Hsin-Jung, , et al. (2017). Human SHMT inhibitors reveal defective glycine import as a targetable metabolic vulnerability of diffuse large B-cell lymphoma. *Proc. Natl. Acad. Sci.* 114, 11404–11409. doi:10.1073/pnas.1706617114

Ducker, G. S., and Rabinowitz, J. D. (2017). One-carbon metabolism in health and disease. *Cell Metab.* 25 (1), 27–42. doi:10.1016/j.cmet.2016.08.009

Friso, S., Udali, S., De Santis, D., and Choi, S. W. (2017). One-carbon metabolism and epigenetics. *Mol. Asp. Med.* 54, 28–36. doi:10.1016/j.mam.2016.11.007

Gao, Y., Feng, L., Zhang, L., Geng, J., and Zhang, E. (2022). ATF4/MYC regulates MTHFD2 to promote NSCLC progression by mediating redox homeostasis. *Dis. Markers* 2022, 7527996. doi:10.1155/2022/7527996

Garcia-Canaveras, J. C., Lancho, O., Ducker, G. S., Ghergurovich, J. M., Xu, X., da Silva-Diz, V., et al. (2021). SHMT inhibition is effective and synergizes with methotrexate in T-cell acute lymphoblastic leukemia. *Leukemia* 35, 377–388. doi:10.1038/s41375-020-0845-6

Green, N. H., Galvan, D. L., Badal, S. S., Chang, B. H., LeBleu, V. S., Long, J., et al. (2019). MTHFD2 links RNA methylation to metabolic reprogramming in renal cell carcinoma. *Oncogene* 38 (34), 6211–6225. doi:10.1038/s41388-019-0869-4

Gu, Y., Si, J., Xiao, X., Tian, Y., and Yang, S. (2017). miR-92aInhibitsProliferationandInducesApoptosisbyRegulatingMethylenetetrahydrofolate Dehydrogenase2(MTHFD2)ExpressioninAcuteMyeloidLeukemia. *Oncol. Res.* 25 (7), 1069–1079. doi:10.3727/096504016X14829256525028

Gustafsson, R., Jemth, A. S., Gustafsson, N. M., Färnegårdh, K., Loseva, O., Wiita, E., et al. (2017). Crystal structure of the emerging cancer target MTHFD2 in complex with a substrate-based inhibitor. *Cancer Res.* 77 (4), 937–948. doi:10.1158/0008-5472.CAN-16-1476

He, L., Endress, J., Cho, S., Li, Z., Zheng, Y., Asara, J. M., et al. (2022). Suppression of nuclear GSK3 signaling promotes serine/one-carbon metabolism and confers metabolic vulnerability in lung cancer cells. *Sci. Adv.* 8 (20), eabm8786. doi:10.1126/sciadv.abm8786

Jin, M., Lee, W. K., You, M. H., Jang, A., Cheng, S. Y., Kim, W. G., et al. (2021). SHMT2 expression as a diagnostic and prognostic marker for thyroid cancer. *Endocr. Connect.* 10 (6), 630–636. doi:10.1530/EC-21-0135

Ju, H. Q., Lu, Y. X., Chen, D. L., Zuo, Z. X., Liu, Z. X., Wu, Q. N., et al. (2019). Modulation of redox homeostasis by inhibition of MTHFD2 in colorectal cancer: mechanisms and therapeutic implications. *J. Natl. Cancer Inst.* 111 (6), 584–596. doi:10.1093/jnci/djy160

Kawai, J., Ota, M., Ohki, H., Toki, T., Suzuki, M., Shimada, T., et al. (2019). Structure-based design and synthesis of an isozyme-selective MTHFD2 inhibitor with a tricyclic coumarin scaffold. *ACS Med. Chem. Lett.* 10 (6), 893–898. doi:10.1021/acsmedchemlett.9b00069

Kim, D., Fiske, B. P., Birsoy, K., Freinkman, E., Kami, K., Possemato, R. L., et al. (2015). SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance. *Nature* 520 (7547), 363–367. doi:10.1038/nature14363

Kurniawan, H., Franchina, D. G., Guerra, L., Bonetti, L., Baguet, L. S., Grusdat, M., et al. (2020). Glutathione restricts serine metabolism to preserve regulatory T cell function. *Cell Metab.* 31 (5), 920–936. doi:10.1016/j.cmet.2020.03.004

Li, G., Wu, J., Li, L., and Jiang, P. (2021a). p53 deficiency induces MTHFD2 transcription to promote cell proliferation and restrain DNA damage. *Proc. Natl. Acad. Sci. U. S. A.* 118 (28), e2019822118. doi:10.1073/pnas.2019822118

Li, Q., Yang, F., Shi, X., Bian, S., Shen, F., Wu, Y., et al. (2021b). MTHFD2 promotes ovarian cancer growth and metastasis via activation of the STAT3 signaling pathway. *FEBS Open Bio* 11 (10), 2845–2857. doi:10.1002/2211-5463.13249

Lin, H., Huang, B., Wang, H., Liu, X., Hong, Y., Qiu, S., et al. (2018). MTHFD2 overexpression predicts poor prognosis in renal cell carcinoma and is associated with cell proliferation and vimentin-modulated migration and invasion. *Cell Physiol. Biochem.* 51 (2), 991–1000. doi:10.1159/000495402

Liu, C., Wang, L., Liu, X., Tan, Y., Tao, L., Xiao, Y., et al. (2021a). Cytoplasmic SHMT2 drives the progression and metastasis of colorectal cancer by inhibiting β-catenin degradation. *Theranostics* 11 (6), 2966–2986. doi:10.7150/thno.48699

Liu, X., Liu, S., Piao, C., Zhang, Z., Zhang, X., Jiang, Y., et al. (2021b). Non-metabolic function of MTHFD2 activates CDK2 in bladder cancer. *Cancer Sci.* 112 (12), 4909–4919. doi:10.1111/cas.15159

Löwenberg, B., Downing, J. R., and Burnett, A. (1999). Acute myeloid leukemia. *N. Engl. J. Med.* 341 (14), 1051–1062. doi:10.1056/NEJM199909303411407

Macfarlane, A. J., Perry, C. A., McEntee, M. F., Lin, D. M., and Stover, P. J. (2011). Shmt1 heterozygosity impairs folate-dependent thymidylate synthesis capacity and modifies risk of Apc(min)-mediated intestinal cancer risk. *Cancer Res.* 71 (6), 2098–2107. doi:10.1158/0008-5472.CAN-10-1886

Meng, Q., Lu, Y. X., Wei, C., Wang, Z. X., Lin, J. F., Liao, K., et al. (2022). Arginine methylation of MTHFD1 by PRMT5 enhances anoikis resistance and cancer metastasis. *Oncogene* 41 (32), 3912–3924. doi:10.1038/s41388-022-02387-7

Miyo, M., Konno, M., Colvin, H., Nishida, N., Koseki, J., Kawamoto, K., et al. (2016). The importance of mitochondrial folate enzymes in human colorectal cancer. *Oncol. Rep.* 37 (1), 417–425. doi:10.3892/or.2016.5264

Mo, J., Gao, Z., Zheng, L., Yan, M., Xue, M., Xu, J., et al. (2022). Targeting mitochondrial one-carbon enzyme MTHFD2 together with pemetrexed confers therapeutic advantages in lung adenocarcinoma. *Cell Death Discov.* 8 (1), 307. doi:10.1038/s41420-022-01098-y

Morscher, R. J., Ducker, G. S., Li, S. H., Mayer, J. A., Gitai, Z., Sperl, W., et al. (2018). Mitochondrial translation requires folate-dependent tRNA methylation. *Nature* 554 (7690), 128–132. doi:10.1038/nature25460

Newman, A. C., and Maddocks, O. D. K. (2017). One-carbon metabolism in cancer. *Br. J. Cancer* 116 (12), 1499–1504. doi:10.1038/bjc.2017.118

Nilsson, R., Jain, M., Madhusudhan, N., Sheppard, N. G., Strittmatter, L., Kampf, C., et al. (2014). Metabolic enzyme expression highlights a key role for MTHFD2 and the mitochondrial folate pathway in cancer. *Nat. Commun.* 5, 3128. doi:10.1038/ncomms4128

Nishimura, T., Nakata, A., Chen, X., Nishi, K., Meguro-Horike, M., Sasaki, S., et al. (2019). Cancer stem-like properties and gefitinib resistance are dependent on purine synthetic metabolism mediated by the mitochondrial enzyme MTHFD2. *Oncogene* 38 (14), 2464–2481. doi:10.1038/s41388-018-0589-1

Pällmann, N., Deng, K., Livgård, M., Tesikova, M., Jin, Y., Frengen, N. S., et al. (2021). Stress-mediated reprogramming of prostate cancer one-carbon cycle drives disease progression. *Cancer Res.* 81 (15), 4066–4078. doi:10.1158/0008-5472.CAN-20-3956

Pan, R., Yuan, Z., Liu, Y., Sun, X., Wang, G., Wang, X., et al. (2021a). A redox probe screens MTHFD1 as a determinant of gemcitabine chemoresistance in cholangiocarcinoma. *Cell Death Discov.* 7 (1), 89. doi:10.1038/s41420-021-00476-2

Pan, S., Fan, M., Liu, Z., Li, X., and Wang, H. (2021b). Serine, glycine and one-carbon metabolism in cancer (Review). *Int. J. Oncol.* 58 (2), 158–170. doi:10.3892/ijo.2020.5158

Paone, A., Marani, M., Fiascarelli, A., Rinaldo, S., Giardina, G., Contestabile, R., et al. (2014). SHMT1 knockdown induces apoptosis in lung cancer cells by causing uracil misincorporation. *Cell Death Dis.* 5 (11), e1525. doi:10.1038/cddis.2014.482

Pikman, Y., Ocasio-Martinez, N., Alexe, G., Dimitrov, B., Kitara, S., Diehl, F. F., et al. (2022). Targeting serine hydroxymethyltransferases 1 and 2 for T-cell acute lymphoblastic leukemia therapy. *Leukemia* 36 (2), 348–360. doi:10.1038/s41375-021-01361-8

Pikman, Y., Puissant, A., Alexe, G., Furman, A., Chen, L. M., Frumm, S. M., et al. (2016). Targeting MTHFD2 in acute myeloid leukemia. *J. Exp. Med.* 213 (7), 1285–1306. doi:10.1084/jem.20151574

Piskounova, E., Agathocleous, M., Murphy, M. M., Hu, Z., Huddlestun, S. E., Zhao, Z., et al. (2015). Oxidative stress inhibits distant metastasis by human melanoma cells. *Nature* 527 (7577), 186–191. doi:10.1038/nature15726

Pranzini, E., Pardella, E., Muccillo, L., Leo, A., Nesi, I., Santi, A., et al. (2022). SHMT2-mediated mitochondrial serine metabolism drives 5-FU resistance by fueling nucleotide biosynthesis. *Cell Rep.* 40 (7), 111233. doi:10.1016/j.celrep.2022.111233

Ron-Harel, N., Santos, D., Ghergurovich, J. M., Sage, P. T., Reddy, A., Lovitch, S. B., et al. (2016). Mitochondrial biogenesis and proteome remodeling promote one-carbon metabolism for T cell activation. *Cell Metab.* 24 (1), 104–117. doi:10.1016/j.cmet.2016.06.007

Shang, M., Yang, H., Yang, R., Chen, T., Fu, Y., Li, Y., et al. (2021). The folate cycle enzyme MTHFD2 induces cancer immune evasion through PD-L1 up-regulation. *Nat. Commun.* 12 (1), 1940. doi:10.1038/s41467-021-22173-5

Shi, Y., Xu, Y., Yao, J., Yan, C., Su, H., Zhang, X., et al. (2021). MTHFD2 promotes tumorigenesis and metastasis in lung adenocarcinoma by regulating AKT/GSK-3β/β-catenin signalling. *J. Cell Mol. Med.* 25 (14), 7013–7027. doi:10.1111/jcmm.16715

Sugiura, A., Andrejeva, G., Voss, K., Heintzman, D. R., Xu, X., Madden, M. Z., et al. (2022). MTHFD2 is a metabolic checkpoint controlling effector and regulatory T cell fate and function. *Immunity* 55 (1), 65–81.e9. doi:10.1016/j.immuni.2021.10.011

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Tibbetts, A. S., and Appling, D. R. (2010). Compartmentalization of Mammalian folate-mediated one-carbon metabolism. *Annu. Rev. Nutr.* 30, 57–81. doi:10.1146/annurev.nutr.012809.104810

Wan, X., Wang, C., Huang, Z., Zhou, D., Xiang, S., Qi, Q., et al. (2020). Cisplatin inhibits SIRT3-deacetylation MTHFD2 to disturb cellular redox balance in colorectal cancer cell. *Cell Death Dis.* 11 (8), 649. doi:10.1038/s41419-020-02825-y

Wei, Y., Liu, P., Li, Q., Du, J., Chen, Y., Wang, Y., et al. (2019). The effect of MTHFD2 on the proliferation and migration of colorectal cancer cell lines. *Onco Targets Ther.* 12, 6361–6370. doi:10.2147/OTT.S210800

Wei, Z., Song, J., Wang, G., Cui, X., Zheng, J., Tang, Y., et al. (2018). Deacetylation of serine hydroxymethyl-transferase 2 by SIRT3 promotes colorectal carcinogenesis. *Nat. Commun.* 9 (1), 4468. doi:10.1038/s41467-018-06812-y

Yan, Y., Zhang, D., Lei, T., Zhao, C., Han, J., Cui, J., et al. (2019). MicroRNA-33a-5p suppresses colorectal cancer cell growth by inhibiting MTHFD2. *Clin. Exp. Pharmacol. Physiol.* 46 (10), 928–936. doi:10.1111/1440-1681.13125

Yang, C., Zhang, J., Liao, M., Yang, Y., Wang, Y., Yuan, Y., et al. (2021). Folate-mediated one-carbon metabolism: a targeting strategy in cancer therapy. *Drug Discov. Today* 26 (3), 817–825. doi:10.1016/j.drudis.2020.12.006

Yang, X., Wang, Z., Li, X., Liu, B., Liu, M., Liu, L., et al. (2018). SHMT2 desuccinylation by SIRT5 drives cancer cell proliferation. *Cancer Res.* 78 (2), 372–386. doi:10.1158/0008-5472.CAN-17-1912

Yang, Y., Zhang, M., Zhao, Y., Deng, T., Zhou, X., Qian, H., et al. (2023). HOXD8 suppresses renal cell carcinoma growth by upregulating SHMT1 expression. *Cancer Sci.* 114, 4583–4595. doi:10.1111/cas.15982

Yu, H., Wang, H., Xu, H. R., Zhang, Y. C., Yu, X. B., Wu, M. C., et al. (2019). Overexpression of MTHFD1 in hepatocellular carcinoma predicts poorer survival and recurrence. *Future Oncol.* 15 (15), 1771–1780. doi:10.2217/fon-2018-0606

Zhang, F., Wang, D., Li, J., Su, Y., Liu, S., Lei, Q. Y., et al. (2022). Deacetylation of MTHFD2 by SIRT4 senses stress signal to inhibit cancer cell growth by remodeling folate metabolism. *J. Mol. Cell Biol.* 14 (4), mjac020. doi:10.1093/jmcb/mjac020

Zhao, L. N., Björklund, M., Caldez, M. J., Zheng, J., and Kaldis, P. (2021). Therapeutic targeting of the mitochondrial one-carbon pathway: perspectives, pitfalls, and potential. *Oncogene* 40 (13), 2339–2354. doi:10.1038/s41388-021-01695-8

Zhao, R., Feng, T., Gao, L., Sun, F., Zhou, Q., Wang, X., et al. (2022). PPFIA4 promotes castration-resistant prostate cancer by enhancing mitochondrial metabolism through MTHFD2. *J. Exp. Clin. Cancer Res.* 41 (1), 125. doi:10.1186/s13046-022-02331-3

Zhu, L., Liu, X., Zhang, W., Hu, H., Wang, Q., and Xu, K. (2022b). MTHFD2 is a potential oncogene for its strong association with poor prognosis and high level of immune infiltrates in urothelial carcinomas of bladder. *BMC Cancer* 22 (1), 556. doi:10.1186/s12885-022-09606-0

Zhu, Z., Kiang, K. M., Li, N., Liu, J., Zhang, P., Jin, L., et al. (2022a). Folate enzyme MTHFD2 links one-carbon metabolism to unfolded protein response in glioblastoma. *Cancer Lett.* 549, 215903. doi:10.1016/j.canlet.2022.215903

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 07 March 2024
DOI 10.3389/fphar.2024.1344317

OPEN ACCESS

EDITED BY
Elisa Giovannetti,
VU Medical Center, Netherlands

REVIEWED BY
Jungshan Chang,
Taipei Medical University, Taiwan
Ketki Bhise,
Biotech Industry, United States

*CORRESPONDENCE
Haitao Ma,
mht7403@163.com
Yu Feng,
fengyu1@suda.edu.cn
Wei Jiang,
jweicat@163.com

†These authors have contributed equally to this work

RECEIVED 25 November 2023
ACCEPTED 21 February 2024
PUBLISHED 07 March 2024

CITATION
Xie K, Chen Z, Feng J, Pan L, Wang N, Luo J, Yao Y, Ma H, Feng Y and Jiang W (2024), Identification and validation of prognostic and immunotherapeutic responses in esophageal squamous carcinoma based on hypoxia phenotype-related genes.
Front. Pharmacol. 15:1344317.
doi: 10.3389/fphar.2024.1344317

Identification and validation of prognostic and immunotherapeutic responses in esophageal squamous carcinoma based on hypoxia phenotype-related genes

Kai Xie[1†], Zhe Chen[1†], Jian Feng[2†], Liangbin Pan[3], Nan Wang[1], Jing Luo[4], Yu Yao[5], Haitao Ma[1,3]*, Yu Feng[3]* and Wei Jiang[1]*

[1]Department of Thoracic Surgery, The Fourth Affiliated Hospital of Soochow University, Suzhou, China, [2]Department of Thoracic Surgery, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, China, [3]Department of Thoracic Surgery, The First Affiliated Hospital of Soochow University, Suzhou, China, [4]Department of Cardiothoracic Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, China, [5]Department of Respiratory Medicine, Nanjing Second Hospital, Nanjing, China

The study aimed to investigate the clinical significance of the interaction between hypoxia and the immune system in esophageal squamous cell carcinoma (ESCC) microenvironment. A comprehensive evaluation of 13 hypoxia phenotype-related genes (HPRs) was conducted using data from TCGA-ESCC and two GEO cohorts. Three distinct HPRclusters were identified, and the HPRscore was established as an independent prognostic factor (p = 0.001), with higher scores indicating poorer prognosis. The HPRscore was validated in various immunotherapy cohorts, demonstrating its efficacy in evaluating immunotherapy and chemotherapy outcomes. Additionally, phenome-wide association study (PheWAS) analysis showed that *PKP1* had no significant correlation with other traits at the gene level. *PKP1* was identified as a potential prognostic marker for ESCC, with upregulated expression observed in ESCC patients. *In vitro* experiments showed that the knockdown of *PKP1* inhibited ESCC cell proliferation and migration. These findings suggest that the novel HPRscore and *PKP1* may serve as prognostic tools and therapeutic targets for ESCC patients.

KEYWORDS

esophageal squamous cell carcinoma, hypoxia, tumor microenvironment, immunotherapy, Prognosis, PKP1, PheWAS

Abbreviations: ESCC, esophageal squamous carcinoma; EC, esophageal cancer; HPRs, hypoxia phenotype-related genes; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; FDR, false discovery rate; MSigDB, the molecular signatures database; DEGs, differentially expressed genes; PCA, principal component analysis; ssGSEA, single-sample gene-set enrichment analysis; GSVA, gene set variation analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PheWAS, phenome-wide association study; TME, tumor immune microenvironment.

1 Introduction

Esophageal cancer (EC) is a prevalent malignancy and ranks seventh in terms of global incidence and sixth in cancer-related mortality (Sung et al., 2021). Among the histological subtypes, esophageal squamous cell carcinoma (ESCC) accounts for more than 85% of cases (Huang and Yu, 2018; Wang et al., 2018). Despite recent therapeutic advancements and improved 5-year survival rates, the prognosis for ESCC patients remains unfavorable, primarily due to delayed clinical presentation and missed treatment opportunities (Rustgi and El-Serag, 2014). This highlights the urgent need for a deeper understanding of the disease and the development of effective therapeutic strategies.

Hypoxia is a common occurrence in various types of solid tumors and has significant implications for both anti-cancer treatment and the malignant progression of cancer. It is increasingly recognized that hypoxia plays a crucial role in contributing to poor prognosis (Jing et al., 2019). The rapid proliferation of cancer cells leads to a high oxygen demand, disrupting the balance between oxygen supply and consumption and resulting in the formation of an anoxic microenvironment within the tumor (Lee et al., 2020). The tissue of ESCC comprises various constituents, such as vasculature, immune cells, fibroblasts, and the extracellular matrix (Becht et al., 2016). The dysregulation of angiogenesis and accelerated cell proliferation in the tumor microenvironment frequently leads to diminished oxygen supply, thereby inducing hypoxia. Hypoxia has been shown to be

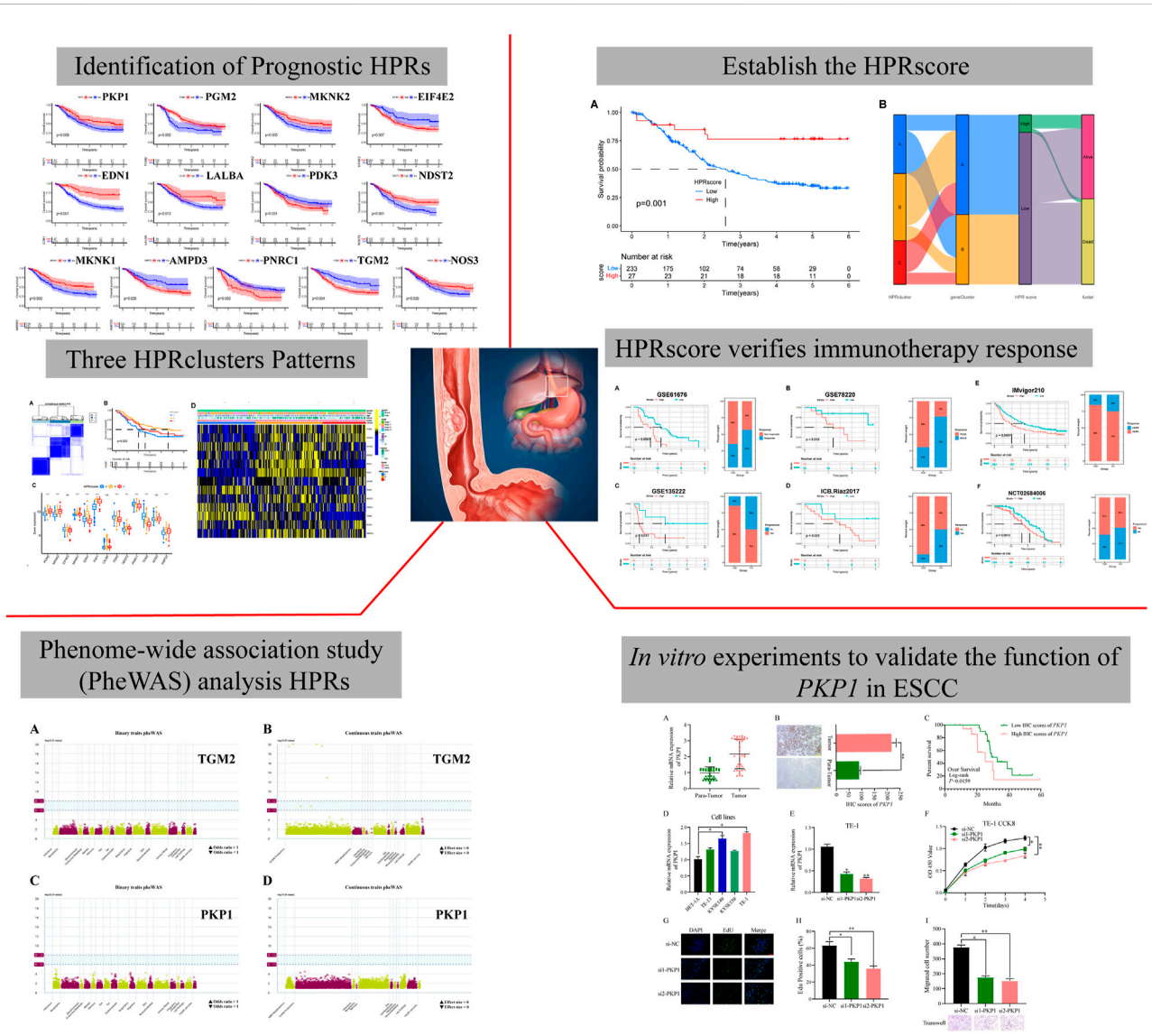


FIGURE 1
The flow diagram of the whole study. A total of 282 hypoxia phenotype-related genes (HPRs) were obtained from the KEGG and MSigDB databases. Univariate Cox combined Kaplan-Meier survival analysis was performed using data from the TCGA-ESCC and two GEO cohorts, identifying 13 independent predictors with a significance level of $p < 0.05$. Unsupervised clustering was used to identify molecular typing, which revealed three biologically unique subtypes based on the expression patterns of 13 prognostic genes. Based on this analysis, the HPR score was constructed and determined to be an independent prognostic factor ($p = 0.001$). The effectiveness of the HPRscore in immunotherapy and chemotherapy outcomes was evaluated by validating it in various immunotherapy cohorts. The potential drug targets of the HPRs and their associated side effects were explored through phenomenon association study (PheWAS) analysis. Finally, the expression and function of *PKP1* were verified *in vitro*.

associated with angiogenesis and poor prognosis in ESCC (Li et al., 2014; Yuan et al., 2023). Furthermore, immune cells play a pivotal role in regulating tumor growth through governing the invasion and metastasis of tumor cells (Lei et al., 2020). Recent studies have also revealed the influence of hypoxia on the tumor immune microenvironment (Palazon et al., 2014). However, the underlying regulatory mechanisms involving hypoxia, immunity, and ESCC remain unclear. Therefore, further studies are needed to investigate the relationship between hypoxia and immunity in ESCC.

In recent years, the application of high-throughput sequencing and public data analysis has become increasingly crucial in the discovery of biomarkers, prognosis prediction, relapse monitoring, and patient stratification (Liu et al., 2023). Several studies have employed multiple biomarkers to establish diagnostic or prognostic models in clinical settings (Xi et al., 2022; He et al., 2023). However, the role of hypoxia phenotype-related genes (HPRs) in the prognosis and response to immunotherapy in ESCC has been largely neglected.

In this study, we utilized HPRs to stratify ESCC samples based on mRNA expression levels from TCGA and GEO cohorts. Subsequently, we developed and validated a novel HPRs model and HPRscore in diverse autonomic immunotherapy cohorts, with HPRscore serving as an independent prognostic factor. Additionally, we assessed the expression and predictive impact of *PKP1* in clinical ESCC tissues. Overall, this study identifies the HPRs model and HPRscore, while also highlighting a potential therapeutic target for ESCC patients.

2 Results

Figure 1 shows the flow diagram of the whole study.

2.1 Expression and prognosis of HPRs in ESCC

This study aimed to investigate the regulatory mechanism of HPRs in ESCC through the analysis of three independent cohorts:

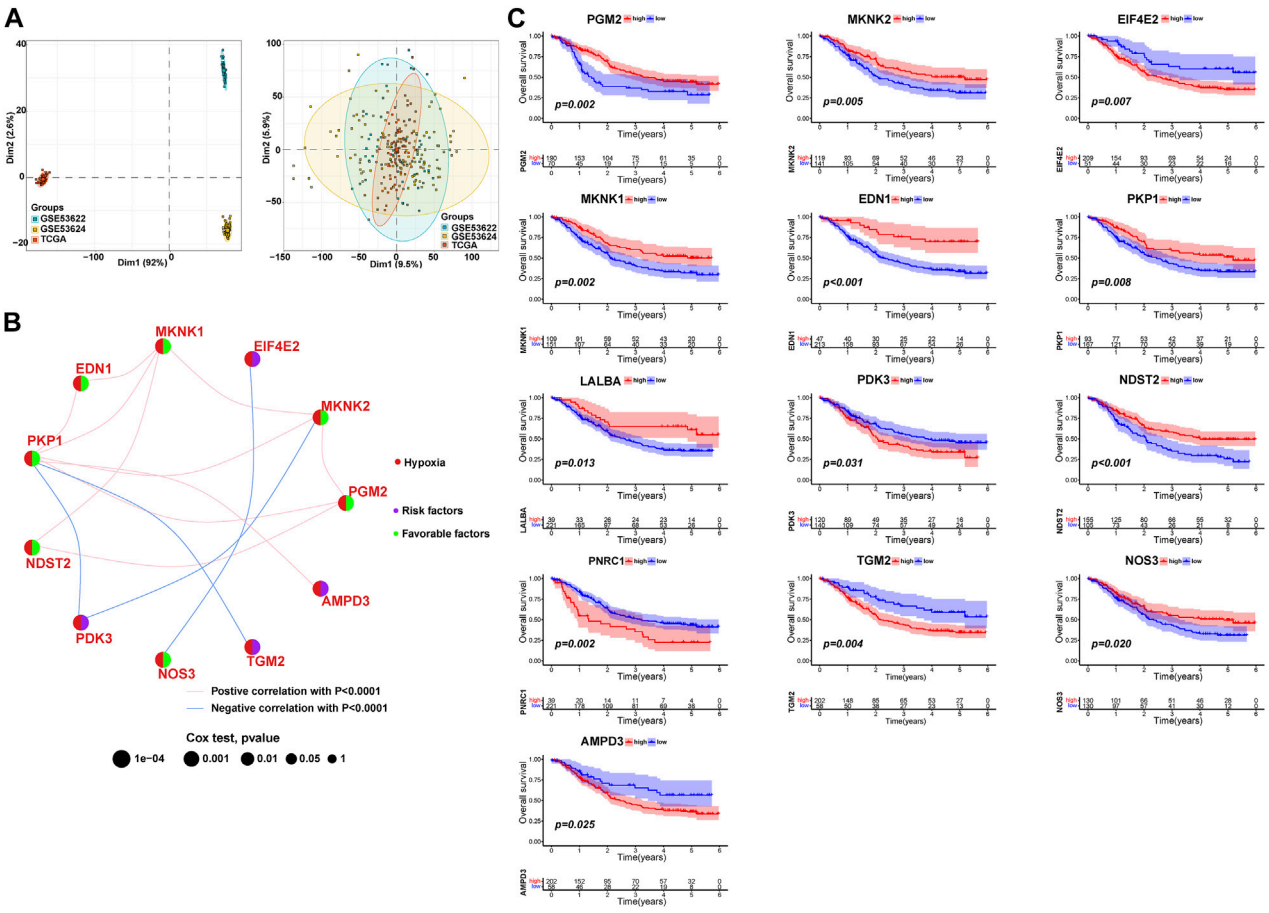


FIGURE 2
Expression and prognosis of hypoxia phenotype-related genes (HPRs) in ESCC. **(A)** Principal component analysis (PCA) showed the distribution of genes expressions in three ESCC cohorts before (left part) and after (right part) the batch effect correction. **(B)** Interaction between HPRs in ESCC. The line connecting HPRs indicated their interaction, and the thickness of the line indicated the correlation strength between HPXs. Purple and green represent negative and positive correlation, respectively. **(C)** Spearman correlation and prognostic values of hypoxia-related genes in ESCC. The circle size represents the range of significance values of each HPRs on the prognosis. The *p*-values were calculated by log-rank test. Green dots represent favorable factors for prognosis, and purple dots represent risk factors for prognosis. The lines linking HPRs represent their correlation. The thickness of the lines represents the strength of correlation between HPRs. Negative and positive correlations were marked with blue and red, respectively.

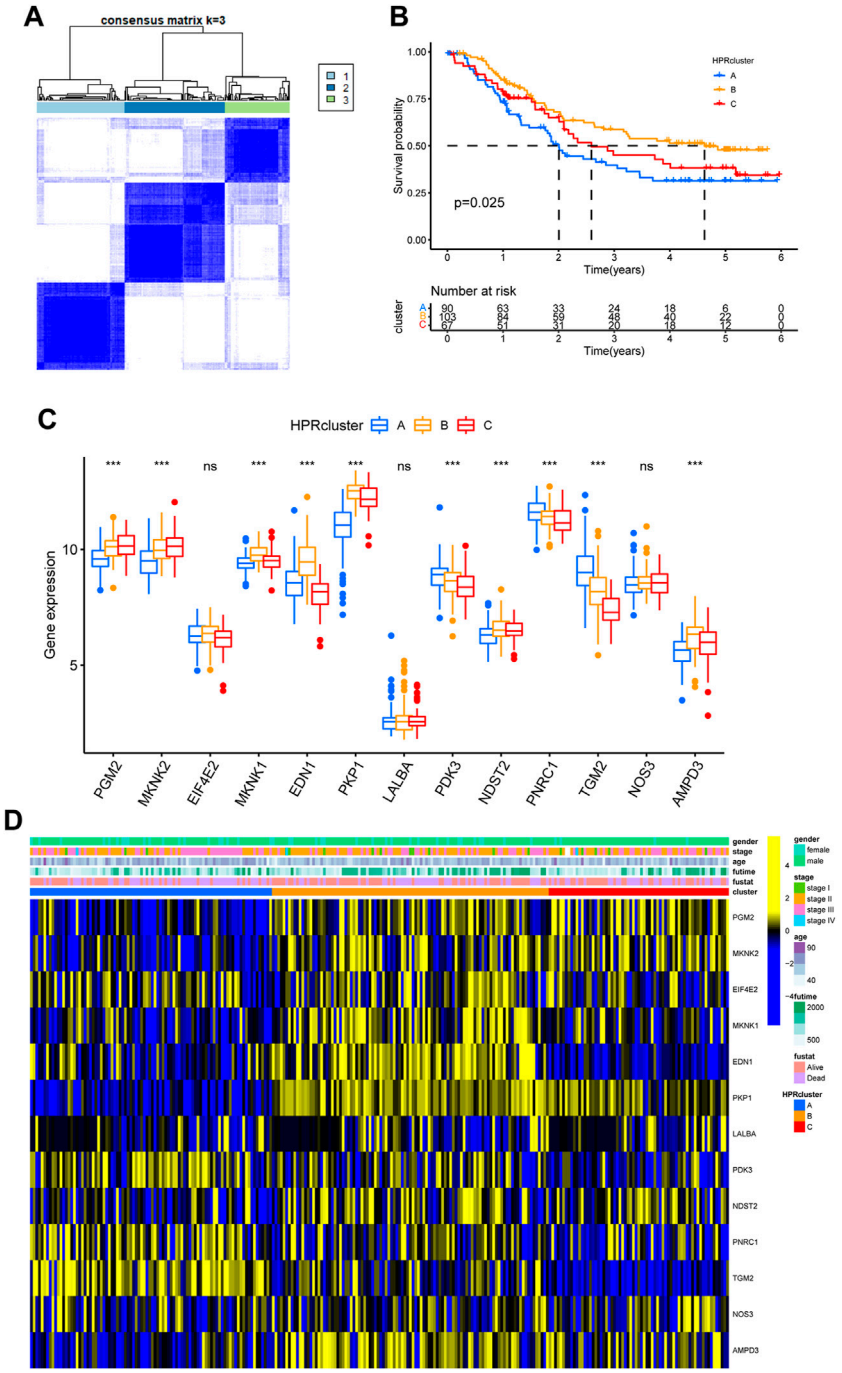


FIGURE 3
Hypoxia patterns mediated by 13 HPRs in ESCC. **(A)** The consensus matrixes for all ESCC samples displayed the clustering stability with 1,000 iterations. All samples were clustered into an appropriate number of subtypes (k = 3). **(B)** Kaplan–Meier curves showed the overall survival difference among the three HPRclusters (p = 0.025). **(C)** Gene expression levels of HPRs in three HPRclusters. **(D)** Differences in clinicopathologic features and expression levels of HPRs among the three HPRclusters. *p < 0.05, **p < 0.01, ***p < 0.001.

TCGA-ESCA (81 ESCC samples), GSE53624 (119 ESCC samples), and GSE53622 (60 ESCC samples). The combined dataset consisted of 14,047 genes and 260 ESCC samples. To effectively eliminate the batch effect across the three gene sets, PCA was conducted (Figure 2A). To identify the regulatory mechanisms underlying hypoxia phenotype-related genes in ESCC, HPRs were obtained from the KEGG *HIF-1* signaling pathway (109 genes) and the Hallmark hypoxia database (200 genes). The dataset, consisting of 282 genes, underwent merging and de-duplication (Supplementary Table S1). To demonstrate the prognostic value of HPRs in ESCC patients, univariate Cox regression and Kaplan-Meier analysis were employed with a screening threshold of $p < 0.05$ (Supplementary Table S2). Thirteen independent predictors, including *PGM2*, *MKNK2*, *EIF4E2*, *MKNK1*, *EDN1*, *PKP1*,

LALBA, *PDK3*, *NDST2*, *PNRC1*, *TGM2*, *NOS3*, and *AMPD3*, were identified. The hypoxia network depicted the integrated landscape of HPRs interactions, regulator associations, and their prognostic value in patients with ESCC (Figure 2B). Additionally, an examination was conducted to determine the correlation between the expression levels of HPRs and patient prognosis. The results revealed that the overall survival rate of thirteen genes was statistically significant between the high expression group and the low expression group (Figure 2C).

2.2 HPRclusters mediated by thirteen HPRs in ESCC

To investigate the expression characteristics of HPRs in patients with ESCC, 260 ESCC samples were analyzed using the unsupervised clustering algorithm "ConsensusClusterPlus" in the R package (Supplementary Figure S1). The clustering analysis revealed the presence of three distinct clusters: HPRcluster A (n = 90), HPRcluster B (n = 103), and HPRcluster C (n = 67) (Figure 3A; Supplementary Table S3). To determine the prognostic significance of these clusters, Kaplan-Meier analysis was performed. The analysis demonstrated that HPRcluster B exhibited a significant prognostic advantage (p = 0.025, Figure 3B), indicating that patients belonging to this cluster had a better overall prognosis compared to the other clusters. We also investigated the alterations in HPRs expression among the clusters. Figures 3C, D depicted the expression patterns, revealing that HPRs were significantly upregulated in HPRcluster B and HPRcluster C, followed by HPRcluster A. Furthermore, Fisher's exact test was employed to examine the distribution of clinicopathologic phenotypes, including age, gender, and pathologic stage, among the clusters (Figure 3D). The results showed that HPRcluster A and HPRcluster B had a higher proportion of female patients. Additionally, patients in advanced stages (Stage III or IV) were predominantly associated with HPRcluster A. In summary, this study provides important insights into the expression characteristics of HPRs in ESCC. The identification of three distinct clusters and the observation of significant upregulation of HPRs in certain clusters, along with the prognostic advantage conferred by HPRcluster B, suggest the potential of HPRs as prognostic markers in ESCC.

To investigate the biological functionalities associated with the three clusters and their impact on prognostic outcomes, a GSVA (Gene Set Variation Analysis) enrichment analysis was conducted using the "GSVA" R package (Hänzelmann et al., 2013). Gene sets derived from the HALLMARK and KEGG pathways obtained from the MSigDB database were utilized. The GSVA enrichment analysis validated the hypothesis that the three clusters possess unique biological functionalities. In HPRcluster A, compared to HPRcluster B and HPRcluster C, significantly higher Hallmark activity was observed in *ALLOGRAFT REJECTION*, *MESENCHYMAL TRANSITION*, *OXYGEN SPECIES PATHWAY*, *MTORC1 SIGNALING*, *ESTROGEN RESPONSE LATE* and *P53 PATHWAY* (Figure 4A; Supplementary Table S4). This indicates that these biological pathways and processes are more active in HPRcluster A. Furthermore, the analysis revealed that tumors in HPRcluster A exhibited a more active *METABOLISM PATHWAY* compared to HPRcluster B, and a greater activity of *BIOSYNTHESIS PATHWAY* than HPRcluster C (Figure 4B; Supplementary Table S5). These findings provided evidence that the three clusters have distinct biological functionalities, as indicated by the enrichment analysis of various pathways. The observed differences in pathway activities among the clusters may contribute to the disparate prognostic outcomes observed in patients subjected to identical treatment protocols.

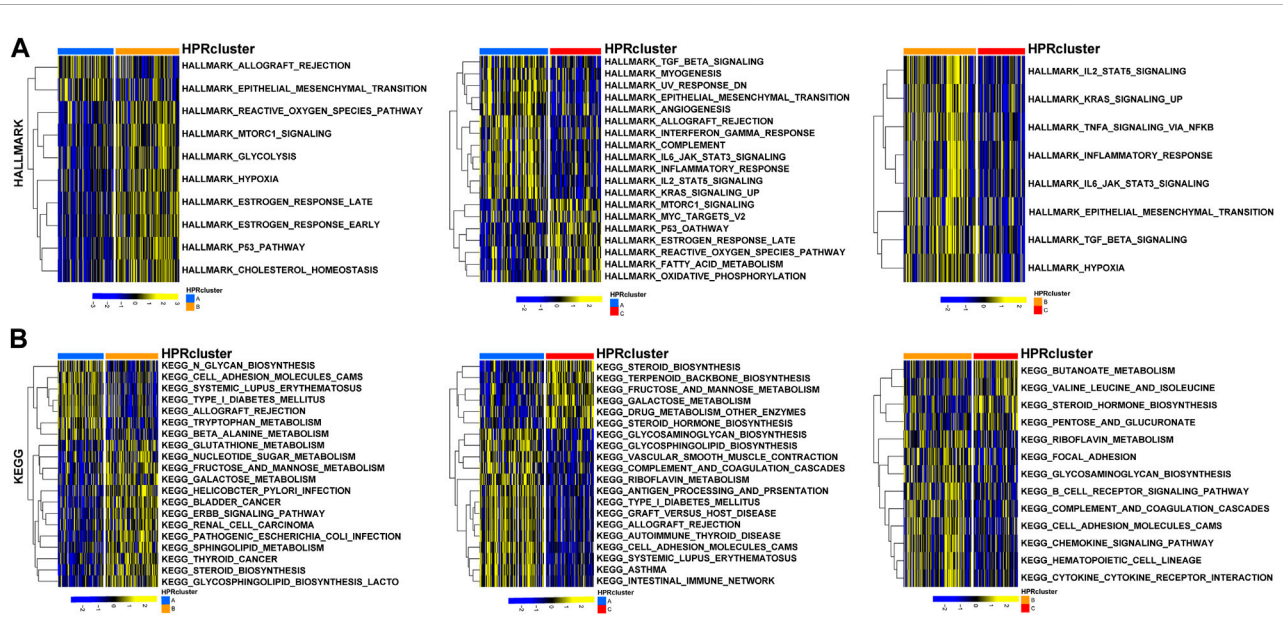


FIGURE 4
GSVA analysis. The **(A)** HALLMARK PATHWAY, **(B)** KEGG PATHWAY were downloaded separately from the Msigdb database and the pathways were scored using the R package GSVA.

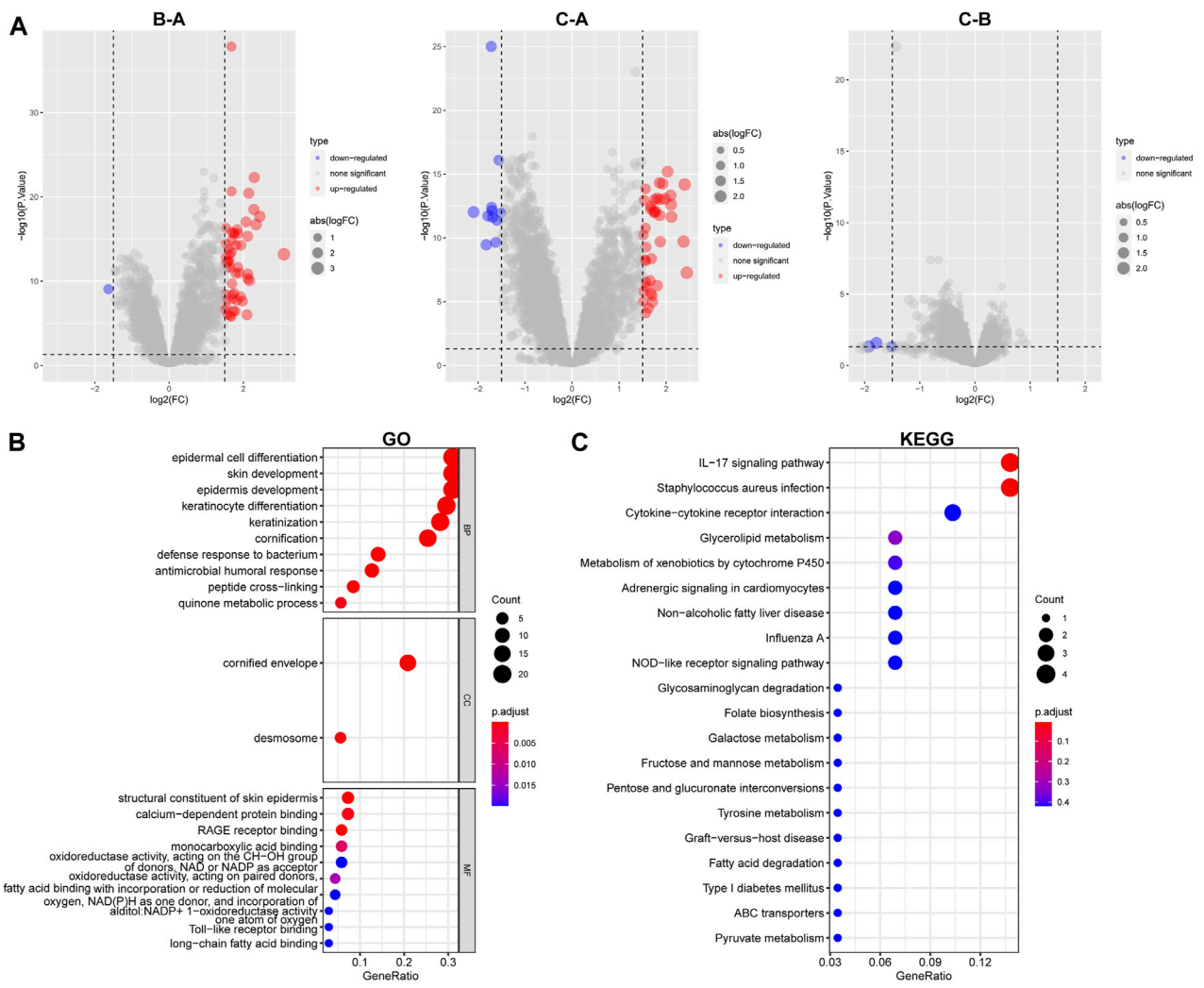


FIGURE 5
GO and KEGG analysis. **(A)** Differential analysis of the three subtypes. **(B)** GO analysis was conducted and visualized. **(C)** KEGG analysis was conducted and visualized.

In preparation for the implementation of HPRscores and the depiction of heat maps showcasing patterns of DEGs between the HPRclusters, several analyses were performed. First, pairwise comparisons of the three HPRclusters were conducted, and volcano plots were generated to visualize the DEGs. The criteria for DEG selection were set at $|\log FC| > 1.5$ and $p < 0.05$ (Figure 5A). To identify co-expressed genes, Venn diagrams were employed, revealing a total of 77 co-expressed genes (Figure 5B; Supplementary Table S6). Subsequently, the R package "ClusterProfiler" was utilized to perform GO and KEGG enrichment analyses (Figures 5C, D). The identified genes showed significant enrichment in biological processes associated with hypoxia and immunity. This finding supported the notion that hypoxia plays a pivotal role in modulating the immune response of TME. These analyses contributed to the understanding of the gene expression patterns between HPRclusters and provide insights into the biological processes influenced by hypoxia and their impact on immune responses within the TME.

2.3 Different TME pattern among the three HPRclusters

After calculating the Stromal Score, Immune Score, and ESTIMATE Score for each cluster using the ESTIMATE algorithm, it was observed that HPRcluster A exhibited the highest scores in all three categories (Figure 6A). This suggested that HPRcluster A has a greater proportion of stromal cells and immune cells compared to the other clusters. The finding of higher scores in HPRcluster A is consistent with the results obtained from CIBERSORTx analysis, which showed a higher degree of infiltration by CD4 T cells, B cells, NK cells, and regulatory T cells in HPRcluster A (Figure 6B). This indicated that HPRcluster A was associated with a more pronounced immune cell infiltration, potentially reflecting a more active immune response within the tumor microenvironment.

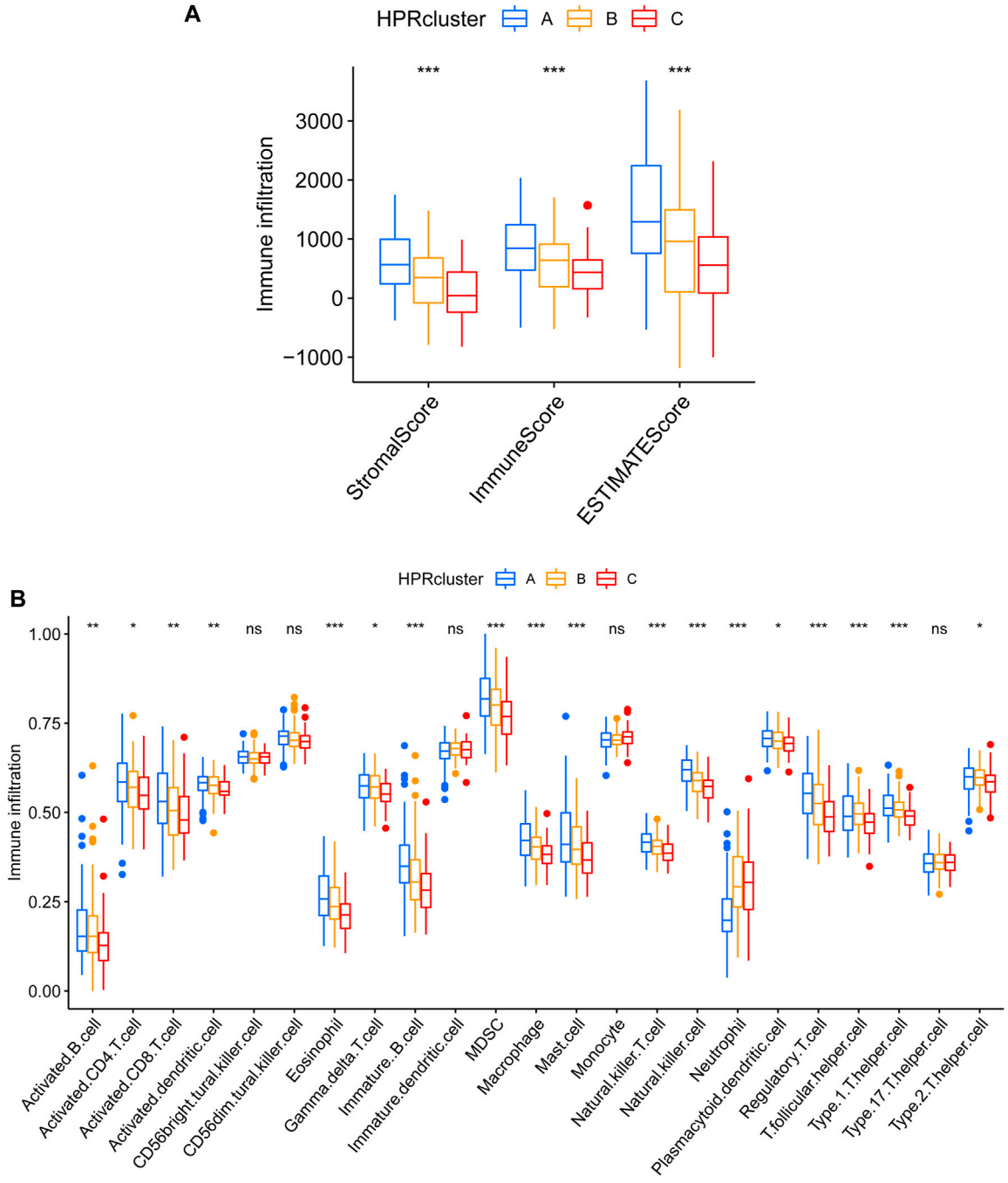


FIGURE 6
Different TME pattern among the three HPRclusters. **(A)** Differences between Stromal Score, Immune Score and ESTIMATE Score in different typologies. **(B)** Differences in immune cell infiltration between different subtypes. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns $p > 0.05$.

2.4 Generation of HPRs signatures

To explore the potential biological attributes of HPRs, a univariate Cox regression analysis was performed on the 77 DEGs listed in Supplementary Table S7. The analysis identified 22 DEGs that were significantly associated with survival ($p < 0.05$) and were selected for further investigation (Figure 7A). Subsequently, an unsupervised clustering analysis was conducted on these 22 DEGs to classify the 260 ESCC patients into two distinct geneClusters: geneCluster A (n = 153) and geneCluster B (n = 107) (Figure 7B; Supplementary Table S8). The results revealed that patients in geneCluster B had a survival disadvantage compared to those in geneCluster A (Figure 7C, $p < 0.049$). Furthermore, notable differences in the expression of DEGs were observed between the two geneClusters. Most of the DEGs were upregulated in geneCluster A, with the exception of *RAMP1* and *TGM2*, which showed differential expression patterns (Figure 7D). Lastly, a heat map was generated to highlight the clinical characteristics of the HPRclusters and geneClusters, revealing opposing characteristics

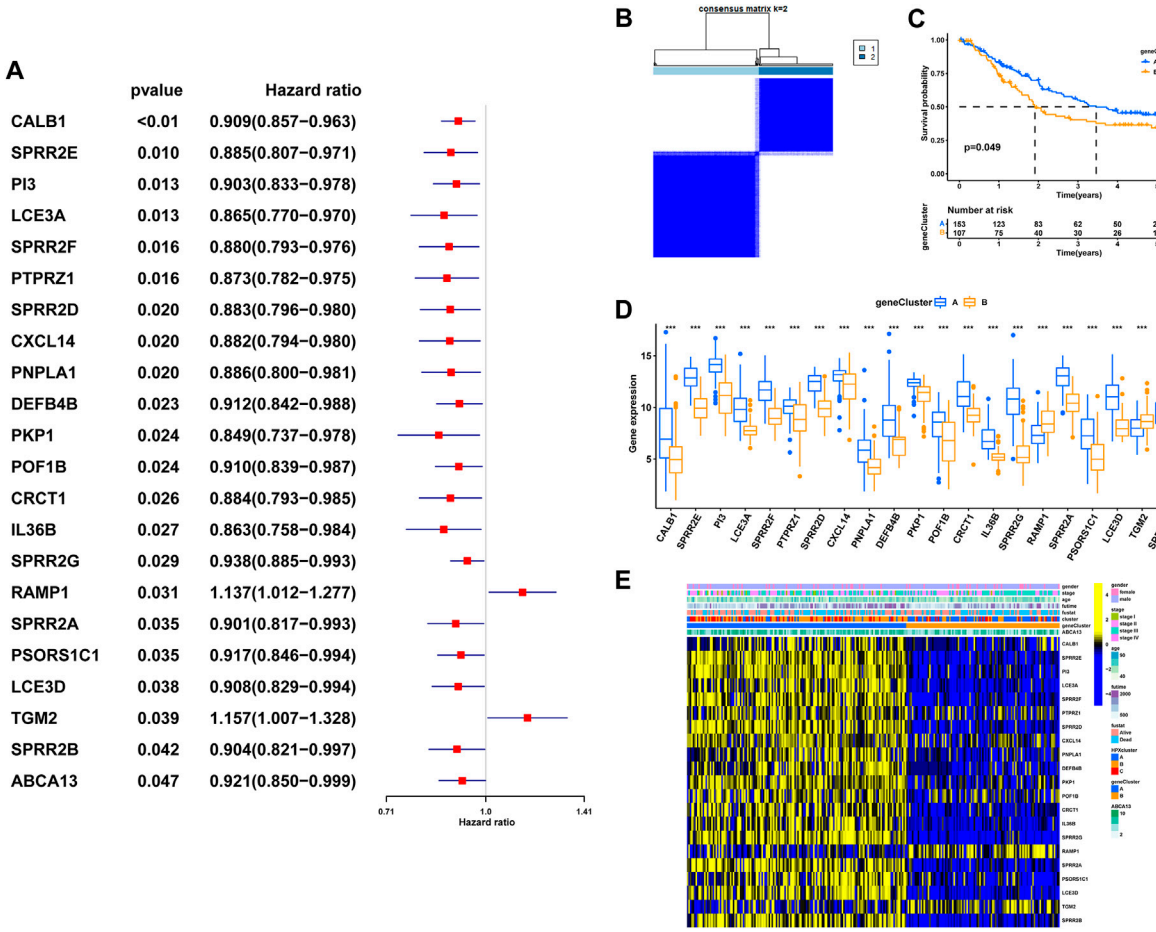


FIGURE 7
The geneCluster and its prognostic value. **(A)** Twenty-two of seventy-seven hub DEGs among the three HPRclusters demonstrated noticeable prognostic power in Cox regression. **(B)** Sub-clusters were performed with differential genes. **(C)** Survival analysis in ESCC. **(D)** Differential expression of hypoxia related genes between geneCluster. **(E)** Heatmap showing the relationship between clinical features, genes expression and sub-clusters. *p < 0.05, **p < 0.01, ***p < 0.001, ns p > 0.05.

between geneCluster A and geneCluster B (Figure 7E). This visualization underscores the distinct biological attributes and potential prognostic implications associated with the two geneClusters.

2.5 Construction of the HPRscore and functional annotation

In order to assess the hypoxia pattern of individual patients with ESCC, a scoring system called HPRscore was developed based on the expression of the 22 DEGs. Utilizing the R package 'GSVA', the 260 ESCC patients were categorized into high or low HPRscore groups using an optimal cut-off value. The prognostic value of the HPRscore was assessed through the log-rank test, which revealed that patients with a low HPRscore exhibited a poor survival outcome (p = 0.001, Figure 8A). An alluvial diagram was employed to visualize the changes in individual patient attributes (Figure 8B). Furthermore, the Kruskal–Wallis test was conducted and showed a significant difference in HPRscore between HPRclusters and geneClusters. HPRcluster A (Figure 8C) and geneCluster B (Figure 8C) exhibited a diminished HPRscore, and both HPRcluster A (Figure 3B) and geneCluster B (Figure 7C) demonstrated an unfavorable prognosis. Additionally, a positive correlation was observed between the HPRscore and the majority of infiltrating immune cells (Figure 8D). This suggested that higher HPRscores were associated with increased immune cell infiltration within TME. Subsequently, an analysis was conducted to determine the relationship between HPRscore and the operation of 50 hallmark pathways using GSVA. The results indicated that the HPRscore exhibited a significant correlation with inflammatory responses, hypoxia, and immune pathway signaling (Figure 9A). Further analysis of the immune activity and chemokine profiles in the high- and low-HPRscore groups revealed that the high HPRscore group was considerably enriched in chemokine-related genes, including chemokines and receptors, interleukins and receptors, interferons and receptors, and other cytokines (Figure 9B). In summary, the HPRscore had potential as a prognostic indicator for ESCC and could have significant implications for the advancement of innovative therapeutic interventions that target hypoxia and immune pathways.

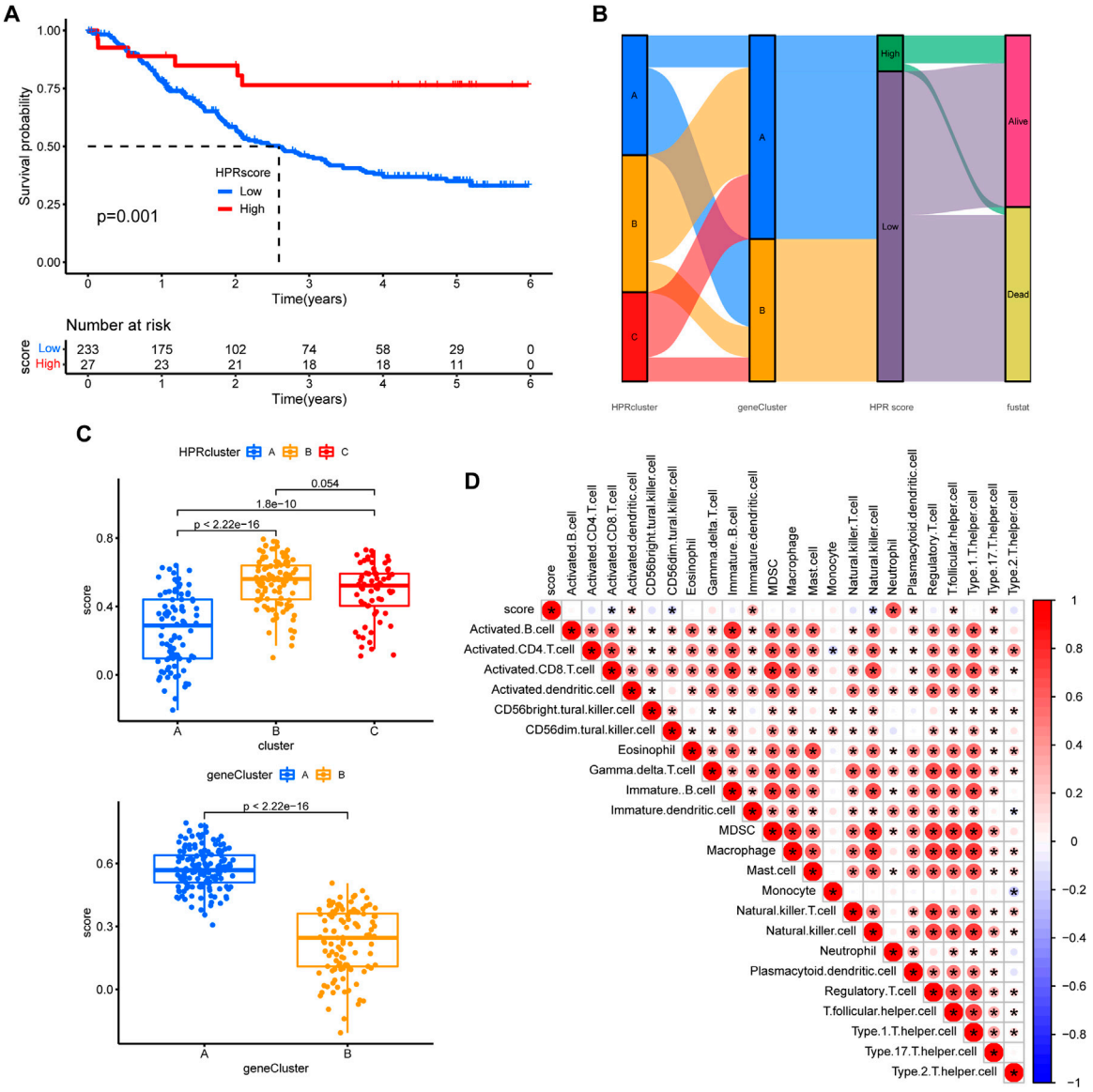


FIGURE 8
Prognostic analysis. **(A)** The overall survival of HPRscores. **(B)** Sankey diagram showing the relationship between staging, scoring and prognostic status. **(C)** Differences in geneCluster scores for HPRclusters and differences cluster scores for Twenty-two hub genes. **(D)** Correlation of immune cell infiltration. Size and color of the circle represent the Pearson correlation coefficients. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns $p > 0.05$.

2.6 Validation of the HPRscore and the role in predicting immunotherapeutic benefits

The utilization of monoclonal antibodies that block inhibitory molecules on T-cells, such as *PD-L1* and *PD-1*, has shown promise in cancer treatment, providing significant survival benefits (Curran et al., 2010). Building upon the findings that HPRscore is associated with inflammatory responses, immune pathway signaling, and chemokine-related genes, which may potentially predict the effectiveness of immunotherapy, a study was conducted to validate the accuracy of HPRscore in predicting immunotherapy efficacy using independent immunotherapy cohorts from published literature. The study included patients diagnosed with advanced non-squamous NSCLC who received a combination of erlotinib and bevacizumab. Additionally, individuals with melanoma who underwent anti-*PD-1* therapy (GSE78220, Figure 10B, $p = 0.012$), advanced NSCLC who received anti-*PD-1*/*PD-L1* antibody (GSE135222, Figure 10C, $p = 0.031$), melanoma who underwent nivolumab therapy (ICB. Riaz 2017, GSE91061, Figure 10D, $p = 0.032$), advanced urothelial cancer who received anti-*PD-L1* therapy (IMvigor210CoreBiologies, Figure 10E, $p = 0.0097$), and advanced renal cell carcinoma who were treated with Avelumab (anti-PD-L1) plus axitinib *versus* sunitinib (The phase III JAVELIN Renal 101 trial, NCT02684006, Figure 10F, $p = 0.012$) were included in the study. The results of the study indicated that patients with low HPRscore experienced significant clinical advantages and extended survival. Moreover, the immune response and favorable therapeutic outcomes observed in patients belonging to the distinct HPRscore cohort who received immune checkpoint blockade

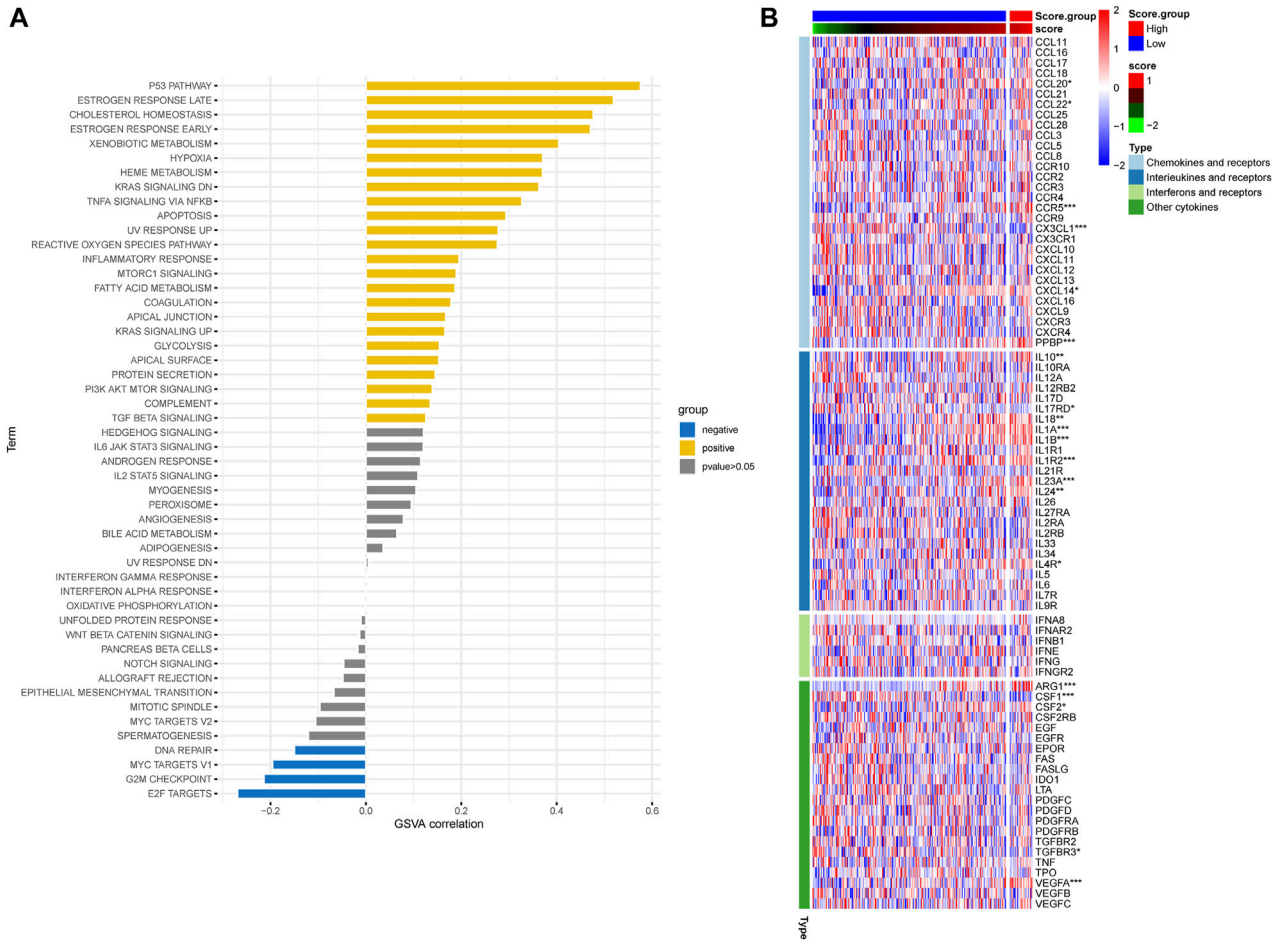


FIGURE 9
Relationship between HPRscore and immune pathways and immune-related chemokines. **(A)** The correlation between HPRscore and the activity of 50 hallmark pathways. **(B)** The immune activity and chemokine profiles in the high- and low- HPRscore groups.

treatment were consistent with the findings. These results provided compelling support for the utilization of HPRscore as a prognostic indicator of immunotherapy effectiveness and patient prognosis.

2.7 Comparison of anticancer drug sensitivity between patients with different HPRscore

Based on the restricted efficacy of immunotherapy in managing ESCC, a tactic was implemented to identify non-immunotherapy medications and assess the vulnerability of low- and high-HPRscore subgroups. This assessment was carried out using the publicly accessible pharmacogenomics database, Genomics of Drug Sensitivity in Cancer (https://www.cancerrxgene.org), with the utilization of the R package 'pRRophetic'. The investigation revealed notable findings. Patients with high HPRscore demonstrated lower IC_{50} values (a measure of drug potency) for the following medications: Bicalutamide, A.443,654, AICAR, AZD6244, Bexarotene, and BIBW2992 (Figures 11A–F). On the other hand, individuals with low HPRscore exhibited significantly reduced IC_{50} values for non-immunotherapy agents, including Axitinib, ABT.888, AG.014699, AMG.706, AP.24534, and AS601245 (Figures 11G–L). These findings suggested a correlation between HPRscore and drug susceptibility. In other words, the HPRscore may serve as an indicator of how susceptible patients with ESCC are to specific medications, both immunotherapy and non-immunotherapy agents.

2.8 PheWAS

We conducted PheWAS analysis on two sets of HPR genes at the gene level, as depicted in Figures 2C, 6A, using a dataset of 17,361 binary phenotypes and 1,419 quantitative phenotypes obtained from the AstraZeneca PheWAS portal database. PheWAS results provide insights into associations between genetically determined protein expression and specific diseases or traits. Except for *TGM2*, *AMPD3*, *PSORS1C1*, *POF1B*, *NOS3*, and *PSORS1C1*, no other genes exhibited significant associations with traits at the gene level, based on the predefined significance threshold ($P < 1E{-}8$) (Supplementary Table S9). This suggests the possibility of potential side effects and horizontal pleiotropy affecting these genes, which may impact drug targeting strategies for these gene targets.

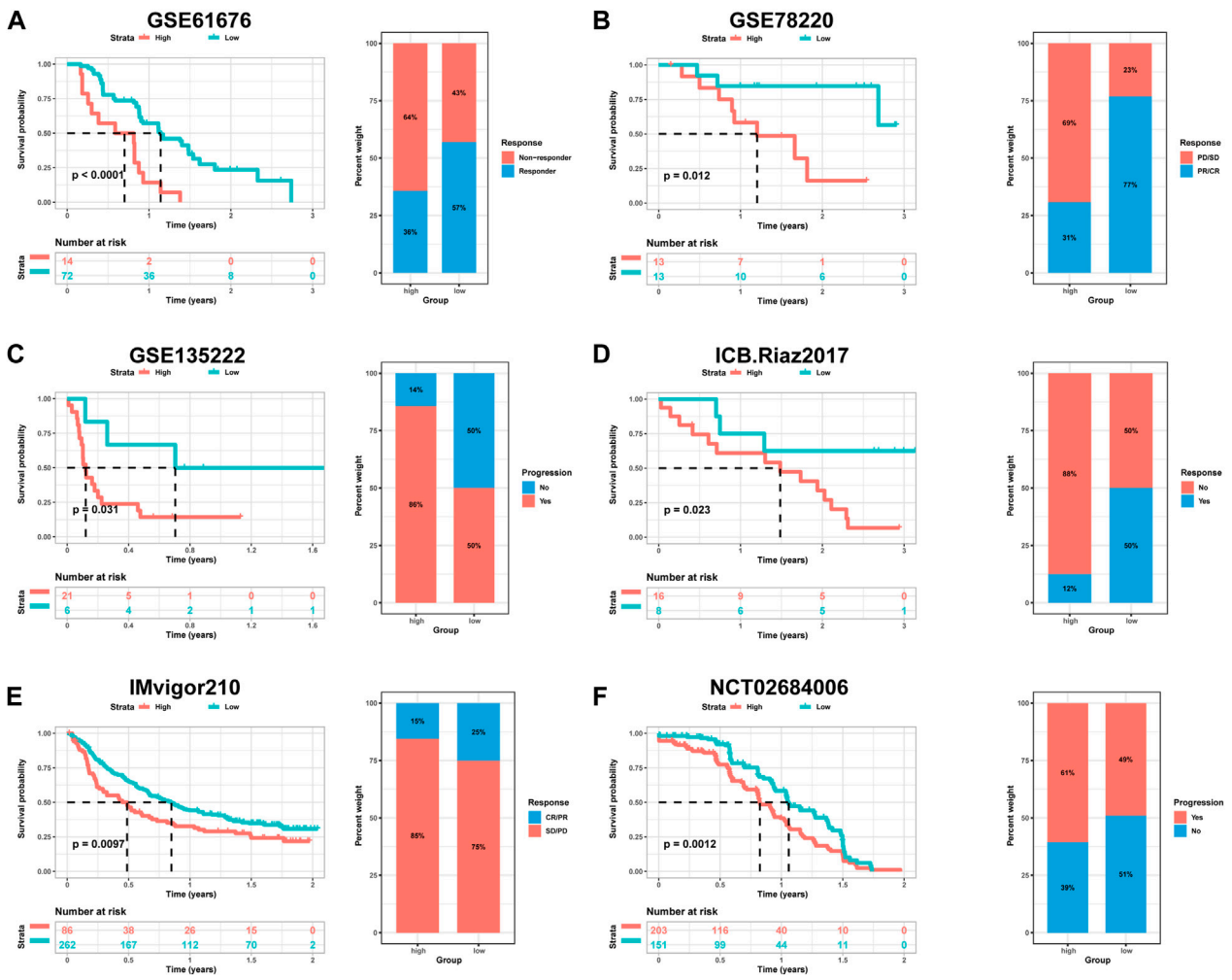


FIGURE 10
The relationship between HPRscore and predicting the benefit of immunotherapy. The relationship between **(A)** late stage non-squamous NSCLC treated with the combination of erlotinib plus bevacizumab (GSE61676), **(B)** melanoma treated with anti-PD-1 therapy (GSE78220), **(C)** advanced NSCLC treated with anti-PD-1/PD-L1 antibody (GSE135222), **(D)** melanoma treated with nivolumab therapy (ICB.Riaz 2017, GSE91061), **(E)** advanced urothelial cancer treated with anti-PD-L1 therapy (IMvigor210CoreBiologies), **(F)** advanced renal cell carcinoma treated with Avelumab (anti-PD-L1) plus axitinib *versus* sunitinib (The phase III JAVELIN Renal 101 trial, NCT02684006).

Notably, the simultaneous occurrence of the *TGM2* and *PKP1* genes in both sets piqued our interest for further exploration. PheWAS results indicated that *TGM2* was primarily associated with Cardiometabolic traits, suggesting that drugs targeting the *TGM2* gene in ESCC may have an impact on these traits (Figures 12A, B). On the other hand, *PKP1* did not show significant associations with other traits at the gene level (Figures 12C, D). Consequently, *PKP1* was selected and validated for further investigation.

2.9 The expression and prognostic value of *PKP1* were evaluated in ESCC samples

PKP1, as a HPR gene, has been documented to exhibit aberrant expression in various cancers (Wang et al., 2020; Boyero et al., 2022). Nevertheless, its involvement in ESCC remains inadequately investigated. *PKP1* expression was significantly downregulated in esophageal squamous cell lines under hypoxia, suggesting that our analysis was reliable (Supplementary Figure S2). The mechanism may be related to the hypoxia inducible factor (*HIF*) signaling pathway or under hypoxia conditions, some mirnas bind to *PKP1* mRNA, inhibit its translation or degradation, and lead to downregulation of its expression.

To further ascertain the potential clinical significance of *PKP1* in ESCC, we conducted qRT-PCR and IHC analyses to investigate the expression levels of *PKP1* in ESCC tissues and adjacent tissues. The results showed a significant upregulation of *PKP1* expression in ESCC tissues compared to adjacent tissues (Figures 13A, B). Based on the IHC scores, patients were divided into two groups: high *PKP1* expression and low *PKP1* expression. The analysis revealed a significant association between elevated *PKP1* expression and various clinicopathological characteristics of ESCC, including tumor size (p = 0.0267), invasion depth (p = 0.0016), lymph node metastasis (p = 0.0251), and clinical

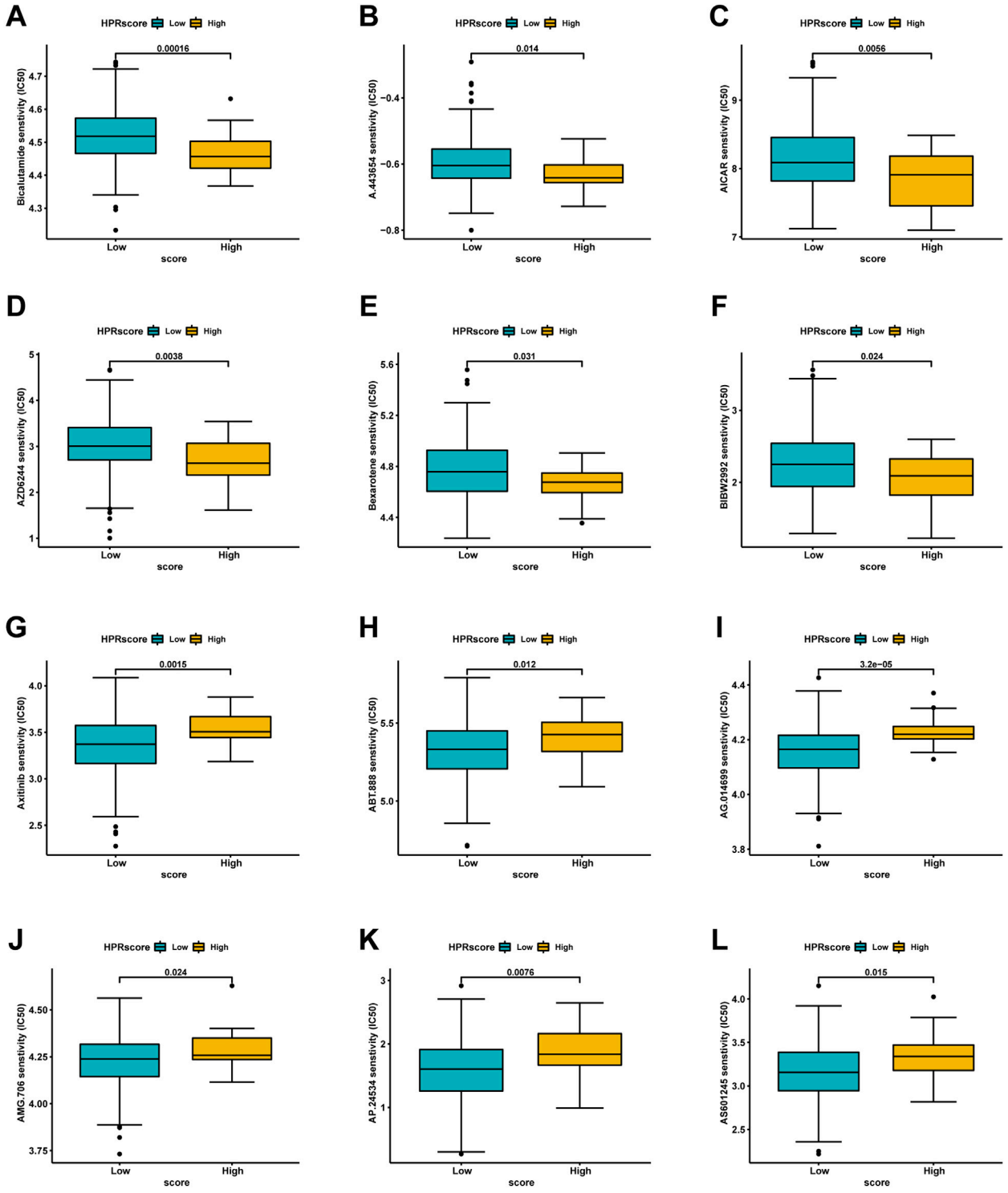


FIGURE 11
Analysis of drug sensitivity associated with HPRscore. Predicting IC50 values for multiple anti-cancer drugs. **(A–F)** High HPRscore exhibited lower IC50 values of Bicalutamide, A.443,654, AICAR, AZD6244, Bexarotene and BIBW2992. **(G–L)** Low HPRscore had significantly reduced IC50 values for non-immunotherapy agents, including Axitinib, ABT.888, AG.014699, AMG.706, AP.24534, and AS601245.

stage (p = 0.0102) (Table 1). Additionally, Kaplan-Meier survival analysis indicated that patients with higher *PKP1* expression had a notable decrease in overall survival (p = 0.0159) (Figure 13C), suggesting that *PKP1* may serve as a prognostic marker in ESCC.

Compared with normal cells, the expression of *PKP1* was significantly upregulated in ESCC cell lines (KYSE140 and TE-1 cell lines) (Figure 13D). To understand the functional role of *PKP1*, its expression was modulated using siRNA-mediated

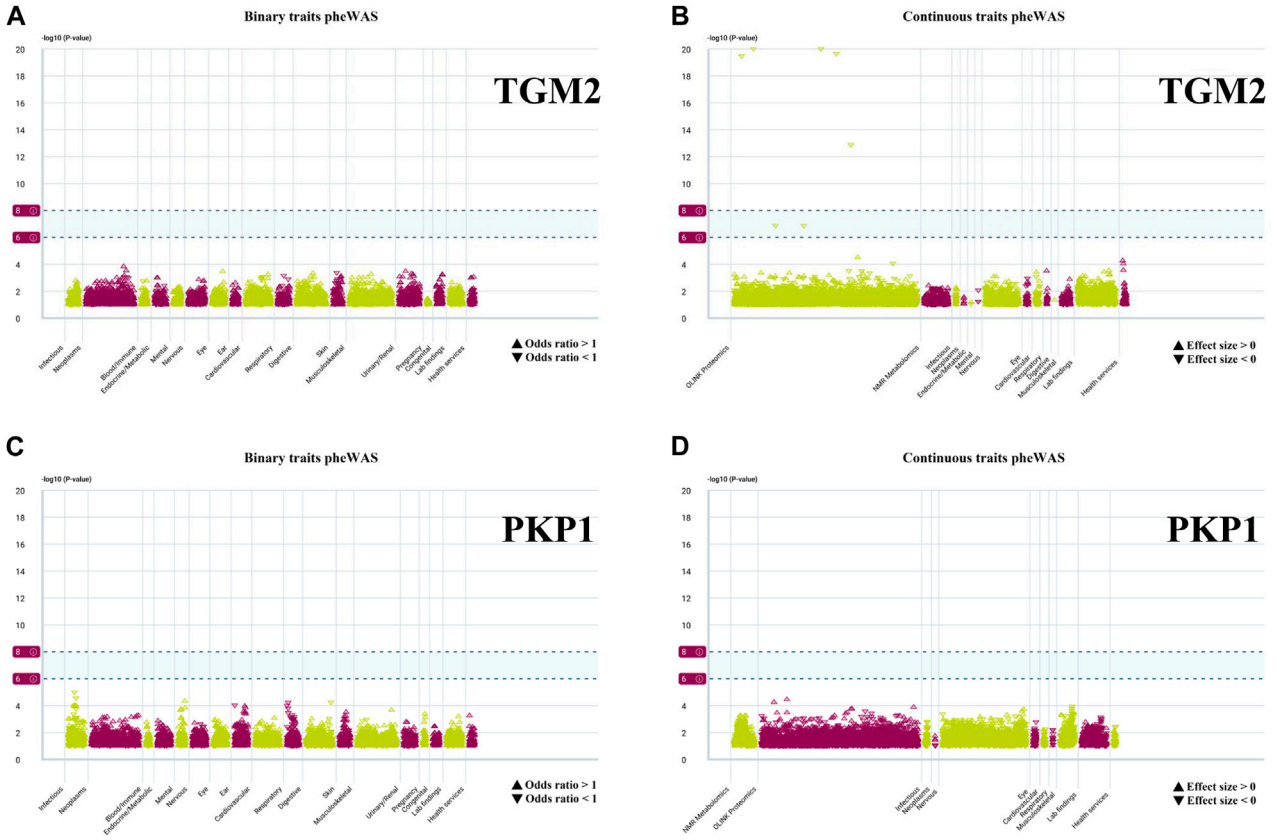


FIGURE 12
PheWAS results for each gene. **(A, B)** Binary traits and continuous traits PheWAS association with TGM2. **(C, D)** Binary traits and continuous traits PheWAS association with PKP1.

knockdown (Figure 13E). The knockdown of *PKP1* expression resulted in a significant inhibition of cell proliferation and migration, as demonstrated by CCK-8, EdU, and Transwell experiments (Figures 13F–I). These findings suggest that *PKP1* plays a role in promoting cell proliferation and migration in ESCC cells.

3 Discussion

ESCC is known to be challenging to treat effectively, and current treatment options have limited success (Codipilly et al., 2018). Immunotherapy, although promising, has shown limited response rates in ESCC patients (Luo et al., 2021; Sun et al., 2021; Doki et al., 2022; Wang et al., 2022). Therefore, there is a need for innovative treatment strategies to improve outcomes.

In recent times, there has been an increased focus on the contribution of TME to the pathogenesis, advancement, and sustenance of diseases. The TME comprises a diverse range of constituents, such as immune cells, stromal cells, chemokines, and cytokines, which can synergize to establish a persistent inflammatory, immunosuppressive, and pro-carcinogenic milieu, thereby evading immune surveillance and bolstering tumor cell viability (Wu and Dai, 2017). Additionally, hypoxia within the tumor microenvironment can modulate gene expression, foster cell survival, and augment resistance to apoptosis induction (Jing et al., 2019). Furthermore, it is postulated that tumor cells enclosed within a hypoxic TME exhibit heightened aggressiveness and resistance to pharmaceutical interventions (Qian and Rankin, 2019). Ye et al. have categorized tumor specimens into high and low hypoxia score groups, identified molecular modifications associated with anticancer drug responses, and illustrated the influence of hypoxia in fostering tumor heterogeneity and viability (Ye et al., 2019). Bhandari et al. have conducted a quantitative analysis of hypoxia across multiple cancer types, revealing a positive correlation between heightened hypoxia and mutational burden (Bhandari et al., 2020).

The study focused on investigating the role of HPRs in ESCC regarding their biological function, prognostic value, correlation with TME, immunotherapy response, and chemotherapy resistance. Through analyzing genetic expression and prognosis in ESCC patient cohorts, we identified three distinct hypoxia clusters characterized by different gene expression patterns. Among these clusters, HPRcluster A exhibited the highest scores for stromal and immune cell infiltration, suggesting a more favorable immune response. The hypoxic microenvironment in tumor cells promotes glucose uptake,

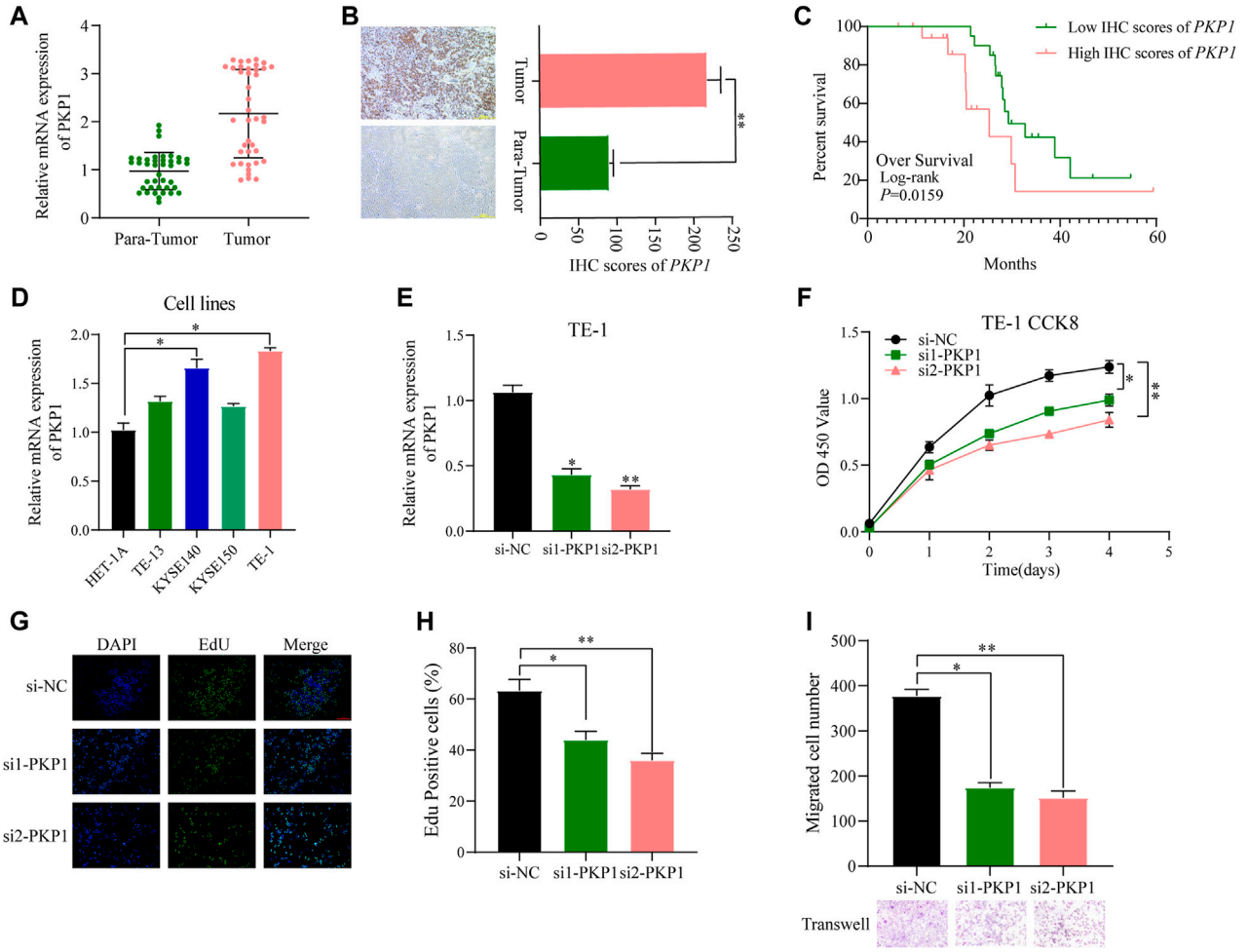


FIGURE 13
Evaluation of *PKP1* expression levels and prognostic value in esophageal samples. **(A, B)** The mRNA and protein expression of *PKP1* was significantly upregulated in ESCC compared with tissues adjacent tissues. **(C)** Kaplan–Meier survival analysis of over survival according IHC scores of *PKP1* in 40 ESCC patients. **(D)** The mRNA level expression of *PKP1* in ESCC cell lines. **(E)** Validation of siRNA knockdown efficiency in TE-1 cells. **(F–I)** Transfection of siRNA into TE-1 cells and CCK-8 **(F)**, EdU **(G, H)**, and Transwell **(I)** assay detected the cell proliferation and migration. *$p < 0.05$, **$p < 0.01$.

which can impact the functionality of crucial immunologically active cells. The high-risk subset showed increased infiltration of macrophages and T cells, known to inhibit the effectiveness of immune checkpoint inhibitor treatment (Samanta and Semenza, 2018). It has been observed that tumor cells have a superior ability to utilize glucose as an energy source compared to T cells, leading to competition for this vital resource (Chang et al., 2015). Consequently, T cells experience hindered nutritional metabolism. Hypoxia intensifies glucose metabolism pathways and enhances glucose absorption by tumor cells, creating an unfavorable nutritional state for T cells, impairing their immune functions and ability to eliminate tumor cells. Additionally, the heightened glycolytic activity of tumor cells in a hypoxic environment generates an acidic microenvironment that further affects T cell functionality (Leone and Powell, 2020). HPRcluster A was identified as an immunoinflammatory phenotype, characterized by the infiltration of adaptive immune cells and immune activation, which correlated with an unfavorable prognosis. Consistent with the clustering results of HPRs, two genomic subtypes associated with immune activation were identified, supporting the crucial role of hypoxia in immune regulation within the TME.

To address individual heterogeneity, we developed a novel scoring system, HPRscore, to evaluate and quantify the hypoxia response pattern in patients with ESCC. Our findings revealed that patients with a low HPRscore experience unfavorable survival outcomes. Interestingly, the low HPRscore group showed enrichment in pathways associated with immune activation, indicating an immune-inflamed phenotype. In contrast, the high HPRscore group was enriched in pathways related to stromal components, suggesting an immune-excluded and immune-desert phenotype. These results were further validated in the IMvigor210 cohort, with the immune-desert and excluded phenotypes exhibiting higher HPRscores, while the immune-inflamed phenotype displaying significantly lower HPRscores.

Furthermore, we have demonstrated the prognostic significance of the HPRscore in relation to checkpoint blockade therapy across multiple patient cohorts. Our analysis included six distinct cohorts, encompassing patients with advanced non-squamous NSCLC treated with erlotinib and bevacizumab (Baty et al., 2017),

TABLE 1 Correlation between *PKP1* expression and clinicopathological features in ESCC patients (n = 40).

Clinicopathological parameters	Numbers	IHC scores of *PKP1*	p-value[a]	
		Low scores	High scores	
Gender				
Male	22 (55%)	9	13	0.2036
Female	18 (45%)	11	7	
Age (year)				
<60	25 (62.5%)	14	11	0.3272
≥60	15 (37.5%)	6	9	
Tumor size				
<5 cm	19 (47.5%)	13	6	0.0267*
≥5 cm	21 (52.5%)	7	14	
Location				
Upper	5 (12.5%)	3	2	0.8854
Middel	23 (57.5%)	11	12	
Low	12 (30%)	6	6	
Invasion depth				
pT 1	15 (37.5%)	13	2	0.0016*
pT 2	11 (27.5%)	3	8	
pT 3	14 (35%)	4	10	
Lymph node metastasis				
Positive	23 (57.5%)	15	8	0.0251*
Negative	17 (42.5%)	5	12	
Differentiation				
Poor	7 (17.5%)	2	5	0.0816
Moderate	28 (70%)	12	14	
Well	7 (17.5%)	6	1	
Clinical stage*				
I	9 (22.5%)	7	2	0.0102*
II	16 (40%)	10	6	
III	15 (37.5%)	3	12	

*$p < 0.05$.
[a]Chi-square test.

melanoma patients receiving anti-PD-1 therapy (Hugo et al., 2016), patients with advanced NSCLC treated with anti-PD-1/PD-L1 antibody (Kim et al., 2020), melanoma patients receiving nivolumab therapy (Riaz et al., 2017), patients with advanced urothelial cancer treated with anti-PD-L1 therapy (Mariathasan et al., 2018), and patients with advanced renal cell carcinoma receiving Avelumab (anti-PD-L1) in combination with axitinib or sunitinib (Motzer et al., 2020). The results consistently demonstrated that patients with a low HPRscore experience greater clinical benefits from checkpoint blockade therapy compared to non-responders.

4 Conclusion

In summary, this study contributes to our understanding of the role of hypoxia-related genes (HPRs) and the TME in ESCC. The HPRscore has potential clinical utility as a prognostic tool and treatment guide, particularly in the context of immunotherapy. These findings may pave the way for personalized approaches in ESCC management and the development of novel therapeutic interventions. Further research is warranted to validate and expand upon these findings for the benefit of ESCC patients. And *PKP1* may be a potential therapeutic target for ESCC.

5 Materials and methods

5.1 Data collection and preprocessing

The present study analyzed RNA expression in ESCC by utilizing data from two databases, namely, The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/) and the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/). RNA expression data were derived from the TCGA cohort as well as two GEO cohorts (GSE53624 and GSE53622), which were subjected to background adjustment and quantile normalization of the raw "CEL" files. The "combat" algorithm was utilized to address potential batch effects, and it involved the use of the limma and sva R packages (Leek et al., 2012; Ritchie et al., 2015). The differential expression of mRNAs was determined based on a false discovery rate (FDR) of less than 0.05 and an absolute $\log_2$ fold change ($|\log_2 FC|$) of at least 1, with the use of R 4.1.1 software and the limma package.

Hypoxia phenotype-related genes (HPRs) were identified from two distinct data-bases, the KEGG database (https://www.kegg.jp/pathway/hsa04066) and the Molecular Signatures Database (MSigDB; http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_HYPOXIA). More specifically, we curated a list of 109 HPRs associated with the *HIF-1* signaling pathway using the KEGG database. Additionally, we included the Hallmark hypoxia gene sets (n = 200) from the MSigDB database (Liberzon et al., 2015). For the complete inventory of these genes, please refer to Table S1.

5.2 Unsupervised clustering based on HPRs

Unsupervised cluster analysis was conducted to distinguish exclusive hypoxia modification patterns and stratify patients for further examination based on the ex-pression levels of thirteen HPRs. The R software package "ConsensusClusterPlus" was utilized for the analysis with 1,000 iterations to guarantee the stability of the clustering results. The optimal number of clusters was determined using the consensus clustering algorithm (Wilkerson and Hayes, 2010).

5.3 Generation of the HPRs signature

To assess the hypoxia modification pattern in ESCC patients, we developed a novel scoring system called HPRscore. This scoring system utilizes a hypoxia gene signature. Initially, we identified differentially expressed genes (DEGs) from individual HPRclusters and standardized them across all ESCC samples. We then performed unsupervised cluster analysis on the overlapping genes to categorize patients into distinct groups for further analysis. The consensus clustering algorithm helped determine the number and stability of gene clusters. Using univariate Cox regression analysis, we identified a prognostic gene within the signature. Principal component analysis (PCA) was then conducted to establish the HPR signature, with the signature scores derived from the main components 1 and 2.

5.4 Estimating of immune infiltration

Single-sample gene-set enrichment analysis (ssGSEA) was utilized to determine the activity levels of specific biological pathways or cell types in individual samples based on their gene expression profiles (Hänzelmann et al., 2013). We applied ssGSEA to assess and quantify immune infiltration in each sample using previously researched immune cell marker gene expression information by Charoen-tong (Charoentong et al., 2017). The enrichment score obtained via ssGSEA represented the relative abundance of infiltration for each immune cell. Additionally, we used the "ESTIMATE" package to calculate ImmuneScore, StromalScore, and ESTIMATEScore. ImmuneScore provided an estimate of immune cell infiltration within the tumor microenvironment, while Stro-malScore indicated the abundance of stromal cells. ESTIMATEScore combined both ImmuneScore and StromalScore, which provided an overall estimate of tumor purity.

5.5 Gene set variation analysis

The R package "GSVA" was used to perform enrichment analysis and investigate differences in biological processes among the HPR subtypes (Hänzelmann et al., 2013). Gene set variation analysis (GSVA), a non-parametric and unsupervised method, was employed to assess pathway and biological process activities across different expression datasets. For the GSVA analysis, gene sets including Gene Ontology (GO) and KEGG were obtained from the MSigDB database. To visually represent hypoxia-related pathways, heatmaps were generated, highlighting pathways with a significance level of $p < 0.05$.

5.6 The HPRscore generation process

The aim of study was to establish a customized scoring system for assessing hypoxia levels in individual patients with ESCC. We developed this scoring system through a series of steps, which began with normalizing DEGs from different hypoxia clusters across all samples and identifying overlapping genes. We identified 77 common differential genes through differential analysis and Venn diagrams among the three HPRclusters. Next, we performed univariate Cox regression analysis for each gene and 22 genes with significant prognostic value for further analysis. The hypoxia score (HPRscore) was calculated using the "GSVA" R package (Hänzelmann et al., 2013). Using the expression data for HPRclusters, we computed HPRscore through PCA using the formula:

$$\text{HPRscore} = \sum(\text{PC1}_i + \text{PC2}_i),$$

where 'i' represents the expression of HPRs. This customized scoring system has great potential for individualized hypoxia evaluation and prognostic prediction improvement in ESCC patients.

5.7 Phenome-wide association study (PheWAS) analysis

To assess potential drug targets and their associated side effects, PheWAS was conducted using the AstraZeneca PheWAS Portal (https://azphewas.com/) (Wang et al., 2021; Dhindsa et al., 2023). AstraZeneca PheWAS Portal is a repository of gene-phenotype

associations for phenotypes derived from electronic health records, questionnaire data, and continuous traits computed on exomes released by UK Biobank. All genomic coordinates in this Portal are based on GRCh38. Continuous phenotypes are rank-based inverse normal transformed before analysis. To mitigate false positives, we applied multiple corrections and set a significance threshold of 1E-8, following the default setting in the AstraZeneca PheWAS Portal.

5.8 Patient tissue samples

A total of forty pairs of ESCC and adjacent normal tissues were procured from pa-tients who underwent surgery at the First Affiliated Hospital of Soochow University and received a pathological diagnosis. This study was carried out with the explicit in-formed consent of all patients and received approval from the Ethics Committee of the First Affiliated Hospital of Soochow University.

5.9 Cell culture

ESCC cell lines (TE-1, TE-13, KYSE150, KYSE140) and normal human esophageal epithelial cell line (HET-1A) were obtained from the Shanghai Institutes for Biological Sciences (Shanghai, China). These cell lines were cultured in 1,640 medium (supplemented with 10% fetal bovine serum (FBS) (KeyGene, Nanjing, China) and 1% penicillin-streptomycin) at 37°C in a humidified 5% CO_2 atmosphere.

5.10 RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The extracted RNA was then reverse transcribed into complementary DNA by synthesis kit (Takara, Cat: RR036A, KeyGEN). The qRT-PCR experiment was performed in triplicate and the data were normalized to *β-actin* using the $2^{-\Delta\Delta CT}$ method. The primer sequences used for the qRT-PCR analysis were as follows: *ACTIN* forward, 5′-GTCATTCCAAATATGAGATGCGT-3′; *ACTIN* reverse, 5′-GCATTACATAATTTACACGAAAGCA-3′; *PKP1* forward, 5′-TCAGCAACAAGAGCGACAAG-3′; *PKP1* reverse, 5′-TCAGGT AGGTGCGGATGG-3′.

5.11 siRNA construction and cell transfection

The siRNAs that targeted *PKP1* were obtained from RiboBio (Guangzhou, China). The transfection of these siRNAs into cells was performed using Imax (Invitrogen, Carlsbad, CA, United States) in accordance with the guidelines provided by the manufacturer. The siRNA sequences as follows: si1-*PKP1* sense sequence, 5′-GGCUGA CAAUUACAACUAUtt-3′; si1-*PKP1* antisense sequence, 5′-AUA GUUGUAAUUGUCAGCCaa-3′; si2-*PKP1* sense sequence, 5′-GCUUUGCCGUCGGACCAAAtt-3′; si2-*PKP1* antisense sequence, 5′-UUUGGUCCGACGGCAAAGCca-3′; si-NC sense sequence, 5′-UAACGACGCGACGACGUAAtt-3′; si-NC antisense sequence, 5′-UUACGUCGUCGCGUCGUUAtt-3′.

5.12 CCK-8 and EdU assay

After transfection and incubation for 24 h, the cells were inoculated into 96-well plates at a density of 2000 cells per 100 μL. To ensure reproducibility, the same sample was placed in 5 repeated wells. The cells were then incubated at 37°C for 6 h to allow them to attach to the well walls. Next, 10 μL of CCK-8 was added to each well, and the baseline absorbance at 450 nm was recorded. Subsequently, the absorbance at 450 nm was measured every 24 h for a total of 4 days.

EdU cell proliferation staining was conducted utilizing an EdU kit (Cat.C10310-3, Ruibo, China), following the guidelines provided by the manufacturer. To be specific, 1.0×10^5 cells per 100 μL were inoculated in a 96-well plate. To prepare the appropriate amount of 50 μM EdU medium, the EdU solution (reagent A) was diluted in a ratio of 1,000:1 with cell complete medium. Next, 100 μL of the 50 μM EdU medium was added to each well, and the cells were incubated for 2 h. After incubation, the medium was discarded, and the cells were washed twice with PBS for 5 min each time. Subsequently, 50 μL of cell fixative was added to each well and incubated at room temperature for 30 min. Following the incubation, the fixative was discarded, and 50 μL of 2 mg/mL glycine was added to each well. The plate was then incubated in a decolorized shaker for 5 min. The glycine solution was subsequently discarded, and the cells were washed twice with PBS for 5 min each time. Next, 100 μL of PBS containing 0.5% Triton X-100 was added to each well and incubated in a shaker for 10 min. The cells were then rinsed with PBS for 5 min. Each well was stained with 100 μL of 1 Apollo solution and 100 μL of 1 Hoechst 33,342 solution, respectively. Finally, after washing with 100 μL of PBS three times, the images were observed under a fluorescence microscope, and the proliferation rate was calculated.

5.13 Transwell assays

The Transwell experiment was conducted using a 24-well plate with a Transwell insert featuring an 8 μm pore size. The upper cavity of the Transwell insert was supplemented with 300 μL of serum-free medium containing 2.5×10^4 cells, while the lower cavity was supplemented with 700 μL of medium containing 10% fetal bovine serum. The plate was then incubated in an incubator for 24 h. After incubation, the Transwell chamber was removed, and the culture solution was discarded. To remove any remaining matrix glue and cells from the chamber, a PBS-soaked cotton swab or cotton was used to gently wipe the surface. The cells were then fixed with 4% methanol for 30 min and washed with PBS for 5 min. Subsequently, the cells were stained with crystal violet for 10 min, followed by three washes with PBS. After drying, the cells were photographed under a microscope in three to five fields, and the average was quantified using ImageJ.

5.14 Immunohistochemistry (IHC)

The tissue slices were incubated in a 65 incubator for 1 h and then soaked in xylene for three 10-min intervals. Subsequently, they were placed in anhydrous ethanol, followed by 95%, 90%, and 80% ethanol, each for 5 min. The slices were then washed twice with PBS for 5 min

each time. To inactivate endogenous peroxidase activity, the slices were incubated with 3% H_2O_2 deionized water for 10 min. After another two washes with PBS for 5 min each time, the slices were boiled in 0.01 M citric acid buffer (pH = 6.0) at 95 for 15–20 min. The slices were then rapidly cooled in cold water to room temperature for 15 min, followed by two additional washes with PBS for 5 min each time. To prevent non-specific binding, the slices were incubated with a normal goat serum sealer at room temperature for 20 min, and any excess liquid was discarded. Next, 50 μL of the primary antibody was added, and the slices were incubated overnight at 4°C. After washing twice with PBS for 5 min each time, the slices were incubated with a horseradish peroxidase-labeled secondary antibody for 1 h at room temperature. Following another two washes with PBS for 5 min each time, the slices were incubated with Streptavidin-Peroxidase at room temperature for 1 h. The slices were washed twice with PBS for 5 min each time before developing the DAB color for 5–10 min. The staining intensity was assessed under a microscope, considering cells with brown cytoplasm as positive cells. To stop the reaction, the slices were rinsed with cold water for 15 min. For further staining, the slices were briefly re-dyed with hematoxylin for 2 min, differentiated using hydrochloric acid and alcohol, and rinsed with cold water for 15 min. The slices were then conventionally dehydrated, made transparent, and sealed with a neutral gum drop next to the tissue, followed by covering with a cover glass. Finally, the slices were observed and photographed under a microscope.

5.15 Statistical analyses

Statistical analyses were performed using R statistical language (version 4.1.1). The Wilcoxon test and Kruskal–Wallis test were utilized for comparison between two and more than two groups, respectively. To draw the prognostic survival curve, the Kaplan–Meier plotter was employed, and the statistical significance was evaluated through the log-rank test. Spearman's test was utilized for correlation analysis and calculation of correlation coefficient. The statistical significance level was set at $p < 0.05$ for all analyses.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KX: Writing–review and editing, Writing–original draft, Validation, Software, Conceptualization. ZC: Writing–review and editing, Writing–original draft, Data curation. JF: Writing–review and editing, Writing–original draft, Methodology. LP: Writing–review and editing, Writing–original draft, Data curation. NW: Writing–review and editing, Writing–original draft, Methodology. JL: Writing–review and editing, Writing–original draft, Software, Methodology. YY: Writing–review and editing, Writing–original draft, Software, Data curation. HM: Writing–review and editing, Writing–original draft, Supervision, Investigation, Conceptualization. YF: Writing–review and editing, Writing–original draft, Supervision, Investigation, Conceptualization. WJ: Writing–review and editing, Writing–original draft, Supervision, Data curation, Conceptualization.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by the National Natural Science Foundation of China (Nos. 82273325, 82203561), Medical and industrial collaborative innovation research project (No. SZM2021005), Suzhou Ke Jiao Xing Wei Youth Science and Technology Projects (Nos. KJXW2022006, KJXW2022084), Postgraduate Research and Practice Innovation Program of Jiangsu Province (No. KYCX23_3275).

Acknowledgments

We appreciate the efforts and data provided by the TCGA, GEO and KEGG database staff. Thanks to all reviewers and editors for their hard work and sincere comments.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1344317/full#supplementary-material

FIGURE S1
Consensus matrixes of all ESCC samples for each k (k = 2–9), displaying the clustering stability using 1000 iterations of hierarchical clustering.

FIGURE S2
RT-qPCR results for the expression of PKP1 in ESCC cell lines cultured under normal and hypoxic conditions. *P<0.05, ns P >0.05.

References

Baty, F., Joerger, M., Früh, M., Klingbiel, D., Zappa, F., and Brutsche, M. (2017). 24h-gene variation effect of combined bevacizumab/erlotinib in advanced non-squamous non-small cell lung cancer using exon array blood profiling. *J. Transl. Med.* 15 (1), 66. doi:10.1186/s12967-017-1174-z

Becht, E., Giraldo, N. A., Germain, C., de Reyniès, A., Laurent-Puig, P., Zucman-Rossi, J., et al. (2016). Immune contexture, immunoscore, and malignant cell molecular subgroups for prognostic and theranostic classifications of cancers. *Adv. Immunol.* 130, 95–190. doi:10.1016/bs.ai.2015.12.002

Bhandari, V., Li, C. H., Bristow, R. G., Boutros, P. C., and PCAWG Consortium (2020). Divergent mutational processes distinguish hypoxic and normoxic tumours. *Nat. Commun.* 11 (1), 737. doi:10.1038/s41467-019-14052-x

Boyero, L., Martin-Padron, J., Fárez-Vidal, M. E., Rodriguez, M. I., Andrades, Á., Peinado, P., et al. (2022). PKP1 and MYC create a feedforward loop linking transcription and translation in squamous cell lung cancer. *Cell Oncol. (Dordr)* 45 (2), 323–332. doi:10.1007/s13402-022-00660-1

Chang, C. H., Qiu, J., O'Sullivan, D., Buck, M. D., Noguchi, T., Curtis, J. D., et al. (2015). Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* 162 (6), 1229–1241. doi:10.1016/j.cell.2015.08.016

Charoentong, P., Finotello, F., Angelova, M., Mayer, C., Efremova, M., Rieder, D., et al. (2017). Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep.* 18 (1), 248–262. doi:10.1016/j.celrep.2016.12.019

Codipilly, D. C., Qin, Y., Dawsey, S. M., Kisiel, J., Topazian, M., Ahlquist, D., et al. (2018). Screening for esophageal squamous cell carcinoma: recent advances. *Gastrointest. Endosc.* 88 (3), 413–426. doi:10.1016/j.gie.2018.04.2352

Curran, M. A., Montalvo, W., Yagita, H., and Allison, J. P. (2010). PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc. Natl. Acad. Sci. U. S. A.* 107 (9), 4275–4280. doi:10.1073/pnas.0915174107

Dhindsa, R. S., Burren, O. S., Sun, B. B., Prins, B. P., Matelska, D., Wheeler, E., et al. (2023). Rare variant associations with plasma protein levels in the UK Biobank. *Nature* 622 (7982), 339–347. doi:10.1038/s41586-023-06547-x

Doki, Y., Ajani, J. A., Kato, K., Xu, J., Wyrwicz, L., Motoyama, S., et al. (2022). Nivolumab combination therapy in advanced esophageal squamous-cell carcinoma. *N. Engl. J. Med.* 386 (5), 449–462. doi:10.1056/NEJMoa2111380

Hänzelmann, S., Castelo, R., and Guinney, J. (2013). GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinforma.* 14, 7. doi:10.1186/1471-2105-14-7

He, J. Z., Chen, Y., Zeng, F. M., Huang, Q. F., Zhang, H. F., Wang, S. H., et al. (2023). Spatial analysis of stromal signatures identifies invasive front carcinoma-associated fibroblasts as suppressors of anti-tumor immune response in esophageal cancer. *J. Exp. Clin. Cancer Res.* 42 (1), 136. doi:10.1186/s13046-023-02697-y

Huang, F. L., and Yu, S. J. (2018). Esophageal cancer: risk factors, genetic association, and treatment. *Asian J. Surg.* 41 (3), 210–215. doi:10.1016/j.asjsur.2016.10.005

Hugo, W., Zaretsky, J. M., Sun, L., Song, C., Moreno, B. H., Hu-Lieskovan, S., et al. (2016). Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* 165 (1), 35–44. doi:10.1016/j.cell.2016.02.065

Jing, X., Yang, F., Shao, C., Wei, K., Xie, M., Shen, H., et al. (2019). Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Mol. Cancer* 18 (1), 157. doi:10.1186/s12943-019-1089-9

Kim, J. Y., Choi, J. K., and Jung, H. (2020). Genome-wide methylation patterns predict clinical benefit of immunotherapy in lung cancer. *Clin. Epigenetics* 12 (1), 119. doi:10.1186/s13148-020-00907-4

Lee, P., Chandel, N. S., and Simon, M. C. (2020). Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nat. Rev. Mol. Cell Biol.* 21 (5), 268–283. doi:10.1038/s41580-020-0227-y

Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E., and Storey, J. D. (2012). The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* 28 (6), 882–883. doi:10.1093/bioinformatics/bts034

Lei, X., Lei, Y., Li, J. K., Du, W. X., Li, R. G., Yang, J., et al. (2020). Immune cells within the tumor microenvironment: biological functions and roles in cancer immunotherapy. *Cancer Lett.* 470, 126–133. doi:10.1016/j.canlet.2019.11.009

Leone, R. D., and Powell, J. D. (2020). Metabolism of immune cells in cancer. *Nat. Rev. Cancer* 20 (9), 516–531. doi:10.1038/s41568-020-0273-y

Li, Y., Fu, L., Li, J. B., Qin, Y., Zeng, T. T., Zhou, J., et al. (2014). Increased expression of EIF5A2, via hypoxia or gene amplification, contributes to metastasis and angiogenesis of esophageal squamous cell carcinoma. *Gastroenterology* 146 (7), 1701–1713. doi:10.1053/j.gastro.2014.02.029

Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, J. P., and Tamayo, P. (2015). The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst.* 1 (6), 417–425. doi:10.1016/j.cels.2015.12.004

Liu, Z., Zhao, Y., Kong, P., Liu, Y., Huang, J., Xu, E., et al. (2023). Integrated multi-omics profiling yields a clinically relevant molecular classification for esophageal squamous cell carcinoma. *Cancer Cell* 41 (1), 181–195.e9. doi:10.1016/j.ccell.2022.12.004

Luo, H., Lu, J., Bai, Y., Mao, T., Wang, J., Fan, Q., et al. (2021). Effect of camrelizumab vs placebo added to chemotherapy on survival and progression-free survival in patients with advanced or metastatic esophageal squamous cell carcinoma: the ESCORT-1st randomized clinical trial. *Jama* 326 (10), 916–925. doi:10.1001/jama.2021.12836

Mariathasan, S., Turley, S. J., Nickles, D., Castiglioni, A., Yuen, K., Wang, Y., et al. (2018). TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* 554 (7693), 544–548. doi:10.1038/nature25501

Motzer, R. J., Robbins, P. B., Powles, T., Albiges, L., Haanen, J. B., Larkin, J., et al. (2020). Avelumab plus axitinib versus sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. *Nat. Med.* 26 (11), 1733–1741. doi:10.1038/s41591-020-1044-8

Palazon, A., Goldrath, A. W., Nizet, V., and Johnson, R. S. (2014). HIF transcription factors, inflammation, and immunity. *Immunity* 41 (4), 518–528. doi:10.1016/j.immuni.2014.09.008

Qian, J., and Rankin, E. B. (2019). Hypoxia-Induced phenotypes that mediate tumor heterogeneity. *Adv. Exp. Med. Biol.* 1136, 43–55. doi:10.1007/978-3-030-12734-3_3

Riaz, N., Havel, J. J., Makarov, V., Desrichard, A., Urba, W. J., Sims, J. S., et al. (2017). Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell* 171 (4), 934–949. doi:10.1016/j.cell.2017.09.028

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., et al. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 43 (7), e47. doi:10.1093/nar/gkv007

Rustgi, A. K., and El-Serag, H. B. (2014). Esophageal carcinoma. *N. Engl. J. Med.* 371 (26), 2499–2509. doi:10.1056/NEJMra1314530

Samanta, D., and Semenza, G. L. (2018). Metabolic adaptation of cancer and immune cells mediated by hypoxia-inducible factors. *Biochim. Biophys. Acta Rev. Cancer* 1870 (1), 15–22. doi:10.1016/j.bbcan.2018.07.002

Sun, J. M., Shen, L., Shah, M. A., Enzinger, P., Adenis, A., Doi, T., et al. (2021). Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study. *Lancet* 398 (10302), 759–771. doi:10.1016/s0140-6736(21)01234-4

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Wang, Q., Dhindsa, R. S., Carss, K., Harper, A. R., Nag, A., Tachmazidou, I., et al. (2021). Rare variant contribution to human disease in 281,104 UK Biobank exomes. *Nature* 597 (7877), 527–532. doi:10.1038/s41586-021-03855-y

Wang, Q. L., Xie, S. H., Wahlin, K., and Lagergren, J. (2018). Global time trends in the incidence of esophageal squamous cell carcinoma. *Clin. Epidemiol.* 10, 717–728. doi:10.2147/clep.S166078

Wang, W. F., Zhao, N., Li, Y. Q., Luo, M. Y., Xiao, S. Q., and Yang, W. (2020). LncRNA APPAT regulated miR-328a/Pkp1 signal pathway to participate in breast cancer. *Eur. Rev. Med. Pharmacol. Sci.* 24 (10), 5493–5503. doi:10.26355/eurrev_202005_21334

Wang, Z. X., Cui, C., Yao, J., Zhang, Y., Li, M., Feng, J., et al. (2022). Toripalimab plus chemotherapy in treatment-naïve, advanced esophageal squamous cell carcinoma (JUPITER-06): a multi-center phase 3 trial. *Cancer Cell* 40 (3), 277–288.e3. doi:10.1016/j.ccell.2022.02.007

Wilkerson, M. D., and Hayes, D. N. (2010). ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. *Bioinformatics* 26 (12), 1572–1573. doi:10.1093/bioinformatics/btq170

Wu, T., and Dai, Y. (2017). Tumor microenvironment and therapeutic response. *Cancer Lett.* 387, 61–68. doi:10.1016/j.canlet.2016.01.043

Xi, Y., Lin, Y., Guo, W., Wang, X., Zhao, H., Miao, C., et al. (2022). Multi-omic characterization of genome-wide abnormal DNA methylation reveals diagnostic and prognostic markers for esophageal squamous-cell carcinoma. *Signal Transduct. Target Ther.* 7 (1), 53. doi:10.1038/s41392-022-00873-8

Ye, Y., Hu, Q., Chen, H., Liang, K., Yuan, Y., Xiang, Y., et al. (2019). Characterization of hypoxia-associated molecular features to aid hypoxia-targeted therapy. *Nat. Metab.* 1 (4), 431–444. doi:10.1038/s42255-019-0045-8

Yuan, H., Zhao, Z., Xu, J., Zhang, R., Ma, L., Han, J., et al. (2023). Hypoxia-induced TMTC3 expression in esophageal squamous cell carcinoma potentiates tumor angiogenesis through Rho GTPase/STAT3/VEGFA pathway. *J. Exp. Clin. Cancer Res.* 42 (1), 249. doi:10.1186/s13046-023-02821-y

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 27 May 2024
DOI 10.3389/fphar.2024.1379508

OPEN ACCESS

EDITED BY
Valentina Audrito,
Università del Piemonte Orientale, Italy

REVIEWED BY
Mario Morales,
National Autonomous University of Mexico, Mexico
Weiping Wang,
Peking Union Medical College Hospital (CAMS), China

*CORRESPONDENCE
Juan Li,
236406024@qq.com
Xiaoying Xu,
xiaoyingxu73@aliyun.com

†These authors have contributed equally to this work

RECEIVED 31 January 2024
ACCEPTED 08 May 2024
PUBLISHED 27 May 2024

CITATION
Ping P, Li J and Xu X (2024), The value of plasma omega-3 polyunsaturated fatty acids in predicting the response and prognosis of cervical squamous cell carcinoma patients to concurrent chemoradiotherapy.
Front. Pharmacol. 15:1379508.
doi: 10.3389/fphar.2024.1379508

The value of plasma omega-3 polyunsaturated fatty acids in predicting the response and prognosis of cervical squamous cell carcinoma patients to concurrent chemoradiotherapy

Pengbin Ping, Juan Li*† and Xiaoying Xu*†

Department of Radiotherapy Oncology, The Second Affiliated Hospital of Dalian Medical University, Dalian, China

Background: In recent years, abnormalities in plasma omega-3 polyunsaturated fatty acids (omega-3 PUFAs) have been proven to be related to the risk of cancer, but their prognostic value for cancer is unclear. The purpose of this study was to retrospectively evaluate the response and prognostic significance of plasma omega-3 PUFAs in patients with cervical squamous cell carcinoma (CSCC) treated with concurrent chemoradiotherapy (CCRT). Spearman rank correlation analysis was used to analyze the correlation between omega-3 PUFAs and squamous cell carcinoma antigen (SCC-Ag) levels.

Methods: A total of 89 patients with CSCC who underwent CCRT were evaluated retrospectively. Binary logistic regression analysis was used to analyze the independent predictors related to complete response (CR) after CCRT. A Cox proportional hazard model and Kaplan-Meier analysis were utilized to perform survival analysis.

Results: According to multivariate logistic regression analyses, a high level of plasma EPA was independently correlated with an increased incidence of CR after CCRT (odds ratio (OR), 0.980; 95% confidence interval (CI), 0.962–0.999, p = 0.038). With a median follow-up of 41.3 months, the CSCC patients in the high EPA (≥46.0 nmol/mL) group exhibited longer OS and PFS. According to our multivariate analysis, pretreatment plasma EPA level was an independent prognostic factor for PFS in patients with CSCC who underwent CCRT (hazard ratio (HR), 0.263; 95% CI, 0.089–0.782, p = 0.016). However, it was not an independent prognostic factor of OS. Spearman rank correlation analysis revealed was a negative correlation between pretreatment SCC-Ag (pre SCC-Ag) levels and EPA levels (r = −0.305, p = 0.004), and a weak negative correlation between posttreatment SCC-Ag (post SCC-Ag) levels and EPA levels (r = −0.251, p = 0.018).

Conclusion: Plasma omega-3 PUFAs are related to the response and survival outcome of patients with CSCC who underwent CCRT. Pretreatment plasma EPA

levels may be a promising biomarker for predicting the response and prognosis of patients with CSCC who undergo CCRT. In addition, the pretreatment plasma EPA levels presented a negative correlation with the SCC-Ag levels.

KEYWORDS

cervical squamous cell carcinoma, concurrent chemoradiotherapy, omega-3 polyunsaturated fatty acids, eicosapentaenoic acid, response, prognosis

1 Introduction

Currently, According to GLOBOCAN2020 data, cervical cancer (CC) accounts for approximately 6.5% of all malignant tumors in women worldwide, making it the fourth most common cancer among women (Sung et al., 2021). Cervical squamous cell carcinoma (CSCC) and cervical adenocarcinoma are the most common subtypes of CC, accounting for 70% and 25% of all cases, respectively (Guo et al., 2018; Jenkins et al., 2020). It is undeniable that newer screening, awareness and technology have reduced the growth of CC in the past decade (Sung et al., 2021). Due to the low coverage of CC screening in middle-and low-income countries, a considerable number of patients are diagnosed with locally advanced cervical cancer (LACC) at the first diagnosis, including patients with stage IB3-IVA disease (Serkies and Jassem, 2018). Since 1999, cisplatin-based CCRT has become the standard treatment for patients with LACC, for which the total effective rate is more than 90% (Rose et al., 1999). However, approximately 30%–50% of patients with CC still experience local recurrence or distant metastasis after undergoing CCRT (Rose et al., 1999; Kumar and Gupta, 2016). Therefore, determining the prognostic factors of patients with LACC treated with CCRT is highly important.

Many studies have shown that PUFAs act vital roles in the occurrence of malignant tumors, including in the regulation of apoptosis (Haycock et al., 2023; Montecillo-Aguado et al., 2023). Omega-3 PUFAs are one of the polysaturated fatty acids, that are derived mainly from deep-sea fishes, such as sardines and herring, thus, they are also known as marine PUFAs (Montecillo-Aguado et al., 2023). It has been widely reported in epidemiological studies because of its remarkable anti-inflammatory effects, direct inhibition of cancer expansion and tumorigenesis (Vaughan et al., 2013; Wei et al., 2022). Omega-3 PUFAs mainly include eicosapentaenoic acid (C20:5, EPA), docosahexaenoic acid (C22:6, DHA) and docosapentaenoic acid (C22:5, DPA), among them, EPA and DHA have the highest biological activity. DPA are the intermediates of DHA and EPA and may also have potential anticancer properties (Vaughan et al., 2013).

Andrew et al. reported that EPA has significant anti-angiogenic effects on colorectal cancer, and that high levels of EPA could improve overall survival (OS) (Cockbain et al., 2014). A meta-analysis of 20 studies showed that omega-3 PUFAs significantly improved nutritional status and immune function in patients with colorectal cancer after radical resection after strict control of bias risk (Yue et al., 2022). Endogenous omega-3 PUFAs enrichment can reduce cisplatin-induced myelosuppression by regulating the NRF2-MDM2-p53 signaling pathway (Xu et al., 2023). Interestingly, the introduction of omega-3 PUFAs in esophageal, colorectal and breast cancer has been gradually proven to enhance the cytotoxicity of some chemotherapeutic drugs and enhance the sensitivity of tumors to ionizing radiation, but does not increase the toxicity to normal tissues (Cai et al., 2014; Shin et al., 2019; Yang et al., 2022). Kai and other researchers have showen that the unsaturation of exogenous fatty acids is positively correlated with ferroptosis sensitivity. DHA can effectively promote ferroptosis by mediating intracellular lipid peroxidation to kill CC HeLa cells (Shan et al., 2022). Supplementation with omega-3 PUFAs is effective at maintaining skeletal muscle mass, nutritional status and reducing toxicity from radiotherapy in CC patients (Aredes et al., 2019). However, the effect of pretreatment plasma omega-3 PUFAs levels on the clinical outcome of patients with CSCC who underwent CCRT has not been reported thus far. Therefore, predicting the survival rate by measuring the levels of omega-3 PUFAs before treatment in patients with CSCC who underwent CCRT may be helpful for evaluating patient prognosis.

The purpose of this experiment was to assess the predictive value of omega-3 PUFAs levels for early treatment response, progression-free survival (PFS), and overall survival (OS) in patients with CSCC who were undergoing CCRT.

2 Materials and methods

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Dalian Medical University.

2.1 Study population

This study retrospectively included 89 patients with CSCC (stages IB3-IIIC2) who underwent CCRT at the Department of Radiotherapy Oncology, The Second Affiliated Hospital of Dalian Medical University from September 2019 to November 2021. The inclusion criteria of this study were as follows: (1) all patients were older than 30 years and were pathologically confirmed to have CSCC. Each patient was staged according to FIGO 2018. (2) Patients had no history of cancer or coexisting cancer. (3) Complete laboratory examination and clinical data, especially the serum SCC-Ag level before treatment and 1–3 months after CCRT. (4) Patients did not receive radiotherapy, chemotherapy, targeting, immunity and other treatments before collecting plasma omega-3PUFAs levels. Patients with the following characteristics were excluded from this study: (1) previous surgery for CC or cervical adenocarcinoma and adenosquamous carcinoma. (2) Patients who failed to complete CCRT for any reason. (3) Patients with any liver disease.

2.2 Clinical parameters and laboratory results

All the laboratory test and clinical data of the subjects, including age at diagnosis, BMI, tumor stage, maximum tumor diameter, lymph node metastasis, history of HPV infection, hypertension, history of diabetes and some routine laboratory examination data were obtained from the hospital electronic medical record database. Blood samples were collected on an empty stomach on the day of treatment, and 10 mL of blood was collected with an anticoagulant tube containing ethylenediaminetetraacetic acid (EDTA). The samples were centrifuged within 4 h to obtain plasma and a brownish yellow coating. Plasma samples were then collected and immediately stored at −80°C. The levels of EPA, DHA and DPA were detected via liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Zhang et al., 2023). In our institution, the normal reference range for SCC-Ag is ≤ 2.5 ng/mL. In this study, patients with SCC-Ag>2.5 ng/mL, were divided into a group with high SCC-Ag levels. In contrast, when was SCC-Ag≤2.5 ng/mL, patients were classified into the low SCC-Ag subgroup.

2.3 CCRT treatment

All patients received external beam radiation therapy (EBRT) and brachytherapy. The EBRT dose was 45–50.4 Gy/25 to 28 fractions (1.8–2.0 Gy per fraction, 5 days per week). The irradiation area included the primary site, uterus, para-uterine region, and partial/full vaginal and pelvic lymph node metastasis/drainage areas. Brachytherapy was initiated near the end of EBRT as defined by the American Society for Brachytherapy guidelines. Brachytherapy was initiated at a dose of 6 Gy per fraction, two fractions per week, for a total dose of 24–30 Gy. Brachytherapy was not available on the same day as EBRT. CCRT was fully completed within 8 weeks. Chemotherapy consisted of weekly intravenous infusions of cisplatin or cisplatin combined with albumin-bound paclitaxel, administered every 3 weeks during radiotherapy.

2.4 Follow-up

The first follow-up examination was performed 1 month after the end of treatment. The data were reviewed every 3 months for the first 2 years after the completion of treatment, then every 6 months thereafter, and annually after 5 years. Routine follow-up included gynecological examinations, transvaginal color ultrasound, SCC-Ag measurements, enhanced chest CT and enhanced pelvic MRI, and enhanced upper and lower abdominal CT and so on. If recurrence is suspected in some patients, further pathologic biopsies and positron emission tomography-CT examinations will also be permitted. The primary endpoint was PFS, defined as the time from diagnosis of CC to local recurrence, distant metastasis, or last follow-up, and the secondary endpoint was OS, the time interval between diagnosis of CC and death from any cause or the date of last follow-up. Clinical response based on MRI scans was determined 3 months after the end of CCRT according to the RECIST 1.1 criteria (Schwartz et al., 2016). The response was divided into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). The patients were divided into two groups: the CR group and the non-CR group. The non-CR group included PR, PD and SD patients.

2.5 Statistical analysis

Student's *t*-test was used to compare continuous variables with a normal distribution, expressed as the average ± standard deviation, while the Mann-Whitney U test was used for data with a nonnormal distribution, expressed as the range of the median (range). The chi-square test or Fisher's exact test was used to compare classified variables in terms of quantities and percentages, and linear regression was used to check for potential collinearity between independent variables. Independent predictors associated with CR after CCRT were analyzed by including indicators that were meaningful as a single factor in a multifactorial binary logistic regression analysis. The OS and PFS curves were calculated by the Kaplan-Meier method with the log-rank test. Variables with $p < 0.05$ in univariate analysis were selected for multivariate Cox regression analysis to determine the independent predictors of survival outcome. Spearman rank correlation was used to analyze the correlation between significant FFAs and SCC-Ag. A p-value < 0.05 was considered to indicate statistical significance. The SPSS 26.0 software package was used for data processing.

3 Results

3.1 Study participant characteristics

The final study cohort consisted of 89 patients with CSCC, whose clinicopathologic characteristics are shown in Table 1, with a median follow-up of 41.3 months (range: 7.7–61.9 months). The median age at diagnosis of CC was 61 years (range: 30–81 years). The patients were staged according to the FIGO 2018 criteria, and the number of patients with stage IB3-II and III CC were 6 (6.7%), 38 (42.7%) and 45 (50.6%), respectively. In this study, lymph node metastasis was determined by a short diameter of lymph nodes ≥1 cm on MRI, of which 28 patients (31.5%) had lymph node metastasis and 61 patients (68.5%) were lymph node-negative. The median values of pre SCC-Ag and post SCC-Ag were 14.2 ng/mL (range: 5.0–52.2 ng/mL) and 1.7 ng/mL (range: 1.2–2.3 ng/mL) respectively. The plasma samples of 89 patients were evaluated by LC-MS/MS. The median level of omega-3 PUFAs in the whole cohort was as follows: EPA of 58.0 nmol/mL (range:42.5–86.0 nmol/mL); DHA of 301.0 nmol/mL (range: 223.0–364.0 nmol/mL), and DPA of 69.0 nmol/mL (range: 55.0–89.5 nmol/mL). MRI scans 3 months after the end of CCRT showed that 63 patients (70.8%) achieved CR and 26 patients (29.2%) did not achieve CR. The non-CR group included 24 (27.0%) patients with PR and 2 (2.2%) patients with PD or SD.

3.2 Factors associated with CR after CCRT

CR after CCRT has been shown to be a reliable surrogate endpoint for survival in patients with LACC. Table 2 shows the

10.3389/fphar.2024.1379508

TABLE 1 The clinical characteristics of all patients (*n* = 89).

Factor	Total (N = 89)	%
Age (years)	61(53-68)	
BMI (kg/m^2)		
<25	51	57.3
≥25	38	42.7
Hypertension history		
Yes	25	28.1
No	64	71.9
Diabetes history		
Yes	11	12.4
No	78	87.6
Gravidity		
0–2	48	53.9
≥3	28	31.5
Unknown	13	14.6
FIGO stage		
IB3	6	6.7
II	38	42.7
III	45	50.6
Size (cm)		
<4	26	29.2
≥ 4	63	70.8
Lymph nodes metastasis		
Positive	28	31.5
Negative	61	68.5
Pre SCC-Ag (ng/mL)	14.15(5.03-52.17)	
≤2.5	9	10.1
>2.5	80	89.9
Post SCC-Ag (ng/mL)	1.70(1.17-2.28)	
≤2.5	74	83.1
>2.5	15	16.9
HPV Status		
16+	35	39.3
Others	6	6.7
Negative	5	5.6
Unknown	43	48.3
HGB (g/L)	122.50±18.30	

(Continued on following page)

TABLE 1 (*Continued*) The clinical characteristics of all patients (*n* = 89).

Factor	Total (N = 89)	%
EPA (nmol/mL)	58.00(42.50-86.00)	
DHA (nmol/mL)	301.00(223.00-364.00)	
DPA (nmol/mL)	69.00(55.00-89.50)	

Abbreviations: BMI, body mass index; HGB, hemoglobin; FIGO, international federation of gynecology and obstetrics; SCC-Ag, squamous cell carcinoma antigen; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

relationship between the clinical response of CCRT and clinicopathologic characteristics, and univariate analysis showed that the response to CCRT was significantly correlated with the post SCC-Ag level ($p = 0.010$). The proportion of post SCC-Ag levels that were low (≤2.5 ng/mL) (90.5%) was much higher in the CCRT CR group than the proportion of post SCC-Ag levels that were high (>2.5 ng/mL) (9.5%). In the CCRT non-CR cohort, the proportion of patients with low levels of post SCC-Ag (≤2.5 ng/mL) (65.4%) was also greater than that of patients with high levels of post SCC-Ag (>2.5 ng/mL) (34.6%), but the difference was not as great as that in the effective group (Table 1). High levels of SCC-Ag after CCRT may be a risk factor for poor CCRT outcomes. Similarly, we also found that pretreatment plasma EPA levels were markedly higher in patients in the CCRT CR group than in the non-CR group, and that low levels of EPA were considerably linked with a reduced likelihood of CR (66.0 (48.0–90.0) nmol/mL vs 48.5 (31.8–69.3) nmol/mL, $p = 0.018$). DHA levels were slightly higher in the CR group than in the non-CR group, however, the results were not statistically different ($p = 0.246$). Similarly, the levels of DPA in the two groups were similar ($p = 0.601$). There were no differences between the two groups in term of BMI, age, tumor stage, incidence of positive lymph nodes, tumor size, and HPV type. We included factors significantly associated with response to CCRT in the univariate analysis via multifactorial binary logistic regression analysis to further identify factors influencing the prediction of CR after CCRT. Multivariate analysis revealed that post SCC-Ag levels >2.5 ng/mL (OR, 4.752; 95% CI, 1.431–15.786, $p = 0.011$) were independently associated with a reduced incidence of CR after CCRT. In contrast, high levels of plasma EPA were independently associated with an increased incidence of CR after CCRT (OR, 0.980; 95% CI, 0.962–0.999, $p = 0.038$), as shown in Table 3.

3.3 Optimal cut-off value for omega −3 PUFAs in plasma before CCRT

Optimal AUC and cut-off value for plasma omega-3 PUFAs parameters prior to CCRT in patients with CSSS were determined by plotting ROC curves for survival outcomes. The cut-off value for the joint maximum sensitivity and specificity of EPA was 46.0 nmol/mL ($p = 0.029$, AUC = 0.713, 95% CI = 0.561–0.865), that of DHA was 308.0 nmol/mL ($p = 0.036$, AUC = 0.704, 95% CI = 0.549–0.858) and that of DPA was 69.0 nmol/mL ($p = 0.740$, AUC = 0.532, 95% CI = 0.363–0.702). For further analysis, patients were categorized into high EPA or low EPA group (≥46.0 nmol/mL or <46.0 nmol/mL, respectively), high DHA or low DHA group (≥308.0 nmol/mL or <308.0 nmol/mL, respectively) and high DPA or low DPA group (≥69.0 nmol/mL or <69.0 nmol/mL, respectively).

3.4 Survival analysis

Of the 89 patients, 10 (37.0%) of 27 patients with an EPA level <46.0 nmol/mL were diagnosed with locally recurrent or metastatic disease, and 7 (11.3%) of 62 patients with an EPA level ≥46.0 nmol/mL were diagnosed with locally recurrent or metastatic disease ($p = 0.003$). The PFS rates of the low EPA group at 1 and 3 years were 85.2% and 62.6%, respectively, and the PFS rates of the high EPA group at 1 and 3 years were 96.8% and 88.1%, respectively. The PFS data are shown in Figure 1A. Patients with EPA levels ≥46.0 nmol/mL had significantly better PFS than patients with EPA levels <46.0 nmol/mL. In terms of OS, 7 (25.9%) of 27 patients with EPA levels <46.0 nmol/mL died, and 3 (4.8%) of 62 patients with EPA levels ≥46.0 nmol/mL died ($p = 0.006$). OS was 96.3% and 81.1% at 1 and 3 years in the low EPA group and 98.4% and 94.8% at 1 and 3 years in the high EPA group, respectively. The OS data are shown in Figure 1B. Patients with EPA levels <46.0 nmol/mL had relatively poor OS. Similarly, the differences in 3-year PFS and OS between patients with post SCC-Ag levels ≤2.5 ng/mL and those with >2.5 ng/mL were statistically significant in Figure 1C,D.

Table 4 and Table 5 summarize the univariate and multivariate HR and 95% CI for PFS and OS (HPV status was not included in the survival analysis because of missing data). Univariate analysis revealed that pretreatment plasma EPA ≥46.0 nmol/mL levels and DHA≥308.0 nmol/mL levels were considerably connected with improvement in PFS (HR, 0.259; 95% CI, 0.099–0.682, $p = 0.006$) and (HR, 0.309; 95% CI, 0.101–0.949, $p = 0.040$). Other clinicopathologic variables that were dramatically linked with improvement in PFS included achievement of CR after CCRT (HR, 0.238; 95% CI, 0.090–0.625, $p = 0.004$). Lymph node metastasis, later FIGO staging, and post SCC-Ag level >2.5 ng/mL were significantly linked with decreased PFS (all $p < 0.05$). In the Multivariate analysis, pretreatment plasma EPA level ≥46.0 nmol/mL remained an independent prognostic factor for improved PFS (HR, 0.263; 95% CI, 0.089–0.782, $p = 0.016$). Other independent factors associated with reduced PFS included lymph node metastasis (HR, 4.678; 95% CI, 1.576–13.886, $p = 0.005$), and post SCC-Ag level >2.5 ng/mL (HR, 3.148; 95% CI, 1.138–8.706, $p = 0.027$).

Univariate analysis showed that achieving CR after CCRT was significantly associated with improved OS (HR, 0.225; 95% CI,

TABLE 2 Univariate analysis of clinical variables with response to CCRT.

	CR (n=63)	Non-CR (n=26)	*P*
Age (years)	61(53-69)	60(52-66)	0.838
<60	30(47.6)	13(50.0)	
≥60	33(52.4)	13(50.0)	
BMI (kg/m^2)			
<25	34(54)	17(65.4)	0.322
≥25	29(46)	9(34.6)	
Hypertension history			
Yes	19(30.2)	6(23.1)	0.499
No	44(69.8)	20(76.9)	
Diabetes history			
Yes	5(7.9)	6(23.1)	0.105
No	58(92.1)	20(76.9)	
Gravidity			
0–2	35(55.6)	13(50)	0.636
≥3	18(28.6)	10(38.5)	
Unknown	10(15.9)	3(11.5)	
FIGO stage			
IB3	6(9.5)	0(0)	0.173
II	28(44.4)	10(38.5)	
III	29(46.0)	16(61.5)	
Size (cm)			
<4	19(20.2)	7(26.9)	0.760
≥ 4	44(69.8)	19(73.1)	
Lymph nodes metastasis			
Positive	17(27.0)	11(42.3)	0.157
Negative	46(73.0)	15(57.7)	
Pre SCC-Ag (ng/mL)	12.95(6.93-40.76)	16.58(4.38-54.46)	
≤2.5	6(9.5)	3(11.5)	1.000
>2.5	57(90.5)	23(88.5)	
Post SCC-Ag (ng/mL)	1.60(1.16-2.05)	2.00(1.24-2.80)	
≤2.5	57(90.5)	17(65.4)	0.010
>2.5	6(9.5)	9(34.6)	
HPV Status			
16+	26(41.3)	9(34.6)	0.871
Others	4(6.3)	2(7.7)	
Negative	4(6.3)	1(3.8)	
Unknown	29(46)	14(53.8)	
HGB (g/L)	123.80±17.40	119.50±20.50	0.319

(Continued on following page)

TABLE 2 (*Continued*) Univariate analysis of clinical variables with response to CCRT.

	CR (n=63)	Non-CR (n=26)	*P*
EPA (nmol/mL)	66.00 (48.00-90.00)	48.50 (31.75-69.25)	0.018
DHA (nmol/mL)	303.00 (246.00-375.00)	289.00 (192.80-354.50)	0.246
DPA (nmol/mL)	69.00 (55.00-91.00)	71.00 (53.30-89.25)	0.601

Abbreviations: BMI, body mass index; HGB, hemoglobin; FIGO, international federation of gynecology and obstetrics; SCC-Ag, squamous cell carcinoma antigen; CR, complete response; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

TABLE 3 Multivariate analysis of clinical variables with response to CCRT.

	β	OR (95% CI)	*P*
Post SCC-Ag	1.559	4.752 (1.431-15.786)	0.011
EPA	-0.020	0.980 (0.962-0.999)	0.038

Abbreviations: SCC-Ag, squamous cell carcinoma antigen; OR, odds ratio; 95% CI, 95% confidence interval; EPA, eicosapentaenoic acid.

0.063–0.800, $p = 0.021$). Pretreatment plasma EPA ≥46.0 nmol/mL level and DHA≥308.0 nmol/mL level were considerably connected with improvement in OS (HR, 0.182; 95% CI, 0.047–0.705, $p = 0.014$) and (HR, 0.109; 95% CI, 0.014–0.862, $p = 0.036$). Similarly, we also observed that lymph node metastasis (HR, 6.951; 95% CI, 1.787–27.040, $p = 0.005$), and post SCC-Ag level >2.5 ng/mL (HR, 5.307; 95% CI, 1.530–18.405, $p = 0.009$) were significantly associated with decreased OS. Through multivariate analysis, only lymph node metastasis was the independent predictor of OS reduction (HR, 7.409; 95% CI, 1.760–31.194, $p = 0.006$). CR after CCRT was an independent prognostic factor for improved OS (HR, 0.232; 95% CI, 0.060–0.896, $p = 0.034$.). Multivariate analysis showed that pretreatment EPA ≥46.0 nmol/mL level was not an independent predictor of OS (HR, 0.288; 95% CI, 0.063–1.313, $p = 0.108$). Similarly, pretreatment DHA ≥308.0 nmol/mL level was not an independent predictor of OS (HR, 0.146; 95% CI, 0.015–1.391, $p = 0.094$).

3.5 Relationship between the EPA level and other clinical characteristics

The clinicopathological features of the two groups were compared, as shown in Table 6. The statistical analysis showed that EPA level was significantly correlated with age ($p = 0.022$), BMI ($p = 0.002$), and CR after CCRT ($p = 0.037$). However, there was no significant relationship between EPA level and other parameters.

3.6 Correlation of SCC-Ag levels with plasma EPA levels

To confirm the relationship between SCC-Ag levels and plasma EPA levels, we performed Spearman rank correlation analysis. The results showed that pre SCC-Ag and EPA were negatively correlated ($r = -0.305$, $p = 0.004$), and post SCC-Ag and EPA were weakly negatively correlated ($r = -0.251$, $p = 0.018$), as shown in Figures 2A,B.

3.7 Prognostic significance of combining plasma EPA and post-SCC ag

The prognostic significance of combining EPA and post SCC-Ag levels in the entire cohort is shown in Figure 3. There was a significant difference in the 3-year OS rate among the four groups ($p = 0.002$). The pretreatment plasma EPA levels ≥46.0 nmol/mL and post SCC-Ag levels ≤2.5 ng/mL groups had significantly greater survival probabilities than the other three groups. In contrast, patients with pretreatment plasma EPA levels <46.0 nmol/mL and post SCC-Ag levels >2.5 ng/mL had the lowest survival rate among the four groups in Figure 3.

4 Discussion

In view of the poor prognosis of patients with CC and the high tumor recurrence rate after CCRT, it is necessary to screen the risk factors of tumor prognosis and recurrence in order to support early intervention, improve monitoring, reduce tumor recurrence rate and prolong patient survival time. As lipids are closely related to tumorigenesis or tumor recurrence, marine polyunsaturated fatty acids (omega-3 PUFAs) as an important lipid, it is necessary to understand whether there is a relationship between omega-3PUFAs and the response and prognosis of patients with CC who underwent CCRT. This is the first study to evaluate the association between omega-3 PUFAs and response and prognosis in patients with CSCC who received CCRT.

Previous *in vivo* and *in vitro* studies have found that in addition to its direct cytotoxic effects on tumor cells, EPA can increase the cytotoxicity of anticancer drugs to tumors or cancer cells, even at doses that have no substantial effect on tumor growth or cell viability (Bégin et al., 1986; Bégin et al., 1988). Early experiments have proven that omega-3 PUFAs represented by EPA can enhance the sensitivity of breast, colorectal and ovarian cancers and other tumors to anticancer drugs, and have cytotoxicity to both vincristine-sensitive and drug-resistant human CC cells, which can increase the killing effect of human CC cells by 30%, and increase cisplatin's cytotoxicity against cytotoxicity of cisplatin on

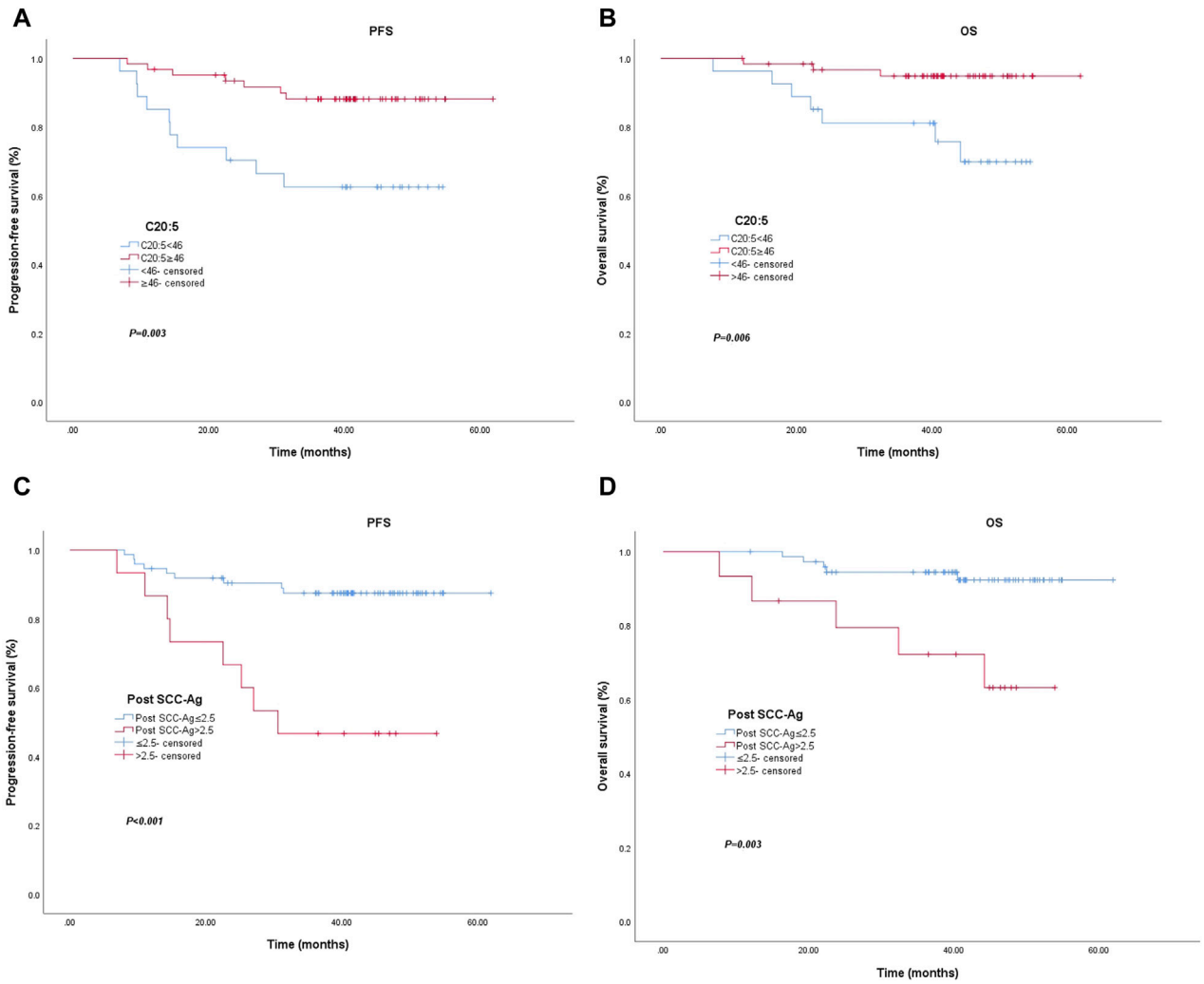


FIGURE 1
Kaplan–Meier curve for progression-free survival and overall survival for patients with low vs. high C20:5 levels **(A)** (p = 0.003) and **(B)** (p = 0.006). Kaplan–Meier curve for progression-free survival and overall survival patients with low vs. high post SCC-Ag levels **(C)** (p < 0.001) and **(D)** (p = 0.003). Abbreviations: Post SCC-Ag, posttreatment squamous cell carcinoma antigen; C20:5, eicosapentaenoic acid, EPA.

human CC cells by 36%–60% (Das et al., 1998; Hajjaji and Bougnoux, 2013). Consistent with these findings, our study found that both univariate and multivariate analysis results showed that patients who achieved CR in patients with CSCC who underwent CCRT had higher pretreatment levels of EPA. This finding suggested that a high level of EPA before treatment is an independent predictor of CR after CCRT. We suspect the mechanism might be EPA is the main starting substance of lipid peroxidation. With the increase in EPA concentration, the level of lipid peroxide produced by cells increased significantly, which was related to the loss of cell vitality and the significant inhibition of cell growth (Falconer et al., 1994). EPA treatment promotes the production of large amounts of ROS, which are highly peroxidizable and therefore may enhance the efficacy of cytotoxic ROS-inducing drugs against tumors (such as anthracycline, mitomycin, etc.) (Das et al., 1998; Colquhoun, 2009; Moon, 2023). Since ionizing radiation also generates ROS, which leads to lipid peroxidation of PUFAs, lipid peroxidation may be further enhanced in the membranes of EPA-rich tumor cells, and thus the combination of EPA and radiotherapy may cause additional damage to tumor cells (Manda et al., 2011; Zaloga, 2021). This seems to provide a strong explanation for the fact that EPA can enhance the sensitivity of radiotherapy and chemotherapy.

Regarding the relationship between EPA and tumor prognosis has rarely been investigated, a phase II randomized, double-blind trial demonstrated that oral EPA significantly slowed the growth of colorectal liver metastases, the reason for which may be that EPA has antiangiogenic properties and limited preoperative EPA exposure may prolong disease-free survival (DFS) and OS (Cockbain et al., 2014). Murphy et al. also demonstrated that NSCLC patients treated with platinum-based chemotherapy taking 2.5 g of EPA + DHA supplementation per day had a twofold increase in clinical benefit and treatment response rate, and a higher 1-year survival rate for patients in the supplementation group (60% vs 38.7%), than patients who received standard treatment but no additional supplementation (Murphy et al., 2011). These studies are consistent with our results. We found that in patients with CSCC treated with CCRT, the level of

TABLE 4 Univariate and multivariate analyses of prognostic factors for PFS among patients with CC.

Characteristics	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years)				
(<60 vs. ≥60)	0.829(0.319-2.145)	0.697		
BMI (kg/m^2)				
(≥25 vs. <25)	0.525(0.185-1.490)	0.226		
Hypertension history				
(yes vs. no)	0.520(0.149-1.810)	0.520		
Diabetes history				
(yes vs. no)	0.391(0.052-2.949)	0.362		
Lymph nodes metastasis				
(yes vs. no)	5.378(1.982-14.594)	0.001	4.678(1.576-13.886)	0.005
FIGO stage				
IB3	3.508(1.213-10.144)	0.021	1.992(0.604-6.568)	0.257
II				
III				
Pre SCC-Ag (ng/mL)				
(>2.5 vs.≤2.5)	1.957(0.259-14.760)	0.515		
Post SCC-Ag (ng/mL)				
(>2.5 vs.≤2.5)	5.296(2.036-13.776)	0.001	3.148(1.138-8.706)	0.027
Size (cm)				
(<4 vs. ≥4)	0.989(0.348-2.808)	0.984		
CR achieved				
(yes vs. no)	0.238(0.090-0.625)	0.004	0.466(0.167-1.306)	0.146
HGB (g/L)				
(≥110 vs.<110)	0.631(0.222-1.794)	0.388		
EPA (nmol/mL)				
(≥46 vs.<46)	0.259(0.099-0.682)	0.006	0.263(0.089-0.782)	0.016
DPA (nmol/mL)				
(≥69 vs.<69)	0.463(0.171-1.254)	0.130		
DHA (nmol/mL)				
(≥308 vs.<308)	0.309(0.101-0.949)	0.040	0.525(0.157-1.759)	0.296

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; BMI, body mass index; HGB, hemoglobin; FIGO, international federation of gynecology and obstetrics; SCC-Ag, squamous cell carcinoma antigen; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

plasma EPA ≥46.0 nmol/mL before treatment was significantly correlated with PFS (HR, 0.263; 95% CI, 0.089–0.782, $p = 0.016$). In multivariate Cox regression analysis, it was found that plasma EPA ≥46.0 nmol/mL before treatment was not an independent predictor of OS (HR, 0.288; 95% CI, 0.063–1.313, $p = 0.108$). However, the Kaplan-Meier survival curve showed that the OS of patients with plasma EPA ≥46.0 nmol/mL was better than that of patients with plasma EPA <46.0 nmol/mL before treatment. The reason for this phenomenon may be due to the small sample size and insufficient follow-up time. We will strive to collect more samples and conduct long-term follow-up for further research. However, in the univariate analysis of PFS and OS, the plasma DHA

TABLE 5 Univariate and multivariate analyses of prognostic factors for OS among patients with CC.

Characteristics	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years)				
(<60 vs. ≥60)	1.428(0.402-5.073)	0.581		
BMI (kg/m^2)				
(≥25 vs. <25)	0.339(0.072-1.597)	0.171		
Hypertension history				
(yes vs. no)	0.262(0.033-2.070)	0.204		
Diabetes history				
(yes vs. no)	0.792(0.100-6.285)	0.825		
Lymph nodes metastasis				
(yes vs. no)	6.951(1.787-27.040)	0.005	7.409(1.760-31.194)	0.006
FIGO stage				
IB3	3.957(0.898-17.439)	0.069		
II				
III				
Post SCC-Ag (ng/mL)				
(>2.5 vs.≤2.5)	5.307(1.530-18.405)	0.009	2.916(0.802-10.605)	0.104
Size (cm)				
(<4 vs. ≥4)	0.634(0.179-2.248)	0.480		
CR achieved				
(yes vs. no)	0.225(0.063-0.800)	0.021	0.232(0.060-0.896)	0.034
HGB (g/L)				
(≥110 vs.<110)	0.604(0.156-2.341)	0.466		
EPA (nmol/mL)				
(≥46 vs.<46)	0.182(0.047-0.705)	0.014	0.288(0.063-1.313)	0.108
DPA (nmol/mL)				
(≥69 vs.<69)	0.521(0.147-1.856)	0.315		
DHA (nmol/mL)				
(≥308 vs.<308)	0.109(0.014-0.862)	0.036	0.146(0.015-1.391)	0.094

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; BMI, body mass index; HGB, hemoglobin; FIGO, international federation of gynecology and obstetrics; SCC-Ag, squamous cell carcinoma antigen; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

level ≥308.0 nmol/mL before treatment was statistically significant, $p = 0.040$ and 0.036, respectively, but in multivariate analysis, DHA level was not an independent prognostic factor for PFS and OS.

This study suggested that plasma EPA is a valid pretreatment biomarker for predicting survival in CSCC patients who underwent CCRT. However, the possible mechanism by which pretreatment plasma EPA reflects the prognosis of patients with CC as a new indicator for assessing OS and PFS is unclear. The preliminary explanation can only be given by considering previous studies. The anticancer effects of EPA are mainly attributed to its ability to modulate cell death pathways, inhibit cell proliferation and induce the expression of anti-inflammatory mediators (D'Angelo et al., 2020; Montecillo-Aguado et al., 2023). The mechanism by which EPA promotes apoptosis alone involves the alteration of multiple complex cellular pathways; for example, EPA was able to induce cell cycle arrest in breast cancer cell lines (Zhu et al., 2018). In particular, the progression of the cell cycle from the S to the G2-M phase was blocked in BT20 breast cancer cells, and this apoptotic effect was shown in a concentration- and time-dependent manner (Nappo

TABLE 6 Relationships between clinicopathological data and the different levels of pretreatment C20:5 in CC.

Characteristics	C20:5<46	C20:5≥46	
	N=27	N=62	*P*
Age (years)			
<60	18(66.7)	25(40.3)	0.022
≥60	9(33.3)	37(59.7)	
BMI (kg/m^2)			
<25	22(81.5)	29(46.8)	0.002
≥25	5(18.5)	33(53.2)	
Hypertension history			
yes	6(22.2)	19(30.6)	0.416
no	21(77.8)	43(69.4)	
Diabetes history			
yes	1(3.7)	10(16.1)	0.198
no	26(96.3)	52(83.9)	
Lymph nodes metastasis			
yes	8(29.6)	20(32.3)	0.806
no	19(70.4)	42(67.7)	
FIGO stage			
IB3	1(3.7)	5(8.1)	0.483
II	10(37.0)	28(45.2)	
III	16(59.3)	29(46.8)	
Pre SCC-Ag (ng/mL)			
≤2.5	1(3.7)	8(12.9)	0.347
>2.5	26(96.3)	54(87.1)	
Post SCC-Ag (ng/mL)			
≤2.5	21(77.8)	53(85.5)	0.559
>2.5	6(22.2)	9(14.5)	
Size(cm)			
<4	7(25.9)	19(30.6)	0.653
≥4	20(74.1)	43(69.4)	
CR achieved			
yes	15(55.6)	48(77.4)	0.037
no	12(44.4)	14(22.6)	
HGB (g/L)			
<110	8(29.6)	12(19.4)	0.286
≥110	19(70.4)	50(80.6)	

Abbreviations: BMI, body mass index; HGB, hemoglobin; FIGO, international federation of gynecology and obstetrics; SCC-Ag, squamous cell carcinoma antigen; C20:5, eicosapentaenoic acid, EPA.

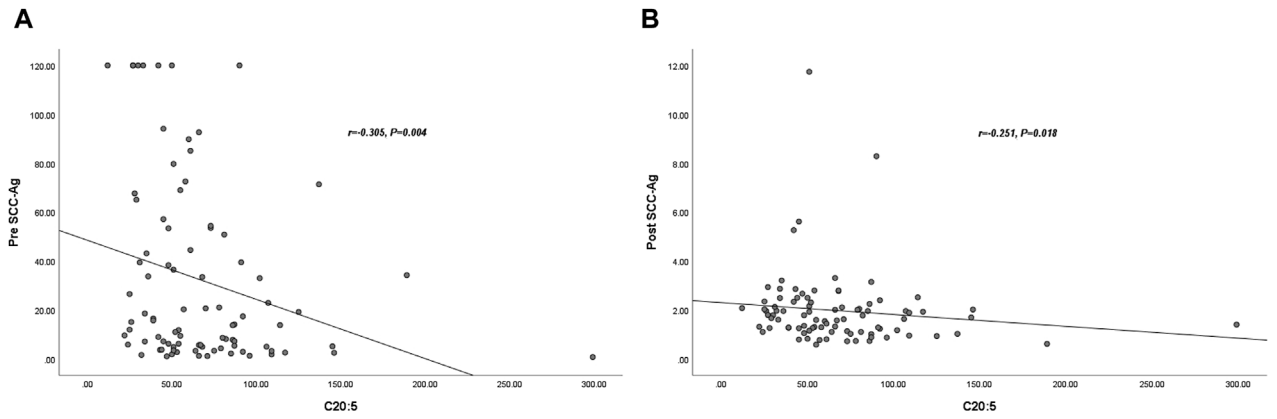


FIGURE 2
Correlation between plasma C20:5 and Post- SCC-Ag levels in 89 patients with CC. The plasma C20:5 levels were negatively correlated with pre SCC-Ag ($r = -0.305$, $p = 0.004$) **(A)**. The plasma C20:5 levels were negatively correlated with post SCC-Ag levels ($r = -0.251$, $p = 0.018$) **(B)**. Abbreviations: SCC-Ag, squamous cell carcinoma antigen; C20:5, eicosapentaenoic acid, EPA; Pre SCC-Ag, pretreatment squamous cell carcinoma antigen; Post SCC-Ag, posttreatment squamous cell carcinoma antigen.

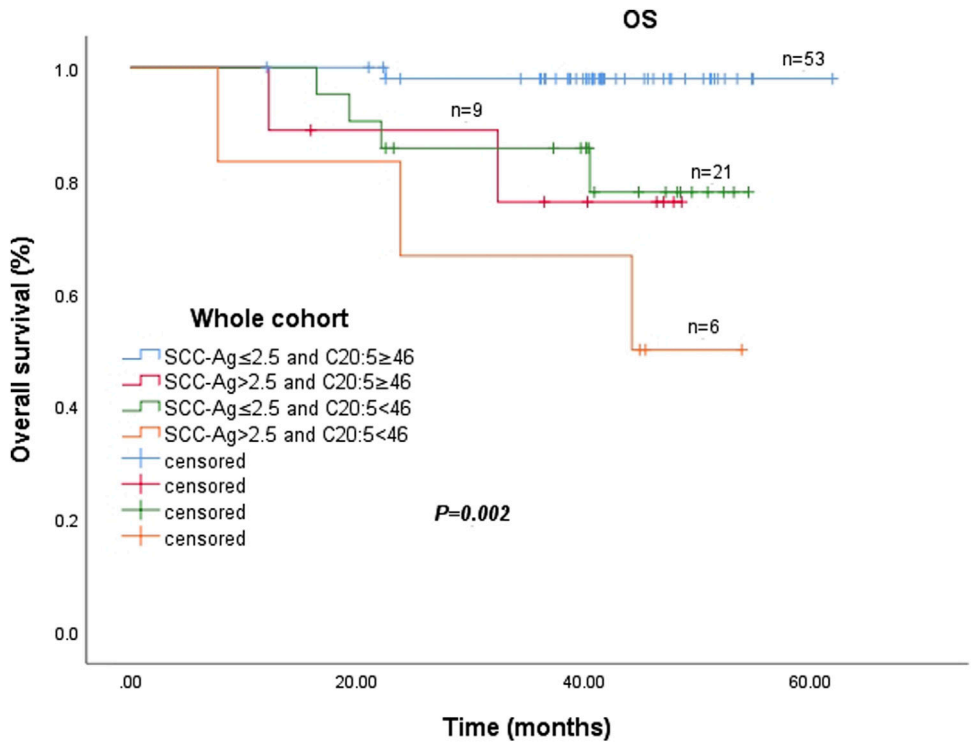


FIGURE 3
Abbreviations: SCC-Ag, squamous cell carcinoma antigen; C20:5, eicosapentaenoic acid, EPA.

et al., 2012). Similarly, it inhibits cholesterol efflux channel protein (ABCA1), leading to intracellular cholesterol accumulation and thus dysregulation of cholesterol homeostasis, which in turn increases cell membrane polarity lethality in triple-negative breast cancer cells (Torres-Adorno et al., 2019).

As a tumor marker, SCC-Ag is widely used in CSCC, and the increase of SCC-Ag before treatment is associated with larger tumor size, late tumor stage, regional lymph node involvement and deep stroma involvement, so it is widely used in monitoring CC recurrence (Wang et al., 2019). However, there are few studies on the relationship between SCC-Ag and the prognosis of CC. Wang et al. included 559 patients with CSCC. Multivariate analysis showed that SCC-Ag did not decrease to normal after CCRT was an independent prognostic factor for DFS (HR, 5.10; 95%CI, 3.31–7.88, $p < 0.001$) (Wang et al., 2019). This is highly consistent with our results, which show that post SCC-Ag level>2.5 ng/mL is an independent risk factor for 3-year PFS

(HR, 3.148; 95%CI, 1.138–8.706, $p = 0.027$). Interestingly, we found that there was a negative correlation between pre SCC-Ag and EPA ($r = -0.305$, $p = 0.004$),a weak negative correlation between post SCC-Ag and EPA ($r = -0.251$, $p = 0.018$) and the survival probability of patients in the pretreatment plasma EPA≥46.0 nmol/mL and post SCC-Ag≤2.5 ng/mL groups had the highest probability of survival, and patients with pretreatment plasma EPA<46.0 nmol/mL and post SCC-Ag>2.5 ng/mL had the lowest survival rate among the four groups. These results further confirmed the inverse relationship between EPA and SCC-Ag. Recent studies have also shown that EPA can significantly reduce the levels of serum TNF- α and IL-6 in tumor patients. At the same time, EPA can reduce the level of inflammatory reaction and improve body immune function by reducing the production of C-reactive protein and limiting the release of IL-6 (Liu et al., 2023). It is suggested that the expression levels of EPA and post SCC-Ag may be auxiliary markers for monitoring disease progression and tumor malignancy. However, we found that FIGO staging was a significant predictor of PFS in univariate analysis, but its significance was not significant in multivariate analysis. One of the reasons for this phenomenon may be the insufficient number of cases studied, and the lack of stage IVA patients in our group may also be.

EPA has a wide range of molecular targets in cancer cells and tumors. To sum up, the results show that EPA can activate a variety of molecular biological processes, including classical and alternative apoptosis pathways, and regulate survival and cell growth signals. Causing cell death in a variety of cells and animal models. However, further experiments are needed to explore the specific details of external approaches, especially upstream events. These findings suggest that EPA is expected to be a potential new treatment for cancer and can complement existing treatments. However, our clinical study further validates these basic experiments. In conclusion, the above mechanisms may partially explain why pretreatment plasma EPA may be a biomarker of outcome and prognosis in patients with CSCC who underwent CCRT. This study showed that the abnormal level of plasma EPA before treatment is directly related to the response and survival outcome of patients with CSCC receiving CCRT, and the level of plasma EPA before treatment can be used as a biomarker to assess the response and prognosis of CSCC patients.

However, our study also has some limitations. First, as a small-sample retrospective study, we regret that we were unable to accurately and dynamically track the level of changes in omega-3 PUFAs during and at the end of CCRT. Second, this study did not examine the relationship between nutritional support therapy and omega-3 PUFAs levels. Finally, the patients we enrolled were all patients with CSCC, and the results may be biased when it comes to patients with types of cervical adenocarcinoma and adenosquamous carcinoma. In the future, it is necessary to further elucidate the specific molecular mechanisms of the regulation of apoptosis, proliferation, survival, migration and drug resistance of omega-3 PUFAs, including the specific pathways involved and the interactions between different PUFAs. In addition, more research is needed to investigate the effects of omega-3 PUFAs on different types of cancer cells and animal models, and to evaluate their potential synergies with existing cancer therapies. However, it is unclear whether there is a clear benefit of elevated EPA levels after supplemental therapy, and if their beneficial effect is confirmed to improve local control of cervical cancer through dietary lipids as an adjunctive to CCRT, the prospects for systemic supplementation during CSCC treatment would be considerable.

5 Conclusion

In conclusion, the present study demonstrated that pretreatment plasma omega-3 PUFAs are associated with survival outcomes in patients with CSCC treated with CCRT. Pretreatment plasma EPA may be a promising biomarker for predicting recent response and PFS in patients with CSCC who underwent CCRT. The use of plasma EPA level before treatment and serum SCC-Ag level after treatment increased the prognostic significance of serum SCC-Ag level alone. Perhaps it is preferable to use a system of two or more determinants to create new prognostic tools for clinicians in CSCC. As an independent factor, EPA can be used as a new and promising prognostic index, which can be detected routinely and conveniently and cheaply, which will provide potential prognostic information for clinicians to promote individualized treatment of patients with CSCC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Ethics Committee of the Second Affiliated Hospital of Dalian Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

PP: Formal Analysis, Methodology, Validation, Writing–original draft, Writing–review and editing. JL: Data curation, Supervision, Validation, Writing–original draft, Writing–review and editing. XX: Formal Analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We would like to thank the patients and all participant have consented to the acknowledgement.

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.

Publisher's note

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1379508/full#supplementary-material

References

Aredes, M. A., da Camara, A. O., de Paula, N. S., Fraga, K. Y. D., do Carmo, M., and Chaves, G. V. (2019). Efficacy of ω-3 supplementation on nutritional status, skeletal muscle, and chemoradiotherapy toxicity in cervical cancer patients: a randomized, triple-blind, clinical trial conducted in a middle-income country. *Nutrition* 67-68, 110528. doi:10.1016/j.nut.2019.06.009

Bégin, M. E., Ells, G., Das, U. N., and Horrobin, D. F. (1986). Differential killing of human carcinoma cells supplemented with n-3 and n-6 polyunsaturated fatty acids. *J. Natl. Cancer Inst.* 77 (5), 1053–1062.

Bégin, M. E., Ells, G., and Horrobin, D. F. (1988). Polyunsaturated fatty acid-induced cytotoxicity against tumor cells and its relationship to lipid peroxidation. *J. Natl. Cancer Inst.* 80 (3), 188–194. doi:10.1093/jnci/80.3.188

Cai, F., Sorg, O., Granci, V., Lecumberri, E., Miralbell, R., Dupertuis, Y. M., et al. (2014). Interaction of ω-3 polyunsaturated fatty acids with radiation therapy in two different colorectal cancer cell lines. *Clin. Nutr.* 33 (1), 164–170. doi:10.1016/j.clnu.2013.04.005

Cockbain, A. J., Volpato, M., Race, A. D., Munarini, A., Fazio, C., Belluzzi, A., et al. (2014). Anticolorectal cancer activity of the omega-3 polyunsaturated fatty acid eicosapentaenoic acid. *Gut* 63 (11), 1760–1768. doi:10.1136/gutjnl-2013-306445

Colquhoun, A. (2009). Mechanisms of action of eicosapentaenoic acid in bladder cancer cells *in vitro*: alterations in mitochondrial metabolism, reactive oxygen species generation and apoptosis induction. *J. Urol.* 181 (4), 1885–1893. doi:10.1016/j.juro.2008.11.092

D'Angelo, S., Motti, M. L., and Meccariello, R. (2020). ω-3 and ω-6 polyunsaturated fatty acids, obesity and cancer. *Nutrients* 12 (9), 2751. doi:10.3390/nu12092751

Das, U. N., Madhavi, N., Sravan Kumar, G., Padma, M., and Sangeetha, P. (1998). Can tumour cell drug resistance be reversed by essential fatty acids and their metabolites? *Prostagl. Leukot. Essent. Fat. Acids* 58 (1), 39–54. doi:10.1016/s0952-3278(98)90128-4

Falconer, J. S., Ross, J. A., Fearon, K. C., Hawkins, R. A., O'Riordain, M. G., and Carter, D. C. (1994). Effect of eicosapentaenoic acid and other fatty acids on the growth *in vitro* of human pancreatic cancer cell lines. *Br. J. Cancer* 69 (5), 826–832. doi:10.1038/bjc.1994.161

Guo, F., Cofie, L. E., and Berenson, A. B. (2018). Cervical cancer incidence in young U.S. Females after human papillomavirus vaccine introduction. *Am. J. Prev. Med.* 55 (2), 197–204. doi:10.1016/j.amepre.2018.03.013

Hajjaji, N., and Bougnoux, P. (2013). Selective sensitization of tumors to chemotherapy by marine-derived lipids: a review. *Cancer Treat. Rev.* 39 (5), 473–488. doi:10.1016/j.ctrv.2012.07.001

Haycock, P. C., Borges, M. C., Burrows, K., Lemaitre, R. N., Burgess, S., Khankari, N. K., et al. (2023). The association between genetically elevated polyunsaturated fatty acids and risk of cancer. *EBioMedicine* 91, 104510. doi:10.1016/j.ebiom.2023.104510

Jenkins, D., Molijn, A., Kazem, S., Pirog, E. C., Alemany, L., de Sanjosé, S., et al. (2020). Molecular and pathological basis of HPV-negative cervical adenocarcinoma seen in a global study. *Int. J. Cancer* 147 (9), 2526–2536. doi:10.1002/ijc.33124

Kumar, L., and Gupta, S. (2016). Integrating chemotherapy in the management of cervical cancer: a critical appraisal. *Oncology* 91 (Suppl. 1), 8–17. doi:10.1159/000447576

Liu, H., Chen, J., Shao, W., Yan, S., and Ding, S. (2023). Efficacy and safety of Omega-3 polyunsaturated fatty acids in adjuvant treatments for colorectal cancer: a meta-analysis of randomized controlled trials. *Front. Pharmacol.* 14, 1004465. doi:10.3389/fphar.2023.1004465

Manda, K., Kriesen, S., Hildebrandt, G., Fietkau, R., and Klautke, G. (2011). Omega-3 fatty acid supplementation in cancer therapy: does eicosapentanoic acid influence the radiosensitivity of tumor cells? *Strahlenther Onkol.* 187 (2), 127–134. doi:10.1007/s00066-010-2166-6

Montecillo-Aguado, M., Tirado-Rodriguez, B., and Huerta-Yepez, S. (2023). The involvement of polyunsaturated fatty acids in apoptosis mechanisms and their implications in cancer. *Int. J. Mol. Sci.* 24 (14), 11691. doi:10.3390/ijms241411691

Moon, Y. A. (2023). Emerging roles of polyunsaturated fatty acid synthesis pathway in colorectal cancer. *Anim. Cells Syst. Seoul.* 27 (1), 61–71. doi:10.1080/19768354.2023.2189933

Murphy, R. A., Mourtzakis, M., Chu, Q. S., Baracos, V. E., Reiman, T., and Mazurak, V. C. (2011). Nutritional intervention with fish oil provides a benefit over standard of care for weight and skeletal muscle mass in patients with nonsmall cell lung cancer receiving chemotherapy. *Cancer* 117 (8), 1775–1782. doi:10.1002/cncr.25709

Nappo, M., Berkov, S., Massucco, C., Di Maria, V., Bastida, J., Codina, C., et al. (2012). Apoptotic activity of the marine diatom Cocconeis scutellum and eicosapentaenoic acid in BT20 cells. *Pharm. Biol.* 50 (4), 529–535. doi:10.3109/13880209.2011.611811

Rose, P. G., Bundy, B. N., Watkins, E. B., Thigpen, J. T., Deppe, G., Maiman, M. A., et al. (1999). Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. *N. Engl. J. Med.* 340 (15), 1144–1153. doi:10.1056/nejm199904153401502

Schwartz, L. H., Litière, S., de Vries, E., Ford, R., Gwyther, S., Mandrekar, S., et al. (2016). RECIST 1.1-Update and clarification: from the RECIST committee. *Eur. J. Cancer* 62, 132–137. doi:10.1016/j.ejca.2016.03.081

Serkies, K., and Jassem, J. (2018). Systemic therapy for cervical carcinoma - current status. *Chin. J. Cancer Res.* 30 (2), 209–221. doi:10.21147/j.issn.1000-9604.2018.02.04

Shan, K., Feng, N., Zhu, D., Qu, H., Fu, G., Li, J., et al. (2022). Free docosahexaenoic acid promotes ferroptotic cell death via lipoxygenase dependent and independent pathways in cancer cells. *Eur. J. Nutr.* 61 (8), 4059–4075. doi:10.1007/s00394-022-02940-w

Shin, J. I., Jeon, Y. J., Lee, S., Lee, Y. G., Kim, J. B., and Lee, K. (2019). G-Protein-Coupled receptor 120 mediates DHA-induced apoptosis by regulating IP3R, ROS and, ER stress levels in cisplatin-resistant cancer cells. *Mol. Cells* 42 (3), 252–261. doi:10.14348/molcells.2019.2440

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Torres-Adorno, A. M., Vitrac, H., Qi, Y., Tan, L., Levental, K. R., Fan, Y. Y., et al. (2019). Eicosapentaenoic acid in combination with EPHA2 inhibition shows efficacy in preclinical models of triple-negative breast cancer by disrupting cellular cholesterol efflux. *Oncogene* 38 (12), 2135–2150. doi:10.1038/s41388-018-0569-5

Vaughan, V. C., Hassing, M. R., and Lewandowski, P. A. (2013). Marine polyunsaturated fatty acids and cancer therapy. *Br. J. Cancer* 108 (3), 486–492. doi:10.1038/bjc.2012.586

Wang, W., Liu, X., Hou, X., Lian, X., Liu, Z., Shen, J., et al. (2019). Posttreatment squamous cell carcinoma antigen predicts treatment failure in patients with cervical squamous cell carcinoma treated with concurrent chemoradiotherapy. *Gynecol. Oncol.* 155 (2), 224–228. doi:10.1016/j.ygyno.2019.09.003

Wei, L., Wu, Z., and Chen, Y. Q. (2022). Multi-targeted therapy of cancer by omega-3 fatty acids-an update. *Cancer Lett.* 526, 193–204. doi:10.1016/j.canlet.2021.11.023

Xu, Q., Zhang, Z., Tang, M., Xing, C., Chen, H., Zheng, K., et al. (2023). Endogenous production of ω-3 polyunsaturated fatty acids mitigates cisplatin-induced myelosuppression by regulating NRF2-MDM2-p53 signaling pathway. *Free Radic. Biol. Med.* 201, 14–25. doi:10.1016/j.freeradbiomed.2023.03.005

Yang, Y., Xu, Y., Zhao, C., Zhang, L., Nuerbol, A., Wang, L., et al. (2022). Pronounced enhancement in radiosensitization of esophagus cancer cultivated in docosahexaenoic acid via the PPAR -γ activation. *Front. Med. (Lausanne)* 9, 922228. doi:10.3389/fmed.2022.922228

Yue, T., Xiong, K., Deng, J., Hu, W., Tan, T., Li, S., et al. (2022). Meta-analysis of omega-3 polyunsaturated fatty acids on immune functions and nutritional status of patients with colorectal cancer. *Front. Nutr.* 9, 945590. doi:10.3389/fnut.2022.945590

Zaloga, G. P. (2021). Narrative review of n-3 polyunsaturated fatty acid supplementation upon immune functions, resolution molecules and lipid peroxidation. *Nutrients* 13 (2), 662. doi:10.3390/nu13020662

Zhang, Z., Li, L., Zhang, C., Zhang, P., Fang, Z., Li, J., et al. (2023). Relationship between plasma amino acid and carnitine levels and primary angle-closure glaucoma based on mass spectrometry metabolomics. *Exp. Eye Res.* 227, 109366. doi:10.1016/j.exer.2022.109366

Zhu, S., Feng, N., Lin, G., Tong, Y., Jiang, X., Yang, Q., et al. (2018). Metabolic shift induced by ω -3 PUFAs and rapamycin lead to cancer cell death. *Cell Physiol. Biochem.* 48 (6), 2318–2336. doi:10.1159/000492648

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 31 May 2024
DOI 10.3389/fonc.2024.1402351

OPEN ACCESS

EDITED BY
Valentina Audrito,
Università del Piemonte Orientale, Italy

REVIEWED BY
Yingkun Xu,
Chongqing Medical University, China
Ritika Tiwari,
University of Miami, United States

*CORRESPONDENCE
Xiaohong Zhang
zxhong@fjmu.edu.cn

RECEIVED 17 March 2024
ACCEPTED 13 May 2024
PUBLISHED 31 May 2024

CITATION
Chen Z and Zhang X (2024) The role of metabolic reprogramming in kidney cancer. *Front. Oncol.* 14:1402351. doi: 10.3389/fonc.2024.1402351

The role of metabolic reprogramming in kidney cancer

Ziyi Chen[1] and Xiaohong Zhang[2,3,4]*

[1]The First Clinical College of Fujian Medical University, Fuzhou, China, [2]Department of Nephrology, Blood Purification Research Center, The First Affiliated Hospital, Fujian Medical University, Fuzhou, China, [3]Fujian Clinical Research Center for Metabolic Chronic Kidney Disease, The First Affiliated Hospital, Fujian Medical University, Fuzhou, China, [4]Department of Nephrology, National Regional Medical Center, Binhai Campus of the First Affiliated Hospital, Fujian Medical University, Fuzhou, China

Metabolic reprogramming is a cellular process in which cells modify their metabolic patterns to meet energy requirements, promote proliferation, and enhance resistance to external stressors. This process also introduces new functionalities to the cells. The 'Warburg effect' is a well-studied example of metabolic reprogramming observed during tumorigenesis. Recent studies have shown that kidney cells undergo various forms of metabolic reprogramming following injury. Moreover, metabolic reprogramming plays a crucial role in the progression, prognosis, and treatment of kidney cancer. This review offers a comprehensive examination of renal cancer, metabolic reprogramming, and its implications in kidney cancer. It also discusses recent advancements in the diagnosis and treatment of renal cancer.

KEYWORDS

metabolism reprogramming, renal cancer, treatment, glucose metabolism, amino acid metabolism

1 Introduction

Metabolic reprogramming is a hallmark of malignancy first discovered a century ago. Reprogrammed metabolic activity has the potential to be utilized in the detection, surveillance, and management of cancer (1). Kidney cancer (KC) is predicted to be the 14th most common cancer globally by 2020, with 431,288 new cases reported, according to the Global Cancer Observatory (2). Despite the ongoing rise in incidence of KC, mortality estimates have reached a plateau (3). Significant roles are played by metabolic reprogramming in the prognosis and progression of kidney disease (4). By providing fresh perspectives on the diagnosis and treatment of metabolic reprogramming in renal cancer, this article examines the function of metabolic reprogramming in kidney cancer.

2 Metabolic reprogramming

In recent years, metabolic reprogramming has been defined as "changes in the bioenergetics of tumor cells" (5); "some metabolic phenomena of cancer cell reprogramming (6)or "mechanisms by which cells reconnect their metabolism to promote proliferation and cell growth (7) ". The proposition is predicated on alterations in lactic acidosis and heightened glucose consumption in specific critical tumor regions (Warburg effect) (8). Primarily, cellular energy production (as ATP), amino acid synthesis, and the surrounding microenvironment are impacted by these modifications.

2.1 Metabolic reprogramming and tumors

Metabolic reprogramming is a phenomenon observed in malignant cells as they advance in development, adjusting their metabolic pathways. Mutations that lead to cancer formation allow nascent tumor cells to acquire metabolic traits that support cell survival, immune evasion, and rapid growth, making it a defining feature of cancer. This concept applies to classic oncogenes like MYC and KRAS, which can independently control cellular metabolism (1). Additionally, metabolic reprogramming influences the treatment of tumors. Given the distinct metabolic attributes exhibited by tumor cells in comparison to normal cells, it is possible to devise therapeutic approaches that specifically target the metabolic deficiencies of tumor cells. Additionally, one potential approach to treating tumors could involve manipulating the metabolic pathways of the cells containing the tumors. This could be achieved through specific metabolic inhibitors or by modifying the nutrient supply.

Tumor cells generate adenosine triphosphate (ATP) via glycolysis, as opposed to the oxidative phosphorylation (OXPHOS) by which normal cells generate energy (5). Tumor cells exhibit a unique capability to consume large quantities of glucose for energy production through glycolysis, even in oxygen-rich conditions, known as the Warburg effect or aerobic glycolysis. Furthermore, metabolic reprogramming involves not only the Warburg effect but also various other metabolic alterations to adjust to different environmental conditions (4), including enhanced lipid synthesis, abnormal amino acid metabolism and altered lactate metabolism. Specific facets pertaining to metabolic reprogramming in tumors are as follows (Figure 1).

2.1.1 Increased glycolysis

Normal cells typically metabolize glucose through oxidative phosphorylation in the presence of oxygen, resulting in the production of significant amounts of ATP. In contrast, tumor cells, even when provided with sufficient oxygen, tend to favor the glycolytic metabolic pathway, also known as 'aerobic fermentation,' to convert glucose into lactate. However, this conversion occurred at a reduced rate of OXPHOS. An excessive amount of lactate produced may cause the tumor microenvironment (TME) to become acidic. In addition to increasing the availability of ATP, lactic acid accumulation may also influence the ability of tumors to

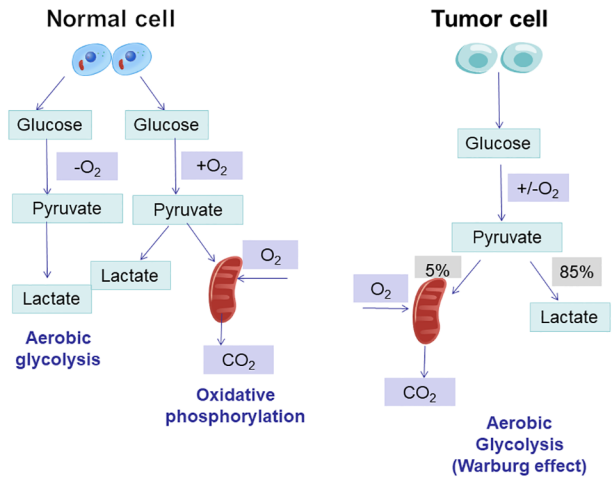


FIGURE 1
Regulation of glucose metabolism in cancer cells. Glucose metabolism in mitochondria mainly consists of glycolysis and the tricarboxylic acid cycle, and tumour cells enhance the conversion of glucose to lactate pathway through the glycolytic metabolic pathway.

invade and metastasize (9). Proliferating cells prevent the accumulation of cytosolic NADPH and reduce ATP production by converting excess pyruvate to lactate. This promotes sustained cytosolic glucose metabolism and helps avoid feedback inhibition caused by 'overproduction' of mitochondrial ATP (10).

2.1.2 Increased lipid synthesis

Glucose is the primary carbon source in most tumor microenvironments (TMEs) and is used for lipid synthesis through citrate. Conversely, cancer cells generate energy by oxidizing fatty acids in a lipid-rich TME. Tumor cells typically demonstrate increased resynthesis of fatty acids, redirecting energy production towards anabolic pathways that create phospholipids for cell membranes and signaling molecules (11). An abundance of evidence suggests that the lipid metabolism of immune cells and tumor cells in tumor microenvironments (TMEs) is essential for coordinating immunosuppression (9).

2.1.3 Alterations in the metabolism of amino acids

Malignant cells often exhibit irregular amino acid metabolism patterns. For instance, specific tumors meet the metabolic needs of cancer cells by consuming a significant amount of glutamine. This process, known as glutamine anaplerosis or glutamine backfilling, leads to increased ammonia release. Exposure to ammonia can trigger autophagy in nearby cells, including cancer-associated fibroblasts (CAFs). Moreover, the activation of autophagy in CAFs by ammonia promotes the release of glutamine, which in turn supports the proliferation of tumor cells. Additionally, byproducts like aspartate and glutamate from glutamine metabolism play crucial roles in regulating tumor cell epigenetics, nucleotide synthesis, redox homeostasis, and overall metabolism (9). In addition to prostaglandin E2 (PGE2) and cyclooxygenase,

the aforementioned pathways also involve adenosine signaling mechanisms. In the hours following tissue injury, adenosine concentrations in hypoxic tissues and TMEs increased significantly (12). Cell surface molecules CD73 and CD39 serve as nucleotide metabolizing enzymes, respectively. Adenosine synthesis is regulated by their conversion of ATP to AMP and AMP to adenosine, respectively (13). A correlation has been identified between heightened expression of CD39 and CD73 in tumors and an unfavorable prognosis in patients with non-small cell lung cancer, gastrointestinal cancer, and gynecological cancer (14). Cyclooxygenase 2 (COX2) overexpression is observed in a multitude of cancers (15). This overexpression is significantly associated with immunosuppression within the tumor microenvironment (TME) and substantial production of PGE2. Inhibiting the production of PGE2 and its associated signaling cascade has been shown to improve numerous components of the immune response against tumors, with colorectal cancer receiving the most attention (16).

The aforementioned attributes of metabolic reprogramming provide tumor cells with advantages in terms of proliferation and survival. Metabolic pathway modifications in tumor cells augment their resistance to the arduous microenvironment present within the tumor.

2.2 Metabolic reprogramming and immunity

The immune system consists of a variety of immune cells such as macrophages, neutrophils, monocytes, eosinophils, basophils, lymphocytes, and natural killer cells. While these cells are inactive during normal conditions, they quickly become activated and respond when exposed to infections, inflammation, or external triggers.

T cells exhibit completely different metabolic patterns depending on their activation state (17). The metabolism of naïve T cells is essentially static, with zero proliferation, and therefore requires only minimal nutrient intake, minimal glycolysis rate and minimal biosynthesis to be maintained, and their ATP is mainly produced by OXPHOS (18). Once activated by an external stimulus to effector T cells (Teff), it exhibits a state of metabolic activation, increased nutrient uptake, increased rate of glycolysis, and accumulation of protein, lipid and nucleotide synthesis (19). At the same time, mitochondrial oxygen consumption is reduced, and eventually T cells gain the ability to grow and proliferate, generating progeny cells that perform effector killing functions (18).The metabolic pattern of memory T cells is similar to that of naïve T cells, maintaining a basic nutrient intake, a lower rate of glycolysis, and a dependence on OXPHOS to provide ATP (19). Enhanced glycolysis and mitochondrial metabolism are observed following B-lymphocyte activation induced by LPS or antigenic stimulation. It is worth noting that glycolysis serves as the primary metabolic pathway for activated B lymphocytes. In contrast, regulatory T cells (Treg cells) and M2 macrophages predominantly rely on oxidative phosphorylation (OXPHOS) generated through fatty acid oxidation (FAO) to meet their energy demands (20) (Table 1 and Figure 2).

3 Metabolic reprogramming in renal cancer

Clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (pRCC), and smoky renal cell carcinoma (chRCC) are the three primary subtypes of renal cell carcinomas as determined by histological examination (21). Furthermore, renal cell carcinoma (RCC), collecting duct renal cell carcinoma, medullary renal cell carcinoma, and hereditary smooth muscle tumor disease are uncommon subtypes (21).CcRCC is the most prevalent subtype of RCC, comprising over 75% of all reported cases (21).Renal cell carcinoma (RCC) is sometimes referred to as a 'metabolic disease' due to the disruptions and alterations that occur in various metabolic pathways. Metabolic reprogramming in renal cancer is mainly triggered by the activation of the Ras-PI3K-AKT-mTOR pathway and the inactivation of the von Hippel-Lindau (VHL) gene (22). Myc and hypoxia-inducible factor (HIF) play vital roles in the metabolic reprogramming of renal cell carcinoma. This reprogramming affects glucose, fatty acid metabolism, and the TCA cycle across all RCC types. Furthermore, renal cancer involves the alteration of glutamine,

TABLE 1 Changes in energy metabolism in glycolysis, oxidative phosphorylation, fatty acid oxidation, and glutamine catabolism in different T cells.

Type	Changing of energy metabolism			
	Glycolysis	OXPHOS	FAO	Glutaminolysis
Naïve T cell	↓	↑	↑↑	↓
Treg cell	↓	↑	↑↑	↓
Teff cell	↑↑	↑		↓
Th1 cell	↑↑	↓	↓	↑
Memory T cell	↓	↑	↑↑	↓
Cytotoxic T cell	↑↑	↓	↓	↑

↓: decreased, ↑↑: Significantly increased, ↑: increased.

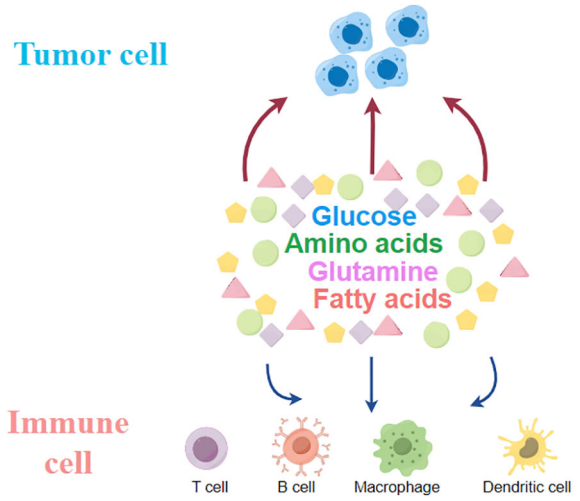


FIGURE 2
Tumour cells compete with immune cells within the tumour for glucose, glutamine, fatty acids and other amino acids.

tryptophan, and arginine metabolism to support tumor growth and tumorigenesis (21).

3.1 Reprogramming of metabolic genes

Essential genes involved in the regulation of metabolic reprogramming in renal cancer are VHL, PTEN, Akt, mTOR, TSC1/2 and Myc (22, 23). The tumor suppressor gene von Hippel-Lindau (VHL) is particularly important for ccRCC, and its frequent mutation or deletion causes dysregulation of several hypoxia-inducible factor (HIF) transcription factor families and their associated pro-oncogenic mediators (24, 25). Inactivation of VHL leads to activation of two VHL E3 ubiquitin ligase complex targets, HIF1α and HIF2α (encoded by HIF1A and EPAS1) (21, 24, 25). Under hypoxia in cancer cells, HIF1α and HIF2α are upregulated, and the transcription of several low-responsive genes involved in tumor growth, angiogenesis and metastasis, as well as genes related to glucose transport and metabolism, are transcribed (21, 26). HIF can drive the expression of several proteins and enzymes involved in glucose uptake and glycolysis, such as GLUT1 (glucose transporter-1), PGK (phosphoglycerate kinase), LDHA (lactate dehydrogenase), PDK1 (pyruvate dehydrogenase kinase) and HK (hexokinase) (27). HIF also inhibits the tricarboxylic acid cycle and oxidative phosphorylation (28).

Frequent mutations in Ras-PI3K-Akt-mTOR pathway genes (including PTEN, mTOR and PIK3CA) were also observed in RCC cells (29, 30). TCGA studies of ccRCC also detected mutations in several genes in the PI3K-AKT-mTOR pathway, PTEN, TSC1/2 and PIK3CA (31–33). TSC1 and TSC2 encode heparin and nodulin to form a complex that inhibits mTORC1 activation (34). Furthermore, inhibition of tumor suppressor 4EBP1 by mTORC1 enhances the expression of HIF-1 and HIF-2 (35, 36). Myc is a proto-oncogenic transcription factor, often overexpressed in renal cell carcinoma cells (37, 38), which plays an important role in reprogramming glutamine metabolism and fatty acid synthesis (37, 39).

3.2 Alterations in glucose metabolism

The presence of HIF in cancer cells is not correlated with the availability of oxygen (26). Increased expression of lactate dehydrogenase A (LDHA), the enzyme responsible for converting pyruvate to lactate, was observed in response to elevated levels of HIF (40). In healthy cells, glucose catabolism to lactate generates less energy than oxidative phosphorylation (41). Therefore, in order to meet the energy demands of cancer cells, they must consume a great deal of glucose. Elevated glucose transporter expression on the membranes of cancer cells is a contributing factor to elevated glucose consumption (42). The metabolic transformation referred to as aerobic glycolysis or the “Wartburg effect” is responsible for this (5). An increase in aerobic glycolysis expedites the provision of carbon intermediates required for the biosynthesis of amino acids, lipids, and nucleic acids (43). Conversely, monocarboxylic acid transporters (MCTs) remove lactate, the principal byproduct of glycolysis, from cancer cells in order to facilitate a positive glucose flux via glycolysis (44).

3.3 Alterations in the pentose phosphate pathway and the tricarboxylic acid cycle

The rate-limiting enzyme of the pentose phosphate (PPP) pathway, glucose-6-phosphate dehydrogenase (G6PD) is frequently upregulated in cancer cells (45).The pentose phosphate pathway, which is up-regulated, supplies ribose precursors to satisfy the high demand for 5-carbon sugars for nucleotide biosynthesis and to maintain intracellular redox homeostasis for growth and proliferation (46, 47). Reducing equivalents (NADPH) are utilized to impede oxidative stress. A concurrent elevation in lactate efflux fosters the development of an immunosuppressive microenvironment within the tumor.

HIF downregulates the tricarboxylic acid cycle in renal cancer cells by inhibiting metabolic fluxes to the TCA cycle through transcriptional activation of PDK1. This results in a decreased conversion of pyruvate to acetyl coenzyme A and the suppression of intermediates such as fumaric acid and β-ketoglutarate. Pyruvate carboxylase (PC) converts acetyl coenzyme A to oxaloacetate, which is the primary stable intermediate of the tricarboxylic acid cycle (48, 49). The neurotransmitter GABA, which is produced as a byproduct of glutamine metabolism, is converted to succinate, another tricarboxylic acid cycle intermediate, via γ-aminobutyric acid transaminase (48, 49). A constituent of the alpha-ketoglutarate dehydrogenase complex, dihydrolipoamide acetyltransferase controls the recycling of alpha-ketoglutarate (48). Experimental investigations have demonstrated that renal cell carcinoma cells exhibit a downregulation of these enzymes in comparison to normal renal cells (50).

3.4 Alterations in fatty acid metabolism

Renal cell carcinoma often associated with obesity (51).In renal cell carcinoma, lipid synthesis exceeds lipid degradation. Expression of enzymes involved in fatty acid oxidation is down-regulated in ccRCC cells compared to normal renal cells (52, 53). SCD1 is the enzyme responsible for lipid storage and is highly expressed in ccRCC (53). The β-oxidation pathway of lipids was down-regulated, however, the synthesis of carnitine, fatty acids, phospholipids and cholesterol were all expressed up-regulated in renal cell carcinoma (21). Higher levels of cholesteryl ester accumulation have been reported in the kidneys of patients with ccRCC. The accumulation of "lipid droplets" is considered a hallmark of clear cell renal cell carcinoma (ccRCC). Accumulation of lipid droplets near the endoplasmic reticulum (ER) contributes to the maintenance of ER integrity in ccRCC cells. Storage of lipid droplets is induced by the gene periplasmic protein 2 (PLIN2), which is upregulated in a HIF2-dependent pathway to maintain endoplasmic reticulum homeostasis and withstand cytotoxic stresses (54).

Reprogramming of glycerophospholipid metabolism and arachidonic acid metabolism is characteristic of renal cancer (55). Glycerophospholipids are a source of phosphatidic acid (PA), lysophosphatidic acid (LPA) and triacylglycerol, which are forms of lipid storage (55). Arachidonic acid is an important derivative of membrane phospholipids, the synthesis of which involves a number of inflammatory enzymes such as lipoxygenases (LOXs) and cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (55). Increased expression of the enzymes 5-LOX and 15-LOX2 and 15-hydroxyeicosatetraenoic acid, an immunosuppressive arachidonic acid, in renal cancer cells compared with normal renal cells (56). In RCC cells, LOX also promotes the secretion of the immunosuppressive chemokine CXCL2 and the cytokine IL10 and regulates immune escape from RCC cells (56). Also, the COX pathway of arachidonic acid metabolism is involved in the tumor-promoting pathway (57). Prostaglandin E2 (PGE2), a product of COX-2, promotes renal cell carcinoma invasion (57). COX-2 in renal cell carcinoma correlates with tumor size, stage and grade, suggesting that it may be a potential target in renal cancer cells (Figure 3).

3.5 Alterations in glutamine metabolism

Glutamate is an essential nutrient utilized by cancer cells for the maintenance of cellular bioenergetics and biomass. Additionally, it is a constituent of both protein and lipid synthesis (21). Glutamine acts as a precursor for the synthesis of glutathione (GSH), an antioxidant that operates within cells, as well as a metabolic intermediate in the form of α-ketoglutarate, which plays an indirect role in the TCA cycle. The conversion of glutamine to α-ketoglutarate is facilitated by the enzyme glutaminase (GLS) (58).

Metabolomic analysis of postoperatively resected tissues from patients diagnosed with clear cell renal cell carcinoma (ccRCC) showed a significant rise in glutamine utilization and uptake by tumor tissues compared to normal paired renal tissues. Additionally, an elevated glutathione content in tumor tissues was positively correlated with the clinical progression of ccRCC patients, including tumor stage and prognosis (21). GSH, an amino acid tripeptide consisting of glutamate, cysteine, and glycine, is present in numerous prokaryotic cells and nearly all eukaryotic cells (22). An important metabolic process involving glutamine is the metabolism of GSH. In the human body, the main forms of GSH are oxidized glutathione (GSSG) and reduced glutathione. Reduced GSH acts as an intracellular antioxidant and is transformed into GSSG under the influence of glutathione peroxidase. The balance between glutathione and oxidized glutathione (GSH/GSSG) is tightly regulated in renal cell carcinoma (21). Glutamine regulates redox processes in cancer cells via GSH as well. As a ROS substrate, glutathione is oxidized to GSSG to reduce ROS levels. This indicates that glutamine and glutathione act as the cell's internal antioxidant system to maintain the survival of healthy tumor cells. The oncogene c-Myc can upregulate glutaminase expression, impacting the glutamine metabolism of cancer cells (59). The conversion of glutamine to glutamate is catalyzed by glutaminase. Inhibiting glutaminase or depriving ccRCC cells of glutamine in the culture medium reduces cell survival, revealing a dependency on exogenous glutamine. This highlights the importance of exogenous glutamine and GLS in the proliferation of tumor cells.

Furthermore, renal cancer cells exhibit a downregulation of the urea cycle, which impedes the catabolism of glutamine and arginine, among other amino acids (21). Increased tryptophan metabolism via the xanthine (KN) pathway leads to enhanced immunosuppression. By reprogramming metabolic pathways, energy (ATP) and other molecules necessary for cell proliferation (lipids, phospholipids, and ribose) are produced, allowing renal cancer cells to evade the immune system and withstand hypoxia, nutrient depletion, and oxidative stress (Table 2).

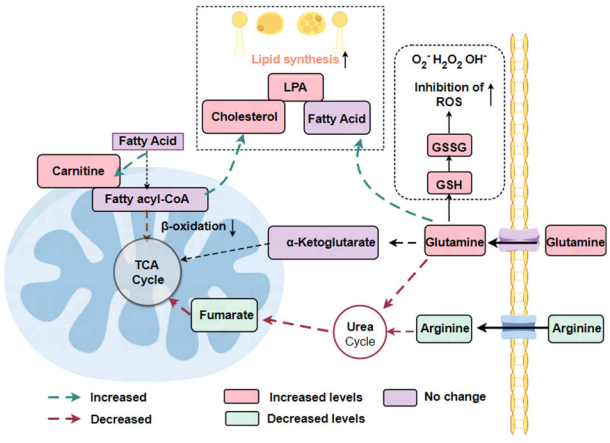


FIGURE 3
Reprogramming of fatty acid metabolism and glutamine metabolism in RCC. In renal cell carcinoma, where lipid synthesis predominantly exceeds lipid degradation, the β-oxidation pathway of lipids is down-regulated whereas the synthesis of carnitine, fatty acids, phospholipids, and cholesterol are all expressed up-regulated. The urea cycle is down-regulated, reducing the catabolism of amino acids such as arginine and glutamine.

TABLE 2 Findings related to glutamine metabolism in renal tumor cells.

Year of study publication	metabolic mechanism	research object	The main findings of the study	reference
2011	Upregulation of free fatty acids in renal cell carcinoma	Patient-derived renal cancer cells	Elevated glutamine levels in kidney cancer	(60)
2015/2016	Increased expression of glutathione peroxidase 1 (GPX1) in ccRCC cells; Inhibition of glutamine-depleted enzyme expression via the GSH/GSSG pathway	138 matched pairs of clear cell ccRCC and normal tissue/ RCC cells	Glutamine maintains cellular redox homeostasis by scavenging ROS; high-grade, high-stage and metastatic ccRCC are associated with elevated glutamine levels and the GSH/GSSG pathway	(48, 49)
2019	HSP60 silencing activated the MEK/ERK/c-Myc pathway to enhance glutamine-directed metabolism	Clear cell renal cell carcinoma 786-O and 769-P cell lines	Low expression of HSP60 enhances cell growth in ccRCC	(61)
2019	Macrophage-secreted IL-23 enhanced Treg functions in glutamine addicted tumors	ccRCC patients tumors from a Shanghai cohort and ccRCC tumor data from The Cancer Genome Atlas (TCGA) cohort; fresh human ccRCC tumors and murine tumor cells	IL-23 is a promising target for immunotherapy in ccRCC	(62)
2023	PHF8 is recruited by c-MYC to the promoter regions of TEA domain transcription factor 1 (TEAD1) to transcriptionally up-regulate TEAD1 then TEAD1 up-regulates GLUL transcriptionally	786-O cells (VHL-null cells)	PHF8-GLUL axis plays an essential role in ccRCC tumor growth and lipid depositionPHF8-GLUL	(63)

4 Metabolic reprogramming in the treatment of ccRCC

ccRCC is frequently associated with mutations in genes that cause hypoxic alterations, the most common of which is VHL (64). VHL mutations lead to the accumulation of HIF-a in cells, which in turn upregulates the expression of vascular endothelial growth factors (VEGFs) (65). Prior to this, the mainstay of treatment for ccRCC was the use of VEGF receptors (VEGFR) or inhibitors such as sunitinib leading to regeneration of the target vessel (66), but inhibitors have limited efficacy and can cause many adverse effects such as vascular toxicity and off-target effects (67). Based on metabolic reprogramming, in ccRCC, we can follow the therapeutic approach of hepatocellular carcinoma and use glycolysis inhibitors to suppress tumor cells (68), while early clinical studies have also demonstrated that targeting the glycolytic pathway can effectively inhibit cancer progression (69).

4.1 HIF-2a inhibitors

HIF-2a is a key downstream effector protein of the VHL tumor suppressor protein, which is frequently mutated in ccRCC (70), and promotes tumorigenesis and metastasis by regulating angiogenesis, cell proliferation and metabolism; therefore targeting the HIF-2a pathway could be used to treat ccRCC (71). First-generation drug PT2399 shows superior activity to sunitinib and is effective against sunitinib-resistant tumors (71) (NCT02293980). Second-generation drugs such as PT2977 (MK- 6482, Belzutivan) can overcome some of the limitations of first-generation compounds (72).

4.2 FAS inhibitors

Upregulation of FAS expression in ccRCC increases fatty acid levels and provides energy for cancer cells and post-translationally modified proteins (71). Preclinical experiments show that the FAS inhibitor C75 inhibits invasiveness and proliferation of ccRCC (73). TVB-2640 is a novel FAS inhibitor that demonstrated promising clinical activity and safety in a phase I clinical trial (74) (NCT02293980).

4.3 Glutaminase inhibitors

Glutamine is essential for energy production, redox stability maintenance, and macromolecule synthesis in cancer cells (71). In colorectal cancer, glutamine-like substance (GLS) functions as a compensatory mechanism to partially stimulate cell proliferation and restore the tricarboxylic acid cycle (75). CB-839, a GLS inhibitor, has demonstrated encouraging outcomes in preclinical investigations and augments antitumor functionality in animal models when combined with everolimus, a frequently utilized mTOR inhibitor for the treatment of ccRCC (71).

4.4 IDO inhibitors

IDO is an enzyme involved in the role of tryptophan catabolism via the renal urinary alkaline pathway (71). IDO promotes tumor metastasis by depleting tryptophan and activating T cells, inhibiting immunosuppression in the local tumor microenvironment and suppressing anti-tumor T cells. Thus, IDO has emerged as a potential therapeutic target for cancer. Epacaostat, a selective IDO-targeting inhibitor, showed promising results in preclinical trials by improving lysis of tumor antigen-specific T cells; however, side effects such as toxicity and lack of efficacy were identified in clinical trials (76). Some IDO inhibitors, such as KHK2455, LY3381916 and MK-7162, are undergoing clinical trials to assess their safety, tolerability and antitumor activity (77).

4.5 Reduction of arginine

In ccRCC, the use of the polyethylene glycol form of arginine deaminase (ADI-PEG20) can limit tumor growth by reducing circulating levels of arginine by catabolizing it to citrulline, however this treatment may be limited by ASS1 re-expression (75).Clinical trials have demonstrated the safety, tolerability and clinical efficacy of ADI-PEG20 in reversing drug resistance in patients with arginine dystrophy tumors (78).

This article focuses on the anoxic processes involved in glucose metabolism in cancer cells, specifically highlighting the pentose phosphate pathway and tricarboxylic acid cycle. It also emphasizes the connection between renal cell carcinoma and obesity. Additionally, it provides detailed descriptions of the specific changes in enzymes related to the fatty acid oxidation pathway and outlines the developmental course of glutamate metabolism in renal carcinoma. However, this article does not delve into the relevant metabolic pathways for tryptophan and arginine, only mentioning them in relation to specific treatment protocols. Another article (75) also discusses kidney cancer-related genes, fatty acids, glucose metabolism, tricarboxylic acid cycle, glutamic acid metabolism, and specific processes. The literature proposes using radionuclide imaging for diagnosing renal cell carcinoma. Furthermore, in this paper new drugs such as KHK2455, LY3381916 and MK-7162 are suggested for treating IDO inhibitors. Second-generation drugs like PT2977 (MK-6482) or Belzutivan are proposed as HIF-2α antagonists that can overcome certain defects of first-generation drugs.

5 Conclusion and future directions

Metabolomics studies have provided a number of small molecules that may be used to diagnose and predict kidney cancer, and which hold promise as biomarkers of kidney cancer. However, these interpretations are limited to mapping identified metabolites to pathways, while many important features remain undefined. Subsequent experimental work is required to demonstrate causal inferences arising from genomic analyses. The dynamic nature of the metabolome means that it may be difficult to identify the direction of protein/metabolite ←→ disease.

By analyzing indications such as metabolites, subgroups of patients with similar metabolic characteristics can be more accurately identified. This contributes to a deeper understanding of the heterogeneity of the disease and provides a basis for personalized treatment. Moreover, therapeutic strategies targeting metabolic vulnerability are based on targeted interventions to the weakness of specific metabolic pathways or links.

In the future, we can improve our understanding of disease progression at the individual level by integrating biological data from multiple genomics and combining a multilevel approach to observe the biological effects of different therapeutic pathways, which will ultimately improve the cure rate and reduce the mortality rate of renal cancer through other approaches such as targeted therapies.

Author contributions

ZC: Writing – original draft, Writing – review & editing. XZ: Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Natural Science Foundation of Fujian Province under Grant (No.2022J01212).

Acknowledgments

Thanks to the related institutions for their help.

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.

Publisher's note

References

1. Faubert B, Solmonson A, DeBerardinis RJ. Metabolic reprogramming and cancer progression. *Science*. (2020) 368:1. doi: 10.1126/science.aaw5473

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660

3. Bukavina L, Bensalah K, Bray F, Carlo M, Challacombe B, Karam JA, et al. Epidemiology of renal cell carcinoma: 2022 update. *Eur Urol*. (2022) 82:529–42. doi: 10.1016/j.eururo.2022.08.019

4. Li Y, Sha Z, Peng H. Metabolic reprogramming in kidney diseases: evidence and therapeutic opportunities. *Int J Nephrol*. (2021) 2021, 1–6. doi: 10.1155/2021/5497346

5. Yoshida GJ. Metabolic reprogramming: The emerging concept and associated therapeutic strategies. *J Exp Clin Cancer*. (2015) 34:111. doi: 10.1186/s13046-015-0221-y

6. Li H, Zhou J, Sun H, Qiu Z, Gao X, Xu Y, et al. CaMeRe: A novel tool for inference of cancer metabolic reprogramming. *Front Oncol*. (2020) 10. doi: 10.3389/fonc.2020.00207

7. Carvalho-Santos Z, Cardoso-Figueiredo R, Elias AP, Tastekin I, Baltazar C, Ribeiro C, et al. Cellular metabolic reprogramming controls sugar appetite in Drosophila. *Nat Metab*. (2020) 2:958–73. doi: 10.1038/s42255-020-0266-x

8. Jacquet P, Stéphanou A. Metabolic reprogramming, questioning, and implications for cancer. *Biology*. (2021) 10:2. doi: 10.3390/biology10020129

9. Li X, Wenes M, Romero P, Huang SC-C, Fendt S-M, Ho P-C, et al. Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. *Nat Rev Clin Oncol*. (2019) 16:425–41. doi: 10.1038/s41571-019-0203-7

10. Stine ZE, Schug ZT, Salvino JM, Dang CV. Targeting cancer metabolism in the era of precision oncology. *Nat Rev Drug Discovery*. (2021) 21:141–62. doi: 10.1038/s41573-021-00339-6

11. Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr, et al. Cellular fatty acid metabolism and cancer. *Cell Metab*. (2013) 18:153–61. doi: 10.1016/j.cmet.2013.05.017

12. Miller WL, Thomas RA, Berne RM, Rubio R. Adenosine production in the ischemic kidney. *Circ*. (1978) p:390–7. doi: 10.1161/01.RES.43.3.390

13. Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive adenosine in cancer. *Nat Rev Cancer*. (2017) 17:709–24. doi: 10.1038/nrc.2017.86

14. Cai X-Y, Wang X-F, Li J, Dong J-N, Liu J-Q, Li N-P, et al. High expression of CD39 in gastric cancer reduces patient outcome following radical resection. *Oncol Lett*. (2016) 12:4080–6. doi: 10.3892/ol.2016.5189

15. Zelenay S, Veen der van AG, Böttcher JP, Snelgrove KJ, Rogers N, Acton SE, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell*. (2015) 162:1257–70. doi: 10.1016/j.cell.2015.08.015

16. Drew DA, Cao Y, Chan AT. Aspirin and colorectal cancer: the promise of precision chemoprevention. *Nat Rev Cancer*. (2016) 16:173–86. doi: 10.1038/nrc.2016.4

17. Ricciardi S, Manfrini N, Alfieri R, Calamita P, Crosti MC, Gallo S, et al. The translational machinery of human CD4+ T cells is poised for activation and controls the switch from quiescence to metabolic remodeling. *Cell Metab*. (2018) 28:895–906.e5. doi: 10.1016/j.cmet.2018.08.009

18. Xia L, Oyang L, Lin J, Tan S, Han Y, Wu N, et al. The cancer metabolic reprogramming and immune response. *Mol Cancer*. (2021) 20:3–4. doi: 10.1186/s12943-021-01316-8

19. Pearce EL, Poffenberger MC, Chang C-H, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. *Science*. (2013) 342. doi: 10.1126/science.1242454

20. Koye DN, Magliano DJ, Nelson RG, Pavkov ME. The global epidemiology of diabetes and kidney disease. *Adv Chronic Kidney Dis*. (2018) 25:121–32. doi: 10.1053/j.ackd.2017.10.011

21. Linehan WM, Ricketts CJ. The Cancer Genome Atlas of renal cell carcinoma: findings and clinical implications. *Nat Rev Urol*. (2019) 16:539–52. doi: 10.1038/s41585-019-0211-5

22. Lien EC, Lyssiotis CA, Juvekar A, Hu H, Asara JM, Cantley LC, et al. Glutathione biosynthesis is a metabolic vulnerability in PI(3)K/Akt-driven breast cancer. *Nat Cell Biol*. (2016) 18:572–8. doi: 10.1038/ncb3341

23. Pearce EL PM, Chang CH, Jones RG. Fueling immunity, Insights into the genetic basis of the renal cell carcinomas from the cancer genome atlas. *Science*. (2013) 14:4–6. doi: 10.1158/1541-7786.MCR-16-0115

24. Shen C, Kaelin WG. The VHL/HIF axis in clear cell renal carcinoma. *Semin Cancer Biol*. (2013) 23:18–25. doi: 10.1016/j.semcancer.2012.06.001

25. Jr. WGK. The von Hippel-Lindau tumor suppressor gene and kidney cancer. *Clin Cancer*. (2004) 10:2–3. doi: 10.1158/1078-0432.CCR-sup-040025

26. Kaelin WG. Molecular basis of the VHL hereditary cancer syndrome. *Nat Rev Cancer*. (2002) 2:673–82. doi: 10.1038/nrc885

27. Schönenberger D, Harlander S, Rajski M, Jacobs RA, Lundby A-K, Adlesic M, et al. Formation of renal cysts and tumors in vhl/trp53-deficient mice requires HIF1α and HIF2α. *Cancer Res*. (2016) 76:2025–36. doi: 10.1158/0008-5472.CAN-15-1859

28. LaGory EL, Wu C, Taniguchi CM, Ding C-KC, Chi J-T, Eyben von R, et al. Suppression of PGC-1α Is critical for reprogramming oxidative metabolism in renal cell carcinoma. *Cell Rep*. (2015) 12:116–27. doi: 10.1016/j.celrep.2015.06.006

29. Sato Y, Yoshizato T, Shiraishi Y, Maekawa S, Okuno Y, et al. Integrated molecular analysis of clear-cell renal cell carcinoma. *Nat Genet*. (2013) 45:860–7. doi: 10.1038/ng.2699

30. Voss MH, Hakimi AA, Pham CG, Brannon AR, Chen Y-B, Cunha LF, et al. Tumor genetic analyses of patients with metastatic renal cell carcinoma and extended benefit from mTOR inhibitor therapy. *Clin Cancer Res*. (2014) 20:1955–64. doi: 10.1158/1078-0432.CCR-13-2345

31. Dibble CC, Cantley LC. Regulation of mTORC1 by PI3K signaling. *Trends Cell Biol*. (2015) 25:545–55. doi: 10.1016/j.tcb.2015.06.002

32. Inoki K, Li Y, Zhu T, Wu J, Guan K-L. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol*. (2002) 4:648–57. doi: 10.1038/ncb839

33. Wee S, D.W. SM, Loo A, Miller C, de Beaumont R, Stegmeier F, et al. PTEN-deficient cancers depend on PIK3CB. *Proc Natl Acad Sci*. (2008) 105:1–2. doi: 10.1073/pnas.0802655105

34. Yang P, Cornejo KM, Sadow PM, Cheng L, Wang M, Xiao Y, et al. Renal cell carcinoma in tuberous sclerosis complex. *Am J Surg Pathol*. (2014) 38:895–909. doi: 10.1097/PAS.0000000000000237

35. Yu HB, Kielczewska A, Rozek A, Takenaka S, Li Y, Thorson L, et al. Sequestosome-1/p62 is the key intracellular target of innate defense regulator peptide. *J Biol Chem*. (2009) 284:36007–11. doi: 10.1074/jbc.C109.073627

36. Düvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, 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

37. Shroff EH, Eberlin LS, Dang VM, Gouw AM, Gabay M, Adam SJ, et al. MYC oncogene overexpression drives renal cell carcinoma in a mouse model through glutamine metabolism. *Proc Natl Acad Sci*. (2015) 112:6539–44. doi: 10.1073/pnas.1507228112

38. Tang S-W, Chang W-H, Su Y-C, Chen Y-C, Lai Y-H, Wu P-T, et al. MYC pathway is activated in clear cell renal cell carcinoma and essential for proliferation of clear cell renal cell carcinoma cells. *Cancer Lett*. (2009) 273:35–43. doi: 10.1016/j.canlet.2008.07.038

39. Shi W, Xu X, Yan F, Wang B, Zhao H, Chan A, et al. N–Myc downstream –regulated gene 2 restrains glycolysis and glutaminolysis in clear cell renal cell carcinoma. *Oncol Lett*. (2017) 14:6882–3. doi: 10.3892/ol

40. Kim Y, Choi J-W, Lee J-H, Kim Y-S. Expression of lactate/H+ symporters MCT1 and MCT4 and their chaperone CD147 predicts tumor progression in clear cell renal cell carcinoma: immunohistochemical and The Cancer Genome Atlas data analyses. *Hum Pathol*. (2015) 46:104–12. doi: 10.1016/j.humpath.2014.09.013

41. Doherty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. *J Clin Invest*. (2013) 123:3685–92. doi: 10.1172/JCI69741

42. Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB, et al. Akt-directed glucose metabolism can prevent bax conformation change and promote growth factor-independent survival. *Mol Cell Biol*. (2023) 23:7315–28. doi: 10.1128/MCB.23.20.7315-7328.2003

43. Liberti MV, Locasale JW. The warburg effect: how does it benefit cancer cells? *Trends Biochem Sci*. (2016) 41:211–8. doi: 10.1016/j.tibs.2015.12.001

44. Wahlström T, Arsenian Henriksson M. Impact of MYC in regulation of tumor cell metabolism. *Biochim Biophys Acta (BBA) Gene Regul Mech*. (2015) 1849:563–9. doi: 10.1016/j.bbagrm.2014.07.004

45. Nogueira V, Hay N. Molecular pathways: reactive oxygen species homeostasis in cancer cells and implications for cancer therapy. *Clin Cancer Res*. (2013) 19:4309–14. doi: 10.1158/1078-0432.CCR-12-1424

46. Jiang P, Du W, Wu M. Regulation of the pentose phosphate pathway in cancer. *Protein Cell*. (2014) 5:592–602. doi: 10.1007/s13238-014-0082-8

47. Mannava S, Grachtchouk V, Wheeler LJ, Im M, Zhuang D, Slavina EG, et al. Direct role of nucleotide metabolism in C-MYC-dependent proliferation of melanoma cells. *Cell Cycle*. (2014) 7:2392–400. doi: 10.4161/cc.6390

48. Hakimi AA, Reznik E, Lee C-H, Creighton CJ, Brannon AR, Luna A, et al. An integrated metabolic atlas of clear cell renal cell carcinoma. *Cancer Cell*. (2016) 29:104–16. doi: 10.1016/j.ccell.2015.12.004

49. Wettersten HI, Hakimi AA, Morin D, Bianchi C, Johnstone ME, Donohoe DR, et al. Grade-dependent metabolic reprogramming in kidney cancer revealed by combined proteomics and metabolomics analysis. *Cancer Res*. (2015) 75:2541–52. doi: 10.1158/0008-5472.CAN-14-1703

50. Chakraborty S, Balan M, Sabarwal A, Choueiri TK, Pal S. Metabolic reprogramming in renal cancer: Events of a metabolic disease. *Biochim Biophys Acta (BBA) Rev Cancer*. (2021) 1876:3–4. doi: 10.1016/j.bbcan.2021.188559

51. Albiges L, Hakimi AA, Xie W, McKay RR, Simantov R, Lin X, et al. Body mass index and metastatic renal cell carcinoma: clinical and biological correlations. *J Clin Oncol*. (2016) 34:3655–63. doi: 10.1200/JCO.2016.66.7311

52. Horiguchi A, Asano T, Asano T, Ito K, Sumitomo M, Hayakawa M, et al. Fatty acid synthase over expression is an indicator of tumor aggressiveness and poor prognosis in renal cell carcinoma. *J Urol*. (2008) 180:1137–40. doi: 10.1016/j.juro.2008.04.135

53. von Roemeling CA, Marlow LA, Wei JJ, Cooper SJ, Caulfield TR. Stearoyl-coA desaturase 1 is a novel molecular therapeutic target for clear cell renal cell carcinoma. *Clin Cancer Res*. (2013) 19:2368–80. doi: 10.1158/1078-0432.CCR-12-3249

54. Qiu B, Ackerman D, Sanchez DJ, Li B, Ochocki JD, Grazioli A, et al. HIF2α-dependent lipid storage promotes endoplasmic reticulum homeostasis in clear-cell renal cell carcinoma. *Cancer Discovery*. (2015) 5:652–67. doi: 10.1158/2159-8290.CD-14-1507

55. Wettersten HI. Reprogramming of metabolism in kidney cancer. *Semin Nephrol*. (2020) 40:2–13. doi: 10.1016/j.semnephrol.2019.12.002

56. Daurkin I, Eruslanov E, Stoffs T, Perrin GQ, Algood C, Gilbert SM, et al. Tumor-associated macrophages mediate immunosuppression in the renal cancer microenvironment by activating the 15-lipoxygenase-2 pathway. *Cancer Res*. (2011) 71:6400–9. doi: 10.1158/0008-5472.CAN-11-1261

57. Wu J, Zhang Y, Frilot N, Kim JI, Kim W-J, Daaka Y, et al. Prostaglandin E2 regulates renal cell carcinoma invasion through the EP4 receptor-rap GTPase signal transduction pathway. *J Biol Chem*. (2011) 286:33954–62. doi: 10.1074/jbc.M110.187344

58. Conrad M, Sato H. The oxidative stress-inducible cystine/glutamate antiporter, system x c –: cystine supplier and beyond. *Amino Acids*. (2011) 42:231–46. doi: 10.1007/s00726-011-0867-5

59. Sabharwal SS, Schumacker PT. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? *Nat Rev Cancer*. (2014) 14:709–21. doi: 10.1038/nrc3803

60. Mullen AR, Wheaton WW, Jin ES, Chen P-H, Sullivan LB, Cheng T, et al. Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature*. (2011) 481:385–8. doi: 10.1038/nature10642

61. Teng R , Liu Z, Tang H, Zhang W, Chen Y, Xu R, et al. HSP60 silencing promotes Warburg-like phenotypes and switches the mitochondrial function from ATP production to biosynthesis in ccRCC cells. *Redox Biol*. (2019) 24:2–3. doi: 10.1016/j.redox.2019.101218

62. Fu Q, Xu L, Wang Y, Jiang Q, Liu Z, Zhang J, et al. Tumor-associated macrophage-derived interleukin-23 interlinks kidney cancer glutamine addiction with immune evasion. *Eur Urol*. (2019) 75:752–63. doi: 10.1016/j.eururo.2018.09.030

63. Peng S, Wang Z, Tang P, Wang S, Huang Y, Xie Q, et al. PHF8-GLUL axis in lipid deposition and tumor growth of clear cell renal cell carcinoma. *Sci Adv*. (2023) 9:2–5. doi: 10.1126/sciadv.adf3566

64. Hsieh JJ, Le VH, Oyama T, Ricketts CJ, Ho TH, Cheng EH. Chromosome 3p loss–orchestrated VHL, HIF, and epigenetic deregulation in clear cell renal cell carcinoma. *J Clin Oncol*. (2018) 36:3534. doi: 10.1200/JCO.2018.79.2549

65. Keith B, Johnson RS, Simon MC. HIF1α and HIF2α: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer*. (2011) 12:9–22. doi: 10.1038/nrc3183

66. Roskoski R. Vascular endothelial growth factor (VEGF) and VEGF receptor inhibitors in the treatment of renal cell carcinomas. *Pharmacol Res*. (2017) 120:116–32. doi: 10.1016/j.phrs.2017.03.010

67. Keefe D, Bowen J, Gibson R, Tan T, Okera M, Stringer A, et al. Noncardiac vascular toxicities of vascular endothelial growth factor inhibitors in advanced cancer: A review. *Oncol*. (2011) 16:432–44. doi: 10.1634/theoncologist.2010-0271

68. Cassim S, Raymond V-A, Dehbidi-Assadzadeh L, Lapierre P, Bilodeau M. Metabolic reprogramming enables hepatocarcinoma cells to efficiently adapt and survive to a nutrient-restricted microenvironment. *Cell Cycle*. (2018) 17:903–16. doi: 10.1080/15384101.2018.1460023

69. Abdel-Wahab AF, Mahmoud W, Al-Harizy RM. Targeting glucose metabolism to suppress cancer progression: prospective of anti-glycolytic cancer therapy. *Pharmacol Res*. (2019) 150:14–5. doi: 10.1016/j.phrs.2019.104511

70. Kondo1 K, Kim WY, Lechpammer M, Kaelin WG Jr. Inhibition of HIF2a is sufficient to suppress pVHL-defective tumor growth. *PloS Biol*. (2003) 1:E83. doi: 10.1371/journal.pbio.0000083

71. Zhu H, Wang X, Lu S, Ou K. Metabolic reprogramming of clear cell renal cell carcinoma. *Front Endocrinol*. (2023) 14. doi: 10.3389/fendo.2023.1195500

72. Xu R, Wang K, Rizzi JP, Huang H, Grina JA, Schlachter ST, et al. 3-[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methylsulfonylindan-4-yl]oxy-5-fluorobenzonitrile (PT2977), a hypoxia-inducible factor 2α (HIF-2α) inhibitor for the treatment of clear cell renal cell carcinoma. *J Med Chem*. (2019) 62:6876–93. doi: 10.1021/acs.jmedchem.9b00719

73. Horiguchi A, Asano T, Asano T, Ito K, Sumitomo M, Hayakawa M, et al. Pharmacological inhibitor of fatty acid synthase suppresses growth and invasiveness of renal cancer cells. *J Urol*. (2008) 180:729–36. doi: 10.1016/j.juro.2008.03.186

74. Falchook G, Infante J, Arkenau H-T, Patel MR, Dean E, Borazanci E, et al. First-in-human study of the safety, pharmacokinetics, and pharmacodynamics of first-in-class fatty acid synthase inhibitor TVB-2640 alone and with a taxane in advanced tumors. *EClinicalMedicine*. (2021) 34:8–9. doi: 10.1016/j.eclinm.2021.100797

75. Wettersten HI, Aboud OA, Lara PN Jr, Weiss RH. Metabolic reprogramming in clear cell renal cell carcinoma. *Nat Rev Nephrol*. (2017) 13:410–9. doi: 10.1038/nrneph.2017.59

76. Jochems C, Fantini M, Fernando RI, Kwilas AR, Donahue RN, Lepone LM, et al. The IDO1 selective inhibitor epacadostat enhances dendritic cell immunogenicity and lytic ability of tumor antigen-specific T cells. *Oncotarget*. (2016) 7:37767. doi: 10.18632/oncotarget.v7i25

77. Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA, et al. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. *Nat Rev Drug Discovery*. (2019) 18:379–401. doi: 10.1038/s41573-019-0016-5

78. Hall PE, Lewis R, Syed N, Shaffer R, Evanson J, Ellis S, et al. A phase I study of pegylated arginine deiminase (Pegargiminase), cisplatin, and pemetrexed in argininosuccinate synthetase 1-deficient recurrent high-grade glioma. *Clin Cancer Res*. (2019) 25:2708–16. doi: 10.1158/1078-0432.CCR-18-3729

frontiers | Frontiers in Pharmacology

TYPE Mini Review
PUBLISHED 26 June 2024
DOI 10.3389/fphar.2024.1423629

OPEN ACCESS

EDITED BY
Elisa Giovannetti,
VU Medical Center, Netherlands

REVIEWED BY
Qiang Wang,
First Affiliated Hospital of Anhui Medical University, China
Baharan Fekry,
University of Texas Health Science Center at Houston, United States

*CORRESPONDENCE
Wenya Li,
saint5288@hotmail.com
Yan Xu,
yanxu@cmu.edu.cn

RECEIVED 26 April 2024
ACCEPTED 06 June 2024
PUBLISHED 26 June 2024

CITATION
Wang Z, Sun X, Li Z, Yu H, Li W and Xu Y (2024), Metabolic reprogramming in esophageal squamous cell carcinoma.
Front. Pharmacol. 15:1423629.
doi: 10.3389/fphar.2024.1423629

Metabolic reprogramming in esophageal squamous cell carcinoma

Ziyi Wang[1,2,3], Xiangyu Sun[4], Zehui Li[1], Huidong Yu[3], Wenya Li[3]* and Yan Xu[1]*

[1]Department of Surgical Oncology and General Surgery, First Hospital of China Medical University, Shenyang, Liaoning, China, [2]Department of Thoracic Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [3]Department of Thoracic Surgery, First Hospital of China Medical University, Shenyang, Liaoning, China, [4]Department of Breast Surgery, Liaoning Cancer Hospital and Institute, Cancer Hospital of Dalian University of Technology, Cancer Hospital of China Medical University, Shenyang, Liaoning, China

Esophageal squamous cell carcinoma (ESCC) is a malignancy with high incidence in China. Due to the lack of effective molecular targets, the prognosis of ESCC patients is poor. It is urgent to explore the pathogenesis of ESCC to identify promising therapeutic targets. Metabolic reprogramming is an emerging hallmark of ESCC, providing a novel perspective for revealing the biological features of ESCC. In the hypoxic and nutrient-limited tumor microenvironment, ESCC cells have to reprogram their metabolic phenotypes to fulfill the demands of bioenergetics, biosynthesis and redox homostasis of ESCC cells. In this review, we summarized the metabolic reprogramming of ESCC cells that involves glucose metabolism, lipid metabolism, and amino acid metabolism and explore how reprogrammed metabolism provokes novel opportunities for biomarkers and potential therapeutic targets of ESCC.

KEYWORDS

metabolic reprogramming, glucose, fatty acid, amino acid, esophageal cancer

Introduction

Esophageal cancer is a common malignancy of the digestive tract. The new cases of esophageal cancer have exceeded 6,00,000 annually, ranking the seventh among all cancers. The death cases of esophageal cancer are about 5,40,000 annually, ranking the sixth among all cancer types (Sung et al., 2021). Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two main histological subtypes of esophageal cancer (Rogers et al., 2022). As a high-risk country of esophageal cancer, more than 90% of the cases diagnosed in China are ESCC (Liu CC et al., 2019). With the improvement of early diagnosis and comprehensive therapeutic strategies for ESCC, the therapeutic effect of esophageal cancer has been improved, but the 5-year overall survival rate of ESCC is only 15%~20% (Huang and Yu, 2018; Zhu et al., 2023).

Metabolic reprogramming is an emerging hallmark of tumors (Kroemer and Pouyssegur, 2008). To adapt to hypoxia and nutrient deprivation, proliferating tumor cells need to reprogram metabolic pathways to fulfill the increasing demands of energetic production, cellular building components and redox balance of tumor cells (Pavlova and Thompson, 2016). A series of genetic alteration and cellular signaling are involved in the regulation of diverse aspects of cellular metabolism (DeBerardinis and Chandel, 2016). Studies have identified profound metabolic reprogramming in aggressive ESCC that

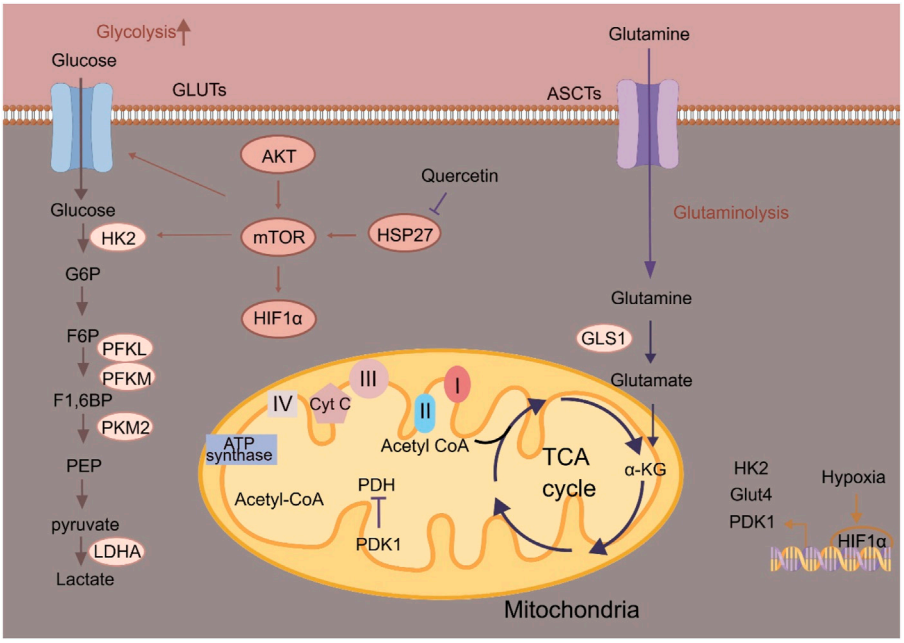


FIGURE 1
Schematic illustration of glucose metabolism in ESCC cells. α-KG, α-ketoglutarate; ASCT, Alanine serine cysteine transporter; Cyt C, Cytochrome c; F-1, 6-BP, Fructose-1, 6-bisphosphatase; G6P, Glucose-6-phosphate; Glut, Glucose transporter; GLS1, Glutaminase 1; HK2, Hexokinase 2; HIF-1α, Hypoxia-inducible factor 1α; HSP27, Heat shock protein 27; LDHA, Lactate dehydrogenase A; PDK1, 3-phosphoinositide-dependent kinase 1; PFK, Phosphofructokinase; PKM2, Pyruvate kinase M2.

involves glycolysis, tricarboxylic acid (TCA) cycle, lipid metabolism, and glutamine addiction, etc. Spatially resolved metabolomics in 256 ESCC patients revealed that multiple altered metabolic pathways, including proline biosynthesis, glutamine metabolism, uridine metabolism, histidine metabolism, fatty acid biosynthesis and polyamine biosynthesis participate in the pathogenesis of ESCC. Notably, metabolic enzymes pyrroline-5-carboxylate reductase 2 and uridine phosphorylase 1 are therefore identified as potential therapeutic targets. Huang et al. (2024) integrated the single cell RNA-seq with metabolomics of ESCC tissues and plasma samples, indicating that nicotinate and nicotinamide metabolism pathway was dysregulated in ESCC patients with lymphatic metastasis with significant 1-methylnicotinamide upregulation. In this review, we primarily discussed the advantages of metabolic reprogramming for ESCC cells and explored how reprogrammed metabolism provokes novel opportunities for biomarkers and potential therapeutic targets of ESCC.

Reprogrammed metabolic pathways in ESCC

Glucose metabolism and ESCC

Altered glucose metabolism is commonly observed in ESCC cells (Figure 1). Like other tumors, glycolysis is the preferred energetic pathway in ESCC cells (Liu et al., 2021; Su et al., 2022). Depending on glycolysis, ESCC cells convert glucose to lactate to rapidly produce ATP and promote tumor progression (Wang et al., 2021; Li et al., 2023). The glycolytic pathway plays an essential role in ESCC metabolism, as shown by the deregulation of multiple metabolic enzymes. As the first rate-limiting enzyme in glycolysis, hexokinases (HKs) catalyze the phosphorylation of glucose to glucose-6-phosphate. Among the four isoforms, HK2 exerts greater effect in promoting aerobic glycolysis than other isoforms (Ciscato F et al., 2021). Liu K et al. (2019) found that HK2 is essential for maintaining esophageal cancer stem cell (CSC) phenotypes. Esophageal CSCs exhibit higher glycolysis, which are regulated via the small heat shock protein 27 (Hsp27)-AKT-HK2 axis. Hsp27 could increase the expression of HK2 through activating AKT-mTOR in esophageal CSCs. Quercetin, the Hsp27 inhibitor, could augment the anti-ESCC effects of adjuvant chemotherapy. Phosphofructokinases (PFKs) catalyze the phosphorylation of fructose-6-phosphate to fructose-1, 6-bisphosphate, and has three PFK isozymes including liver type (PFKL), platelet type (PFKP) and muscle type (PFKM) (Feng et al., 2020). PFKL overexpression is associated with reduced survival of ESCC patients, and its inhibition suppresses ESCC growth (Zheng et al., 2022). Antipsychotic drug penfluridol directly targets PFKL to impair glycolytic pathway and only exhibits its anti-tumor effect in PFKL-proficient tumors (Zheng et al., 2022). Under hypoxia, GHRH-R splice variant 1 (SV1) is elevated to upregulate PFKM to promote glycolytic pathway and enhance development of ESCC (Gu et al., 2022). MIA-602 (a SV1 inhibitor) could block the oncogenic role of PFKM in ESCC. 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3 (PFKFB3) mediates the synthesis and degradation of fructose 2, 6-bisphosphate. PFKFB3 could increase glycolytic flux in ESCC cells, as well as ESCC cell cycle progression and growth. lncRNA Actin Gamma 1 Pseudogene (AGPG) has been identified to bind to and stabilize PFKFB3 by preventing APC/C-mediated

ubiquitination of PFKFB3 and stabilizing PFKFB3 protein (Liu et al., 2020). Pyruvate kinases (PKs), the last rate-limiting enzymes in glycolysis, catalyze the conversion of phosphoenolpyruvate to pyruvate. There are four isoforms of PKs, including PKL (found in liver), PKR (red blood cell), PKM1 and PKM2 (found in muscle) (Israelsen and Vander Heiden, 2015). The critical role of glycolysis in ESCC development is further supported by the correlation between increased PKM2 expression and poor prognosis. PKM2 has been found to be upregulated in ESCC patients (Ma et al., 2019). circCYP24A1 interacts with PKM2 to enhance C-C chemokine ligand 5 (CCL5) production and development of ESCC (Gu et al., 2022). Methyltransferase-like 3 induces the m6A modification of APC by recruiting YTH domain family (YTHDF) for APC mRNA degradation, which further upregulates β-catenin and its downstream targets PKM2 to enhance aerobic glycolysis and promote ESCC progression. PKM2 has also been found to be a target of photodynamic therapy (PDT) (Li B et al., 2021). PDT inhibits PKM2 and activates caspase-3/8 to release Gasdermin E-N (N-GSDME) and induce pyroptosis in ESCC cells. Lysyl-oxidase like-2 (LOXL2) and its catalytically inactive L2Δ13 splice variant interact physically with aldolase A, glyceraldehyde-3-phosphate dehydrogenase and enolase to boost glycolysis of ESCC cells, therefore promoting ESCC progression (Jiao et al., 2022). Pyruvate dehydrogenase kinase 1 (PDK1) is a Ser/Thr kinase that inactivates mitochondrial pyruvate dehydrogenase, leading to a metabolic reprogramming from oxidative phosphorylation (OXPHOS) to glycolytic pathway. PDK1 is significantly overexpressed in ESCC tissues and cell lines compared with the normal tissues or cells (Ma et al., 2023). PDK1 could suppress cell proliferation of ESCC by blocking glycolytic pathway (Ma et al., 2023). Hypoxia-inducible factor 1α (HIF-1α) is the key transcriptional regulator of glycolysis, mediating cellular adaptation to hypoxia. In ESCC, long intergenic noncoding RNA for kinase activation (LINK-A) disrupts the interaction between MCM3 and HIF-1α, reducing MCM3-mediated HIF-1α transcriptional inhibition to enhance glycolytic phenotype and chemoresistance. m6A demethylase fat mass and obesity-associated protein (FTO) could stabilize LINK-A, making the FTO/LINK-A/MCM3/HIF-1α axis as a potential target for anti-ESCC strategy (Nan et al., 2023). LncRNA MALR upregulation leads to ILF3 liquid-liquid phase separation and activates HIF1α to promote ESCC development (Liu et al., 2023). Hypoxia-induced lncRNA G077640 upregulation promotes ESCC progression by stabilizing HIF1α to upregulate glucose transporter 4 (GLUT4), HK2 and PDK1 (Huang et al., 2023). Targeting glycolytic rate-limiting enzymes, glucose transporters and other metabolic enzymes are considered targets for screening potential anti-tumor drugs. However, based on *in vitro* or *in vivo* experiments, most anti-ESCC drugs that target glucose metabolism are still in the development stage.

The TCA cycle is indispensable for OXPHOS to fulfill the needs of bioenergetics and biosynthesis (Nolfi-Donegan et al., 2020). The regulation of mitochondrial biogenesis is essential for sustaining redox homeostasis to boost anti-immunity and inhibit tumor progression (Zong et al., 2016). Mitochondrial transcription factor A (TFAM) has been found to be downregulated in ESCC, and TFAM downregulation is correlated with poor survival of ESCC patients. TFAM is essential for replication and stability of mitochondrial genome, and TFAM inhibition induces mitochondrial DNA (mtDNA) release into the cytosol and activates the cGAS-STING signaling to induce autophagy and ESCC proliferation. Further, STING abrogation or mtDNA degradation by DNase I impairs TFAM-depleted ESCC cell proliferation (Li et al., 2022). CircPUM1 is positively associated with the expression of HIF1α under hypoxia in ESCC cells. CircPUM1 regulates the mitochondrial complex III assembly through interacting with mitochondrial ubiquinol-cytochrome c reductase core protein (UQCRC), which ultimately promotes OXPHOS for ATP production to facilitate proliferation and inhibit pyroptosis of ESCC cells (Gong et al., 2022). In ESCC, oncogenic STAT3 signaling pathway participates in regulating the activity of electron transport chain (ETC). STAT3β could inhibit the phosphorylation of STAT3α at S727 in mitochondria via ERK1/2 to impair the activity of ETC, therefore activating GSDME for pyroptosis and sensitizing ESCC cells to cisplatin (Zheng et al., 2021). Targeting OXPHOS in ESCC cells as an anti-ESCC strategy deserves further investigations.

The pentose phosphate pathway is a branch from glycolysis and serves as a major source of ribonucleotides and NADPH. In ESCC, downregulation of polo like kinase 1 (PLK1) could block the pentose phosphate pathway to impair the production of NADPH, thereby enhancing ferroptosis and promoting the sensitivity of ESCC cells to chemotherapy (Zhao et al., 2023).

Lipid metabolism and ESCC

Altered lipid metabolism is a prominent metabolic reprogramming in ESCC (Figure 2). Enhanced lipid biosynthesis or uptake is required for ESCC progression (Cheng et al., 2018). Fatty acid synthesis is a process that begins with the carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase. Malonyl-CoA is further committed to fatty acid synthesis by producing mainly 16-C palmitate via fatty acid synthase (FASN). FASN has been found to promote the progression of ESCC. circHIPK3 acts as a ceRNA by sponging miR-637 to upregulate FASN expression and fatty acid biosynthesis of ESCC cells, indicating circHIPK3/miR-637/FASN axis as a promising therapeutic target for anti-ESCC strategy (Cao et al., 2024). *De novo* lipogenesis is transcriptionally regulated by sterol regulatory element binding protein (SREBP) (Eberlé et al., 2004). Overexpression of SREBP1 is correlated with poor prognosis in ESCC patients, and supports ESCC progression by enhancing fatty acid biosynthesis. In ESCC, pre-mRNA processing factor 19 (PRP19) enhances the stability of SREBP1 mRNA in an N6-methyladenosine-dependent manner to mediate SREBP-dependent fatty acid synthesis and ESCC progression. SREBP could also cooperate with TP63/Kruppel like factor 5 (KLF5) to regulate the biosynthesis of fatty acids (Zhang et al., 2023).

Fatty acid oxidation is the process involving shortening of fatty acids and producing in acetyl-CoA, NADH and FADH2 (Ma et al., 2018). Carnitine O-palmitoyl transferase 1 (CPT1A), the key rate-limiting enzyme in fatty acid oxidation, is upregulated in ESCC, which is correlated with poor survival of ESCC patients (Tian et al., 2022). In ESCC, CPT1A could inhibit cellular apoptosis by

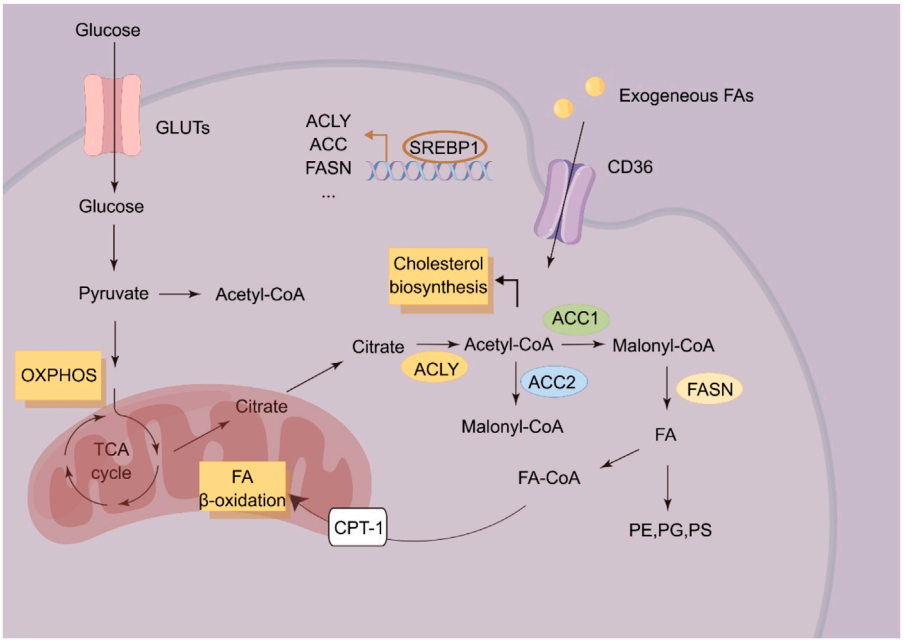


FIGURE 2
Schematic illustration of lipid metabolism in ESCC cells. ACC, acetyl-CoA; ACLY, ATP citrate lyase carboxylase; CPT-1, Carnitine palmitoyl transferase 1; FA, Fatty acid; FASN, Fatty acid synthase; PE, Phosphatidylethanolamine; PS, Phosphatidylserine; PG, Phosphatidylglycerol; SREBP1, Sterol response element binding protein-1.

providing GSH and NADPH to sustain redox homeostasis. Genetic or pharmacologic inhibition of CPT1A reduces the NADPH supply to impair anchorage-independent growth of ESCC cells *in vitro* and *in vivo* (Tian et al., 2022).

Cholesterol is an important component of cell and organelle membranes and a precursor of steroid hormones and bile acids (Huang et al., 2020). The rapid proliferation of tumor cells requires enhanced biosynthesis of cell and organelle membranes, and aberrant cholesterol metabolism therefore plays a pivotal role in tumor initiation and progression (Xu et al., 2020). Metastatic ESCC cells display elevated cholesterol accumulation via upregulating the expression of anoctamin 1 (ANO1). ANO1 results in cholesterol accumulation by blocking LXR signaling and inhibiting cholesterol hydroxylation by downregulating hydroxylase CYP27A1 (Singh and Mehla, 2023). Lysophosphatidylcholine acyltransferase 1 (LPCAT1) has been found to be upregulated in ESCC tissues, and LPCAT1 could rewire cholesterol metabolism of ESCC cells. LPCAT1 could upregulate the expression of SQLE to promoting the entry of SP1/SREBP2 into the nucleus via activating PI3K. LPCAT1 also promotes the entry of SREBP1 into the nucleus by EGFR-mediated INSIG-1 downregulation, leading to enhance cholesterol biosynthesis (Tao et al., 2021).

Phospholipid is composed of phosphoglyceride and sphingomyelin. Phosphoglyceride includes phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol. In ESCC, DNA hypermethylation of otubain 2 (OTUB2) has been found to induce tumor initiation and chemoresistance by regulating biosynthesis of phosphatidylserine. Mechanistically, OTUB2 mediates the deubiquitination and phosphorylation of STAT1 and further promotes calmodulin-like protein 3 (CALML3) upregulation. Subsequently, CALML3 regulates mitochondrial calcium signaling to promote OXPHOS and biosynthesis of phosphatidylserine. More importantly, orally-administered phosphatidylserine impairs tumor initiation of OTUB2-low ESCC cells in mouse models, making phosphatidylserine administration as a potential anti-ESCC strategy (Chang et al., 2022). Fatty acid 2-hydroxylase (FA2H), catalyzing the hydroxylation of fatty acids, has been found to be significantly enriched in a subpopulation of ESCC cells with high metastatic potential, and that FA2H inhibition significantly blocks metastatic potential of ESCC cells. Notably, Ceramide (d18:0/24:0) and Ceramide (d18:0/24:1) are increased in FA2H-knockdown ESCC cells. Upon administration, Cer (d18:0/24:0) and Cer (d18:0/24:1) significantly blocks tumor metastasis in mouse models (Zhou et al., 2022).

Currently, multiple studies have revealed that lipid metabolism in ESCC tissues is significantly altered compared with normal esophageal tissues, which may lead to the occurrence and development of ESCC. Therefore, targeting lipid metabolism as a potential therapeutic approach will provide new opportunities for the treatment of ESCC. However, there are still challenges to overcome in techniques of lipid detection. The accuracy of mass spectrometry-based lipidomics is required to be improved for more accurate qualitative and quantitative analysis. The combined analysis of multi-omics could reveal the biological feature of the occurrence and development of ESCC comprehensively. The combination of lipidomics with genomics, transcriptomics and proteomics may provide more insights for the treatment ESCC.

TABLE 1 Targeting cellular metabolism for potential anti-ESCC strategy.

Drug	Target	Metabolic effects	Anti-tumor effects	Reference
Targeting glucose metabolism				
Quercetin	Hsp27	Suppressing Hsp27-AKT-HK2 axis to block glycolysis	Increasing sensitivity to chemotherapy	Liu CC et al. (2019)
Penfluridol	PFKL	Suppressing glycolysis	Inhibiting proliferation *in vitro* and growth *in vivo*	Zheng et al. (2022)
MIA-602	SV1	Suppressing SV1-PFKM axis to block glycolysis	Inhibiting cell cycle progression and growth	Gu et al. (2022)
Cloperastine	—	Suppressing OXPHOS	Inhibiting proliferation *in vitro* and growth *in vivo*	Li L et al. (2021)
DNase I	mtDNA	Suppressing OXPHOS	Inhibiting TFAM-depleted ESCC cell proliferation	Li et al. (2022)
Sulconazole	—	Suppressing glycolysis, inducing oxidative stress	Inducing PANoptosis, increasing radiosensitivity Inhibiting cell proliferation and migration	Liu et al. (2023)
Targeting lipid metabolism				
Perhexiline	CPT1A	Suppressing fatty acid oxidation	Inhibiting the anchorage-independent growth of ESCC cells *in vitro* and lung metastases of xenografted tumor models *in vivo*	Tian et al. (2022)
Phosphatidylserine	OTUB2-low	DNA hypermethylation of OTUB2 promotes OXPHOS and biosynthesis of phosphatidylserine	Impairing tumor initiation and chemoresistance of OTUB2-low ESCC cells in mouse models	Chang et al. (2022)
Ceramide (d18:0/24:0) and Ceramide (d18:0/24:1)	FA2H-low	—	Blocking metastatic potential of ESCC *in vitro* and vivo	Zhou et al. (2022)
Targeting amino acid metabolism				
CB-839	GLS1	Suppressing glutamine addiction	Combined with metformin to overcome acquired resistance to CDK4/6 inhibitors *in vitro* and *in vivo*	Qie et al. (2019)
	GLS1	Suppressing glutamine addiction	Inducing cellular senescence and sustaining cell proliferation	Chen et al. (2023)

Amino acid metabolism and ESCC

Cancer cells exhibit elevated demand for amino acids to support their fast proliferation (Sivanand and Vander Heiden, 2020). It has been found that tumor cells exhibit greater dependence on glutamine (Cluntun et al., 2017). Glutamine participates in cellular bioenergetics through α-ketoglutarate in TCA cycle and serves as a major source of nitrogen for nucleotide biosynthesis. In ESCC, genetic alterations including Fbxo4 loss and hyperactivation of cyclin D1-CDK4/6 kinases have been frequently observed, leading to glutamine addiction (Qie et al., 2019). The Fbxo4-cyclin D1 axis could regulate glutamine consumption and mitochondrial dysfunction by suppressing Rb activity and activating mTORC1 to promote ESCC development. Glutaminase 1 (GLS1) is a rate-limiting glutaminolysis enzyme that transforms glutamine into glutamate to support proliferation of ESCC cells. Combined treatment of CB-839 (GLS1 inhibitor) and metformin could synergistically impair proliferation of ESCC cells, providing promising therapeutic strategy for anti-ESCC treatment. Glutamine metabolism is also regulated by RNA binding motif protein 4 (RBM4)-LKB1 axis to enhance ESCC cell survival. RBM4 binds with LKB1 to impair the LKB1/STRAD/MO25 complex and promote TRIM26-mediated LKB1 ubiquitination and degradation to overcome cell senescence (Chen et al., 2023). NEDD4 like E3 ubiquitin protein ligase (NEDD4L) blocks glutamine metabolism in ESCC by ubiquitination of c-Myc to downregulate GLS1 and SLC1A5, which suppresses tumor progression (Cheng et al., 2022). Collectively, targeting glutamine metabolism in cancer therapy brings new opportunities for anti-ESCC strategy.

Arginine functions as the precursor for synthesis of protein, nitric oxide, polyamines, agmatine, creatine, and urea (Chen et al., 2021). Of the enzymes catalyzing rate-controlling steps in arginine metabolism, argininosuccinate synthetase 1 (ASS1) and argininosuccinate lyase (ASL) levels were increased in ESCC tissues, but reduced in metastatic ESCC tissues (Sun et al., 2024). Blocking ASS1 or ASL could impair ESCC growth at the primary site and promote distant metastasis (Sun et al., 2024). The gene encoding 26S proteasome non-ATPase regulatory subunit 2 (PSMD2) activates the mTOR pathway by upregulating ASS1 to inhibit autophagy and promote ESCC progression (Liu et al., 2023). Under hypoxia, receptor tyrosine kinase IGF1R is upregulated in ESCC to elevate the transcription of ASS1 through c-MYC to reprogram arginine metabolism (Fang et al., 2023).

Although nutritional epidemiological study has shown that high methionine intake is not associated with ESCC risk, Jin et al. (2024) found that methionine cycling has been hyperactivated in ESCC tissues and correlated with poor survival outcome. ESCC cells prefers to utilize exogenous methionine to produce S-adenosine methionine (SAM), leading to enhanced ESCC cell proliferation. Mechanistically, methionine enhances METTL3-mediated RNA m6A methylation through SAM and upregulates NR4A2 expression. Celecoxib has been identified as a potent NR4A2 inhibitor with promising anti-ESCC potential.

More comprehensive analysis of amino acid metabolic reprogramming and its related metabolic pathways in ESCC cells should be conducted to assist developing more effective anti-ESCC strategies. However, there are still questions waiting for answer. For example, whether metabolic reprogramming in amino acid cooperates with other related pathways to sustain tumor development? How other metabolic pathways compensate to promote ESCC cell proliferation once amino acid metabolism is dysregulated in ESCC cells? What is the therapeutic effect of the usage of specific amino acid-limited diet to treat ESCC?

Clinical implications of metabolism-targeted therapy in ESCC

Metabolomics cooperates with genomics, transcriptomics and proteomics to form "systems biology," which plays an important role in cancer research. Metabolomics mainly includes targeted metabolomics and non-targeted metabolomics. Untargeted metabolomics works by detecting all metabolites in the samples to obtain quantitative information to decipher the differences in metabolites between groups. Targeted metabolomics analyzes a limited number of metabolites associated with biological process (Schrimpe-Rutledge et al., 2016). Based on the tissue metabolic profiles consistently identified by nuclear magnetic resonance and targeted mass spectrometry techniques, Zhao et al. (2024) developed nuclear magnetic resonance -based serum and urine metabolic profiles and optimized to reliably reflect the metabolic profiles of ESCC. Due to the metabolic adaptability during tumor progression and treatment, it is important to track and monitor metabolic adaptability of ESCC cells during tumor progression and treatment. Therefore, it is necessary to dynamically and accurately observe metabolic alterations and change treatment strategies accordingly. Optimization of existing detection methods and searching for alternative non-invasive detection methods are key research directions (Xiao et al., 2023).

One strategy for discovering anti-ESCC therapy is to screen FDA-approved drugs. Recent study reported that antitussive agent cloperastine inhibits the proliferation of ESCC *in vivo* and *in vitro* by impairing mitochondrial OXPHOS (Li L et al., 2021; Li LY et al., 2021). Liu Y et al. (2023) found that sulconazole can effectively block the growth of ESCC cells by inducing mitochondrial oxidative stress and inhibiting glycolysis. The current reported metabolism-targeted therapy has been summarized in Table 1. Metabolic enzymes are attractive therapeutic targets in anti-tumor therapy, but new drugs targeting metabolism have been limited due to toxicity to normal tissues. And more studies have recognized that some metabolic enzymes drive tumor progression through non-catalytic mechanisms (Pan et al., 2021). Preclinical studies have identified several metabolic molecules that inhibit tumor progression as potential therapeutic targets, and some are already in clinical trials (Xiao et al., 2023). However, at present, metabolic therapy for ESCC patients is still stuck in pre-clinical research, and it is urgent to further explore new targets and optimal treatment strategies. The goal of identifying and tracking metabolic targets is to enable precision therapy. Considering the heterogeneity of metabolism in tumors, individualized metabolism-targeted therapy will be the future development direction.

Collectively, exploring the anti-ESCC strategy in the context of tumor metabolism has unveiled novel and promising opportunities, as well as more comprehensive understandings of metabolic reprogramming of ESCC cells. In the future, more knowledge of the metabolic reprogramming of ESCC cells are required to refine rational anti-ESCC strategies that target tumor metabolism.

Author contributions

ZW: Writing–original draft, Writing–review and editing. XS: Writing–original draft, Writing–review and editing. ZL: Writing–original draft, Writing–review and editing. HY: Writing–original draft, Writing–review and editing. WL: Writing–original draft, Writing–review and editing. YX: Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of China (82072733) and Technology Program Joint Fund of Liaoning Province (2023-BSBA-207).

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.

Publisher's note

References

Cao, S. Q., Xue, S. T., Li, W. J., Hu, G. S., Wu, Z. G., Zheng, J. C., et al. (2024). CircHIPK3 regulates fatty acid metabolism through miR-637/FASN axis to promote esophageal squamous cell carcinoma. *Cell Death Discov.* 10 (1), 110. doi:10.1038/s41420-024-01881-z

Chang, W., Luo, Q., Wu, X., Nan, Y., Zhao, P., Zhang, L., et al. (2022). OTUB2 exerts tumor-suppressive roles via STAT1-mediated CALML3 activation and increased phosphatidylserine synthesis. *Cell Rep.* 41 (4), 111561. doi:10.1016/j.celrep.2022.111561

Chen, C. L., Hsu, S. C., Ann, D. K., Yen, Y., and Kung, H. J. (2021). Arginine signaling and cancer metabolism. *Cancers (Basel)* 13 (14), 3541. doi:10.3390/cancers13143541

Chen, L., Zhang, W., Chen, D., Yang, Q., Sun, S., Dai, Z., et al. (2023). RBM4 dictates ESCC cell fate switch from cellular senescence to glutamine-addiction survival through inhibiting LKB1-AMPK-axis. *Signal Transduct. Target Ther.* 8 (1), 159. doi:10.1038/s41392-023-01367-x

Cheng, C., Geng, F., Cheng, X., and Guo, D. (2018). Lipid metabolism reprogramming and its potential targets in cancer. *Cancer Commun. (Lond).* 38 (1), 27. doi:10.1186/s40880-018-0301-4

Cheng, W., Li, G., Ye, Z., Hu, J., Gao, L., Jia, X., et al. (2022). NEDD4L inhibits cell viability, cell cycle progression, and glutamine metabolism in esophageal squamous cell carcinoma via ubiquitination of c-Myc. *Acta Biochim. Biophys. Sin. (Shanghai)* 54 (5), 716–724. doi:10.3724/abbs.2022048

Ciscato, F., Ferrone, L., Masgras, I., Laquatra, C., and Rasola, A. (2021). Hexokinase 2 in cancer: a prima donna playing multiple characters. *Int. J. Mol. Sci.* 22 (9), 4716. doi:10.3390/ijms22094716

Cluntun, A. A., Lukey, M. J., Cerione, R. A., and Locasale, J. W. (2017). Glutamine metabolism in cancer: understanding the heterogeneity. *Trends Cancer* 3 (3), 169–180. doi:10.1016/j.trecan.2017.01.005

DeBerardinis, R. J., and Chandel, N. S. (2016). Fundamentals of cancer metabolism. *Sci. Adv.* 2 (5), e1600200. eCollection 2016 May. doi:10.1126/sciadv.1600200

Eberlé, D., Hegarty, B., Bossard, P., Ferré, P., and Foufelle, F. (2004). SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 86 (11), 839–848. doi:10.1016/j.biochi.2004.09.018

Fang, K., Sun, M., Leng, Z., Chu, Y., Zhao, Z., Li, Z., et al. (2023). Targeting IGF1R signaling enhances the sensitivity of cisplatin by inhibiting proline and arginine metabolism in oesophageal squamous cell carcinoma under hypoxia. *J. Exp. Clin. Cancer Res.* 42 (1), 73. doi:10.1186/s13046-023-02623-2

Feng, J., Li, J., Wu, L., Yu, Q., Ji, J., Wu, J., et al. (2020). Emerging roles and the regulation of aerobic glycolysis in hepatocellular carcinoma. *J. Exp. Clin. Cancer Res.* 39 (1), 126. doi:10.1186/s13046-020-01629-4

Gong, W., Xu, J., Wang, Y., Min, Q., Chen, X., Zhang, W., et al. (2022). Nuclear genome-derived circular RNA circPUM1 localizes in mitochondria and regulates oxidative phosphorylation in esophageal squamous cell carcinoma. *Signal Transduct. Target Ther.* 7 (1), 40. doi:10.1038/s41392-021-00865-0

Gu, L., Sang, Y., Nan, X., Zheng, Y., Liu, F., Meng, L., et al. (2022). circCYP24A1 facilitates esophageal squamous cell carcinoma progression through binding PKM2 to regulate NF-κB-induced CCL5 secretion. *Mol. Cancer* 21 (1), 217. doi:10.1186/s12943-022-01686-7

Huang, B., Song, B. L., and Xu, C. (2020). Cholesterol metabolism in cancer: mechanisms and therapeutic opportunities. *Nat. Metab.* 2 (2), 132–141. Epub 2020 Feb 10. doi:10.1038/s42255-020-0174-0

Huang, F. L., and Yu, S. J. (2018). Esophageal cancer: risk factors, genetic association, and treatment. *Asian J. Surg.* 41 (3), 210–215. doi:10.1016/j.asjsur.2016.10.005

Huang, Q., Chen, H., Yin, D., Wang, J., Wang, S., Yang, F., et al. (2024). Multi-omics analysis reveals NNMT as a master metabolic regulator of metastasis in esophageal squamous cell carcinoma. *NPJ Precis. Oncol.* 8 (1), 24. doi:10.1038/s41698-024-00509-w

Huang, X., Liu, C., Li, H., Dai, T., Luo, G., Zhang, C., et al. (2023). Hypoxia-responsive lncRNA G077640 promotes ESCC tumorigenesis via the H2AX-HIF1α-glycolysis axis. *Carcinogenesis* 44 (5), 383–393. doi:10.1093/carcin/bgad036

Israelsen, W. J., and Vander Heiden, M. G. (2015). Pyruvate kinase: function, regulation and role in cancer. *Semin. Cell Dev. Biol.* 43, 43–51. Epub 2015 Aug 13. doi:10.1016/j.semcdb.2015.08.004

Jiao, J. W., Zhan, X. H., Wang, J. J., He, L. X., Guo, Z. C., Xu, X. E., et al. (2022). LOXL2-dependent deacetylation of aldolase A induces metabolic reprogramming and tumor progression. *Redox Biol.* 57, 102496. Epub 2022 Oct 3. doi:10.1016/j.redox.2022.102496

Jin, X., Liu, L., Liu, D., Wu, J., Wang, C., Wang, S., et al. (2024). Unveiling the methionine cycle: a key metabolic signature and NR4A2 as a methionine-responsive oncogene in esophageal squamous cell carcinoma. *Cell Death Differ.* 31 (5), 558–573. Epub 2024 Apr 3. doi:10.1038/s41418-024-01285-7

Kroemer, G., and Pouyssegur, J. (2008). Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell* 13 (6), 472–482. doi:10.1016/j.ccr.2008.05.005

Li, B., Yu, Y., Jiang, Y., Zhao, L., Li, A., Li, M., et al. (2021). Cloperastine inhibits esophageal squamous cell carcinoma proliferation *in vivo* and *in vitro* by suppressing mitochondrial oxidative phosphorylation. *Cell Death Discov.* 7 (1), 166. doi:10.1038/s41420-021-00509-w

Li, L., Jiang, D., Zhang, Q., Liu, H., Xu, F., Guo, C., et al. (2023). Integrative proteogenomic characterization of early esophageal cancer. *Nat. Commun.* 14 (1), 1666. doi:10.1038/s41467-023-37440-w

Li, L., Song, D., Qi, L., Jiang, M., Wu, Y., Gan, J., et al. (2021). Photodynamic therapy induces human esophageal carcinoma cell pyroptosis by targeting the PKM2/caspase-8/caspase-3/GSDME axis. *Cancer Lett.* 520, 143–159. Epub 2021 Jul 10. doi:10.1016/j.canlet.2021.07.014

Li, L. Y., Yang, Q., Jiang, Y. Y., Yang, W., Jiang, Y., Li, X., et al. (2021). Interplay and cooperation between SREBF1 and master transcription factors regulate lipid metabolism and tumor-promoting pathways in squamous cancer. *Nat. Commun.* 12 (1), 4362. doi:10.1038/s41467-021-24656-x

Li, Y., Yang, Q., Chen, H., Yang, X., Han, J., Yao, X., et al. (2022). TFAM downregulation promotes autophagy and ESCC survival through mtDNA stress-mediated STING pathway. *Oncogene* 41, 3735–3746. doi:10.1038/s41388-022-02365-z

Liu, C. C., Chou, K. T., Hsu, J. W., Lin, J. H., Hsu, T. W., Yen, D. H., et al. (2019). High metabolic rate and stem cell characteristics of esophageal cancer stem-like cells depend on the Hsp27-AKT-HK2 pathway. *Int. J. Cancer* 145 (8), 2144–2156. Epub 2019 Apr 10. doi:10.1002/ijc.32301

Liu, H., Zhang, Q., Song, Y., Hao, Y., Cui, Y., Zhang, X., et al. (2021). Long non-coding RNA SLC2A1-AS1 induced by GLI3 promotes aerobic glycolysis and progression in esophageal squamous cell carcinoma by sponging miR-378a-3p to enhance Glut1 expression. *J. Exp. Clin. Cancer Res.* 40 (1), 287. doi:10.1186/s13046-021-02081-8

Liu, J., Liu, Z. X., Wu, Q. N., Lu, Y. X., Wong, C. W., Miao, L., et al. (2020). Long noncoding RNA AGPG regulates PFKFB3-mediated tumor glycolytic reprogramming. *Nat. Commun.* 11 (1), 1507. doi:10.1038/s41467-020-15112-3

Liu, K., Zhao, T., Wang, J., Chen, Y., Zhang, R., Lan, X., et al. (2019). Etiology, cancer stem cells and potential diagnostic biomarkers for esophageal cancer. *Cancer Lett.* 458, 21–28. doi:10.1016/j.canlet.2019.05.018

Liu, L. X., Heng, J. H., Deng, D. X., Zhao, H., Zheng, Z. Y., Liao, L. D., et al. (2023). Sulconazole induces PANoptosis by triggering oxidative stress and inhibiting glycolysis to increase radiosensitivity in esophageal cancer. *Mol. Cell Proteomics* 22 (6), 100551. Epub 2023 Apr 17. doi:10.1016/j.mcpro.2023.100551

Liu, Y., Wu, M., Xu, S., Niu, X., Liu, W., Miao, C., et al. (2023). PSMD2 contributes to the progression of esophageal squamous cell carcinoma by repressing autophagy. *Cell Biosci.* 13 (1), 67. doi:10.1186/s13578-023-01016-4

Ma, R., Liu, Q., Zheng, S., Liu, T., Tan, D., and Lu, X. (2019). PKM2-regulated STAT3 promotes esophageal squamous cell carcinoma progression via TGF-β1-induced EMT. *J. Cell Biochem.* 120 (7), 11539–11550. Epub 2019 Feb 12. doi:10.1002/jcb.28434

Ma, Y., Temkin, S. M., Hawkridge, A. M., Guo, C., Wang, W., Wang, X. Y., et al. (2018). Fatty acid oxidation: an emerging facet of metabolic transformation in cancer. *Cancer Lett.* 435, 92–100. Epub 2018 Aug 10. doi:10.1016/j.canlet.2018.08.006

Ma, Y., Zhang, X., Han, X., Hao, Z., Ji, R., Li, J., et al. (2023). PDK1 regulates the progression of esophageal squamous cell carcinoma through metabolic reprogramming. *Mol. Carcinog.* 62 (6), 866–881. Epub 2023 Mar 29. doi:10.1002/mc.23531

Nan, Y., Liu, S., Luo, Q., Wu, X., Zhao, P., Chang, W., et al. (2023). m^{6}A demethylase FTO stabilizes LINK-A to exert oncogenic roles via MCM3-mediated cell-cycle progression and HIF-1α activation. *Cell Rep.* 42 (10), 113273. Epub 2023 Oct 19. doi:10.1016/j.celrep.2023.113273

Nolfi-Donegan, D., Braganza, A., and Shiva, S. (2020). Mitochondrial electron transport chain: oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biol.* 37, 101674. Epub 2020 Aug 6. doi:10.1016/j.redox.2020.101674

Pan, C., Li, B., and Simon, M. C. (2021). Moonlighting functions of metabolic enzymes and metabolites in cancer. *Mol. Cell* 81 (18), 3760–3774. doi:10.1016/j.molcel.2021.08.031

Pavlova, N. N., and Thompson, C. B. (2016). The emerging hallmarks of cancer metabolism. *Cell Metab.* 23 (1), 27–47. doi:10.1016/j.cmet.2015.12.006

Qie, S., Yoshida, A., Parnham, S., Oleinik, N., Beeson, G. C., Beeson, C. C., et al. (2019). Targeting glutamine-addiction and overcoming CDK4/6 inhibitor resistance in human esophageal squamous cell carcinoma. *Nat. Commun.* 10 (1), 1296. doi:10.1038/s41467-019-09179-w

Rogers, J. E., Sewastjanow-Silva, M., Waters, R. E., and Ajani, J. A. (2022). Esophageal cancer: emerging therapeutics. *Expert Opin. Ther. Targets* 26 (2), 107–117. doi:10.1080/14728222.2022.2036718

Schrimpe-Rutledge, A. C., Codreanu, S. G., Sherrod, S. D., and McLean, J. A. (2016). Untargeted metabolomics strategies-challenges and emerging directions. *J. Am. Soc. Mass Spectrom.* 27 (12), 1897–1905. Epub 2016 Sep 13. doi:10.1007/s13361-016-1469-y

Singh, P. K., and Mehla, K. (2023). LXR signaling-mediated cholesterol metabolism reprogramming regulates cancer cell metastasis. *Cancer Res.* 83 (11), 1759–1761. doi:10.1158/0008-5472.CAN-23-0624

Sivanand, S., and Vander Heiden, M. G. (2020). Emerging roles for branched-chain amino acid metabolism in cancer. *Cancer Cell* 37 (2), 147–156. doi:10.1016/j.ccell.2019.12.011

Su, X., Xue, C., Xie, C., Si, X., Xu, J., Huang, W., et al. (2022). lncRNA-LET regulates glycolysis and glutamine decomposition of esophageal squamous cell carcinoma through miR-93-5p/miR-106b-5p/SOCS4. *Front. Oncol.* 12, 897751. eCollection 2022. doi:10.3389/fonc.2022.897751

Sun, W., Kou, H., Fang, Y., Xu, F., Xu, Z., Wang, X., et al. (2024). FOXO3a-regulated arginine metabolic plasticity adaptively promotes esophageal cancer proliferation and metastasis. *Oncogene* 43 (3), 216–223. Epub 2023 Dec 4. doi:10.1038/s41388-023-02906-0

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Tao, M., Luo, J., Gu, T., Yu, X., Song, Z., Jun, Y., et al. (2021). LPCAT1 reprogramming cholesterol metabolism promotes the progression of esophageal squamous cell carcinoma. *Cell Death Dis.* 12 (9), 845. doi:10.1038/s41419-021-04132-6

Tian, T., Lu, Y., Lin, J., Chen, M., Qiu, H., Zhu, W., et al. (2022). CPT1A promotes anoikis resistance in esophageal squamous cell carcinoma via redox homeostasis. *Redox Biol.* 58, 102544. Epub 2022 Nov 15. doi:10.1016/j.redox.2022.102544

Wang, W., Shao, F., Yang, X., Wang, J., Zhu, R., Yang, Y., et al. (2021). METTL3 promotes tumour development by decreasing APC expression mediated by APC mRNA N6-methyladenosine-dependent YTHDF binding. *Nat. Commun.* 12 (1), 3803. doi:10.1038/s41467-021-23501-5

Xiao, Y., Yu, T. J., Xu, Y., Ding, R., Wang, Y. P., Jiang, Y. Z., et al. (2023). Emerging therapies in cancer metabolism. *Cell Metab.* 35 (8), 1283–1303. doi:10.1016/j.cmet.2023.07.006

Xu, H., Zhou, S., Tang, Q., Xia, H., and Bi, F. (2020). Cholesterol metabolism: new functions and therapeutic approaches in cancer. *Biochim. Biophys. Acta Rev. Cancer* 1874 (1), 188394. doi:10.1016/j.bbcan.2020.188394

Zhang, G. C., Yu, X. N., Guo, H. Y., Sun, J. L., Liu, Z. Y., Zhu, J. M., et al. (2023). PRP19 enhances esophageal squamous cell carcinoma progression by reprogramming SREBF1-dependent fatty acid metabolism. *Cancer Res.* 83 (4), 521–537. doi:10.1158/0008-5472.CAN-22-2156

Zhao, M., Lu, T., Bi, G., Hu, Z., Liang, J., Bian, Y., et al. (2023). PLK1 regulating chemoradiotherapy sensitivity of esophageal squamous cell carcinoma through pentose phosphate pathway/ferroptosis. *Biomed. Pharmacother.* 168, 115711. Epub 2023 Oct 24. doi:10.1016/j.biopha.2023.115711

Zhao, Y., Ma, C., Cai, R., Xin, L., Li, Y., Ke, L., et al. (2024). NMR and MS reveal characteristic metabolome atlas and optimize esophageal squamous cell carcinoma early detection. *Nat. Commun.* 15 (1), 2463. doi:10.1038/s41467-024-46837-0

Zheng, C., Yu, X., Liang, Y., Zhu, Y., He, Y., Liao, L., et al. (2022). Targeting PFKL with penfluridol inhibits glycolysis and suppresses esophageal cancer tumorigenesis in an AMPK/FOXO3a/BIM-dependent manner. *Acta Pharm. Sin. B* 12 (3), 1271–1287. Epub 2021 Sep 11. doi:10.1016/j.apsb.2021.09.007

Zheng, Z. Y., Yang, P. L., Li, R. Y., Liu, L. X., Xu, X. E., Liao, L. D., et al. (2021). STAT3β disrupted mitochondrial electron transport chain enhances chemosensitivity by inducing pyroptosis in esophageal squamous cell carcinoma. *Cancer Lett.* 522, 171–183. Epub 2021 Sep 25. doi:10.1016/j.canlet.2021.09.035

Zhou, X., Huang, F., Ma, G., Wei, W., Wu, N., and Liu, Z. (2022). Dysregulated ceramides metabolism by fatty acid 2-hydroxylase exposes a metabolic vulnerability to target cancer metastasis. *Signal Transduct. Target Ther.* 7 (1), 370. doi:10.1038/s41392-022-01199-1

Zhu, H., Ma, X., Ye, T., Wang, H., Wang, Z., Liu, Q., et al. (2023). Esophageal cancer in China: practice and research in the new era. *Int. J. Cancer* 152 (9), 1741–1751. Epub 2022 Oct 5. doi:10.1002/ijc.34301

Zong, W. X., Rabinowitz, J. D., and White, E. (2016). Mitochondria and cancer. *Mol. Cell.* 61 (5), 667–676. doi:10.1016/j.molcel.2016.02.011

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 27 June 2024
DOI 10.3389/fphar.2024.1367981

OPEN ACCESS

EDITED BY
Elisa Giovannetti,
VU Medical Center, Netherlands

REVIEWED BY
Vasudevarao Penugurti,
Duke University, United States
Shrikant Anant,
University of Kansas Medical Center,
United States

*CORRESPONDENCE
Juan Du,
jindalai66@163.com

†These authors have contributed equally to this work

RECEIVED 09 January 2024
ACCEPTED 10 June 2024
PUBLISHED 27 June 2024

CITATION
Du J and Qin H (2024), Lipid metabolism dynamics in cancer stem cells: potential targets for cancers.
Front. Pharmacol. 15:1367981.
doi: 10.3389/fphar.2024.1367981

Lipid metabolism dynamics in cancer stem cells: potential targets for cancers

Juan Du[1*†] and Hai Qin[2†]

[1]Department of Pharmacy, The Affiliated Cancer Hospital of Zhengzhou University and Henan Cancer Hospital, Zhengzhou, China, [2]Department of Clinical Laboratory, Beijing Jishuitan Hospital Guizhou Hospital, Guiyang, China

Cancer stem cells (CSCs) represent a small subset of heterogeneous cells within tumors that possess the ability to self-renew and initiate tumorigenesis. They serve as potential drivers for tumor initiation, metastasis, recurrence, and drug resistance. Recent research has demonstrated that the stemness preservation of CSCs is heavily reliant on their unique lipid metabolism alterations, enabling them to maintain their own environmental homeostasis through various mechanisms. The primary objectives involve augmenting intracellular fatty acid (FA) content to bolster energy supply, promoting β-oxidation of FA to optimize energy utilization, and elevating the mevalonate (MVA) pathway for efficient cholesterol synthesis. Additionally, lipid droplets (LDs) can serve as alternative energy sources in the presence of glycolysis blockade in CSCs, thereby safeguarding FA from peroxidation. Furthermore, the interplay between autophagy and lipid metabolism facilitates rapid adaptation of CSCs to the harsh microenvironment induced by chemotherapy. In this review, we comprehensively review recent studies pertaining to lipid metabolism in CSCs and provide a concise overview of the indispensable role played by LDs, FA, cholesterol metabolism, and autophagy in maintaining the stemness of CSCs.

KEYWORDS

cancer stem cells, lipid metabolism, fatty acid, lipid droplets, autophagy

1 Introduction

The global burden of cancer in 2019 was estimated at 23.6 million new cases (95% UI, 22.2–24.9 million) and 10 million deaths (95% UI, 9.36–10.6 million), making it the leading cause of mortality worldwide (Global Burden of Disease Cancer et al., 2022). Despite advancements in cancer prevention, it remains a formidable challenge for medical professionals due to the scarcity of cures and the prevalence of metastasis or recurrence among patients. The term "stem cells" refers to a unique population of cells that possess the remarkable abilities of self-renewal and differentiation, enabling them to give rise to diverse cell lineages (Al-Hajj and Clarke, 2004). The majority of cancers are composed of heterogeneous cell populations with varying capacities to induce tumor growth. Only the less differentiated and distinct cell populations within the tumor exhibit a high ability to self-renew and initiate tumorigenesis, which are commonly known as cancer stem cells (CSCs), tumor-propagating cells, or tumor initiating cells (TICs), possessing pluripotency and the capacity to repopulate tumors (Reya et al., 2001) (Figure 1). The CSCs represent a limited population of tumor cells exhibiting stem cell characteristics. CSCs serve as the primordial cells responsible for tumorigenesis, recurrence, and metastasis, with numerous CSCs or CSC-like cells having been identified and isolated from diverse

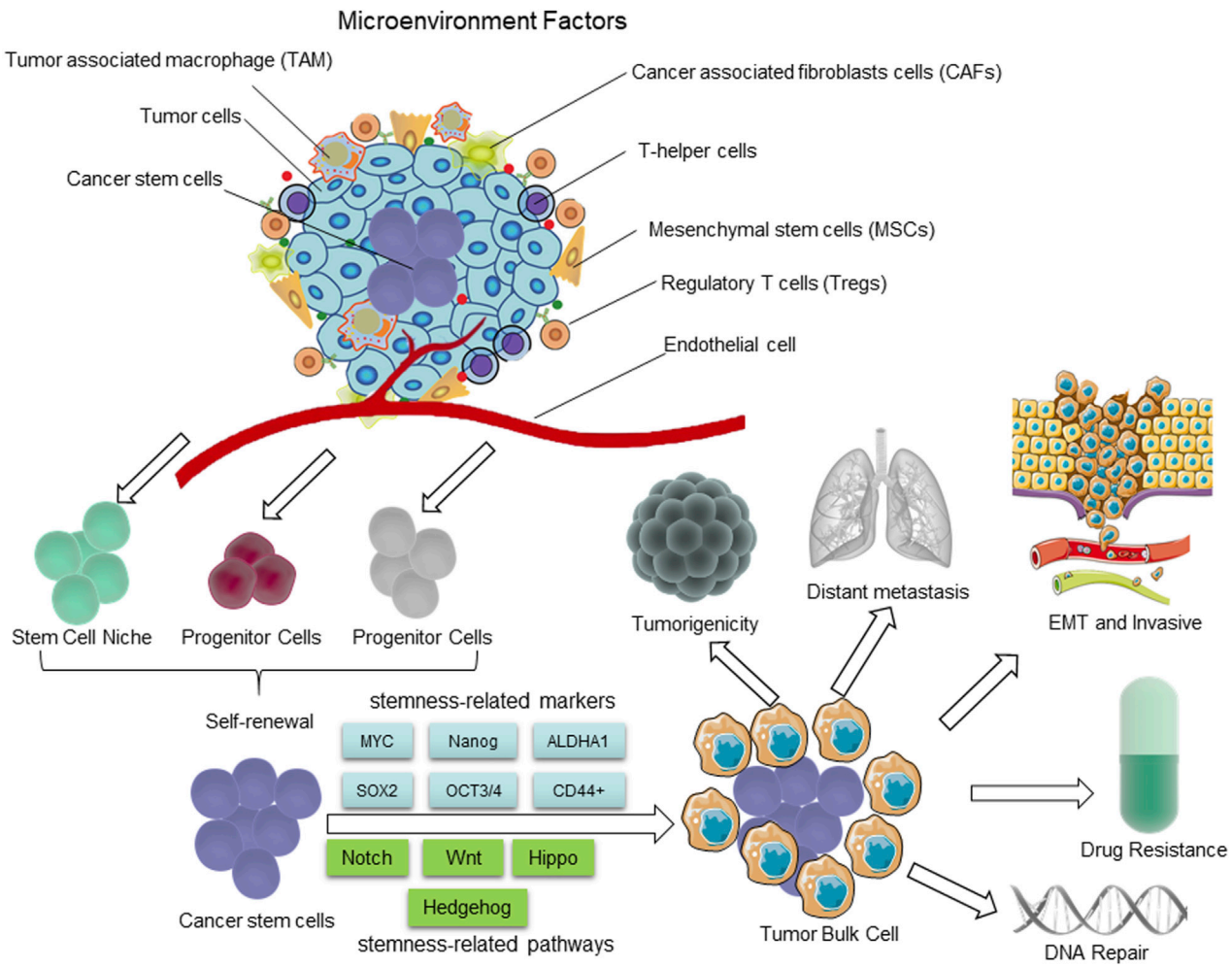


FIGURE 1
The distinguishing characteristics of CSCs. The presence of CSCs is closely linked to the tumor microenvironment (TME). The self-renewal capability of CSCs is significantly enhanced to certain degrees, comparable to or even surpassing that of normal pluripotent and multipotent stem cells; however, there is a partial loss in their differentiation capacity. During the process of epithelial-mesenchymal transition (EMT), cancer cells acquire characteristics resembling those of stem cells, enabling them to migrate and invade foreign tissues. The hierarchical plasticity subpopulations of CSCs are the primary drivers of tumorigenesis, EMT, tumor progression, metastasis, and drug resistance. The regulation of these CSC characters is mediated by multiple signaling pathways, including Notch, Wnt, Hippo cascades, Hh, *etc.*

tumors (Prasetyanti and Medema, 2017).The identification of CSCs in leukemia was initially achieved through the experimental technique of xenotransplantation (Bonnet and Dick, 1997). The presence of distinct populations of CSCs has been demonstrated in the majority of blood cancers and solid tumors, including breast, brain, colon cancers, and melanoma (Al-Hajj et al., 2003; Singh et al., 2004; Galli et al., 2004; OBrien et al., 2007; Schatton et al., 2008). The presence of CSCs renders them resistant to cytotoxic treatment and significantly contributes to the tumor's resistance against radio/chemotherapy (Shiozawa et al., 2013). CSCs also initiate metastasis and drive cancer relapse by their capacity for self-renewal and proliferation, enabling them to expand into the bulk of the tumor (Reya et al., 2001). The pressing demand for the advancement of innovative therapies that efficiently eliminate CSCs remains paramount.

The metabolic reprogramming has emerged as a pivotal hallmark of cancer in recent years (Hanahan and Weinberg, 2011). It has been demonstrated that the initiation and progression of cancer are frequently accompanied by profound metabolic alterations (Cao and Yan, 2020; Martinez-Reyes and Chandel, 2021). Notably, the biological characteristics of TICs and CSCs differ from those of non-CSCs (Dando et al., 2015). The metabolic regulation of ATP synthesis and bioconstruct formation in CSCs differs from that of non-stem cancer cells, but resembles that of stem cells derived from normal tissue (Sancho et al., 2016). The role of metabolism in the biology of CSCs has emerged as a prominent area of research over the past decade, with particular emphasis on lipid metabolism. Various studies have demonstrated the crucial role of lipid metabolism in preserving the stemness of CSCs and fulfilling their energy demands, ultimately contributing to cancer progression (Martinez-Outschoorn et al., 2017). For example, the overexpression of acetyl coenzyme a synthase (ACSL1 and ACSL4) and sterol coenzyme a desaturase (SCD) induces epithelial-mesenchymal transition (EMT) in colorectal cancer, thereby enhancing the migratory and invasive capabilities of tumor cells (Sanchez-Martinez et al., 2015). The

overexpression of CD36 enhances the uptake of fatty acids and activates Wnt-dependent EMT in hepatocellular carcinoma (HCC) (Nath et al., 2015). Notably, CSCs may originate from normal stem cells or tissue progenitor cells due to stochastic genetic mutations and epigenetic alterations, with tumor progression associated with genome-wide epigenetic regulation influencing CSC maintenance and survival via diverse pathways (Peiris-Pages et al., 2016). The occurrence of abnormal epigenetic modifications can induce the conversion of normal stem cells into CSCs, for example, the processes of DNA methylation and histone modification play crucial roles in guiding the differentiation of stem cells into specific cell and tissue types (Toh et al., 2017). The differentiation of CSCs can be inhibited through the suppression of gene expression via H3K27me3 modification and/or DNA methylation, in a manner analogous to that observed in ESCs (Easwaran et al., 2012). The crucial role of DNA methylation in maintaining the properties of CSCs in leukemia, lung, and colon has been extensively reported (Broske et al., 2009; Brunetti et al., 2017; Maiuri et al., 2019; Liang et al., 2021). Moreover, DNA methylation plays a pivotal role in facilitating this transformation process through the involvement of DNA methyltransferases (Wongtrakoongate, 2015). Notably, these CSC can maintain their stemness through their specific epigenetic alteration by regulating lipid metabolism, for example, CSCs can sustain their stemness through their super-enhancers by promoting polyunsaturated fatty acid (FA) (PUFA) synthesis (Gimple et al., 2019).

Besides, the epigenetic mechanisms also play a crucial role in regulating several key pathways of CSCs, including the Wnt/β-catenin, Hedgehog (Hh), and Notch signaling pathways. These pathways play a pivotal role in the development and maintenance of normal tissues, as well as in the self-renewal and differentiation of hematopoietic stem cells (Hoffmeyer et al., 2012; Myant et al., 2013) (Figure 1). Additionally, they also regulate the proliferation and maintain the stemness of progenitor cells and CSCs in a variety of tissues through modulating lipid metabolic process (Beachy et al., 2004; Yang et al., 2020; Wang et al., 2022). Furthermore, these pathways through which stem cells can be derived via genetic mutations and epigenetic alterations have a significant potential to be exploited for the maintenance of unrestricted proliferation, invasion, and drug resistance (Reya et al., 2001; Wang et al., 2022). This review presents a comprehensive overview of the metabolism of lipid droplets (LDs), FA, and cholesterol, as well as the impact of autophagy on maintaining stemness in CSCs. Furthermore, we investigate the characteristics and mechanisms of lipid metabolism in CSCs and their role in conferring resistance to radiotherapy.

2 Biological properties of CSCs

Over the past decade, numerous studies have been conducted to assess the expression profiles of cancer cells exhibiting stem cell properties in various solid tumors, leading to the identification of a plethora of biomarkers, pathways, and therapeutic targets against CSCs (Medema, 2013; Wang et al., 2014). The principal characteristics of CSCs encompass cell surface adhesion molecules, cytoprotective enzymes, transcription factors, and drug efflux pumps (Medema, 2013). However, the markers of CSCs in one organ or tissue differ from those in other organs or tissues, with only a few shared markers between them. Table 1 provides an overview of the most prevalent molecules that can serve as CSC biomarkers. Recently, the involvement of CSCs is pivotal in driving cancer progression, facilitating metastasis, promoting recurrence, and conferring resistance to cytotoxic therapies. The primary targets of classical radiotherapy and chemotherapy are predominantly fast-proliferating cells (Gerlinger et al., 2012). Unlike normal stem cells, cancer stem cells are believed to be responsible for tumor growth, recurrence, and drug resistance. One of the key characteristics of stem cells is their quiescent or dormant state, indicating infrequent division and prolonged periods in a dormant state. This attribute renders them less susceptible to conventional cancer therapies that primarily target rapidly dividing cells. However, recent studies have indicated that certain CSCs expressing leucine-rich repeat G protein-coupled receptor 5 (LGR5+) may not exhibit complete quiescence (Shiokawa et al., 2020). LGR5+ CSCs have been identified in various types of cancers, including colorectal, liver, and pancreatic cancers. It is believed that these cells undergo regular cell division cycles, indicating their cyclic nature. These findings hold significant implications as they challenge the previous notion of cancer stem cells being quiescent and suggest that targeting these cells may be more effective than conventional therapies aimed at rapidly dividing cells (Higa and Nakayama, 2024). Furthermore, it underscores the importance of comprehending the heterogeneity within the CSC population since different subpopulations may exhibit varying degrees of quiescence and sensitivity to treatment. In conclusion, while the concept of CSCs existing in a dormant state has long been fundamental in cancer research, recent evidence suggests that at least some CSCs, such as LGR5+ CSCs, are not entirely dormant. This discovery carries crucial implications for developing novel therapeutic strategies to target these cells and enhance cancer prognosis.

It is widely recognized that one of the primary challenges in cancer treatment lies in the development of drug resistance. Cellular plasticity has been identified as a pivotal factor contributing to the emergence of drug resistance (Boumahdi and de Sauvage, 2020). Cancer cell plasticity refers to a state wherein non-transformed differentiated cells exhibit adaptive plastic behavior under oncogenic stress (Figure 2). This phenomenon promotes diversity and heterogeneity among cancer cells within tumors, serving as a bypass mechanism to evade therapeutic agents. In the context of Darwinian selection, a vast number of non-CSCs conceals a small population of CSCs. According to the coexisting model theory, dynamic transcriptional fluctuations in individual cells give rise to therapeutic resistance markers, leading to resistance against treatment. The Lamarckian induction concept suggests that epigenetic modifications occurring in a small subset of cancer cells result in alterations of their drug-refractory phenotype and subsequently enhance therapeutic resistance. These changes in phenotype are commonly referred to as cellular plasticity (Vergara et al., 2019; Paul et al., 2022). This phenomenon promotes diversity and heterogeneity of CSCs within the tumor as a bypass mechanism to evade therapeutic agents.

In addition, CSCs express high levels of ATP-binding cassette transporters (ABC transporters), which contribute to the efflux of chemotherapeutic agents, leading to multidrug resistance

TABLE 1 The summary of diverse markers utilized for the identification of CSCs.

Tumor type	CSCs markers	Biological function	Reference
hepatocellular carcinoma, lung, melanoma, pancreatic	ABCG2	The ABCG2 protein is an ATP-binding cassette transporter primarily involved in drug metabolism and cellular drug resistance, potentially contributing to the development of chemotherapeutic drug resistance in tumor cells	Herpel et al. (2011), Jia et al. (2013), Su et al. (2016)
Breast, Colorectal, Esophageal, Glioblastoma, Liver/Lung adenocarcinoma, Nasopharyngeal	SOX2	The dysregulated expression of SOX2 has been implicated in various cancer types, and research studies have demonstrated that SOX2 exerts a positive influence on key characteristics of cancer cells, including proliferation, migration, invasion, and metastasis. In addition, SOX2 mediates resistance to existing cancer therapies and is expressed in CSCs	Novak et al. (2020)
leukemia, liver, colorectal, prostate, ovarian, lung, head and neck, brain, pancreatic, gastric and breast cancer	Nanog	The transcription factor Nanog is widely recognized as a pivotal marker for the identification of CSCs. The activation of Nanog via distinct signaling pathways, such as JAK/STAT and Wnt/β-catenin cascades, elicits stemness, self-renewal capacity, metastatic potential, invasiveness, and chemoresistance in cancer cells	Vasefifar et al. (2022)
Colorectal, head and neck, Lung, pancreatic cancer	CD166 (ALCAM)	CD166 (ALCAM) is a cell surface molecule that is a member of the immunoglobulin superfamily. CD166 is widely expressed in various tumors, and its biological functions in tumor stem cells primarily encompass the facilitation of tumor growth and metastasis, enhancement of tumor cell survival, modulation of immune response, involvement in the formation of the tumor microenvironment, as well as regulation of self-renewal and differentiation processes within tumor stem cells	Jiao et al. (2012), Yan et al. (2013a), Margaritescu et al. (2014)
Breast, lung, colorectal, liver, gastric, cervical, esophageal, ovarian, head, and neck cancer	ALDH1	The expression of ALDH1 is considered as a reliable indicator for the presence of cancer stem cells (CSCs), which play a crucial role in tumorigenesis by preserving CSC properties, modulating cellular metabolism, and facilitating DNA repair mechanisms	Wei et al. (2022)
Breast, colorectal, glioma, liver, lung, ovarian, pancreatic, prostate	CD44	The cell surface glycoprotein functions as a receptor for various extracellular matrix components, such as acid hyaluronic, collagen, integrins, and metalloproteinases, thereby facilitating cellular migration and self-renewal	Todaro et al. (2014), Yan et al. (2015), Erhart et al. (2019), Herreros-Pomares et al. (2019)
Breast, glioma, liver, lung	CD90 (THY1)	The involvement of a highly conserved glycophosphatidylinositol (GPI)-anchored cell-surface eggplant protein in T cell adhesion and signaling, promotion of tumor growth and metastasis, as well as regulation of self-renewal and differentiation of tumor stem cells has been observed	Yan et al. (2013b)
Colorectal, liver, lung, ovarian, prostate	CD326	The transmembrane glycoprotein CD326, also known as EpCAM (Epithelial Cell Adhesion Molecule), is predominantly expressed on normal epithelial cells and functions as a homotypic calcium-independent cell adhesion molecule. It plays crucial roles in tumor stem cells by promoting cell proliferation, inhibiting apoptosis (programmed cell death), and maintaining stem cell properties. This makes CD326 an important therapeutic target.	Sayd et al. (2014)

(Continued on following page)

TABLE 1 (*Continued*) The summary of diverse markers utilized for the identification of CSCs.

Tumor type	CSCs markers	Biological function	Reference
Colorectal, Breast, lung, prostate	Integrinα6β4	A cell adhesion molecule that specifically binds to laminin in the extracellular matrix and initiates the formation of semimembranous vesicles, thereby facilitating cellular migration and invasion, has been found to be closely associated with the proliferation, invasion, and metastasis of tumor stem cells. Integrin α6β4 interacts with the extracellular matrix, promoting survival and proliferation of tumor stem cells while aiding in evading immune system surveillance by tumor cells	Subramaniam et al. (2018)

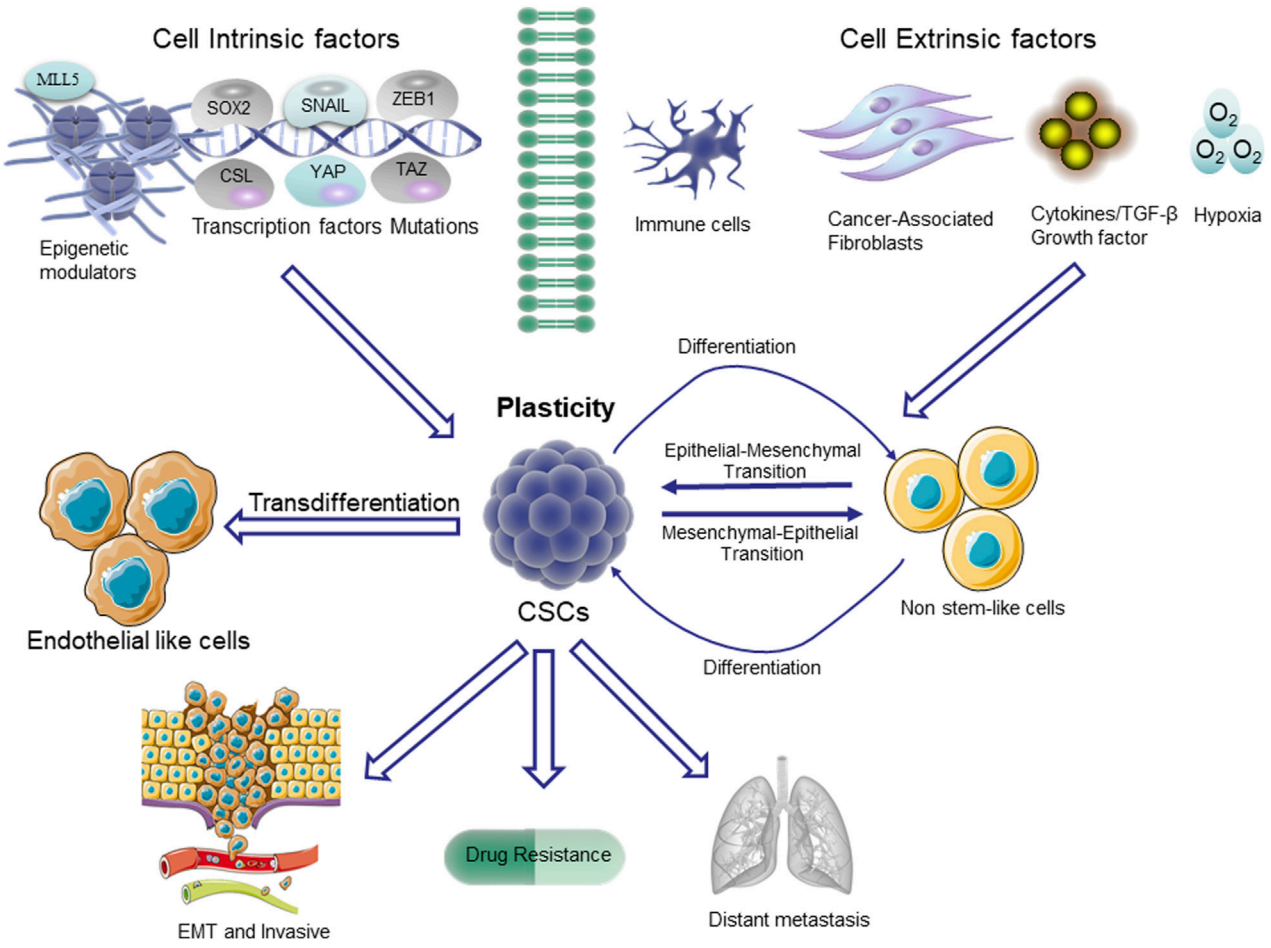


FIGURE 2
Plasticity in CSCs. The non-stem cells depicted in the figure typically exhibit restricted tumorigenic capacity and maintain their differentiated state. The upregulation of the stem cell pathway in these cells enables them to undergo a transition to a higher level of cellular state, referred to as plasticity. The pathways that trigger stem cell differentiation are mediated through transcription factors involved in epithelial-mesenchymal transition (EMT), such as SNAIL and ZEB1, as well as stem cell-specific transcription factors like SOX2. Additionally, the Hippo/YES-associated protein (YAP)/tafazzin (TAZ) signaling pathway, NOTCH/CSL pathway, and the epigenetic regulator MLL5 also play crucial roles in this process.

(Dean et al., 2005; Robey et al., 2018). In order to develop novel and efficacious therapeutic strategies targeting the stem cell-like subpopulations of tumor cells, it is imperative to gain comprehensive insights into the characteristics of CSCs and elucidate the underlying mechanisms responsible for their acquired resistance and stem cell-like properties, which are closely associated with CSCs plasticity, senescence, and quiescence. The concept of plasticity refers to the phenomenon that stem cells can generate tumor cells through asymmetric division (Vlashi and Pajonk, 2015). However, this hierarchy is not unidirectional; tumor cells have the ability to undergo de-differentiation and acquire stem-like properties (Figure 2). The

occurrence of plasticity can be attributed to genetic and epigenetic alterations. For example, the non-CSC tumor cells in the basal-like subtype of breast cancer have the ability to undergo a transition to a CSC-like state through ZEB1 (Chaffer et al., 2013). In glioblastoma (GBM), cancer cells expressing CSC markers do not represent a functionally distinct clonal entity but rather exhibit a phenotypic plasticity that can be induced by microenvironmental cues (Dirkse et al., 2019). Recent findings from single-cell RNA sequencing analyses of human GBM tumors have revealed intratumoral heterogeneity, indicating the simultaneous presence of three cancer grades and cellular plasticity within the tumors (Patel et al., 2014; Neftel et al., 2019; Couturier et al., 2020; Lopes and Vinga, 2020). The study conducted by Neftel reveals that plasticity encompasses not only the process of de-cellularization but also the dynamic transition between distinct cell states, including intratumoral heterogeneity. This is exemplified by the simultaneous presence of 4 cell states within a single GBM, namely, neural progenitor cells, oligodendrocytes, astrocytes, and mesenchymal-like cells, which can interconvert among each other (Neftel et al., 2019). The rapid changes in cellular state can significantly impede therapies aimed at targeting specific tumor cell states. Cellular senescence is a stress response triggered by various molecular damages, resulting in cell cycle arrest, and characterized by diverse phenotypic alterations, including the secretion of bioactive molecules. Senescent cells progressively accumulate during the aging process and are observed in cancerous and fibrotic lesions (Bousset and Gil, 2022). The process of cellular senescence exerts a well-established tumor-suppressive effect by constraining the tumorigenic potential of cancer cells and enhancing the efficacy of cytotoxic therapies (Braig et al., 2005; Michaloglou et al., 2005; Dorr et al., 2013). However, the co-regulation of senescence and stemness functions through overlapping signaling pathways such as p16, p21, and p53 suggests that senescence may trigger genetic reprogramming and activate stemness, thereby contributing to CSC-mediated tumor progression, metastasis, and therapy resistance (Zon, 2008; Milanovic et al., 2018). A recent study has also provided insights into dysfunctional aging from a molecular perspective: in response to genotoxic damage or oncogenic stresses, tumor cells activate extensive chromatin remodeling that involves the addition of repressive methylation marks. The aforementioned marks exert a stable repression on S-phase promoter genes, while concurrently enhancing the secretion of pro-tumorigenic factors and activating stem cell transcription factors such as WNT/lymphocyte enhancer factor 1 (LEF1) (Lecot et al., 2016; Milanovic et al., 2018). The elucidation of the pathways involved in this reprogramming mechanism during cellular senescence will facilitate the overcoming of challenges posed by current therapeutic approaches through targeted CSCs.

The ability of CSCs to transition into a dormant or quiescent state, specifically entering the reversible G0 phase of the cell cycle and remaining dormant, is another distinguishing characteristic (Chen W. et al., 2016). This state transition is typically induced by the microenvironment, such as hypoxia, nutrient deprivation, or oxidative stress, or by selective pressure from chemotherapeutic agents. The fact that most conventional cancer treatments primarily target actively dividing cells allows quiescent CSCs to evade the effects of such therapies and tend to transition back into a proliferative state when favorable conditions arise (Sosa et al., 2014; De Angelis et al., 2019). The resistance of slow-circulating CSCs to temozolomide treatment in GBM was determined through strain tracing. Interestingly, the restoration of sensitivity to temozolomide was observed upon ablation of this specific population (Chen et al., 2012). The future plays a crucial role in the successful treatment of cancer through the identification of CSCs derived from quiescent or dormant cell populations and their associated maintenance pathways.

3 Metabolic properties of CSCs

Due to the heterogeneity of tumor cells, cancer cells heavily rely on glucose and aerobic oxidation for energy supply, distinguishing their energy metabolism from that of normal cells (Liberti and Locasale, 2016). The reprogramming of metabolism is a hallmark feature exhibited by cancer cells (Hanahan and Weinberg, 2011). The metabolic characteristics of cancer cells differ significantly from those of normal cells. Given the hypoxic, highly oxidized, acidic, and nutrient-deprived TME resulting from rapid tumor cell proliferation and inadequate blood vessel formation, cancer cells must effectively adapt their cellular bioenergetics to survive in this unfavorable milieu (Viale et al., 2014; Lue et al., 2017). This adaptive process is commonly referred to as metabolic reprogramming. Metabolic reprogramming becomes indispensable for sustaining cancer cell proliferation and survival when oncogenic signaling is obstructed. The majority of human cancers exhibit aerobic glycolysis even in the presence of abundant oxygen, a phenomenon commonly referred to as the Warburg effect (Danhier et al., 2017; Strickland and Stoll, 2017). The Warburg effect characterizes the metabolic shift from oxidative phosphorylation (OXPHOS) to glycolysis in the pentose phosphate pathway, accompanied by the substitution of lactate accumulation for sustained ATP production in the TME (Koren and Fuchs, 2016). The metabolic rewiring not only fulfills the energy demands for sustained proliferation, but also generates a substantial amount of substrate for cellular compartments. It has been shown that poorly differentiated cancers exhibit higher glucose uptake than differentiated cancers, suggesting that the high glycolytic flux in tumor tissues arises mainly from impaired differentiation of CSCs (Riester et al., 2018). The activation of mitochondrial metabolism, in contrast, results in the loss of pluripotent potential and triggers the differentiation of P19 embryonal carcinoma stem cells (Vega-Naredo et al., 2014). The emerging evidence suggests that the Warburg effect of glycolytic metabolism is implicated in the processes of stemness and EMT (Aguilar et al., 2016). Further findings also provided compelling evidence that the regulation of stem cell metabolism plays a pivotal role in governing the control of stem cell fate. For instance, the compound R406 functions as a Syk inhibitor in immune thrombocytopenia by inducing a metabolic shift from glycolysis to OXPHOS in glioma stem cells (GSCs). This metabolic alteration subsequently leads to an excessive production of reactive oxygen species (ROS), ultimately triggering apoptosis in GBM cells (Sun et al., 2019). Peng et al. demonstrated that the overexpression of pyruvate dehydrogenase kinase 1 (PDK1) in breast CSCs (BCSCs) leads to the inhibition of aerobic glycolysis in mitochondria. The depletion of PDK1 resulted in a significant reduction in ALDH1-positive BCSCs, thereby impairing their ability

to form spheroids (Peng et al., 2018). During the process of EMT in basal-like breast cancer, SNAIL-mediated methylation of the fructose-1,6-bisphosphatase promoter enhances the characteristics of CSCs and tumorigenicity by increasing glucose uptake and macromolecule biosynthesis. Additionally, it inhibits oxygen consumption through the inhibition of mitochondrial complex I activity (Dong et al., 2013). The presence of mutations in the internal tandem duplication (ITD) of the Fms-like tyrosine kinase 3 gene (FLT3/ITD) in acute myeloid leukemia (AML) is considered an unfavorable genetic alteration associated with a poor prognosis (Burchert, 2021). H-Q Ju et al. reported that FLT3/ITD induces a significant increase in aerobic glycolysis through akt-mediated upregulation of mitochondrial hexokinase 2, leading to heightened reliance on glycolysis and enhanced sensitivity of leukemic cells to pharmacological inhibition of glycolytic activity. The preferential inhibition of glycolysis results in severe depletion of ATP and extensive cell death in FLT3/ITD leukemia cells (Ju et al., 2017).

Notably, although the utilization of glycolysis for survival has been reported in some studies, others have suggested that CSCs may also rely on OXPHOS for their survival (Ciavardelli et al., 2014; Pasto et al., 2014). The transfer of electrons and H+ from the donor molecules, reduced nicotinamide adenine dinucleotide or reduced flavin adenine dinucleotide, to the acceptor molecule O_2 is accompanied by the liberation of energy for ATP synthesis, involving a series of protein complexes located in the inner mitochondrial membrane. LAGADINOU et al. discovered that AML cells enriched with low levels of ROS from leukemia stem cells (LSCs) exhibited elevated expression of B-cell lymphocytoma-2 (Bcl-2), an antiapoptotic protein involved in mitochondrial regulation. Furthermore, Bcl-2 inhibition hampers ATP production in leukemia stem cells (LSCs) by hindering oxidative phosphorylation. Unlike AML cells and normal $CD34^+$ cells, LSCs lack efficient backup glycolysis, making this metabolic vulnerability a promising target for selective elimination in clinical applications (Lagadinou et al., 2013). Additionally, through isotope tracing combined with metabolomics, researchers have demonstrated that LSCs exhibit enhanced efficiency in converting stearic acid and glucose into intermediates of the tricarboxylic acid cycle compared to other chronic myeloid leukemia cells, indicating a high dependence on OXPHOS (Kuntz et al., 2017). Similarly, the crucial significance of OXPHOS in solid tumors persists for CSCs. Ciavardelli et al., 2014 demonstrated that the proliferation of CD44+/CD24-breast CSCs could be reduced by inhibiting their glycolysis, indicating a glycolytic nature of this specific population. The proto-oncogene-encoded transcription factor MYC and the anti-apoptotic protein MCL1 synergistically enhance OXPHOS in CSCs, thereby promoting chemoresistance maintenance in triple-negative breast cancer (TNBC) (Lee et al., 2017). The results of clinical studies also indicate that well-differentiated tumors exhibit decreased uptake levels of 18F-fluorodeoxyglucose, whereas poorly differentiated tumors demonstrate elevated uptake levels of 18F-fluorodeoxyglucose (Riester et al., 2018). Additionally, the levels of oxidative metabolism and ATP in GSCs are higher compared to differentiated tumor cells and inhibiting OXPHOS, but not glycolysis, significantly impairs the tumorigenic potential and survival ability of GSCs in xenograft models (Minami et al., 2021). Notably, the nutrient-deprived CSCs in GBMs preferentially utilize the pentose phosphate shunt, thereby facilitating the self-renewal, proliferation, and survival of CSCs (Kathagen et al., 2013). Furthermore, the regulation of OXPHOS in GSCs is mediated by endogenous insulin-like growth factor 2 mRNA binding protein 2 (IMP2), which plays a crucial role in the transportation of mRNAs encoding respiratory chain-associated components to the mitochondria and facilitates OXPHOS maintenance through its involvement in the assembly of respiratory chain complexes (Janiszewska et al., 2012). In another study, based on the energy metabolism characteristics of pancreatic CSCs, a shift in the carbon source was employed by replacing glucose with galactose to induce enhanced OXPHOS activity in pancreatic cancer cells under *ex vivo* conditions. Consequently, this approach led to the enrichment of pancreatic CSCs, which exhibit upregulated expression of CSC surface antigens, heightened tumorigenicity, and immune evasion properties (Valle et al., 2020). Importantly, nasopharyngeal carcinoma, ovarian cancer, osteosarcoma, GBM, and colon cancer heavily rely on mitochondrial OXPHOS for energy generation (Zhou et al., 2011; Liao et al., 2014; Palorini et al., 2014; Shen et al., 2015; Song et al., 2015). These findings suggest that targeting aerobic glycolysis or OXPHOS could be a potential strategy for eradicating CSCs.

4 Key modulators of lipid metabolism in CSCs

The alteration of cellular metabolism, particularly in lipid metabolism, has recently been acknowledged as a distinctive characteristic of various cancer cells (Yi et al., 2018). The lipid category encompasses various types of lipids, such as phospholipids, cholesterol and cholesterol esters, while fats primarily refer to triglycerides (TG). Lipids play a crucial role in numerous cellular functions, including membrane formation, signaling pathways, and other biological activities, while TG serves as a significant source of cellular energy (Lingwood and Simons, 2010). A growing body of evidence suggests that cancer cells undergo alterations in various aspects of cell membrane formation, lipid synthesis and degradation, as well as lipid-driven cellular signaling. A hallmark of cancer metabolism is the upregulation of *de novo* lipogenesis (Kinlaw et al., 2016), as illustrated in (Figure 3). The energy requirements of cancer cells are primarily met through *de novo* lipogenesis, as dietary lipids are limited in availability, unlike most non-malignant cells. The metabolic intermediates of glycolysis may be redirected towards enhanced lipid biosynthesis by CSCs, thereby promoting self-renewal growth (Corominas-Faja et al., 2014). The expression of various lipid synthases is elevated in cancer cells, including sterol regulatory element binding proteins (SREBPs), ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl-CoA desaturase 1 (SCD1) (Currie et al., 2013; Sun et al., 2016; Geng et al., 2016; Rohrig and Schulze, 2016; Shimano and Sato, 2017). The citric acid (TCA) cycle also plays a crucial role in lipid metabolism by providing acetyl groups for FA synthesis, thereby contributing to the maintenance of malignant biological behavior in cancer cells (Williams and

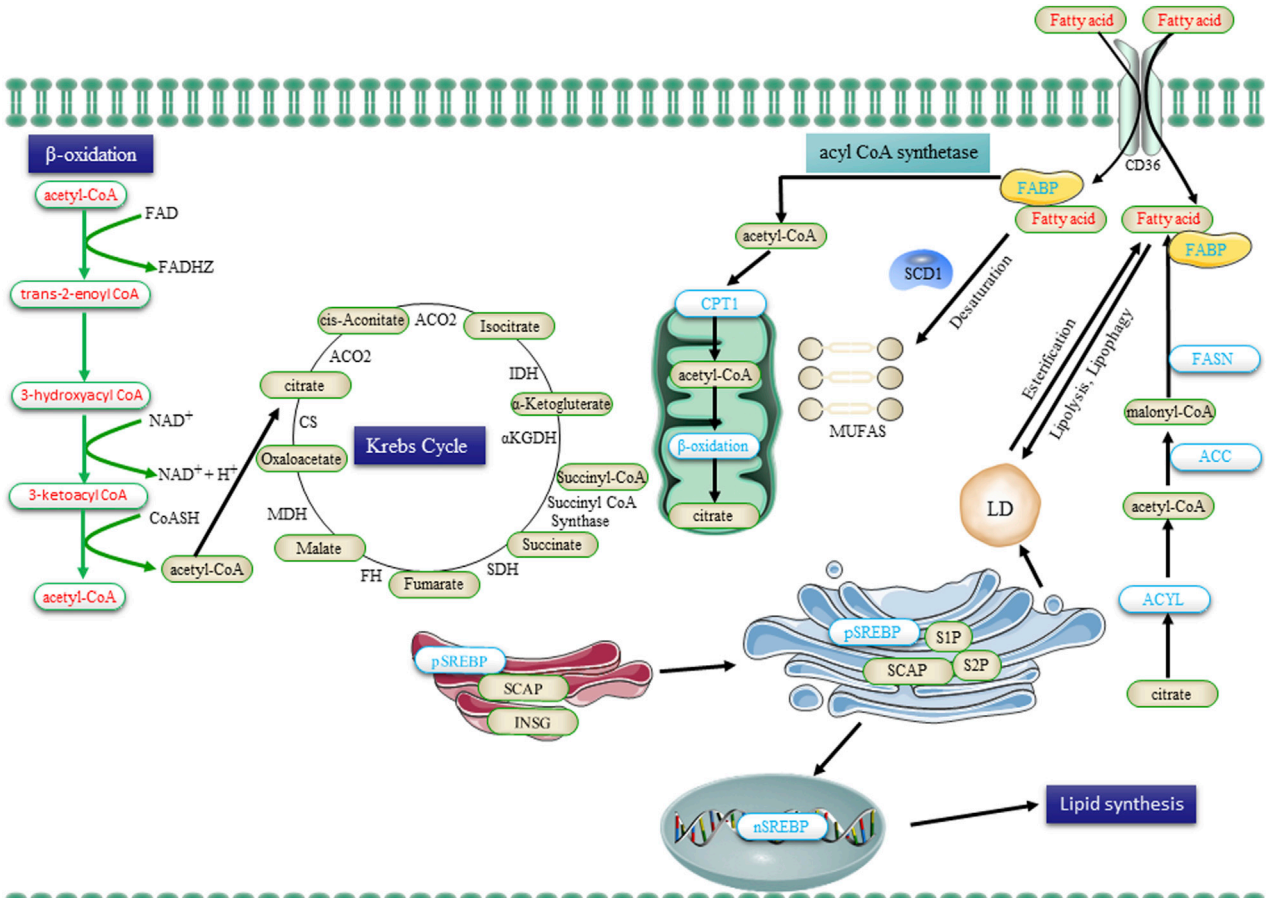


FIGURE 3
The lipid metabolism in CSCs, encompassing uptake, lipogenesis, and utilization, undergoes alterations. Extracellular FA is transported into cells via the CD36 receptor and subsequently undergone β-oxidation in mitochondria to generate acetyl-CoA. Acetyl-CoA is then converted to citrate by the enzyme citrate synthase, which enters the Krebs cycle for complete oxidation. The *de novo* synthesis of FA initiates with acetyl-CoA and proceeds through the sequential addition of two-carbon units, a process tightly regulated by the key enzyme FASN. Transcriptional control exerted by SREBPs governs the majority of enzymes involved in FA synthesis. Saturated FA undergoes desaturation to form monounsaturated FA (MUFA) catalyzed by SCD1. Additionally, FA is esterified to glycerol and subsequently stored as triglycerides in LDs. FA, obtained from the uptake of exogenous lipids or through *de novo* lipogenesis, is released to provide cellular fuel via FAO in the mitochondria, with CPTI catalyzing the rate-limiting step. Abbreviations: FA, fatty acid; FABP, FA binding protein; FASN, FA synthase; ACC, acetyl-CoA carboxylase; SREBP, sterol regulatory element binding protein; SCAP, SREBP cleavage-activating protein; INSG, insulin-induced gene protein; SCD1, stearoyl-CoA desaturase 1; MUFAs, mono-unsaturated FA; CPT1, carnitine palmitoyltransferase 1; and LDs, lipid droplets.

ONeill, 2018). Here, we will concentrate the roles of these master regulatory elements in CSC progression.

4.1 ACC

The activation of ACC leads to the catalysis of the conversion from acetyl coenzyme A (AcCoA) to malonyl-coenzyme A, which plays a crucial role in *ab initio* FA synthesis, particularly when cells require additional FA for energy demands or other biosynthetic processes. CSCs typically exhibit a heightened capacity for FA synthesis owing to their substantial energy demands required to sustain their highly active metabolic state (Liu et al., 2022). Additionally, ACC exhibits high expression levels in induced pluripotent stem cells (iPSCs) and suppression of ACC expression significantly diminishes the reprogramming efficiency of iPSCs (Vazquez-Martin et al., 2013). As normal stem cells always share the similar reprogramming procedure with that of CSCs (Zheng et al., 2019), this pronounced upregulation of ACC and FASN in iPSC underscores the significance of adipogenesis in stem cells and paves the way for potential therapeutic applications for CSCs. Thus, targeting ACC has been shown to suppress CSC progression, such as the ACC inhibitors *in vitro* reinstated the tumor cells to a histological epithelial phenotype (Petrova et al., 2017), and the inhibition of ACC in pancreatic cancer cells effectively suppresses both *in vivo* and *in vitro* pancreatic tumor growth by attenuating the ligand palmitoylation of Wnt and Hh, thereby inhibiting the signaling pathways of Wnt and Hh (Petrova et al., 2017), both of which are also implicated in the regulation of ACC in CSCs, as evident by that β-catenin knockdown leads to an upregulation of ACC expression (Vergara et al., 2017). Additionally, the inhibition of ACC activation effectively restored intracellular

lipid levels, attenuated EMT, and suppressed the characterization of CSCs (Bort et al., 2020). Currently, given its pivotal role in lipid metabolism within CSCs, ACC represents a promising target for potential therapeutic interventions against numerous tumors (Moncur et al., 1998; Fang et al., 2014; Svensson et al., 2016).

4.2 ACLY

Located predominantly in the cytoplasm, ACLY facilitates the enzymatic conversion of citric acid into AcCoA and oxaloacetate, thereby playing a crucial role in FA and cholesterol synthesis within CSCs (Feng et al., 2020). AcCoA serves as a crucial substrate not only for FA and cholesterol synthesis, but also plays an essential role in protein acetylation reactions, consequently emerging as a pivotal enzyme in lipid synthesis and connecting catabolic pathways with biosynthesis (Metallo et al., 2011). The upregulation and activation of ACLY have been extensively documented in various malignancies, as evidenced by the association with an increased malignant phenotype and a poor prognosis (Hatzivassiliou et al., 2005; Qian et al., 2015). Additionally, ACLY can facilitate tumor stemness through the downstream effectors, such as overexpression of ACLY enhances the expression of Snail, an EMT master regulator, thereby promoting EMT and stemness (Hanai et al., 2013). Conversely, inhibition of ACLY diminishes the invasiveness of breast cancer cells, while targeting ACLY reduces the proliferative potential and cisplatin resistance of ovarian cancer cells (Lucenay et al., 2016; Wei et al., 2021). Notably, Migita T et al. discovered a positive correlation between phosphorylation of ACLY in the PI3K/AKT pathway and the stage, grade of tumor differentiation, as well as poor prognosis in non-small cell lung cancer. This particular form of phosphorylation is believed to significantly enhance the role of ACLY in CSCs (Migita et al., 2008).

4.3 CD36

The transmembrane glycolipid protein CD36 is extensively expressed in various cell types, encompassing adipocytes, myocytes, endothelial cells, macrophages, and hepatocytes (Yang P. et al., 2022). CD36 protein exhibits numerous biological functions, encompassing lipid metabolism, inflammatory response, apoptosis, cell migration, and tumorigenesis (Zhao L. et al., 2018; Wang and Li, 2019). Elevated CD36 expression in tumor cells plays a pivotal role in lipid metabolism by facilitating the uptake and utilization of saturated fatty acids, such as palmitic acid, thereby contributing to both lipid synthesis and catabolism processes and augmenting proliferation, invasion, and metastatic potential (Li et al., 2022; Yuan et al., 2022). Additionally, CD36 is implicated in the crucial FA metabolic pathway known as β-oxidation (Zeng et al., 2022). Consistently, the blockade of β-oxidation through the targeting of CD36 with neutralizing antibodies has the potential to completely eradicate metastasis in melanoma and breast cancer (Pascual et al., 2017). In GSCs, CD36 facilitates the uptake of oxidized phospholipids, thereby promoting the proliferation of GSCs (Hale et al., 2014). Specifically, CD36 recognizes and binds to oxidized phospholipids, internalizing them into the intracellular compartment, thereby facilitating the proliferation and self-renewal of GSCs, this is attributed to the inhibitory effect exerted by elevated CD36 levels on the activation of apoptotic signaling pathways, thereby safeguarding GSCs against death-inducing stimuli. Additionally, with respect to non-solid tumor hematopoietic stem cells, CD36-enriched LSCs derived from gonadal adipose tissue exhibit enhanced survival advantages and resistance to treatment (Ye et al., 2016).

4.4 FASN and FA binding protein (FABP)

Recently, it has been demonstrated that lipids and lipoproteins, both acquired through exogenous (or dietary) uptake and synthesized endogenously, exert a profound influence on the maintenance of CSCs' stemness during tumorigenesis. FASN, a key enzyme in *de novo* lipid synthesis, has consistently been identified as a facilitator in various types of CSCs (Ali et al., 2018; Rabionet et al., 2021; Castagnoli et al., 2023). Interestingly, overexpression of FASN in patient-derived GSCs was significantly diminished during serum-induced differentiation, indicating that augmented *de novo* adipogenesis contributes to the maintenance of the undifferentiated state of GSCs. After treatment with 20 μM cerulenin, a pharmacological inhibitor of FASN, the proliferation and migration of GSCs were significantly suppressed, and *de novo* lipogenesis was reduced. Additionally, the expression levels of nestin, Sox2, and FABP7, which are markers of GCSs, are decreased while the expression level of glial fibrillary acidic protein increased (Yasumoto et al., 2016). However, to date, limited research has been conducted regarding the involvement of FABPs in CSCs. The FABPs, functioning as lipid chaperones, are believed to bind and transport FA across various cellular compartments and organelles including plasma membranes, mitochondria, LDs, endoplasmic reticulum, and nuclei. A notable exception is FABP7, a widely recognized neural stem cell marker that exhibits high enrichment in GSCs and demonstrates significant downregulation in differentiated GSCs (Morihiro et al., 2013). Furthermore, Antonella De Rosa et al. showed that FABP7 downregulation in GSCs is associated with decreased proliferation and migration ability (De Rosa et al., 2012). Through proteomic and metabolomic analyses, Brandi et al. demonstrated that pancreatic CSCs exhibit elevated glycolysis levels and increased *de novo* adipogenic activity, while displaying reduced mitochondrial OXPHOS levels compared to a significant number of parental cancer cells. The authors discovered that FASN exhibited higher expression levels in Panc1 CSCs and displayed increased sensitivity to cerulenin inhibition compared to parental non-stem cell cancer cells (Brandi et al., 2017). Additionally, the expression level of FASN is regulated by β-catenin and exhibits a positive correlation with the expression levels of stem cell markers (SOX2, CD133, and Nestin) in GSCs (Yasumoto et al., 2016; El-Sahli et al., 2019). Importantly, FASN inhibitors have been shown to decrease the expression of stemness markers in GSCs (Yasumoto et al., 2016). For instance, resveratrol has the ability to induce apoptosis in CSCs by inhibiting adipogenesis through the downregulation of FASN expression (Pandey et al., 2011). Notably, CSCs often show a positive correlation between the levels of FASN and ACC expression under specific conditions,

and FASN is more vulnerable to attacks in CSCs compared to regular cancer cells. Ongoing molecular and cell-based preclinical studies have focused on the development and characterization of various FASN blockers. Despite these efforts, translating these promising findings into clinical applications remains a challenging endeavor (Menendez and Lupu, 2017; Schcolnik-Cabrera et al., 2018). Therefore, numerous efforts should be directed towards several key areas. Firstly, there is a need for a comprehensive investigation into the interplay between FASN and ACC expression in CSCs to enhance our understanding of their correlation in CSCs. Additionally, a deeper exploration of the mechanisms underlying the increased vulnerability of FASN in CSCs compared to normal cancer cells is essential for the development of more effective therapeutic strategies. Optimization of existing FASN blockers is crucial, ensuring they exhibit high selectivity and efficacy in inhibiting FASN within CSCs. Moreover, emphasis should be placed on translating molecular and cell-based preclinical research findings into clinical applications by designing improved drug delivery systems and refining precision treatment protocols. Finally, addressing challenges associated with clinical applications, such as formulating suitable treatment regimens, ensuring drug safety and efficacy, and accommodating individual patient variations, is vital for the successful implementation of these research outcomes in the clinical setting. Through these concerted efforts, a better understanding of FASN's role in CSCs can be achieved, leading to improved treatment strategies and ultimately facilitating the translation of these research findings into clinical applications.

4.5 Lipid Stearoyl-CoA desaturase-SCD1

Recently, multiple studies have demonstrated that an elevation in FA within cancer cells may also heighten their reliance on desaturase activity (Peck and Schulze, 2016). The promotion of CSCs through the regulation of unsaturated FA has been demonstrated in several studies, highlighting the significance of signaling pathways in this process, for example, NF-κB, a pivotal regulator of tumors and CSCs, directly governs the expression and activation of lipid desaturases (Li et al., 2017). Moreover, inhibition of adipogenesis through desaturase inactivation effectively disrupts the AKT/ERK-mediated NF-κB signaling pathway (Fritz et al., 2010; Li et al., 2017). Similarly, the levels of SCD-dependent MUFAs directly regulate CSCs through the Wnt/β-catenin pathway, which is a pivotal signaling pathway in stem cells and CSCs (Zheng et al., 2016; Lai et al., 2017). The two isoforms of SCDs in humans are SCD1 and SCD5, with SCD1 being the predominant enzyme responsible for desaturation in all tissues, while SCD5 is primarily expressed in the pancreas and brain (Wang et al., 2005). The expression of SCD-1, is significantly upregulated and contributes to the progression of cancer. It catalyzes the conversion of saturated FA into Δ9-MUFA (AM et al., 2017). Noto et al. demonstrated that the gene encoding SCD1 exhibited the highest level of upregulation in lung tumor spheroidal cells with adherent cultures, and further revealed that SCD1 inhibitors selectively eradicated cells possessing stem-like properties (Noto et al., 2013). Additionally, their subsequent investigation demonstrated that SCD1 governs the regulation of stem cells in lung cancer by stabilizing and localizing transcriptional co-activators of the Hippo pathway effector yes-related proteins and PDZ-binding motifs (Noto et al., 2017a). The Hippo pathway, which is regulated by YAP and TAZ, has been shown to facilitate the renewal and differentiation of both embryonic and somatic stem cells (Cao et al., 2020). Meanwhile, another one of their studies confirmed that the expression of SCD1 was associated with a poor prognosis in lung adenocarcinoma, and inhibiting the activity of SCD1 reversed resistance to cisplatin in lung CSCs (Pisanu et al., 2017). The significance of MUFAs is further underscored by the heightened levels of SCD1 expression in lung, ovarian, breast, and GSCs, which aligns with the presence of MUFAs in CSCs (Li L. et al., 2013; Colacino et al., 2016; Lobello et al., 2016; Noto et al., 2017b). Furthermore, SCD1 also governs the Wnt signaling pathway in CSCs (El-Sahli et al., 2019) and has been observed to play a crucial role in the maintenance of stem cells in various other cancers, including melanoma, hepatocellular carcinoma (HCC), and colon cancer (Pisanu et al., 2018; Choi et al., 2019; Ma et al., 2019). However, it must be noted that certain cancer cells utilize an alternative pathway for FA desaturation and sapienate biosynthesis, bypassing the established SCD-dependent pathway and diminishing the relevance of SCD, thereby questioning its suitability as a therapeutic target (Vriens et al., 2019).

4.6 Transcriptional Induction-SREBPs

The synthesis and activation of FA in cancer cells can be accomplished through various mechanisms, including transcriptional induction. The transcriptional regulation of SREBPs governs the majority of enzymes involved in FA synthesis. The SREBPs are a group of transmembrane transcription factors that activate the expression of genes encoding enzymes essential for cholesterol synthesis and the production of UFA. Human cells contain three isoforms of SREBP, namely, SREBPla, SREBPic, and SREBP2. Among these isoforms, both SREBPla and SREBP1c are derived from individual genes through distinct transcription start sites (Shimano and Sato, 2017; DeBose-Boyd and Ye, 2018). The SREBP1 protein is a member of the SREBP family of transcription factors and serves as a key transcriptional regulator in adipogenesis, controlling the synthesis of FA and cholesterol (Li L. et al., 2013). SREBP1 is essential for maintaining lipogenesis in lipid and hypoxic conditions, and it directly regulates several key lipogenic enzymes, including ACLY, ACC1, and FASN (Pandey et al., 2013; Shimano and Sato, 2017) (Figure 4). Overexpression of SREBP1 has been observed in various human cancers, promoting the growth of a wide range of tumors and playing an essential role in maintaining the stemness of CSCs (Pandey et al., 2013). For instance, PR Pandey et al. demonstrated that the ectopic expression of SREBP1 in MCF10A cells significantly augmented stem cell adipogenesis and facilitated cellular proliferation and mammosphere formation (Pandey et al., 2013). The expression of oncogenic PI3K (H1047R) or K-Ras (G12V) in mammary epithelial cells induces *de novo* synthesis of adipose tissue, which necessitates the activation of sterol-regulatory element binding proteins (SREBP1 and SREBP2) within the PI3K/AKT/mTOR signaling pathway (Ricoult et al., 2016). In addition to promoting lipogenesis,

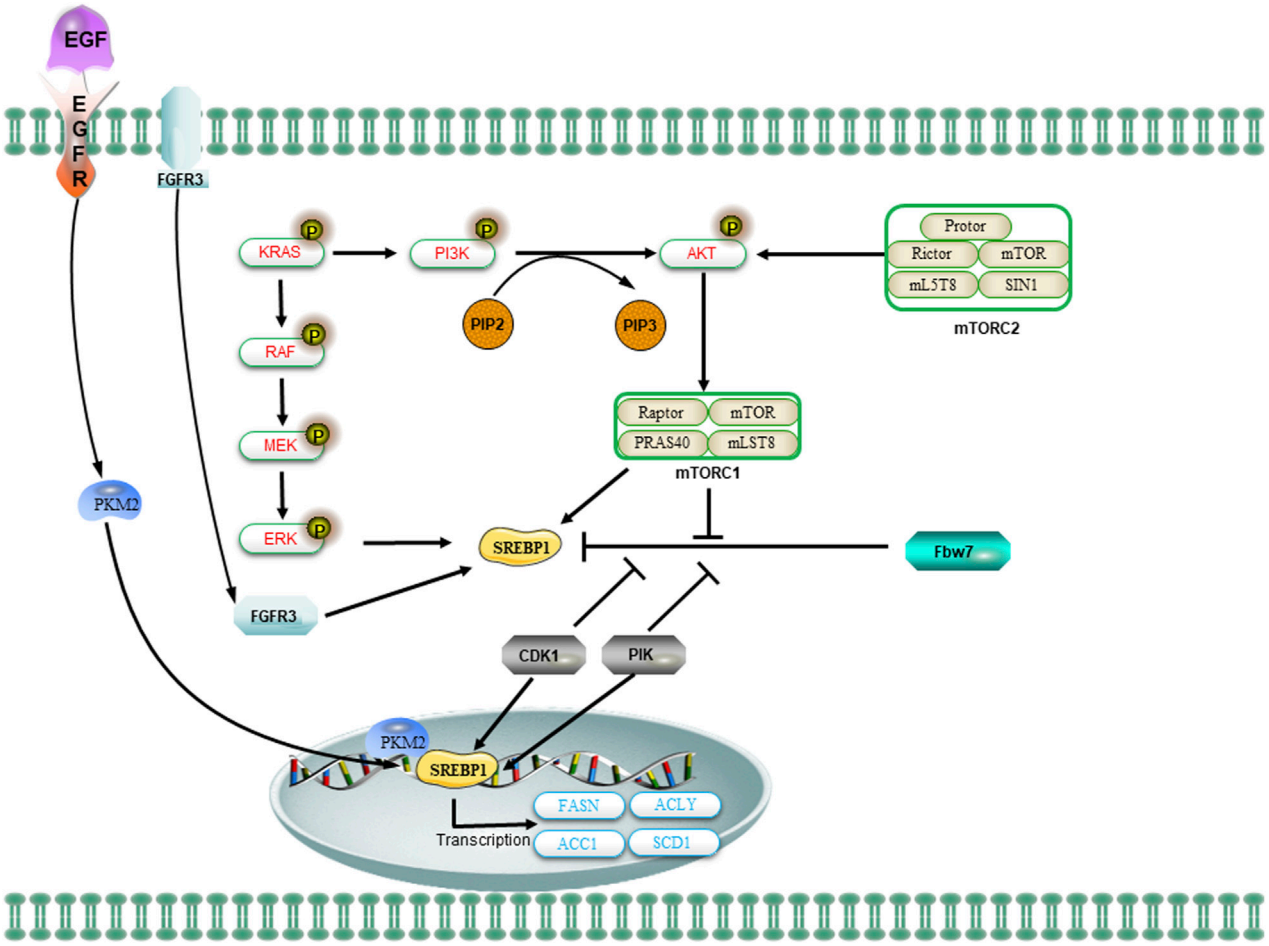


FIGURE 4
The regulation of SREBP1 and lipid metabolism in CSCs is mediated by oncogenic signaling. The oncogenic PI3K/Akt/mTORC1 and KRAS signaling pathways activate SREBP1 to facilitate *de novo* lipid synthesis and promote cellular growth. The mTOR signaling regulates the levels of SREBP1 through transcriptional or translational mechanisms. The activation of the PI3K/AKT/mTOR signaling pathway or FGFR3 results in the stabilization of SREBP1 proteins and facilitates their translocation to the nucleus. Additionally, the nuclear SREBP1 protein interacts with mitotic kinases Cdk1 and Plk1. The sequential phosphorylation of SREBP1 by Cdk1 and Plk1 impedes the interaction between the ubiquitin ligase Fbw7 and SREBP1, thereby attenuating the degradation of SREBP1. Upon activation of EGFR signaling, the nuclear form of PKM2 interacts with SREBP to facilitate the activation of SREBP target gene expression and promote lipid biosynthesis.

SREBP1 also stimulates the expression of SCD1, thereby facilitating the production of MUFA (Lewis et al., 2015) (Figure 4). The silencing of SREBP1 results in the inhibition of proliferation and the induction of apoptosis in pancreatic cancer cells, thereby further suppressing lipid metabolism and impeding tumor growth *in vivo* (Sun et al., 2015). Furthermore, the growth of glioblastoma spheroids was significantly inhibited by blocking SREBP1 (Lewis et al., 2015). Mechanistically, during mitosis, SREBP1 protein hinders the interaction between the ubiquitin ligase Fbw7 and SREBP1, thereby suppressing the phosphorylation-mediated degradation of SREBP1 by Cdk1 and Plk1 (Bengoechea-Alonso and Ericsson, 2006; Bengoechea-Alonso and Ericsson, 2009; Bengoechea-Alonso and Ericsson, 2016). Furthermore, the PI3-kinase/Akt/rapamycin target (mTOR) C1 signaling pathway additionally promotes the nuclear accumulation of mature SREBP1 (Porstmann et al., 2008). Activation of EGFR signaling triggers the translocation of pyruvate kinase M2 (PKM2) into the nucleus, thereby inducing the Warburg effect (Christofk et al., 2008; Yang et al., 2011). Notably, nuclear PKM2 physically engages with SREBP1, contributing to enhanced lipid biosynthesis by stabilizing SREBP-1 proteins (Figure 4) (Zhao X. et al., 2018). These findings provide further evidence for the interplay between glycolysis and FA metabolism.

5 Characterization of FA metabolism in CSCs

Recently, the significance of lipid metabolism in cancer cells has been consistently emphasized, leading to a series of notable advancements that offer valuable reference indices and guidance for cancer therapy (Luo X. et al., 2017). The energy metabolism of CSCs is mainly carried out in mitochondria, and they are able to efficiently utilize nutrients, such as FA, to produce ATP to provide the energy they need in the harsh microenvironment. The high level of UFA in CSCs has been demonstrated, and it has been shown that

inhibiting the activities of SCD1 and acetaldehyde dehydrogenase 1A1 (ALDH1A1), or reducing the level of NF-κB in CSCs, can significantly decrease UFA content, diminish the stemness of CSCs, and impede tumor formation (Sun and Yang, 2019). The level of unsaturated fatty acid (UFA) in ovarian CSCs is significantly elevated compared to non-CSCs, and key lipases involved in FA synthesis, including ACLY, ACC, and FASN, exhibited elevated levels (Li et al., 2017). These lipases were regulated by the lipid-generating transcription factor SREBP1c, which has gradually emerged as a reliable marker of stemness in CSCs (Visweswaran et al., 2020). Additionally, the activity of lipase activity was modulated by other protein kinases, such as the reduction in AMP kinase (AMPK) phosphorylation, and content in CSCs not only elevated lipase activity but also increased malonyl coenzyme A levels, a precursor for fatty acid synthesis, resulting in enhanced fatty acid synthesis and mitochondrial β-oxidation (Bort et al., 2020). Furthermore, increased FA synthesis facilitates the uptake and utilization of lipids by CSCs (Peck and Schulze, 2016). Conversely, inhibition of the NF-κB-regulated lipid desaturase signaling pathway can effectively eradicate CSCs and impede their tumorigenic potential (Li et al., 2017). Additionally, the dynamic equilibrium of FA is pivotal for lipid metabolism in CSCs, and maintaining a stable metabolic state in CSCs contributes to chemoresistance and the acquisition of stem cell-like properties (Wang et al., 2018).

The connection between glycolipid metabolism in CSCs lies in the fact that AcCoA, generated through the oxidation of pyruvate—an intermediate product of glycolipid metabolism—can be utilized for FA synthesis, thereby facilitating the self-renewal of CSCs and contributing to the maintenance of their stemness (Pizer et al., 2000). Studies have demonstrated that maintaining a well-balanced ratio between FA and glycerophospholipids can effectively impede the progression of HCC (Lin et al., 2017). The elevated glucose metabolism and β-oxidation in CSCs of HCC and leukemia ensure the provision of alternative energy sources under extreme conditions, thereby sustaining their stemness (Chen C. L. et al., 2016). The intermediates generated through glycolysis can also serve as substrates for the synthesis of FA, thereby facilitating the self-renewal of CSCs (Corominas-Faja et al., 2014). Thus, the maintenance of pluripotency, self-renewal, proliferation, and formation of CSCs relies on the delicate balance of FA homeostasis or the state of catabolism/anabolism.

6 Characterization of cholesterol metabolism in CSCs

The sources of cholesterol can be categorized as either exogenous or endogenous, with exogenous cholesterol originating from dietary intake and endogenous cholesterol being synthesized within the body. The maintenance of cholesterol homeostasis is dependent on two primary mechanisms (Hafiane et al., 2019). On one hand, cholesterol levels can be elevated through the re-synthesis of AcCoA provided by glycolysis, glutamine metabolism, the TCA cycle, or exogenous uptake of low-density lipoprotein (LDL) receptors. Additionally, peripheral cholesterol can return to the liver in the form of LDL via the cholesterol reverse transporter, as well (Munir et al., 2019). On the contrary, cholesterol levels can be downregulated through inhibition of the MVA pathway or activation of liver X receptors (LXRs). The MVA pathway can be attenuated by protein hydrolysis or nuclear importation of SREBP2, while LXRs can be stimulated by the conversion of cholesterol to oxysterols (Ahmad et al., 2019). The activation of the LXRs/PPAR pathway subsequently induces the transcription of the E3 ubiquitin ligase IDOL, which in turn facilitates the ubiquitination of LDLR and enhances the expression of cholesterol efflux pumps ABCA1 and ABCG1 (Figure 5) (Spite, 2014). The MVA pathway plays a crucial role in the biosynthesis of steroid hormones, cholesterol, and nonsteroidal isoprenoids. The pathway primarily maintains homeostasis in the microenvironment of CSCs through protein geranylgeranylation. Additionally, 3-hydroxy-3-methylglutaryl monoacyl-coenzyme A (HMG-CoA) serves as the rate-limiting enzyme in the MAV pathway and represents a molecular target for statin drugs. The HMG-CoA reductase, which is the rate-limiting enzyme in the MAV pathway, serves as a molecular target for statin-type cholesterol-lowering drugs. The administration of statin drugs disrupts the MAV pathway, thereby inhibiting geranylgeranylation of proteins and disrupting the homeostasis of the microenvironment in CSCs, ultimately leading to their eradication (Mullen et al., 2016). For example, an overexpression of HMG-CoA is revealed in basal-like tumors, and the inhibition of the MAV pathway through simvastatin demonstrated a significant reduction in the number of CSCs within the tumors (Mancini et al., 2018). The combination of valproic acid and simvastatin can concurrently modulate the MAV pathway and AMPK phosphorylation level, thereby inhibiting the YAP oncogene. This enhances the sensitivity of denervation-tolerant prostate cancer cells to doxorubicin while reducing drug resistance caused by CSCs (Iannelli et al., 2020). Furthermore, the treatment of colorectal cancer cell lines with metformin, an AMPK activator, an HMG-CoA reductase inhibitor, or a mammalian target of rapamycin (mTOR) inhibitor significantly decreased the population of CSCs; however, the number of CSCs rebounded after treatment with mevalonic acid, indicating that mevalonic acid attenuates the inhibitory effect of these treatments on CSCs (Sharon et al., 2015). Moreover, bile acids and oxysterols serve as two chemical by-products of the MVA pathway. They function as ligands for a variety of nuclear receptors (including FXR, VDR, LXR, and PXR) as well as G-protein coupled receptors (Joyce and Gahan, 2016; Hafiane et al., 2019). Ting Fu et al. discovered that in colorectal cancer, a high-fat diet and dysregulated WNT signaling pathway led to alterations in bile acid profiles, activation of FXR, and the initiation of malignant transformation in Lgr5+ subpopulation CSCs (Fu et al., 2019). Similarly, the MAV pathway is found to enhance the proliferation of pancreatic CSCs, while the administration of atorvastatin effectively inhibits this proliferative effect (Brandi et al., 2017). Notably, in the MAV pathway, the inhibition of geranylgeranyltransferase can effectively reduce CSCs by suppressing protein isoprenylation. However, it has been observed that inhibiting cholesterol synthesis with squalene synthase inhibitors does not lead to a decrease in the number of CSCs (Brandi et al., 2017). The formation of breast tumorspheres occurs during the culture of breast CSCs, giving rise to both breast cancer cells and breast CSCs (Gupta et al., 2009). The inhibition of

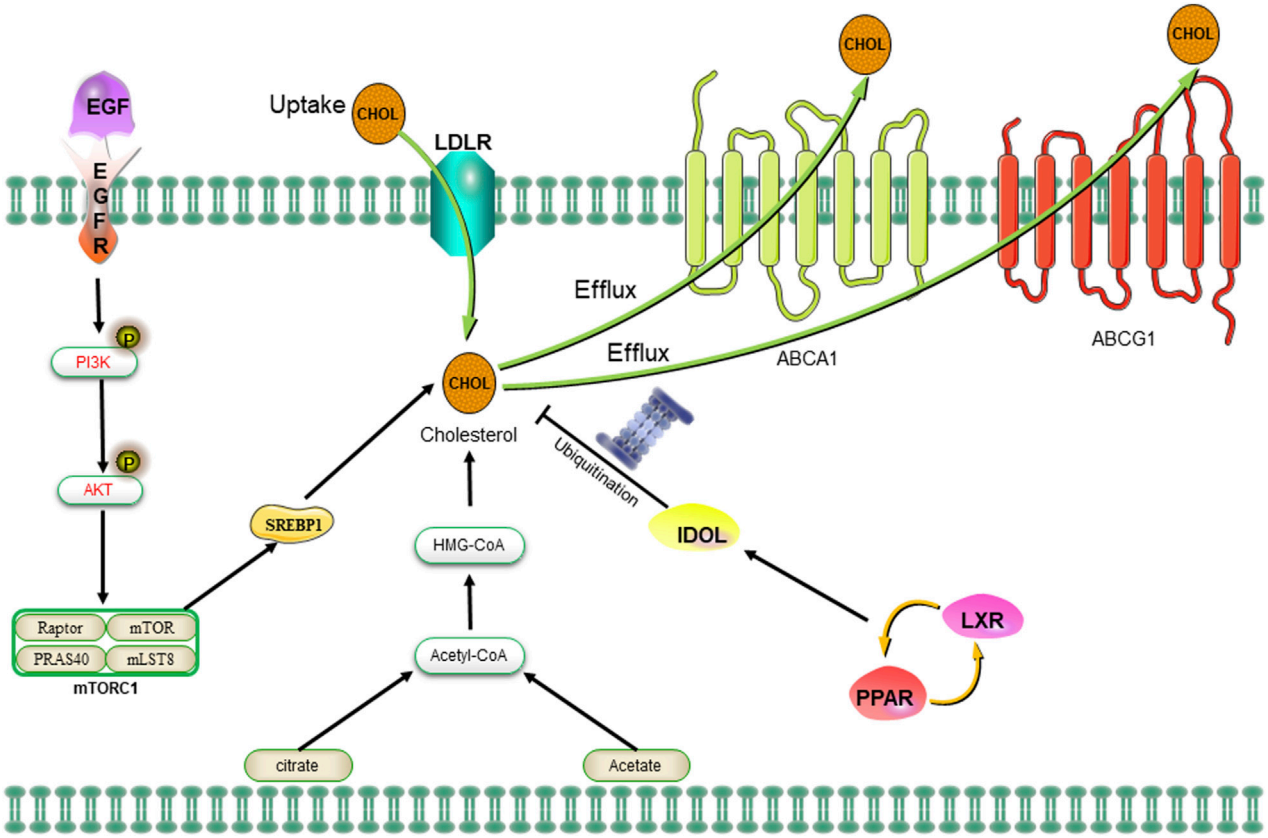


FIGURE 5
The uptake of cholesterol is facilitated by LDLR via the epidermal growth factor receptor-dependent pathway. The MVA pathway is responsible for cholesterol synthesis, while LDLR plays a crucial role in both the negative regulation of cholesterol uptake and the control of cholesterol efflux. Abbreviation: EGFR, epidermal growth factor receptor; LDLR, low density lipoprotein receptors; ABCA1, ATP-binding cassette transporter 1; PI3K/Akt, phosphatidylinositol 3-kinase/protein kinase B; SREBP-1, sterol regulatory element binding proteins; MVA, mevalonate; IDOL, inducible degrader of the low-density lipoprotein receptor; PPAR/LXR, lipid-activated transcription factors/LXRs.

cholesterol synthesis effectively reduces the formation of breast tumorspheres, indicating that targeting the cholesterol synthesis pathway is a promising therapeutic strategy for suppressing the development of breast cancer by specifically targeting CSCs (Ehmsen et al., 2019). Some researchers have also demonstrated that synthetic progestins, such as medroxyprogesterone acetate (MPA), widely utilized in clinical practice, expedite the formation of breast tumorspheres while simultaneously increasing the activity of ALDH1A1 in CSCs and augmenting the tumorigenicity of CSCs (Goyette et al., 2017). Furthermore, it has been found that cholesterol synthesis inhibitors can mitigate this undesirable induction of breast tumorspheres by MPA (Liang et al., 2017).

7 The impact of autophagy on lipid metabolism in CSCs

Autophagy is a natural and highly conserved cellular degradation process that involves the lysosome-mediated breakdown of unwanted or dysfunctional intracellular components, including molecules and organelles (Mahapatra et al., 2021). The process of autophagy is crucial for maintaining cellular homeostasis, and any disruption to this mechanism may potentially facilitate the development of tumorigenesis. The process of lipophagy involves the fusion of LDs phagosomes with lysosomes to form autophagolysosomes, followed by the breakdown of LDs to generate free FA (Zhang et al., 2022). These FAs are then transported to the mitochondria for β-oxidation, resulting in energy production (Singh et al., 2009). The process of autophagy serves as a metabolic adaptation employed by tumor cells to surmount nutrient deprivation (Sinha et al., 2017). Additionally, autophagy facilitates the mobilization of nutrients and confers survival advantages to cancer cells, particularly under conditions of cellular stress such as hypoxia, chemotherapy, and radiotherapy (Lin et al., 2023). It plays a crucial role in maintaining cellular homeostasis and enables cells to withstand disturbances within the TME (Camuzard et al., 2020). The process of autophagy is crucial for maintaining lipid homeostasis, and the inhibition of autophagy leads to a decrease in the rate of β-oxidation in LDs, thereby reducing energy utilization (Li et al., 2018). Furthermore, autophagy facilitates the survival of CSCs by providing energy, enabling them to swiftly adapt to the challenging microenvironment post-chemotherapy, such as CSCs exhibit a higher autophagic rate compared to normal stem cells, and inhibition of autophagy compromises the stemness and tumorigenicity of CSCs (Visweswaran et al., 2020). The induction of autophagy is governed by the UNC-51-like

autophagy-activated kinase 1 (ULK1) complex, and both mTOR and AMPK have the ability to modulate the expression level of ULK1. The activation of AMPK and the inhibition of mTOR can effectively suppress cholesterol synthesis and downregulate ULK1, thereby inducing apoptosis in CSCs (Bu et al., 2020). Moreover, the basal level of autophagy/mitophagy is higher in BCSCs compared to normal tissue-specific stem cells and autophagy induces the upregulation of CD44 and vimentin, both of which are recognized as stem cell markers (Cufi et al., 2011). GSCs also exhibit high expression levels of the autophagy regulators SQSTM1 and DRAM1, which are positively correlated with the expression of mesenchymal factors such as c-MET (Galavotti et al., 2013). The involvement of CD133, another stem cell marker, in the regulation of autophagy in GSC has been observed, as evidenced by the fact that the cytoplasmic localization of CD133 is relatively enhanced under conditions of glucose deprivation, while it remains membrane-bound in the presence of normal glucose levels within the cells (Sun H. et al., 2016). Autophagy involves several specific membrane structures, including phagosomes, autophagolysosomes, and autolysosomes. These structures are capable of engulfing isolated cytoplasmic constituents and subsequently degrading intracellular substances through hydrolytic enzymes (Klionsky et al., 2014). The lipid metabolism alterations in CSCs facilitate the regeneration of these membrane structures, and reciprocally, the regenerated membrane structures exert an influence on the CSCs, thereby establishing a positive feedback loop (Snaebjornsson et al., 2020). Moreover, the maintenance of pluripotency in breast hematopoietic stem cells under various pathophysiological conditions is also attributed to the crucial role played by autophagic homeostasis (Han et al., 2018). Interestingly, in addition to providing energy to CSCs, autophagy also facilitates the lipid peroxidation of UFA, which is abundantly expressed on the cellular membrane, leading to programmed cell death - ferroptosis (Xie et al., 2020). Ours and other studies have indicated that facilitating ferroptosis can specifically kill CSCs (Mai et al., 2017; Polewski et al., 2017; Ni et al., 2021; Yang Y. et al., 2022). However, it must be admitted that the potential benefits and drawbacks of autophagy in promoting the survival of CSCs necessitate further investigation: On the one hand, autophagy may facilitate CSC survival during periods of nutrient deprivation by recycling cellular components, providing an alternative energy source for their maintenance; On the other hand, excessive or dysregulated autophagy could lead to the degradation of essential cellular components in CSCs, compromising their functionality and survival.

8 The involvement of CSCs in crucial signaling pathways of lipid metabolism

The maintenance of stemness, survival, proliferation, and invasion in CSCs involves a variety of lipid metabolic pathways, including the Notch, Hippo, Hh, and Wnt signaling pathways (Wang et al., 2022). The pathways through which stem cells can be derived via genetic mutations and epigenetic alterations have a significant potential to be exploited for the maintenance of unrestricted proliferation, invasion, and drug resistance (Reya et al., 2001; Wang et al., 2022).

8.1 Wnt signaling pathway

The Wnt signaling cascade comprises three primary pathways: the canonical Wnt pathway (which results in β-catenin accumulation, activation of transcription-activation complexes, and involvement in tumorigenesis), the non-canonical planar cell polarity pathway, and the non-canonical Wnt-calcium pathway (Takebe et al., 2015). In the typical Wnt pathway, Wnt ligands bind to the frizzled family of transmembrane receptors, thereby activating disheveled. This activation then collaborates with the T-cell factor/LEF family to induce stabilization and accumulation of nuclear β-catenin transcriptional activity. The Wnt signaling pathway exhibits a high degree of evolutionary conservation during the development of embryonic proliferative tissues, such as the hematopoietic system, skin, and intestines. This conservation is evident in terms of somatotaxis stereotypy, cell fate specification, cell proliferation, and migration (Teo et al., 2006). During tumorigenesis, the Wnt signaling facilitates tumor migration and invasion by upregulating genes involved in cell adhesion, such as Eph/ephrin, E-cadherin, and MMPs (Lang et al., 2019). Thus, the Wnt signaling pathway plays a pivotal role in the regulation of CSCs (Patel et al., 2019; Fendler et al., 2020). The Wnt signaling pathway, for instance, is also implicated in lipid synthesis within CSCs. Specifically, the canonical Wnt/β-catenin pathway governs subordinate lipogenesis and MUFA production (Bagchi et al., 2020). Additionally, the Wnt/β-catenin signaling significantly regulates the process of *de novo* adipogenesis in breast cancer cells, characterized by a substantial upregulation of ACC, FASN, and SREBP1-c expression (Vergara et al., 2017). Another study suggests that SCD potentially links Wnt signaling and lipid metabolism as in mouse liver hematopoietic stem cells, the Wnt/β-catenin pathway regulates SCD expression, and SCD-derived monounsaturated fatty acids create a positive feedback loop, reinforcing Wnt signaling through enhanced Lrp5/6 mRNA stability and expression (Lai et al., 2017). Furthermore, MUFAs play a crucial role in the synthesis and release of Wnt ligands (Rios-Esteves and Resh, 2013). The metabolism of FA in YAP/TAZ signaling, specifically the function of SCD1, is reliant on the activity of the β-catenin pathway in CSCs (Noto et al., 2017a). The dysregulation of the Wnt signaling pathway and a high-fat diet in colorectal cancer lead to alterations in bile acid distribution, activation of FXR, and subsequent malignant transformation of the Lgr5+ subpopulation of CSCs. This process promotes the progression from adenomas to adenocarcinomas (Fu et al., 2019).

8.2 Notch signaling pathway

The Notch signaling pathway comprises of Notch receptors, DSL proteins (Notch ligands), CSLs (CBF-1, Hairless Suppressor Factor, Lag), DNA-binding proteins, other effectors, and Notch-regulated molecules. The Notch signaling pathway is a highly conserved signal transduction pathway that plays a crucial role in various biological processes, including tumor metastasis and immune evasion (Takebe et al., 2015; Bocci et al., 2019). The activation of the Notch pathway in CSCs has been demonstrated by numerous studies to promote cell survival, self-renewal, and metastasis while inhibiting apoptosis. The aberrant activation of

Notch signaling (Notch1 and Notch4) facilitates the self-renewal and metastasis processes in breast cancer and HCC stem cells (Stylianou et al., 2006; Harrison et al., 2010). In lipid metabolism, the Notch signaling pathway regulates the expression of peroxisome proliferator-activated receptor α and lipid oxidation genes to maintain lipid homeostasis and redox homeostasis in lipid metabolism (Song et al., 2016). The selective elimination of colon CSCs through inhibition of Notch signaling is achieved by targeting scd1-dependent lipid desaturation (Yu et al., 2021). Furthermore, in *Drosophila*, the Notch signaling pathway is responsive to environmental sterol levels and its expression is regulated by dietary cholesterol, ultimately leading to the differentiation of enterocytes from a stem-like state (Obniski et al., 2018). The activation of the Notch signaling is also regulated by dietary cholesterol, thereby inducing the differentiation of enterocytes from a stem-like state. Additionally, the Notch pathway plays a crucial role in angiogenesis, EMT, and the proliferation of CSCs within cancer cells. It has been observed that a low-sterol diet can potentially restrict the growth of enteroendocrine tumors by attenuating the Notch response (Obniski et al., 2018). Kalucka et al., 2018 discovered that Notch1 regulates FAO to maintain intermediate lipid homeostasis and redox homeostasis in CSCs. Importantly, the Notch signaling in humans is influenced by the lipid composition of the cellular membrane (Sorrentino et al., 2014).

8.3 Hippo signaling

The evolutionarily conserved Hippo signaling pathway is initiated by Hippo kinase, a serine/threonine kinase. In normal conditions, Hippo kinase is inactive due to phosphorylation. However, external stimuli activate Hippo kinase, leading to the phosphorylation and activation of downstream molecules MST1/2. Activated MST1/2, in turn, phosphorylate LATS1/2, exerting inhibitory effects on the activities of YAP and transcriptional coactivator with PDZ-binding motif (Mo et al., 2014). The activation of YAP or TAZ in CSCs has been confirmed to sustain the self-renewal and tumor-initiating capacity (Bhat et al., 2011), promote cellular pluripotency (Cordenonsi et al., 2011) and drug resistance (Huo et al., 2013), and exhibits a strong correlation with the process of EMT (Kulkarni et al., 2018; Sundqvist et al., 2018). Emerging evidence suggests a close and significant association between the Hippo-YAP/TAZ signaling and the regulation of lipid metabolism in cancer stemness (Shu et al., 2019). For example, SCD1, a major regulator of MUFA, contributes to the maintenance of cancer stemness by modulating the expression and nuclear localization of YAP/TAZ (Noto et al., 2017a). GGPP, an intermediate in the control of the MAV pathway, stabilizes YAP/TAZ (Sorrentino et al., 2014). As previously mentioned, SCD1 plays a crucial role in regulating the stemness of lung CSCs by stabilizing YAP/TAZ and facilitating their nuclear localization (Noto et al., 2017a). Notably, a positive feedback loops involving LATS2 and p53 has been identified to inhibit cholesterol synthesis (Aylon et al., 2016). Additionally, LATS2 binds to endoplasmic reticulum tethered precursors (P-SREBP) of SREBP1 and SREBP2, thereby suppressing the transcription of SREBP mRNAs and subsequently inhibiting cellular SREBP activity (Aylon and Oren, 2016). Recent studies have demonstrated that the pro-carcinogenic properties of YAP/TAZ are contingent upon cholesterol biosynthesis activity and MVA-dependent nuclear localization and activity of YAP/TAZ (Sorrentino et al., 2014). The lipid synthesis mediated by YAP/TAZ may serve as a crucial factor influencing metabolic alterations in CSCs (Koo and Guan, 2018).

8.4 Hh signaling

The Hh signaling network is intricate and encompasses extracellular Hh ligands, the transmembrane protein receptor PTCH, the transmembrane protein SMO, intermediate signal transduction molecules, and the downstream effector molecule GLI (Yang et al., 2020). The membrane protein SMO exerts a positive regulatory function, while the transmembrane protein PTCH plays a negative regulatory role. The GLI protein acts as an effector, and in vertebrates, it exists in three isoforms: Gli1, Gli2, and Gli3. These effector proteins exhibit distinct functionalities: Gli1 exerts a robust transcriptional activation effect, whereas Gli3 functions as a transcriptional inhibitor. On the other hand, Gli2 displays dual functionality by both activating and inhibiting transcription but primarily serves as a transcriptional activator (Sasaki et al., 1999; Li S. H. et al., 2013). The Hh signaling pathway also plays a pivotal role in the regulation of CSCs. It governs the proliferation of postnatal mammary stem cells and orchestrates the intricate ductal architecture formation in the adult mammary gland (Miyazaki et al., 2016). The existing evidence suggests that lipid metabolism also plays a regulatory role in the Hh signaling and its ligand properties, highlighting the crucial involvement of lipids as key regulators in Hh signaling (Blassberg and Jacob, 2017). The covalent modification of SMO by cholesterol is regulated by the Hh signaling pathway and plays a crucial role in mediating Hh signaling and cellular biological functions (Radhakrishnan et al., 2020). PTCH1 inhibits the cholesterol modification of SMO, while SHH overexpression enhances it (Hu and Song, 2019). Furthermore, SMO exerts direct or indirect inhibition on FA and cholesterol synthesis by activating adenosine monophosphate kinase through a non-classical pathway (Blassberg and Jacob, 2017). Notably, recent clinical trials have utilized SMO and GLI inhibitors to target the Hh signaling pathway (Long et al., 2016; Zhang et al., 2016). Thus, the Hh signaling pathway, intricately linked with lipid metabolism and CSCs, demonstrates the regulatory role of lipids in signaling cascades, offering potential therapeutic targets for cancer intervention.

9 Emerging drugs that target lipid metabolism in CSCs

The targeted eradication of CSCs can be accomplished by intervening in various aspects of their lipid metabolism, including lipogenesis, lipid uptake, lipid desaturation, and FA oxidation (Yi et al., 2018). Due to the exorbitant cost and inherent risks associated with the discovery and development of novel therapeutic agents, there has been a growing interest in drug repositioning strategies for hard-to-treat diseases. This approach offers an opportunity to establish effective targeting strategies aimed

TABLE 2 Emerging drugs that target lipid metabolism in CSCs.

Metabolism type	Drug	Targeting enzyme	CSC or tumor type	Metabolic processes or signaling pathways	Clinical trial
Lipogenesis	Resveratrol	FASN	BCSCs (Pandey et al., 2011), GCSCs (Sayd et al., 2014), PCSCs (Subramaniam et al., 2018)	Regulation of FASN	Clinical Trial
	Orlistat	FASN	NSCLC (Ali et al., 2018)	Regulation of FASN	failure
	TVB-2640	FASN	NSCLC and breast cancer (Corominas-Faja et al., 2014)	Regulation of FASN, Inhibitor of HMG-COAR, inhibitor of cholesterol synthesis	Recruiting
	Cerulenin	FASN	PCSCs (Brandi et al., 2017), GCSCs (Yasumoto et al., 2016)	Regulation of FASN	Pre-clinical
	Leptin	ACC	BCSCs (Schug et al., 2015)	TAK1-AMPK signaling pathways	Pre-clinical
	Soraphen A	ACC	BCSCs (Corominas-Faja et al., 2014)	Regulation of FASN	Pre-clinical
	ND-646	ACC	Non-small-cell lung CSCs (Svensson et al., 2016)	Regulation of FASN	Pre-clinical
FAO	ST1326	CPT1A	Lymphoma (Pacilli et al., 2013), AML cells (Kalucka et al., 2018)	Inhibition of FAO	Pre-clinical
	Avocatin B	FABP4	AML cells (Tabe et al., 2018)	enhanced glucose uptake	Pre-clinical
	Etomoxir	CPT1A	TNBC (Camarda et al., 2016), leukemia (Samudio et al., 2010)	Inhibition of FAO	Pre-clinical
Cholesterol synthesis	25-HC or fatostatin, Pyrvinium pamoate	SREBPs	Colon CSCs (Wen et al., 2018), TNBC CSCs (Dattilo et al., 2020)	FA synthesis and cholesterol synthesis	Pre-clinical
	Simvastatin	HMGCR	BCSCs (Dattilo et al., 2020)	Cholesterol synthesis	FDA-approved cardiovascular system drug
Lipid desaturation	MF-438	SCD1	Colon CSCs (Yu et al., 2021), lung CSCs (Pisanu et al., 2017)	Regulation of Wnt, Notch, and YAP/TAZ signaling pathways	Pre-clinical
	A939572	SCD1	CRC (Chen et al., 2016c), clear cell renal cell carcinoma (von Roemeling et al., 2013), Liver cancer (Tracz-Gaszewska and Dobrzyn, 2019)etc.	MUFA synthesis	Pre-clinical
	SSI-4	SCD1	Liver CSCs (Belkaid et al., 2015)	The inhibition of SCD1 compels hepatic CSCs to undergo differentiation by inducing ER stress	Pre-clinical
	CAY10566	SCD1	Ovarian CSCs (Li et al., 2017), glioblastoma CSCs (Valle et al., 2020)	NF-κB pathway, ER stress	Pre-clinical
	PluriSIn#1	SCD1	Colon CSCs (Yuan et al., 2022), liver CSCs (Lobello et al., 2016)	Wnt/β-catenin and Notch signaling	Pre-clinical
	T-3764518	SCD1	CRC (Nishizawa et al., 2017)	Inhibit lipogenesis	Pre-clinical
	SC-26196	Delta 6 desaturase	Ovarian CSCs (Li et al., 2017)	Inhibit polyunsaturated FA synthesis	Pre-clinical
	BetA	SCD1	CRC-CSCs (Potze et al., 2016)	BetA inhibits SCD1-induced rapid death of colonic hematopoietic stem cells	Pre-clinical
Lipid uptake	CD36 Antibody	CD36	OSCC (Pascual et al., 2017)	Inhibit lipogenesis	Pre-clinical

BCSCs: breast cancer CSCs, GCSCs: glioblastoma CSCs, PCSCs: pancreatic CSCs, FASN: FA synthase, NSCLC: non-small cell lung cancer, AML: acute myeloid leukemia, TNBC: triple-negative breast cancer, ER: endoplasmic reticulum, CRC colorectal cancer, OSCC oral squamous cell carcinomas.

at eradicating CSCs. The targeted elimination of CSCs can be achieved by disrupting various aspects of lipid synthesis, including FA synthesis, lipid desaturation, and cholesterol synthesis (Table 2).

The FASN gene is the primary focus among adipogenic genes and the antitumor activity of several FASN inhibitors has been demonstrated in preclinical cancer models. The expression of invasiveness, sphere formation, and stemness markers is

effectively reduced by both FASN inhibitors and RNA silencing, leading to the eradication of various CSCs (Table 2) (Yasumoto et al., 2016; Brandi et al., 2017). The development of novel FASN inhibitors is underway, and preliminary clinical trial data on TVB-2640 demonstrate its potential to mitigate tumor response in patients with non-small cell lung cancer and breast cancer when used in combination with paclitaxel (Jones and Infante, 2015). Similarly, the formation of mammospheres was inhibited by Soraphen A, an inhibitor of ACC (Corominas-Faja et al., 2014). Mechanistically, Soraphen A inhibits the self-renewal and growth of CSC-like cells by blocking FA synthesis and abolishes the promotion of CSC proliferation mediated by human epidermal growth factor receptor 2 (HER2) (Corominas-Faja et al., 2014). The inhibition of ACC not only suppressed tumor growth, metastasis, and recurrence in non-small cell lung and breast cancers, but also underscored the significance and potential of ACC in suppressing CSCs and combating cancer (Schug et al., 2015; Svensson et al., 2016).

Additionally, the selective elimination of CSCs can be achieved by targeting SCD1, an enzyme responsible for the conversion of fully saturated FA into MUFAs. The recent demonstration of SCD in a genetic mouse model is particularly noteworthy, as it is essential for the emergence of tumor-initiating cells (Lai et al., 2017). The significant reliance on UFA renders SCD a promising target for the eradication of CSCs (Peck and Schulze, 2016). The SCD1 inhibitors, such as CAY10566 and A939572, have been documented to effectively suppress cancer stemness, inhibit tumorigenesis, and overcome chemoresistance in cancer cells (Tracz-Gaszewska and Dobrzyn, 2019). Notably, MF-438 and PluriSIn #1, as inhibitors of SCD1, exhibited selective eradication of colonic CSCs while showing no efficacy against cancer cells (Yu et al., 2021). Furthermore, the inhibition of SCD1 expression enhances the sensitivity of CSCs to cisplatin and reduces the occurrence of drug resistance (Pisanu et al., 2017). The SSI-4 compound is a novel inhibitor of SCD1 that effectively overcomes sorafenib resistance in hepatic CSCs. The antitumor activity of SSI-4 against hepatic CSCs was also observed in animal models, with no significant adverse effects reported (Ma et al., 2017). Importantly, the combination of SSI-4 and sorafenib demonstrated the most significant inhibition of tumorigenesis in the sorafenib-resistant PDTX model. The combination of SCD1 inhibitors and chemotherapy may potentially offer a more efficacious therapeutic approach. The effective SCD1 inhibitors utilized in the preclinical phase are summarized in Table 1.

The reliance of CSCs on FAO justifies the targeting of these cells with FAO inhibitors. The compound Etomoxir functions as a highly specific inhibitor of mitochondrial CPT1A (Holubarsch et al., 2007) (as shown in Table 1). The inhibition of FAO by etomoxir in leukemic CSCs leads to the suppression of cell proliferation and enhances the susceptibility of human leukemic cells to apoptosis (Samudio et al., 2010; Estan et al., 2014). ST1326 is a CPT1A inhibitor that suppresses FAO and exhibits cytotoxic activity against leukemia cell lines, while sparing normal $CD34^+$ myeloid cells (Ricciardi et al., 2015) (Table 1). ST1326 also exhibits preventive effects against MYC-driven lymphomagenesis in an Eμ-myc transgenic mouse model (Pacilli et al., 2013).

The activation of cholesterol synthesis has been previously suggested to be linked with the invasive and metastatic capabilities of CSCs. The inhibition of SREBP activation by 25-HC or adiponectin hampers adipogenesis, including FA and cholesterol synthesis, and disrupts the expression of genes associated with CSCs (Sun H. W. et al., 2016). Furthermore, Dattilo R not only significantly inhibits lipid anabolism in CSCs but also exhibits a cytotoxic effect on TNBC -CSCs by suppressing cholesterol synthesis in TNBC (Dattilo et al., 2020). The inhibition of cholesterol biosynthesis by Simvastatin significantly suppresses the formation and growth of mammospheres (Gu et al., 2019). Additionally, statins exert their effects on CSCs by inhibiting signaling pathways associated with protein farnesylation and protein geranylation in the MVA pathway (Ginestier et al., 2012; Iannelli et al., 2020). Similarly, metformin suppresses colorectal CSCs by inhibiting protein prenylation of the MVA pathway (Seo et al., 2020). Taken together, these emerging drugs targeting lipid metabolism in CSCs offer promising avenues for selective elimination of CSCs, focusing on disrupting various aspects such as lipogenesis, lipid uptake, desaturation, and fatty acid oxidation, and presenting potential strategies to suppress CSC proliferation and enhance therapeutic outcomes.

10 Conclusion

In conclusion, the intricate interplay between lipid metabolism dynamics and CSCs has emerged as a pivotal determinant in the initiation, progression, and therapeutic response of various cancers. The preservation of CSC stemness relies heavily on unique alterations in lipid metabolism, encompassing strategies to increase intracellular fatty acid content, promote β-oxidation for optimized energy utilization, and upregulate the MAV pathway for efficient cholesterol synthesis. Additionally, the versatile role of LDs as alternative energy sources in the context of glycolysis blockade underscores their significance in safeguarding FAs from peroxidation. The symbiotic relationship between autophagy and lipid metabolism not only facilitates the rapid adaptation of CSCs to the harsh microenvironment induced by chemotherapy but also presents a complex network of potential therapeutic targets. As we delve into the molecular intricacies of lipid metabolism in CSCs, a myriad of possibilities emerges for innovative cancer therapies. Identifying and targeting key components within this dynamic metabolic landscape holds promise for disrupting CSC functions, curbing tumor initiation, and overcoming drug resistance.

In recent years, the rapid advancement of high-throughput technologies has significantly enhanced our comprehension of the role of metabolic reprogramming in oncogenesis, which is now widely acknowledged as a fundamental characteristic of cancer. CSCs play a pivotal role in the initiation, progression, distant dissemination, and acquisition of drug resistance in tumors. Metabolic alterations have been shown to serve as the primary mechanism through which cancer cells and CSCs evade adverse environmental influences. In lipid metabolism, such as augmented FA uptake, neoadipogenesis, LDL formation, FAO, and lipid desaturation, play a significant role in the generation and sustained maintenance of CSCs. Moreover, lipid synthesis plays a crucial role in the activation of several pivotal oncogenic signaling pathways, including the Notch, Wnt/β-catenin, Hippo, and HH signaling. The targeting of crucial lipid synthesis molecules holds great potential in the elimination of CSCs. In addition, the

heterogeneity and metabolic plasticity of CSCs pose a dilemma, despite the promising opportunity to target cellular metabolism for their elimination (Wang et al., 2013; Thomas and Majeti, 2016; Krstic et al., 2017). The metabolic profiles of CSCs and tumor cells can be modulated in response to nutrient availability. For instance, when cetuximab counteracted the Warburg effect, HNSCC cells exhibited elevated levels of ACC, thereby reprogramming cancer metabolism from glycolysis to adipogenesis in order to support energy demands and facilitate proliferation (Luo J. et al., 2017). The enhanced uptake of FA, neoadipogenesis, LDL formation, FAO, and lipid desaturation has been extensively documented in CSCs across various cancer types. The majority of the targeted compounds, however, have not demonstrated therapeutic efficacy in preclinical cancer models, with only a limited number of inhibitors progressing to clinical trials. The metabolic flexibility of CSCs poses a challenge in effectively eliminating these cells through a single metabolic pathway, necessitating the exploration of synergistic targeting of multiple metabolic pathways in future studies.

In conclusion, the intricate interplay between lipid metabolism dynamics and CSCs has emerged as a critical determinant in various cancers' initiation, progression, and therapeutic response. The preservation of CSC stemness heavily relies on unique alterations in lipid metabolism, including strategies to increase intracellular fatty acid content, promote β-oxidation for optimized energy utilization, and upregulate the mevalonate pathway for efficient cholesterol synthesis. Additionally, the versatile role of LDs as alternative energy sources in the context of glycolysis blockade underscores their significance in safeguarding fatty acids from peroxidation. The symbiotic relationship between autophagy and lipid metabolism not only facilitates the rapid adaptation of CSCs to the harsh microenvironment induced by chemotherapy but also presents a complex network of potential therapeutic targets. As we delve into the molecular intricacies of lipid metabolism in CSCs, a myriad of possibilities emerges for innovative cancer therapies. Identifying and targeting key components within this dynamic metabolic landscape holds promise for disrupting CSC functions, curbing tumor initiation, and overcoming drug resistance.

All in all, this comprehensive review underscores the potential of lipid metabolism as a therapeutic nexus, offering valuable insights into the vulnerabilities of CSCs. As we move forward, unraveling the complexities of lipid-mediated stemness maintenance opens avenues for the development of targeted interventions, marking a significant stride in the pursuit of effective cancer treatments.

Author contributions

JD: Data curation, Funding acquisition, Methodology, Resources, Software, Visualization, Writing–review and editing. HQ: Conceptualization, Formal Analysis, Investigation, Project administration, Supervision, Validation, Writing–original draft.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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.

Publisher's note

References

Aguilar, E., Marin de Mas, I., Zodda, E., Marin, S., Morrish, F., Selivanov, V., et al. (2016). Metabolic reprogramming and dependencies associated with epithelial cancer stem cells independent of the epithelial-mesenchymal transition program. *Stem Cells* 34, 1163–1176. doi:10.1002/stem.2286

Ahmad, F., Sun, Q., Patel, D., and Stommel, J. M. (2019). Cholesterol metabolism: a potential therapeutic target in glioblastoma. *Cancers (Basel)* 11, 146. doi:10.3390/cancers11020146

Al-Hajj, M., and Clarke, M. F. (2004). Self-renewal and solid tumor stem cells. *Oncogene* 23, 7274–7282. doi:10.1038/sj.onc.1207947

Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., and Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. U. S. A.* 100, 3983–3988. doi:10.1073/pnas.0530291100

Ali, A., Levantini, E., Teo, J. T., Goggi, J., Clohessy, J. G., Wu, C. S., et al. (2018). Fatty acid synthase mediates EGFR palmitoylation in EGFR mutated non-small cell lung cancer. *EMBO Mol. Med.* 10, e8313. doi:10.15252/emmm.201708313

Am, A. L., Syed, D. N., and Ntambi, J. M. (2017). Insights into stearoyl-CoA desaturase-1 regulation of systemic metabolism. *Trends Endocrinol. Metab.* 28, 831–842. doi:10.1016/j.tem.2017.10.003

Aylon, Y., Gershoni, A., Rotkopf, R., Biton, I. E., Porat, Z., Koh, A. P., et al. (2016). The LATS2 tumor suppressor inhibits SREBP and suppresses hepatic cholesterol accumulation. *Genes. Dev.* 30, 786–797. doi:10.1101/gad.274167.115

Aylon, Y., and Oren, M. (2016). The Hippo pathway, p53 and cholesterol. *Cell. Cycle* 15, 2248–2255. doi:10.1080/15384101.2016.1207840

Bagchi, D. P., Nishii, A., Li, Z., DelProposto, J. B., Corsa, C. A., Mori, H., et al. (2020). Wnt/β-catenin signaling regulates adipose tissue lipogenesis and adipocyte-specific loss is rigorously defended by neighboring stromal-vascular cells. *Mol. Metab.* 42, 101078. doi:10.1016/j.molmet.2020.101078

Beachy, P. A., Karhadkar, S. S., and Berman, D. M. (2004). Tissue repair and stem cell renewal in carcinogenesis. *Nature* 432, 324–331. doi:10.1038/nature03100

Belkaid, A., Duguay, S. R., Ouellette, R. J., and Surette, M. E. (2015). 17β-estradiol induces stearoyl-CoA desaturase-1 expression in estrogen receptor-positive breast cancer cells. *BMC Cancer* 15, 440. doi:10.1186/s12885-015-1452-1

Bengoechea-Alonso, M. T., and Ericsson, J. (2006). Cdk1/cyclin B-mediated phosphorylation stabilizes SREBP1 during mitosis. *Cell. Cycle* 5, 1708–1718. doi:10.4161/cc.5.15.3131

Bengoechea-Alonso, M. T., and Ericsson, J. (2009). A phosphorylation cascade controls the degradation of active SREBP1. *J. Biol. Chem.* 284, 5885–5895. doi:10.1074/jbc.M807906200

Bengoechea-Alonso, M. T., and Ericsson, J. (2016). The phosphorylation-dependent regulation of nuclear SREBP1 during mitosis links lipid metabolism and cell growth. *Cell. Cycle* 15, 2753–2765. doi:10.1080/15384101.2016.1220456

Bhat, K. P., Salazar, K. L., Balasubramaniyan, V., Wani, K., Heathcock, L., Hollingsworth, F., et al. (2011). The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. *Genes. Dev.* 25, 2594–2609. doi:10.1101/gad.176800.111

Blassberg, R., and Jacob, J. (2017). Lipid metabolism fattens up hedgehog signaling. *BMC Biol.* 15, 95. doi:10.1186/s12915-017-0442-y

Bocci, F., Gearhart-Serna, L., Boareto, M., Ribeiro, M., Ben-Jacob, E., Devi, G. R., et al. (2019). Toward understanding cancer stem cell heterogeneity in the tumor microenvironment. *Proc. Natl. Acad. Sci. U. S. A.* 116, 148–157. doi:10.1073/pnas.1815345116

Bonnet, D., and Dick, J. E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med.* 3, 730–737. doi:10.1038/nm0797-730

Bort, A., Sanchez, B. G., de Miguel, I., Mateos-Gomez, P. A., and Diaz-Laviada, I. (2020). Dysregulated lipid metabolism in hepatocellular carcinoma cancer stem cells. *Mol. Biol. Rep.* 47, 2635–2647. doi:10.1007/s11033-020-05352-3

Boumahdi, S., and de Sauvage, F. J. (2020). The great escape: tumour cell plasticity in resistance to targeted therapy. *Nat. Rev. Drug Discov.* 19, 39–56. doi:10.1038/s41573-019-0044-1

Bousset, L., and Gil, J. (2022). Targeting senescence as an anticancer therapy. *Mol. Oncol.* 16, 3855–3880. doi:10.1002/1878-0261.13312

Braig, M., Lee, S., Loddenkemper, C., Rudolph, C., Peters, A. H., Schlegelberger, B., et al. (2005). Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* 436, 660–665. doi:10.1038/nature03841

Brandi, J., Dando, I., Pozza, E. D., Biondani, G., Jenkins, R., Elliott, V., et al. (2017). Proteomic analysis of pancreatic cancer stem cells: functional role of fatty acid synthesis and mevalonate pathways. *J. Proteomics* 150, 310–322. doi:10.1016/j.jprot.2016.10.002

Broske, A. M., Vockentanz, L., Kharazi, S., Huska, M. R., Mancini, E., Scheller, M., et al. (2009). DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. *Nat. Genet.* 41, 1207–1215. doi:10.1038/ng.463

Brunetti, L., Gundry, M. C., and Goodell, M. A. (2017). DNMT3A in leukemia. *Cold Spring Harb. Perspect. Med.* 7, a030320. doi:10.1101/cshperspect.a030320

Bu, H., Liu, D., Zhang, G., Chen, L., and Song, Z. (2020). AMPK/mTOR/ULK1 axis-mediated pathway participates in apoptosis and autophagy induction by oridonin in colon cancer DLD-1 cells. *Onco Targets Ther.* 13, 8533–8545. doi:10.2147/OTT.S262022

Burchert, A. (2021). Maintenance therapy for FLT3-ITD-mutated acute myeloid leukemia. *Haematologica* 106, 664–670. doi:10.3324/haematol.2019.240747

Camarda, R., Zhou, A. Y., Kohnz, R. A., Balakrishnan, S., Mahieu, C., Anderton, B., et al. (2016). Inhibition of fatty acid oxidation as a therapy for MYC-overexpressing triple-negative breast cancer. *Nat. Med.* 22, 427–432. doi:10.1038/nm.4055

Camuzard, O., Santucci-Darmanin, S., Carle, G. F., and Pierrefite-Carle, V. (2020). Autophagy in the crosstalk between tumor and microenvironment. *Cancer Lett.* 490, 143–153. doi:10.1016/j.canlet.2020.06.015

Cao, J., and Yan, Q. (2020). Cancer epigenetics, tumor immunity, and immunotherapy. *Trends Cancer* 6, 580–592. doi:10.1016/j.trecan.2020.02.003

Cao, X., Wang, C., Liu, J., and Zhao, B. (2020). Regulation and functions of the Hippo pathway in stemness and differentiation. *Acta Biochim. Biophys. Sin. (Shanghai)* 52, 736–748. doi:10.1093/abbs/gmaa048

Castagnoli, L., Corso, S., Franceschini, A., Raimondi, A., Bellomo, S. E., Dugo, M., et al. (2023). Fatty acid synthase as a new therapeutic target for HER2-positive gastric cancer. *Cell. Oncol. (Dordr)* 46, 661–676. doi:10.1007/s13402-023-00769-x

Chaffer, C. L., Marjanovic, N. D., Lee, T., Bell, G., Kleer, C. G., Reinhardt, F., et al. (2013). Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. *Cell.* 154, 61–74. doi:10.1016/j.cell.2013.06.005

Chen, C. L., Uthaya Kumar, D. B., Punj, V., Xu, J., Sher, L., Tahara, S. M., et al. (2016b). NANOG metabolically reprograms tumor-initiating stem-like cells through tumorigenic changes in oxidative phosphorylation and fatty acid metabolism. *Cell. Metab.* 23, 206–219. doi:10.1016/j.cmet.2015.12.004

Chen, J., Li, Y., Yu, T. S., McKay, R. M., Burns, D. K., Kernie, S. G., et al. (2012). A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* 488, 522–526. doi:10.1038/nature11287

Chen, L., Ren, J., Yang, L., Li, Y., Fu, J., Li, Y., et al. (2016c). Stearoyl-CoA desaturase-1 mediated cell apoptosis in colorectal cancer by promoting ceramide synthesis. *Sci. Rep.* 6, 19665. doi:10.1038/srep19665

Chen, W., Dong, J., Haiech, J., Kilhoffer, M. C., and Zeniou, M. (2016a). Cancer stem cell quiescence and plasticity as major challenges in cancer therapy. *Stem Cells Int.* 2016, 1740936. doi:10.1155/2016/1740936

Choi, S., Yoo, Y. J., Kim, H., Lee, H., Chung, H., Nam, M. H., et al. (2019). Clinical and biochemical relevance of monounsaturated fatty acid metabolism targeting strategy for cancer stem cell elimination in colon cancer. *Biochem. Biophys. Res. Commun.* 519, 100–105. doi:10.1016/j.bbrc.2019.08.137

Christofk, H. R., Vander Heiden, M. G., Harris, M. H., Ramanathan, A., Gerszten, R. E., Wei, R., et al. (2008). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 452, 230–233. doi:10.1038/nature06734

Ciavardelli, D., Rossi, C., Barcaroli, D., Volpe, S., Consalvo, A., Zucchelli, M., et al. (2014). Breast cancer stem cells rely on fermentative glycolysis and are sensitive to 2-deoxyglucose treatment. *Cell. Death Dis.* 5, e1336. doi:10.1038/cddis.2014.285

Colacino, J. A., McDermott, S. P., Sartor, M. A., Wicha, M. S., and Rozek, L. S. (2016). Transcriptomic profiling of curcumin-treated human breast stem cells identifies a role for stearoyl-coa desaturase in breast cancer prevention. *Breast Cancer Res. Treat.* 158, 29–41. doi:10.1007/s10549-016-3854-4

Cordenonsi, M., Zanconato, F., Azzolin, L., Forcato, M., Rosato, A., Frasson, C., et al. (2011). The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell.* 147, 759–772. doi:10.1016/j.cell.2011.09.048

Corominas-Faja, B., Cuyas, E., Gumuzio, J., Bosch-Barrera, J., Leis, O., Martin, A. G., et al. (2014). Chemical inhibition of acetyl-CoA carboxylase suppresses self-renewal growth of cancer stem cells. *Oncotarget* 5, 8306–8316. doi:10.18632/oncotarget.2059

Couturier, C. P., Ayyadhury, S., Le, P. U., Nadaf, J., Monlong, J., Riva, G., et al. (2020). Single-cell RNA-seq reveals that glioblastoma recapitulates a normal neurodevelopmental hierarchy. *Nat. Commun.* 11, 3406. doi:10.1038/s41467-020-17186-5

Cufi, S., Vazquez-Martin, A., Oliveras-Ferraros, C., Martin-Castillo, B., Vellon, L., and Menendez, J. A. (2011). Autophagy positively regulates the CD44(+) CD24(-/low) breast cancer stem-like phenotype. *Cell. Cycle* 10, 3871–3885. doi:10.4161/cc.10.22.17976

Currie, E., Schulze, A., Zechner, R., Walther, T. C., and Farese, R. V., Jr. (2013). Cellular fatty acid metabolism and cancer. *Cell. Metab.* 18, 153–161. doi:10.1016/j.cmet.2013.05.017

Dando, I., Dalla Pozza, E., Biondani, G., Cordani, M., Palmieri, M., and Donadelli, M. (2015). The metabolic landscape of cancer stem cells. *IUBMB Life* 67, 687–693. doi:10.1002/iub.1426

Danhier, P., Banski, P., Payen, V. L., Grasso, D., Ippolito, L., Sonveaux, P., et al. (2017). Cancer metabolism in space and time: beyond the Warburg effect. *Biochim. Biophys. Acta Bioenerg.* 1858, 556–572. doi:10.1016/j.bbabio.2017.02.001

Dattilo, R., Mottini, C., Camera, E., Lamolinara, A., Auslander, N., Doglioni, G., et al. (2020). Pyrvinium pamoate induces death of triple-negative breast cancer stem-like cells and reduces metastases through effects on lipid anabolism. *Cancer Res.* 80, 4087–4102. doi:10.1158/0008-5472.CAN-19-1184

Dean, M., Fojo, T., and Bates, S. (2005). Tumour stem cells and drug resistance. *Nat. Rev. Cancer* 5, 275–284. doi:10.1038/nrc1590

De Angelis, M. L., Francescangeli, F., La Torre, F., and Zeuner, A. (2019). Stem cell plasticity and dormancy in the development of cancer therapy resistance. *Front. Oncol.* 9, 626. doi:10.3389/fonc.2019.00626

DeBose-Boyd, R. A., and Ye, J. (2018). SREBPs in lipid metabolism, insulin signaling, and beyond. *Trends Biochem. Sci.* 43, 358–368. doi:10.1016/j.tibs.2018.01.005

De Rosa, A., Pellegatta, S., Rossi, M., Tunici, P., Magnoni, L., Speranza, M. C., et al. (2012). A radial glia gene marker, fatty acid binding protein 7 (FABP7), is involved in proliferation and invasion of glioblastoma cells. *PLoS One* 7, e52113. doi:10.1371/journal.pone.0052113

Dirkse, A., Golebiewska, A., Buder, T., Nazarov, P. V., Muller, A., Poovathingal, S., et al. (2019). Stem cell-associated heterogeneity in Glioblastoma results from intrinsic tumor plasticity shaped by the microenvironment. *Nat. Commun.* 10, 1787. doi:10.1038/s41467-019-09853-z

Dong, C., Yuan, T., Wu, Y., Wang, Y., Fan, T. W., Miriyala, S., et al. (2013). Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell.* 23, 316–331. doi:10.1016/j.ccr.2013.01.022

Dorr, J. R., Yu, Y., Milanovic, M., Beuster, G., Zasada, C., Dabritz, J. H., et al. (2013). Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. *Nature* 501, 421–425. doi:10.1038/nature12437

Easwaran, H., Johnstone, S. E., Van Neste, L., Ohm, J., Mosbruger, T., Wang, Q., et al. (2012). A DNA hypermethylation module for the stem/progenitor cell signature of cancer. *Genome Res.* 22, 837–849. doi:10.1101/gr.131169.111

Ehmsen, S., Pedersen, M. H., Wang, G., Terp, M. G., Arslanagic, A., Hood, B. L., et al. (2019). Increased cholesterol biosynthesis is a key characteristic of breast cancer stem cells influencing patient outcome. *Cell. Rep.* 27, 3927–3938. doi:10.1016/j.celrep.2019.05.104

El-Sahli, S., Xie, Y., Wang, L., and Liu, S. (2019). Wnt signaling in cancer metabolism and immunity. *Cancers (Basel)* 11, 904. doi:10.3390/cancers11070904

Erhart, F., Blauensteiner, B., Zirkovits, G., Printz, D., Soukup, K., Klingenbrunner, S., et al. (2019). Gliomasphere marker combinatorics: multidimensional flow cytometry detects CD44+/CD133+/ITGA6+/CD36+ signature. *J. Cell. Mol. Med.* 23, 281–292. doi:10.1111/jcmm.13927

Estan, M. C., Calvino, E., Calvo, S., Guillen-Guio, B., Boyano-Adanez Mdel, C., de Blas, E., et al. (2014). Apoptotic efficacy of etomoxir in human acute myeloid leukemia cells. Cooperation with arsenic trioxide and glycolytic inhibitors, and regulation by oxidative stress and protein kinase activities. *PLoS One* 9, e115250. doi:10.1371/journal.pone.0115250

Fang, W., Cui, H., Yu, D., Chen, Y., Wang, J., and Yu, G. (2014). Increased expression of phospho-acetyl-CoA carboxylase protein is an independent prognostic factor for

human gastric cancer without lymph node metastasis. *Med. Oncol.* 31, 15. doi:10.1007/s12032-014-0015-7

Fendler, A., Bauer, D., Busch, J., Jung, K., Wulf-Goldenberg, A., Kunz, S., et al. (2020). Inhibiting WNT and NOTCH in renal cancer stem cells and the implications for human patients. *Nat. Commun.* 11, 929. doi:10.1038/s41467-020-14700-7

Feng, X., Zhang, L., Xu, S., and Shen, A. Z. (2020). ATP-citrate lyase (ACLY) in lipid metabolism and atherosclerosis: an updated review. *Prog. Lipid Res.* 77, 101006. doi:10.1016/j.plipres.2019.101006

Fritz, V., Benfodda, Z., Rodier, G., Henriquet, C., Iborra, F., Avances, C., et al. (2010). Abrogation of *de novo* lipogenesis by stearoyl-CoA desaturase 1 inhibition interferes with oncogenic signaling and blocks prostate cancer progression in mice. *Mol. Cancer Ther.* 9, 1740–1754. doi:10.1158/1535-7163.MCT-09-1064

Fu, T., Coulter, S., Yoshihara, E., Oh, T. G., Fang, S., Cayabyab, F., et al. (2019). FXR regulates intestinal cancer stem cell proliferation. *Cell.* 176, 1098–1112. doi:10.1016/j.cell.2019.01.036

Galavotti, S., Bartesaghi, S., Faccenda, D., Shaked-Rabi, M., Sanzone, S., McEvoy, A., et al. (2013). The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells. *Oncogene* 32, 699–712. doi:10.1038/onc.2012.111

Galli, R., Binda, E., Orfanelli, U., Cipelletti, B., Gritti, A., De Vitis, S., et al. (2004). Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res.* 64, 7011–7021. doi:10.1158/0008-5472.CAN-04-1364

Geng, F., Cheng, X., Wu, X., Yoo, J. Y., Cheng, C., Guo, J. Y., et al. (2016). Inhibition of SOAT1 suppresses glioblastoma growth via blocking SREBP-1-mediated lipogenesis. *Clin. Cancer Res.* 22, 5337–5348. doi:10.1158/1078-0432.CCR-15-2973

Gerlinger, M., Rowan, A. J., Horswell, S., Math, M., Larkin, J., Endesfelder, D., et al. (2012). Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* 366, 883–892. doi:10.1056/NEJMoa1113205

Gimple, R. C., Kidwell, R. L., Kim, L. J. Y., Sun, T., Gromovsky, A. D., Wu, Q., et al. (2019). Glioma stem cell-specific superenhancer promotes polyunsaturated fatty-acid synthesis to support EGFR signaling. *Cancer Discov.* 9, 1248–1267. doi:10.1158/2159-8290.cd-19-0061

Ginestier, C., Monville, F., Wicinski, J., Cabaud, O., Cervera, N., Josselin, E., et al. (2012). Mevalonate metabolism regulates Basal breast cancer stem cells and is a potential therapeutic target. *Stem Cells* 30, 1327–1337. doi:10.1002/stem.1122

Global Burden of Disease Cancer, C., Kocarnik, J. M., Compton, K., Dean, F. E., Fu, W., Gaw, B. L., et al. (2022). Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups from 2010 to 2019: a systematic analysis for the global burden of Disease study 2019. *JAMA Oncol.* 8, 420–444. doi:10.1001/jamaoncol.2021.6987

Goyette, S., Liang, Y., Mafuvadze, B., Cook, M. T., Munir, M., and Hyder, S. M. (2017). Natural and synthetic progestins enrich cancer stem cell-like cells in hormone-responsive human breast cancer cell populations *in vitro*. *Breast Cancer (Dove Med. Press)* 9, 347–357. doi:10.2147/BCTT.S135371

Gu, Q., Yang, X., Lv, J., Zhang, J., Xia, B., Kim, J. D., et al. (2019). AIBP-mediated cholesterol efflux instructs hematopoietic stem and progenitor cell fate. *Science* 363, 1085–1088. doi:10.1126/science.aav1749

Gupta, P. B., Onder, T. T., Jiang, G., Tao, K., Kuperwasser, C., Weinberg, R. A., et al. (2009). Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell.* 138, 645–659. doi:10.1016/j.cell.2009.06.034

Hafiane, A., Gasbarrino, K., and Daskalopoulou, S. S. (2019). The role of adiponectin in cholesterol efflux and HDL biogenesis and metabolism. *Metabolism* 100, 153953. doi:10.1016/j.metabol.2019.153953

Hale, J. S., Otvos, B., Sinyuk, M., Alvarado, A. G., Hitomi, M., Stoltz, K., et al. (2014). Cancer stem cell-specific scavenger receptor CD36 drives glioblastoma progression. *Stem Cells* 32, 1746–1758. doi:10.1002/stem.1716

Han, Y., Fan, S., Qin, T., Yang, J., Sun, Y., Lu, Y., et al. (2018). Role of autophagy in breast cancer and breast cancer stem cells (Review). *Int. J. Oncol.* 52, 1057–1070. doi:10.3892/ijo.2018.4270

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell.* 144, 646–674. doi:10.1016/j.cell.2011.02.013

Hanai, J. I., Doro, N., Seth, P., and Sukhatme, V. P. (2013). ATP citrate lyase knockdown impacts cancer stem cells *in vitro*. *Cell. Death Dis.* 4, e696. doi:10.1038/cddis.2013.215

Harrison, H., Farnie, G., Howell, S. J., Rock, R. E., Stylianou, S., Brennan, K. R., et al. (2010). Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. *Cancer Res.* 70, 709–718. doi:10.1158/0008-5472.CAN-09-1681

Hatzivassiliou, G., Zhao, F., Bauer, D. E., Andreadis, C., Shaw, A. N., Dhanak, D., et al. (2005). ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell.* 8, 311–321. doi:10.1016/j.ccr.2005.09.008

Herpel, E., Jensen, K., Muley, T., Warth, A., Schnabel, P. A., Meister, M., et al. (2011). The cancer stem cell antigens CD133, BCRP1/ABCG2 and CD117/c-KIT are not associated with prognosis in resected early-stage non-small cell lung cancer. *Anticancer Res.* 31, 4491–4500.

Herreros-Pomares, A., de-Maya-Girones, J. D., Calabuig-Farinas, S., Lucas, R., Martinez, A., Pardo-Sanchez, J. M., et al. (2019). Lung tumorspheres reveal cancer stem cell-like properties and a score with prognostic impact in resected non-small-cell lung cancer. *Cell. Death Dis.* 10, 660. doi:10.1038/s41419-019-1898-1

Higa, T., and Nakayama, K. I. (2024). Cell cycle heterogeneity and plasticity of colorectal cancer stem cells. *Cancer Sci.* 115, 1370–1377. doi:10.1111/cas.16117

Hoffmeyer, K., Raggioli, A., Rudloff, S., Anton, R., Hierholzer, A., Del Valle, I., et al. (2012). Wnt/β-catenin signaling regulates telomerase in stem cells and cancer cells. *Science* 336, 1549–1554. doi:10.1126/science.1218370

Holubarsch, C. J., Rohrbach, M., Karrasch, M., Boehm, E., Polonski, L., Ponikowski, P., et al. (2007). A double-blind randomized multicentre clinical trial to evaluate the efficacy and safety of two doses of etomoxir in comparison with placebo in patients with moderate congestive heart failure: the ERGO (etomoxir for the recovery of glucose oxidation) study. *Clin. Sci. (Lond)* 113, 205–212. doi:10.1042/CS20060307

Hu, A., and Song, B. L. (2019). The interplay of Patched, Smoothened and cholesterol in Hedgehog signaling. *Curr. Opin. Cell. Biol.* 61, 31–38. doi:10.1016/j.ceb.2019.06.008

Huo, X., Zhang, Q., Liu, A. M., Tang, C., Gong, Y., Bian, J., et al. (2013). Overexpression of Yes-associated protein confers doxorubicin resistance in hepatocellullar carcinoma. *Oncol. Rep.* 29, 840–846. doi:10.3892/or.2012.2176

Iannelli, F., Roca, M. S., Lombardi, R., Ciardiello, C., Grumetti, L., De Rienzo, S., et al. (2020). Synergistic antitumor interaction of valproic acid and simvastatin sensitizes prostate cancer to docetaxel by targeting CSCs compartment via YAP inhibition. *J. Exp. Clin. Cancer Res.* 39, 213. doi:10.1186/s13046-020-01723-7

Janiszewska, M., Suva, M. L., Riggi, N., Houtkooper, R. H., Auwerx, J., Clement-Schatlo, V., et al. (2012). Imp2 controls oxidative phosphorylation and is crucial for preserving glioblastoma cancer stem cells. *Genes. Dev.* 26, 1926–1944. doi:10.1101/gad.188292.112

Jia, Q., Zhang, X., Deng, T., and Gao, J. (2013). Positive correlation of Oct4 and ABCG2 to chemotherapeutic resistance in CD90(+)CD133(+) liver cancer stem cells. *Cell. Reprogr.* 15, 143–150. doi:10.1089/cell.2012.0048

Jiao, J., Hindoyan, A., Wang, S., Tran, L. M., Goldstein, A. S., Lawson, D., et al. (2012). Identification of CD166 as a surface marker for enriching prostate stem/progenitor and cancer initiating cells. *PLoS One* 7, e42564. doi:10.1371/journal.pone.0042564

Jones, S. F., and Infante, J. R. (2015). Molecular pathways: fatty acid synthase. *Clin. cancer Res. official J. Am. Assoc. Cancer Res.* 21, 5434–5438. doi:10.1158/1078-0432.ccr-15-0126

Joyce, S. A., and Gahan, C. G. (2016). Bile acid modifications at the microbe-host interface: potential for nutraceutical and pharmaceutical interventions in host health. *Annu. Rev. Food Sci. Technol.* 7, 313–333. doi:10.1146/annurev-food-041715-033159

Ju, H. Q., Zhan, G., Huang, A., Sun, Y., Wen, S., Yang, J., et al. (2017). ITD mutation in FLT3 tyrosine kinase promotes Warburg effect and renders therapeutic sensitivity to glycolytic inhibition. *Leukemia* 31, 2143–2150. doi:10.1038/leu.2017.45

Kalucka, J., Bierhansl, L., Conchinha, N. V., Missiaen, R., Elia, I., Bruning, U., et al. (2018). Quiescent endothelial cells upregulate fatty acid beta-oxidation for vasculoprotection via redox homeostasis. *Cell. Metab.* 28, 881–894 e813. doi:10.1016/j.cmet.2018.07.016

Kathagen, A., Schulte, A., Balcke, G., Phillips, H. S., Martens, T., Matschke, J., et al. (2013). Hypoxia and oxygenation induce a metabolic switch between pentose phosphate pathway and glycolysis in glioma stem-like cells. *Acta Neuropathol.* 126, 763–780. doi:10.1007/s00401-013-1173-y

Kinlaw, W. B., Baures, P. W., Lupien, L. E., Davis, W. L., and Kuemmerle, N. B. (2016). Fatty acids and breast cancer: make them on site or have them delivered. *J. Cell. Physiol.* 231, 2128–2141. doi:10.1002/jcp.25332

Klionsky, D. J., Eskelinen, E. L., and Deretic, V. (2014). Autophagosomes, phagosomes, autolysosomes, phagolysosomes, autophagolysosomes. wait, I'm confused. *Autophagy* 10, 549–551. doi:10.4161/auto.28448

Koo, J. H., and Guan, K. L. (2018). Interplay between YAP/TAZ and metabolism. *Cell. Metab.* 28, 196–206. doi:10.1016/j.cmet.2018.07.010

Koren, E., and Fuchs, Y. (2016). The bad seed: cancer stem cells in tumor development and resistance. *Drug Resist Updat* 28, 1–12. doi:10.1016/j.drup.2016.06.006

Krstic, J., Trivanovic, D., Jaukovic, A., Santibanez, J. F., and Bugarski, D. (2017). Metabolic plasticity of stem cells and macrophages in cancer. *Front. Immunol.* 8, 939. doi:10.3389/fimmu.2017.00939

Kulkarni, M., Tan, T. Z., Syed Sulaiman, N. B., Lamar, J. M., Bansal, P., Cui, J., et al. (2018). RUNX1 and RUNX3 protect against YAP-mediated EMT, stem-ness and shorter survival outcomes in breast cancer. *Oncotarget* 9, 14175–14192. doi:10.18632/oncotarget.24419

Kuntz, E. M., Baquero, P., Michie, A. M., Dunn, K., Tardito, S., Holyoake, T. L., et al. (2017). Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. *Nat. Med.* 23, 1234–1240. doi:10.1038/nm.4399

Lagadinou, E. D., Sach, A., Callahan, K., Rossi, R. M., Neering, S. J., Minhajuddin, M., et al. (2013). BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. *Cell. Stem Cell.* 12, 329–341. doi:10.1016/j.stem.2012.12.013

Lai, K. K. Y., Kweon, S. M., Chi, F., Hwang, E., Kabe, Y., Higashiyama, R., et al. (2017). Stearoyl-CoA desaturase promotes liver fibrosis and tumor development in mice via a Wnt positive-signaling loop by stabilization of low-density lipoprotein-receptor-related proteins 5 and 6. *Gastroenterology* 152, 1477–1491. doi:10.1053/j.gastro.2017.01.021

Lang, C. M. R., Chan, C. K., Veltri, A., and Lien, W. H. (2019). Wnt signaling pathways in keratinocyte carcinomas. *Cancers (Basel)* 11, 1216. doi:10.3390/cancers11091216

Lecot, P., Alimirah, F., Desprez, P. Y., Campisi, J., and Wiley, C. (2016). Context-dependent effects of cellular senescence in cancer development. *Br. J. Cancer* 114, 1180–1184. doi:10.1038/bjc.2016.115

Lee, K. M., Giltnane, J. M., Balko, J. M., Schwarz, L. J., Guerrero-Zotano, A. L., Hutchinson, K. E., et al. (2017). MYC and MCL1 cooperatively promote chemotherapy-resistant breast cancer stem cells via regulation of mitochondrial oxidative phosphorylation. *Cell. metab.* 26, 633–647. doi:10.1016/j.cmet.2017.09.009

Lewis, C. A., Brault, C., Peck, B., Bensaad, K., Griffiths, B., Mitter, R., et al. (2015). SREBP maintains lipid biosynthesis and viability of cancer cells under lipid- and oxygen-deprived conditions and defines a gene signature associated with poor survival in glioblastoma multiforme. *Oncogene* 34, 5128–5140. doi:10.1038/onc.2014.439

Li, J., Condello, S., Thomes-Pepin, J., Ma, X., Xia, Y., Hurley, T. D., et al. (2017). Lipid desaturation is a metabolic marker and therapeutic target of ovarian cancer stem cells. *Cell. Stem Cell.* 20, 303–314. doi:10.1016/j.stem.2016.11.004

Li, L., Wang, C., Calvisi, D. F., Evert, M., Pilo, M. G., Jiang, L., et al. (2013a). SCD1 Expression is dispensable for hepatocarcinogenesis induced by AKT and Ras oncogenes in mice. *PLoS One* 8, e75104. doi:10.1371/journal.pone.0075104

Li, S. H., Fu, J., Watkins, D. N., Srivastava, R. K., and Shankar, S. (2013b). Sulforaphane regulates self-renewal of pancreatic cancer stem cells through the modulation of Sonic hedgehog-GLI pathway. *Mol. Cell. Biochem.* 373, 217–227. doi:10.1007/s11010-012-1493-6

Li, Y., Chao, X., Yang, L., Lu, Q., Li, T., Ding, W. X., et al. (2018). Impaired fasting-induced adaptive lipid droplet biogenesis in liver-specific atg5-deficient mouse liver is mediated by persistent nuclear factor-like 2 activation. *Am. J. Pathol.* 188, 1833–1846. doi:10.1016/j.ajpath.2018.04.015

Li, Y., Huang, X., Yang, G., Xu, K., Yin, Y., Brecchia, G., et al. (2022). CD36 favours fat sensing and transport to govern lipid metabolism. *Prog. Lipid Res.* 88, 101193. doi:10.1016/j.plipres.2022.101193

Liang, R., Li, X., Li, W., Zhu, X., and Li, C. (2021). DNA methylation in lung cancer patients: opening a "window of life" under precision medicine. *Biomed. Pharmacother.* 144, 112202. doi:10.1016/j.biopha.2021.112202

Liang, Y., Goyette, S., and Hyder, S. M. (2017). Cholesterol biosynthesis inhibitor RO 48-8071 reduces progesterone receptor expression and inhibits progestin-dependent stem cell-like cell growth in hormone-dependent human breast cancer cells. *Breast Cancer (Dove Med. Press)* 9, 487–494. doi:10.2147/BCTT.S140265

Liao, J., Qian, F., Tchabo, N., Mhawech-Fauceglia, P., Beck, A., Qian, Z., et al. (2014). Ovarian cancer spheroid cells with stem cell-like properties contribute to tumor generation, metastasis and chemotherapy resistance through hypoxia-resistant metabolism. *PloS one* 9, e84941. doi:10.1371/journal.pone.0084941

Liberti, M. V., and Locasale, J. W. (2016). The Warburg effect: how does it benefit cancer cells? *Trends Biochem. Sci.* 41, 211–218. doi:10.1016/j.tibs.2015.12.001

Lin, L., Ding, Y., Wang, Y., Wang, Z., Yin, X., Yan, G., et al. (2017). Functional lipidomics: palmitic acid impairs hepatocellular carcinoma development by modulating membrane fluidity and glucose metabolism. *Hepatology* 66, 432–448. doi:10.1002/hep.29033

Lin, Y., Yu, B., Fang, P., and Wang, J. (2023). Inhibiting autophagy before it starts. *Autophagy* 20, 923–924. doi:10.1080/15548627.2023.2197364

Lingwood, D., and Simons, K. (2010). Lipid rafts as a membrane-organizing principle. *Science* 327, 46–50. doi:10.1126/science.1174621

Liu, H., Zhang, Z., Song, L., Gao, J., and Liu, Y. (2022). Lipid metabolism of cancer stem cells. *Oncol. Lett.* 23, 119. doi:10.3892/ol.2022.13239

Lobello, N., Biamonte, F., Pisanu, M. E., Faniello, M. C., Jakopin, Z., Chiarella, E., et al. (2016). Ferritin heavy chain is a negative regulator of ovarian cancer stem cell expansion and epithelial to mesenchymal transition. *Oncotarget* 7, 62019–62033. doi:10.18632/oncotarget.11495

Long, B., Wang, L. X., Zheng, F. M., Lai, S. P., Xu, D. R., Hu, Y., et al. (2016). Targeting GLI1 suppresses cell growth and enhances chemosensitivity in CD34+ enriched acute myeloid leukemia progenitor cells. *Cell. Physiol. Biochem.* 38, 1288–1302. doi:10.1159/000443075

Lopes, M. B., and Vinga, S. (2020). Tracking intratumoral heterogeneity in glioblastoma via regularized classification of single-cell RNA-Seq data. *BMC Bioinforma.* 21, 59. doi:10.1186/s12859-020-3390-4

Lucenay, K. S., Doostan, I., Karakas, C., Bui, T., Ding, Z., Mills, G. B., et al. (2016). Cyclin E associates with the lipogenic enzyme ATP-citrate lyase to enable malignant growth of breast cancer cells. *Cancer Res.* 76, 2406–2418. doi:10.1158/0008-5472.CAN-15-1646

Lue, H. W., Podolak, J., Kolahi, K., Cheng, L., Rao, S., Garg, D., et al. (2017). Metabolic reprogramming ensures cancer cell survival despite oncogenic signaling blockade. *Genes. Dev.* 31, 2067–2084. doi:10.1101/gad.305292.117

Luo, J., Hong, Y., Lu, Y., Qiu, S., Chaganty, B. K., Zhang, L., et al. (2017b). Acetyl-CoA carboxylase rewires cancer metabolism to allow cancer cells to survive inhibition of the Warburg effect by cetuximab. *Cancer Lett.* 384, 39–49. doi:10.1016/j.canlet.2016.09.020

Luo, X., Cheng, C., Tan, Z., Li, N., Tang, M., Yang, L., et al. (2017a). Emerging roles of lipid metabolism in cancer metastasis. *Mol. Cancer* 16, 76. doi:10.1186/s12943-017-0646-3

Ma, M. K. F., Lau, E. Y. T., Leung, D. H. W., Lo, J., Ho, N. P. Y., Cheng, L. K. W., et al. (2017). Stearoyl-CoA desaturase regulates sorafenib resistance via modulation of ER stress-induced differentiation. *J. Hepatol.* 67, 979–990. doi:10.1016/j.jhep.2017.06.015

Ma, X. L., Sun, Y. F., Wang, B. L., Shen, M. N., Zhou, Y., Chen, J. W., et al. (2019). Sphere-forming culture enriches liver cancer stem cells and reveals Stearoyl-CoA desaturase 1 as a potential therapeutic target. *BMC Cancer* 19, 760. doi:10.1186/s12885-019-5963-z

Mahapatra, K. K., Mishra, S. R., Behera, B. P., Patil, S., Gewirtz, D. A., and Bhutia, S. K. (2021). The lysosome as an imperative regulator of autophagy and cell death. *Cell. Mol. Life Sci.* 78, 7435–7449. doi:10.1007/s00018-021-03988-3

Mai, T. T., Hamai, A., Hienzsch, A., Caneque, T., Muller, S., Wicinski, J., et al. (2017). Salinomycin kills cancer stem cells by sequestering iron in lysosomes. *Nat. Chem.* 9, 1025–1033. doi:10.1038/nchem.2778

Maiuri, A. R., Savant, S. S., Podicheti, R., Rusch, D. B., and O'Hagan, H. M. (2019). DNA methyltransferase inhibition reduces inflammation-induced colon tumorigenesis. *Epigenetics* 14, 1209–1223. doi:10.1080/15592294.2019.1634986

Mancini, R., Noto, A., Pisanu, M. E., De Vitis, C., Maugeri-Sacca, M., and Ciliberto, G. (2018). Metabolic features of cancer stem cells: the emerging role of lipid metabolism. *Oncogene* 37, 2367–2378. doi:10.1038/s41388-018-0141-3

Margaritescu, C., Pirici, D., Cherciu, I., Barbalan, A., Cartana, T., and Saftoiu, A. (2014). CD133/CD166/Ki-67 triple immunouorescence assessment for putative cancer stem cells in colon carcinoma*. *J. Gastrointestin Liver Dis.* 23, 161–170. doi:10.15403/jgld.2014.1121.232.cm1

Martinez-Outschoorn, U. E., Peiris-Pages, M., Pestell, R. G., Sotgia, F., and Lisanti, M. P. (2017). Cancer metabolism: a therapeutic perspective. *Nat. Rev. Clin. Oncol.* 14, 11–31. doi:10.1038/nrclinonc.2016.60

Martinez-Reyes, I., and Chandel, N. S. (2021). Cancer metabolism: looking forward. *Nat. Rev. Cancer* 21, 669–680. doi:10.1038/s41568-021-00378-6

Medema, J. P. (2013). Cancer stem cells: the challenges ahead. *Nat. Cell. Biol.* 15, 338–344. doi:10.1038/ncb2717

Menendez, J. A., and Lupu, R. (2017). Fatty acid synthase (FASN) as a therapeutic target in breast cancer. *Expert Opin. Ther. Targets* 21, 1001–1016. doi:10.1080/14728222.2017.1381087

Metallo, C. M., Gameiro, P. A., Bell, E. L., Mattaini, K. R., Yang, J., Hiller, K., et al. (2011). Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* 481, 380–384. doi:10.1038/nature10602

Michaloglou, C., Vredeveld, L. C., Soengas, M. S., Denoyelle, C., Kuilman, T., van der Horst, C. M., et al. (2005). BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 436, 720–724. doi:10.1038/nature03890

Migita, T., Narita, T., Nomura, K., Miyagi, E., Inazuka, F., Matsuura, M., et al. (2008). ATP citrate lyase: activation and therapeutic implications in non-small cell lung cancer. *Cancer Res.* 68, 8547–8554. doi:10.1158/0008-5472.CAN-08-1235

Milanovic, M., Fan, D. N. Y., Belenki, D., Dabritz, J. H. M., Zhao, Z., Yu, Y., et al. (2018). Senescence-associated reprogramming promotes cancer stemness. *Nature* 553, 96–100. doi:10.1038/nature25167

Minami, N., Tanaka, K., Sasayama, T., Kohmura, E., Saya, H., and Sampetrean, O. (2021). Lactate reprograms energy and lipid metabolism in glucose-deprived oxidative glioma stem cells. *Metabolites* 11, 325. doi:10.3390/metabo11050325

Miyazaki, Y., Matsubara, S., Ding, Q., Tsukasa, K., Yoshimitsu, M., Kosai, K., et al. (2016). Efficient elimination of pancreatic cancer stem cells by hedgehog/GLI inhibitor GANT61 in combination with mTOR inhibition. *Mol. Cancer* 15, 49. doi:10.1186/s12943-016-0534-2

Mo, J. S., Park, H. W., and Guan, K. L. (2014). The Hippo signaling pathway in stem cell biology and cancer. *EMBO Rep.* 15, 642–656. doi:10.15252/embr.201438638

Moncur, J. T., Park, J. P., Memoli, V. A., Mohandas, T. K., and Kinlaw, W. B. (1998). The "Spot 14" gene resides on the telomeric end of the 11q13 amplicon and is expressed in lipogenic breast cancers: implications for control of tumor metabolism. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6989–6994. doi:10.1073/pnas.95.12.6989

Morihiro, Y., Yasumoto, Y., Vaidyan, L. K., Sadahiro, H., Uchida, T., Inamura, A., et al. (2013). Fatty acid binding protein 7 as a marker of glioma stem cells. *Pathol. Int.* 63, 546–553. doi:10.1111/pin.12109

Mullen, P. J., Yu, R., Longo, J., Archer, M. C., and Penn, L. Z. (2016). The interplay between cell signalling and the mevalonate pathway in cancer. *Nat. Rev. Cancer* 16, 718–731. doi:10.1038/nrc.2016.76

Munir, R., Lisec, J., Swinnen, J. V., and Zaidi, N. (2019). Lipid metabolism in cancer cells under metabolic stress. *Br. J. Cancer* 120, 1090–1098. doi:10.1038/s41416-019-0451-4

Myant, K. B., Cammareri, P., McGhee, E. J., Ridgway, R. A., Huels, D. J., Cordero, J. B., et al. (2013). ROS production and NF-κB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. *Cell. Stem Cell.* 12, 761–773. doi:10.1016/j.stem.2013.04.006

Nath, A., Li, I., Roberts, L. R., and Chan, C. (2015). Elevated free fatty acid uptake via CD36 promotes epithelial-mesenchymal transition in hepatocellular carcinoma. *Sci. Rep.* 5, 14752. doi:10.1038/srep14752

Neftel, C., Laffy, J., Filbin, M. G., Hara, T., Shore, M. E., Rahme, G. J., et al. (2019). An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell.* 178, 835–849. doi:10.1016/j.cell.2019.06.024

Ni, H., Qin, H., Sun, C., Liu, Y., Ruan, G., Guo, Q., et al. (2021). MiR-375 reduces the stemness of gastric cancer cells through triggering ferroptosis. *Stem Cell. Res. Ther.* 12, 325. doi:10.1186/s13287-021-02394-7

Nishizawa, S., Sumi, H., Satoh, Y., Yamamoto, Y., Kitazawa, S., Honda, K., et al. (2017). *In vitro* and *in vivo* antitumor activities of T-3764518, a novel and orally available small molecule stearoyl-CoA desaturase 1 inhibitor. *Eur. J. Pharmacol.* 807, 21–31. doi:10.1016/j.ejphar.2017.03.064

Noto, A., De Vitis, C., Pisanu, M. E., Roscilli, G., Ricci, G., Catizone, A., et al. (2017a). Stearoyl-CoA-desaturase 1 regulates lung cancer stemness via stabilization and nuclear localization of YAP/TAZ. *Oncogene* 36, 4573–4584. doi:10.1038/onc.2017.75

Noto, A., De Vitis, C., Pisanu, M. E., Roscilli, G., Ricci, G., Catizone, A., et al. (2017b). Stearoyl-CoA-desaturase 1 regulates lung cancer stemness via stabilization and nuclear localization of YAP/TAZ. *Oncogene* 36, 4671–4672. doi:10.1038/onc.2017.212

Noto, A., Raffa, S., De Vitis, C., Roscilli, G., Malpicci, D., Coluccia, P., et al. (2013). Stearoyl-CoA desaturase-1 is a key factor for lung cancer-initiating cells. *Cell. Death Dis.* 4, e947. doi:10.1038/cddis.2013.444

Novak, D., Huser, L., Elton, J. J., Umansky, V., Altevogt, P., and Utikal, J. (2020). SOX2 in development and cancer biology. *Semin. Cancer Biol.* 67, 74–82. doi:10.1016/j.semcancer.2019.08.007

Obniski, R., Sieber, M., and Spradling, A. C. (2018). Dietary lipids modulate notch signaling and influence adult intestinal development and metabolism in Drosophila. *Dev. Cell.* 47, 98–111. doi:10.1016/j.devcel.2018.08.013

OBrien, C. A., Pollett, A., Gallinger, S., and Dick, J. E. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 445, 106–110. doi:10.1038/nature05372

Pacilli, A., Calienni, M., Margarucci, S., D'Apolito, M., Petillo, O., Rocchi, L., et al. (2013). Carnitine-acyltransferase system inhibition, cancer cell death, and prevention of myc-induced lymphomagenesis. *J. Natl. Cancer Inst.* 105, 489–498. doi:10.1093/jnci/djt030

Palorini, R., Votta, G., Balestrieri, C., Monestiroli, A., Olivieri, S., Vento, R., et al. (2014). Energy metabolism characterization of a novel cancer stem cell-like line 3AB-OS. *J. Cell. Biochem.* 115, 368–379. doi:10.1002/jcb.24671

Pandey, P. R., Okuda, H., Watabe, M., Pai, S. K., Liu, W., Kobayashi, A., et al. (2011). Resveratrol suppresses growth of cancer stem-like cells by inhibiting fatty acid synthase. *Breast cancer Res. Treat.* 130, 387–398. doi:10.1007/s10549-010-1300-6

Pandey, P. R., Xing, F., Sharma, S., Watabe, M., Pai, S. K., Iiizumi-Gairani, M., et al. (2013). Elevated lipogenesis in epithelial stem-like cell confers survival advantage in ductal carcinoma *in situ* of breast cancer. *Oncogene* 32, 5111–5122. doi:10.1038/onc.2012.519

Pascual, G., Avgustinova, A., Mejetta, S., Martin, M., Castellanos, A., Attolini, C. S., et al. (2017). Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature* 541, 41–45. doi:10.1038/nature20791

Pasto, A., Bellio, C., Pilotto, G., Ciminale, V., Silic-Benussi, M., Guzzo, G., et al. (2014). Cancer stem cells from epithelial ovarian cancer patients privilege oxidative phosphorylation, and resist glucose deprivation. *Oncotarget* 5, 4305–4319. doi:10.18632/oncotarget.2010

Patel, A. P., Tirosh, I., Trombetta, J. J., Shalek, A. K., Gillespie, S. M., Wakimoto, H., et al. (2014). Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science* 344, 1396–1401. doi:10.1126/science.1254257

Patel, S., Alam, A., Pant, R., and Chattopadhyay, S. (2019). Wnt signaling and its significance within the tumor microenvironment: novel therapeutic insights. *Front. Immunol.* 10, 2872. doi:10.3389/fimmu.2019.02872

Paul, R., Dorsey, J. F., and Fan, Y. (2022). Cell plasticity, senescence, and quiescence in cancer stem cells: biological and therapeutic implications. *Pharmacol. Ther.* 231, 107985. doi:10.1016/j.pharmthera.2021.107985

Peck, B., and Schulze, A. (2016). Lipid desaturation - the next step in targeting lipogenesis in cancer? *FEBS J.* 283, 2767–2778. doi:10.1111/febs.13681

Peiris-Pages, M., Martinez-Outschoorn, U. E., Pestell, R. G., Sotgia, F., and Lisanti, M. P. (2016). Cancer stem cell metabolism. *Breast cancer Res. BCR* 18, 55. doi:10.1186/s13058-016-0712-6

Peng, F., Wang, J. H., Fan, W. J., Meng, Y. T., Li, M. M., Li, T. T., et al. (2018). Glycolysis gatekeeper PDK1 reprograms breast cancer stem cells under hypoxia. *Oncogene* 37, 1062–1074. doi:10.1038/onc.2017.368

Petrova, E., Scholz, A., Paul, J., Sturz, A., Haike, K., Siegel, F., et al. (2017). Acetyl-CoA carboxylase inhibitors attenuate WNT and Hedgehog signaling and suppress pancreatic tumor growth. *Oncotarget* 8, 48660–48670. doi:10.18632/oncotarget.12650

Pisanu, M. E., Maugeri-Sacca, M., Fattore, L., Bruschini, S., De Vitis, C., Tabbi, E., et al. (2018). Inhibition of Stearoyl-CoA desaturase 1 reverts BRAF and MEK inhibition-induced selection of cancer stem cells in BRAF-mutated melanoma. *J. Exp. Clin. Cancer Res.* 37, 318. doi:10.1186/s13046-018-0989-7

Pisanu, M. E., Noto, A., De Vitis, C., Morrone, S., Scognamiglio, G., Botti, G., et al. (2017). Blockade of Stearoyl-CoA-desaturase 1 activity reverts resistance to cisplatin in lung cancer stem cells. *Cancer Lett.* 406, 93–104. doi:10.1016/j.canlet.2017.07.027

Pizer, E. S., Thupari, J., Han, W. F., Pinn, M. L., Chrest, F. J., Frehywot, G. L., et al. (2000). Malonyl-coenzyme-A is a potential mediator of cytotoxicity induced by fatty-acid synthase inhibition in human breast cancer cells and xenografts. *Cancer Res.* 60, 213–218.

Polewski, M. D., Reveron-Thornton, R. F., Cherryholmes, G. A., Marinov, G. K., and Aboody, K. S. (2017). SLC7A11 overexpression in glioblastoma is associated with increased cancer stem cell-like properties. *Stem Cells Dev.* 26, 1236–1246. doi:10.1089/scd.2017.0123

Porstmann, T., Santos, C. R., Griffiths, B., Cully, M., Wu, M., Leevers, S., et al. (2008). SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell. Metab.* 8, 224–236. doi:10.1016/j.cmet.2008.07.007

Potze, L., di Franco, S., Kessler, J. H., Stassi, G., and Medema, J. P. (2016). Betulinic acid kills colon cancer stem cells. *Curr. Stem Cell. Res. Ther.* 11, 427–433. doi:10.2174/1574888x11666151203223512

Prasetyanti, P. R., and Medema, J. P. (2017). Intra-tumor heterogeneity from a cancer stem cell perspective. *Mol. Cancer* 16, 41. doi:10.1186/s12943-017-0600-4

Qian, X., Hu, J., Zhao, J., and Chen, H. (2015). ATP citrate lyase expression is associated with advanced stage and prognosis in gastric adenocarcinoma. *Int. J. Clin. Exp. Med.* 8, 7855–7860.

Rabionet, M., Polonio-Alcala, E., Relat, J., Yeste, M., Sims-Mourtada, J., Kloxin, A. M., et al. (2021). Fatty acid synthase as a feasible biomarker for triple negative breast cancer stem cell subpopulation cultured on electrospun scaffolds. *Mater Today Bio* 12, 100155. doi:10.1016/j.mtbio.2021.100155

Radhakrishnan, A., Rohatgi, R., and Siebold, C. (2020). Cholesterol access in cellular membranes controls Hedgehog signaling. *Nat. Chem. Biol.* 16, 1303–1313. doi:10.1038/s41589-020-00678-2

Reya, T., Morrison, S. J., Clarke, M. F., and Weissman, I. L. (2001). Stem cells, cancer, and cancer stem cells. *Nature* 414, 105–111. doi:10.1038/35102167

Ricciardi, M. R., Mirabilii, S., Allegretti, M., Licchetta, R., Calarco, A., Torrisi, M. R., et al. (2015). Targeting the leukemia cell metabolism by the CPT1a inhibition: functional preclinical effects in leukemias. *Blood* 126, 1925–1929. doi:10.1182/blood-2014-12-617498

Ricoult, S. J., Yecies, J. L., Ben-Sahra, I., and Manning, B. D. (2016). Oncogenic PI3K and K-Ras stimulate *de novo* lipid synthesis through mTORC1 and SREBP. *Oncogene* 35, 1250–1260. doi:10.1038/onc.2015.179

Riester, M., Xu, Q., Moreira, A., Zheng, J., Michor, F., and Downey, R. J. (2018). The Warburg effect: persistence of stem-cell metabolism in cancers as a failure of differentiation. *Ann. Oncol.* 29, 264–270. doi:10.1093/annonc/mdx645

Rios-Esteves, J., and Resh, M. D. (2013). Stearoyl CoA desaturase is required to produce active, lipid-modified Wnt proteins. *Cell. Rep.* 4, 1072–1081. doi:10.1016/j.celrep.2013.08.027

Robey, R. W., Pluchino, K. M., Hall, M. D., Fojo, A. T., Bates, S. E., and Gottesman, M. M. (2018). Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat. Rev. Cancer* 18, 452–464. doi:10.1038/s41568-018-0005-8

Rohrig, F., and Schulze, A. (2016). The multifaceted roles of fatty acid synthesis in cancer. *Nat. Rev. Cancer* 16, 732–749. doi:10.1038/nrc.2016.89

Samudio, I., Harmancey, R., Fiegl, M., Kantarjian, H., Konopleva, M., Korchin, B., et al. (2010). Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. *J. Clin. Investig.* 120, 142–156. doi:10.1172/JCI38942

Sanchez-Martinez, R., Cruz-Gil, S., Gomez de Cedron, M., Alvarez-Fernandez, M., Vargas, T., Molina, S., et al. (2015). A link between lipid metabolism and epithelial-mesenchymal transition provides a target for colon cancer therapy. *Oncotarget* 6, 38719–38736. doi:10.18632/oncotarget.5340

Sancho, P., Barneda, D., and Heeschen, C. (2016). Hallmarks of cancer stem cell metabolism. *Br. J. Cancer* 114, 1305–1312. doi:10.1038/bjc.2016.152

Sasaki, H., Nishizaki, Y., Hui, C., Nakafuku, M., and Kondoh, H. (1999). Regulation of Gli2 and Gli3 activities by an amino-terminal repression domain: implication of Gli2 and Gli3 as primary mediators of Shh signaling. *Development* 126, 3915–3924. doi:10.1242/dev.126.17.3915

Sayd, S., Thirant, C., El-Habr, E. A., Lipecka, J., Dubois, L. G., Bogeas, A., et al. (2014). Sirtuin-2 activity is required for glioma stem cell proliferation arrest but not necrosis induced by resveratrol. *Stem Cell. Rev. Rep.* 10, 103–113. doi:10.1007/s12015-013-9465-0

Schatton, T., Murphy, G. F., Frank, N. Y., Yamaura, K., Waaga-Gasser, A. M., Gasser, M., et al. (2008). Identification of cells initiating human melanomas. *Nature* 451, 345–349. doi:10.1038/nature06489

Schcolnik-Cabrera, A., Chavez-Blanco, A., Dominguez-Gomez, G., Taja-Chayeb, L., Morales-Barcenas, R., Trejo-Becerril, C., et al. (2018). Orlistat as a FASN inhibitor and multitargeted agent for cancer therapy. *Expert Opin. Investig. Drugs* 27, 475–489. doi:10.1080/13543784.2018.1471132

Schug, Z. T., Peck, B., Jones, D. T., Zhang, Q., Grosskurth, S., Alam, I. S., et al. (2015). Acetyl-CoA synthetase 2 promotes acetate utilization and maintains cancer cell growth under metabolic stress. *Cancer Cell.* 27, 57–71. doi:10.1016/j.ccell.2014.12.002

Seo, Y., Kim, J., Park, S. J., Park, J. J., Cheon, J. H., Kim, W. H., et al. (2020). Metformin suppresses cancer stem cells through AMPK activation and inhibition of protein prenylation of the mevalonate pathway in colorectal cancer. *Cancers (Basel)* 12, 2554. doi:10.3390/cancers12092554

Sharon, C., Baranwal, S., Patel, N. J., Rodriguez-Agudo, D., Pandak, W. M., Majumdar, A. P., et al. (2015). Inhibition of insulin-like growth factor receptor/AKT/mammalian target of rapamycin axis targets colorectal cancer stem cells by attenuating mevalonate-isoprenoid pathway *in vitro* and *in vivo*. *Oncotarget* 6, 15332–15347. doi:10.18632/oncotarget.3684

Shen, Y. A., Wang, C. Y., Hsieh, Y. T., Chen, Y. J., and Wei, Y. H. (2015). Metabolic reprogramming orchestrates cancer stem cell properties in nasopharyngeal carcinoma. *Cell. Cycle* 14, 86–98. doi:10.4161/15384101.2014.974419

Shimano, H., and Sato, R. (2017). SREBP-regulated lipid metabolism: convergent physiology - divergent pathophysiology. *Nat. Rev. Endocrinol.* 13, 710–730. doi:10.1038/nrendo.2017.91

Shiokawa, D., Sakai, H., Ohata, H., Miyazaki, T., Kanda, Y., Sekine, S., et al. (2020). Slow-cycling cancer stem cells regulate progression and chemoresistance in colon cancer. *Cancer Res.* 80, 4451–4464. doi:10.1158/0008-5472.CAN-20-0378

Shiozawa, Y., Nie, B., Pienta, K. J., Morgan, T. M., and Taichman, R. S. (2013). Cancer stem cells and their role in metastasis. *Pharmacol. Ther.* 138, 285–293. doi:10.1016/j.pharmthera.2013.01.014

Shu, Z., Gao, Y., Zhang, G., Zhou, Y., Cao, J., Wan, D., et al. (2019). A functional interaction between Hippo-YAP signalling and SREBPs mediates hepatic steatosis in diabetic mice. *J. Cell. Mol. Med.* 23, 3616–3628. doi:10.1111/jcmm.14262

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., et al. (2009). Autophagy regulates lipid metabolism. *Nature* 458, 1131–1135. doi:10.1038/nature07976

Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., et al. (2004). Identification of human brain tumour initiating cells. *Nature* 432, 396–401. doi:10.1038/nature03128

Sinha, R. A., Singh, B. K., and Yen, P. M. (2017). Reciprocal crosstalk between autophagic and endocrine signaling in metabolic homeostasis. *Endocr. Rev.* 38, 69–102. doi:10.1210/er.2016-1103

Snaebjornsson, M. T., Janaki-Raman, S., and Schulze, A. (2020). Greasing the wheels of the cancer machine: the role of lipid metabolism in cancer. *Cell. Metab.* 31, 62–76. doi:10.1016/j.cmet.2019.11.010

Song, I. S., Jeong, Y. J., and Han, J. (2015). Mitochondrial metabolism in cancer stem cells: a therapeutic target for colon cancer. *BMB Rep.* 48, 539–540. doi:10.5483/bmbrep.2015.48.10.179

Song, N. J., Yun, U. J., Yang, S., Wu, C., Seo, C. R., Gwon, A. R., et al. (2016). Notch1 deficiency decreases hepatic lipid accumulation by induction of fatty acid oxidation. *Sci. Rep.* 6, 19377. doi:10.1038/srep19377

Sorrentino, G., Ruggeri, N., Specchia, V., Cordenonsi, M., Mano, M., Dupont, S., et al. (2014). Metabolic control of YAP and TAZ by the mevalonate pathway. *Nat. Cell. Biol.* 16, 357–366. doi:10.1038/ncb2936

Sosa, M. S., Bragado, P., and Aguirre-Ghiso, J. A. (2014). Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat. Rev. Cancer* 14, 611–622. doi:10.1038/nrc3793

Spite, M. (2014). Resolving lipids: lipoxins regulate reverse cholesterol transport. *Cell. Metab.* 20, 935–937. doi:10.1016/j.cmet.2014.11.012

Strickland, M., and Stoll, E. A. (2017). Metabolic reprogramming in glioma. *Front. Cell. Dev. Biol.* 5, 43. doi:10.3389/fcell.2017.00043

Stylianou, S., Clarke, R. B., and Brennan, K. (2006). Aberrant activation of notch signaling in human breast cancer. *Cancer Res.* 66, 1517–1525. doi:10.1158/0008-5472.CAN-05-3054

Su, J., Wu, S., Tang, W., Qian, H., Zhou, H., and Guo, T. (2016). Reduced SLC27A2 induces cisplatin resistance in lung cancer stem cells by negatively regulating Bmi1-ABCG2 signaling. *Mol. Carcinog.* 55, 1822–1832. doi:10.1002/mc.22430

Subramaniam, D., Kaushik, G., Dandawate, P., and Anant, S. (2018). Targeting cancer stem cells for chemoprevention of pancreatic cancer. *Curr. Med. Chem.* 25, 2585–2594. doi:10.2174/0929867324666170127095832

Sun, H., Zhang, M., Cheng, K., Li, P., Han, S., Li, R., et al. (2016b). Resistance of glioma cells to nutrient-deprived microenvironment can be enhanced by CD133-mediated autophagy. *Oncotarget* 7, 76238–76249. doi:10.18632/oncotarget.12803

Sun, H. W., Yu, X. J., Wu, W. C., Chen, J., Shi, M., Zheng, L., et al. (2016a). GLUT1 and ASCT2 as predictors for prognosis of hepatocellular carcinoma. *PLoS One* 11, e0168907. doi:10.1371/journal.pone.0168907

Sun, M., and Yang, Z. (2019). Metabolomic studies of live single cancer stem cells using mass spectrometry. *Anal. Chem.* 91, 2384–2391. doi:10.1021/acs.analchem.8b05166

Sun, S., Xue, D., Chen, Z., Ou-Yang, Y., Zhang, J., Mai, J., et al. (2019). R406 elicits anti-Warburg effect via Syk-dependent and -independent mechanisms to trigger apoptosis in glioma stem cells. *Cell. Death Dis.* 10, 358. doi:10.1038/s41419-019-1587-0

Sun, Y., He, W., Luo, M., Zhou, Y., Chang, G., Ren, W., et al. (2015). SREBP1 regulates tumorigenesis and prognosis of pancreatic cancer through targeting lipid metabolism. *Tumour Biol.* 36, 4133–4141. doi:10.1007/s13277-015-3047-5

Sundqvist, A., Morikawa, M., Ren, J., Vasilaki, E., Kawasaki, N., Kobayashi, M., et al. (2018). JUNB governs a feed-forward network of TGFβ signaling that aggravates breast cancer invasion. *Nucleic Acids Res.* 46, 1180–1195. doi:10.1093/nar/gkx1190

Svensson, R. U., Parker, S. J., Eichner, L. J., Kolar, M. J., Wallace, M., Brun, S. N., et al. (2016). Inhibition of acetyl-CoA carboxylase suppresses fatty acid synthesis and tumor growth of non-small-cell lung cancer in preclinical models. *Nat. Med.* 22, 1108–1119. doi:10.1038/nm.4181

Tabe, Y., Saitoh, K., Yang, H., Sekihara, K., Yamatani, K., Ruvolo, V., et al. (2018). Inhibition of FAO in AML co-cultured with BM adipocytes: mechanisms of survival and chemosensitization to cytarabine. *Sci. Rep.* 8, 16837. doi:10.1038/s41598-018-35198-6

Takebe, N., Miele, L., Harris, P. J., Jeong, W., Bando, H., Kahn, M., et al. (2015). Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. *Nat. Rev. Clin. Oncol.* 12, 445–464. doi:10.1038/nrclinonc.2015.61

Teo, R., Mohrlen, F., Plickert, G., Muller, W. A., and Frank, U. (2006). An evolutionary conserved role of Wnt signaling in stem cell fate decision. *Dev. Biol.* 289, 91–99. doi:10.1016/j.ydbio.2005.10.009

Thomas, D., and Majeti, R. (2016). Burning fat fuels leukemic stem cell heterogeneity. *Cell. Stem Cell.* 19, 1–2. doi:10.1016/j.stem.2016.06.014

Todaro, M., Gaggianesi, M., Catalano, V., Benfante, A., Iovino, F., Biffoni, M., et al. (2014). CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell. Stem Cell.* 14, 342–356. doi:10.1016/j.stem.2014.01.009

Toh, T. B., Lim, J. J., and Chow, E. K. (2017). Epigenetics in cancer stem cells. *Mol. Cancer* 16, 29. doi:10.1186/s12943-017-0596-9

Tracz-Gaszewska, Z., and Dobrzyn, P. (2019). Stearoyl-CoA desaturase 1 as a therapeutic target for the treatment of cancer. *Cancers (Basel)* 11, 948. doi:10.3390/cancers11070948

Valle, S., Alcala, S., Martin-Hijano, L., Cabezas-Sainz, P., Navarro, D., Munoz, E. R., et al. (2020). Exploiting oxidative phosphorylation to promote the stem and immunoevasive properties of pancreatic cancer stem cells. *Nat. Commun.* 11, 5265. doi:10.1038/s41467-020-18954-z

Vasefifar, P., Motafakkerazad, R., Maleki, L. A., Najafi, S., Ghrobaninezhad, F., Najafzadeh, B., et al. (2022). Nanog, as a key cancer stem cell marker in tumor progression. *Gene* 827, 146448. doi:10.1016/j.gene.2022.146448

Vazquez-Martin, A., Corominas-Faja, B., Cufi, S., Vellon, L., Oliveras-Ferraros, C., Menendez, O. J., et al. (2013). The mitochondrial H(+)-ATP synthase and the lipogenic switch: new core components of metabolic reprogramming in induced pluripotent stem (iPS) cells. *Cell. Cycle* 12, 207–218. doi:10.4161/cc.23352

Vega-Naredo, I., Loureiro, R., Mesquita, K. A., Barbosa, I. A., Tavares, L. C., Branco, A. F., et al. (2014). Mitochondrial metabolism directs stemness and differentiation in P19 embryonal carcinoma stem cells. *Cell. Death Differ.* 21, 1560–1574. doi:10.1038/cdd.2014.66

Vergara, D., Simeone, P., Damato, M., Maffia, M., Lanuti, P., and Trerotola, M. (2019). The cancer microbiota: EMT and inflammation as shared molecular mechanisms associated with plasticity and progression. *J. Oncol.* 2019, 1253727. doi:10.1155/2019/1253727

Vergara, D., Stanca, E., Guerra, F., Priore, P., Gaballo, A., Franck, J., et al. (2017). β-Catenin knockdown affects mitochondrial biogenesis and lipid metabolism in breast cancer cells. *Front. Physiol.* 8, 544. doi:10.3389/fphys.2017.00544

Viale, A., Pettazzoni, P., Lyssiotis, C. A., Ying, H., Sanchez, N., Marchesini, M., et al. (2014). Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* 514, 628–632. doi:10.1038/nature13611

Visweswaran, M., Arfuso, F., Warrier, S., and Dharmarajan, A. (2020). Aberrant lipid metabolism as an emerging therapeutic strategy to target cancer stem cells. *Stem cells* 38, 6–14. doi:10.1002/stem.3101

Vlashi, E., and Pajonk, F. (2015). Cancer stem cells, cancer cell plasticity and radiation therapy. *Semin. Cancer Biol.* 31, 28–35. doi:10.1016/j.semcancer.2014.07.001

von Roemeling, C. A., Marlow, L. A., Wei, J. J., Cooper, S. J., Caulfield, T. R., Wu, K., et al. (2013). Stearoyl-CoA desaturase 1 is a novel molecular therapeutic target for clear cell renal cell carcinoma. *Clin. Cancer Res.* 19, 2368–2380. doi:10.1158/1078-0432.CCR-12-3249

Vriens, K., Christen, S., Parik, S., Broekaert, D., Yoshinaga, K., Talebi, A., et al. (2019). Evidence for an alternative fatty acid desaturation pathway increasing cancer plasticity. *Nature* 566, 403–406. doi:10.1038/s41586-019-0904-1

Wang, D., Huang, J., Wang, X., Yu, Y., Zhang, H., Chen, Y., et al. (2013). The eradication of breast cancer cells and stem cells by 8-hydroxyquinoline-loaded hyaluronan modified mesoporous silica nanoparticle-supported lipid bilayers containing docetaxel. *Biomaterials* 34, 7662–7673. doi:10.1016/j.biomaterials.2013.06.042

Wang, J., and Li, Y. (2019). CD36 tango in cancer: signaling pathways and functions. *Theranostics* 9, 4893–4908. doi:10.7150/thno.36037

Wang, J., Li, Z. H., White, J., and Zhang, L. B. (2014). Lung cancer stem cells and implications for future therapeutics. *Cell. Biochem. Biophys.* 69, 389–398. doi:10.1007/s12013-014-9844-4

Wang, J., Yu, L., Schmidt, R. E., Su, C., Huang, X., Gould, K., et al. (2005). Characterization of HSCD5, a novel human stearoyl-CoA desaturase unique to primates. *Biochem. Biophys. Res. Commun.* 332, 735–742. doi:10.1016/j.bbrc.2005.05.013

Wang, S. Y., Hu, Q. C., Wu, T., Xia, J., Tao, X. A., and Cheng, B. (2022). Abnormal lipid synthesis as a therapeutic target for cancer stem cells. *World J. Stem Cells* 14, 146–162. doi:10.4252/wjsc.v14.i2.146

Wang, T., Fahrmann, J. F., Lee, H., Li, Y. J., Tripathi, S. C., Yue, C., et al. (2018). JAK/STAT3-Regulated fatty acid beta-oxidation is critical for breast cancer stem cell self-renewal and chemoresistance. *Cell. metab.* 27, 136–150 e135. doi:10.1016/j.cmet.2017.11.001

Wei, X., Shi, J., Lin, Q., Ma, X., Pang, Y., Mao, H., et al. (2021). Targeting ACLY attenuates tumor growth and acquired cisplatin resistance in ovarian cancer by inhibiting the PI3K-AKT pathway and activating the AMPK-ROS pathway. *Front. Oncol.* 11, 642229. doi:10.3389/fonc.2021.642229

Wei, Y., Li, Y., Chen, Y., Liu, P., Huang, S., Zhang, Y., et al. (2022). ALDH1: a potential therapeutic target for cancer stem cells in solid tumors. *Front. Oncol.* 12, 1026278. doi:10.3389/fonc.2022.1026278

Wen, Y. A., Xiong, X., Zaytseva, Y. Y., Napier, D. L., Vallee, E., Li, A. T., et al. (2018). Downregulation of SREBP inhibits tumor growth and initiation by altering cellular metabolism in colon cancer. *Cell. Death Dis.* 9, 265. doi:10.1038/s41419-018-0330-6

Williams, N. C., and ONeill, L. A. J. (2018). A role for the Krebs cycle intermediate citrate in metabolic reprogramming in innate immunity and inflammation. *Front. Immunol.* 9, 141. doi:10.3389/fimmu.2018.00141

Wongtrakoongate, P. (2015). Epigenetic therapy of cancer stem and progenitor cells by targeting DNA methylation machineries. *World J. Stem Cells* 7, 137–148. doi:10.4252/wjsc.v7.i1.137

Xie, Y., Li, J., Kang, R., and Tang, D. (2020). Interplay between lipid metabolism and autophagy. *Front. Cell. Dev. Biol.* 8, 431. doi:10.3389/fcell.2020.00431

Yan, M., Yang, X., Wang, L., Clark, D., Zuo, H., Ye, D., et al. (2013a). Plasma membrane proteomics of tumor spheres identify CD166 as a novel marker for cancer stem-like cells in head and neck squamous cell carcinoma. *Mol. Cell. Proteomics* 12, 3271–3284. doi:10.1074/mcp.M112.025460

Yan, X., Luo, H., Zhou, X., Zhu, B., Wang, Y., and Bian, X. (2013b). Identification of CD90 as a marker for lung cancer stem cells in A549 and H446 cell lines. *Oncol. Rep.* 30, 2733–2740. doi:10.3892/or.2013.2784

Yan, Y., Zuo, X., and Wei, D. (2015). Concise review: emerging role of CD44 in cancer stem cells: a promising biomarker and therapeutic target. *Stem Cells Transl. Med.* 4, 1033–1043. doi:10.5966/sctm.2015-0048

Yang, L., Shi, P., Zhao, G., Xu, J., Peng, W., Zhang, J., et al. (2020). Targeting cancer stem cell pathways for cancer therapy. *Signal Transduct. Target Ther.* 5, 8. doi:10.1038/s41392-020-0110-5

Yang, P., Qin, H., Li, Y., Xiao, A., Zheng, E., Zeng, H., et al. (2022a). CD36-mediated metabolic crosstalk between tumor cells and macrophages affects liver metastasis. *Nat. Commun.* 13, 5782. doi:10.1038/s41467-022-33349-y

Yang, W., Xia, Y., Ji, H., Zheng, Y., Liang, J., Huang, W., et al. (2011). Nuclear PKM2 regulates β-catenin transactivation upon EGFR activation. *Nature* 480, 118–122. doi:10.1038/nature10598

Yang, Y., Lu, Y., Zhang, C., Guo, Q., Zhang, W., Wang, T., et al. (2022b). Phenazine derivatives attenuate the stemness of breast cancer cells through triggering ferroptosis. *Cell. Mol. Life Sci.* 79, 360. doi:10.1007/s00018-022-04384-1

Yasumoto, Y., Miyazaki, H., Vaidyan, L. K., Kagawa, Y., Ebrahimi, M., Yamamoto, Y., et al. (2016). Inhibition of fatty acid synthase decreases expression of stemness markers in glioma stem cells. *PLoS One* 11, e0147717. doi:10.1371/journal.pone.0147717

Ye, H., Adane, B., Khan, N., Sullivan, T., Minhajuddin, M., Gasparetto, M., et al. (2016). Leukemic stem cells evade chemotherapy by metabolic adaptation to an adipose tissue niche. *Cell. Stem Cell.* 19, 23–37. doi:10.1016/j.stem.2016.06.001

Yi, M., Li, J., Chen, S., Cai, J., Ban, Y., Peng, Q., et al. (2018). Emerging role of lipid metabolism alterations in Cancer stem cells. *J. Exp. Clin. cancer Res. CR* 37, 118. doi:10.1186/s13046-018-0784-5

Yu, Y., Kim, H., Choi, S., Yu, J., Lee, J. Y., Lee, H., et al. (2021). Targeting a lipid desaturation enzyme, SCD1, selectively eliminates colon cancer stem cells through the suppression of Wnt and NOTCH signaling. *Cells* 10, 106. doi:10.3390/cells10010106

Yuan, S. F., Hung, A. C., Hsu, C. W., Lan, T. H., Su, C. W., Chi, T. C., et al. (2022). CD44 mediates oral squamous cell carcinoma-promoting activity of MRE11 via AKT signaling. *J. Pers. Med.* 12, 841. doi:10.3390/jpm12050841

Zeng, S., Wu, F., Chen, M., Li, Y., You, M., Zhang, Y., et al. (2022). Inhibition of fatty acid translocase (FAT/CD36) palmitoylation enhances hepatic fatty acid β-oxidation by increasing its localization to mitochondria and interaction with long-chain acyl-CoA synthetase 1. *Antioxid. Redox Signal* 36, 1081–1100. doi:10.1089/ars.2021.0157

Zhang, S., Peng, X., Yang, S., Li, X., Huang, M., Wei, S., et al. (2022). The regulation, function, and role of lipophagy, a form of selective autophagy, in metabolic disorders. *Cell. Death Dis.* 13, 132. doi:10.1038/s41419-022-04593-3

Zhang, Y., Jin, Z., Zhou, H., Ou, X., Xu, Y., Li, H., et al. (2016). Suppression of prostate cancer progression by cancer cell stemness inhibitor napabucasin. *Cancer Med.* 5, 1251–1258. doi:10.1002/cam4.675

Zhao, L., Varghese, Z., Moorhead, J. F., Chen, Y., and Ruan, X. Z. (2018a). CD36 and lipid metabolism in the evolution of atherosclerosis. *Br. Med. Bull.* 126, 101–112. doi:10.1093/bmb/ldy006

Zhao, X., Zhao, L., Yang, H., Li, J., Min, X., Yang, F., et al. (2018b). Pyruvate kinase M2 interacts with nuclear sterol regulatory element-binding protein 1a and thereby activates lipogenesis and cell proliferation in hepatocellular carcinoma. *J. Biol. Chem.* 293, 6623–6634. doi:10.1074/jbc.RA117.000100

Zheng, B., Jarugumilli, G. K., Chen, B., and Wu, X. (2016). Chemical probes to directly profile palmitoleoylation of proteins. *Chembiochem* 17, 2022–2027. doi:10.1002/cbic.201600403

Zheng, L., Guo, Q., Xiang, C., Liu, S., Jiang, Y., Gao, L., et al. (2019). Transcriptional factor six2 promotes the competitive endogenous RNA network between CYP4Z1 and pseudogene CYP4Z2P responsible for maintaining the stemness of breast cancer cells. *J. Hematol. Oncol.* 12, 23. doi:10.1186/s13045-019-0697-6

Zhou, Y., Zhou, Y., Shingu, T., Feng, L., Chen, Z., Ogasawara, M., et al. (2011). Metabolic alterations in highly tumorigenic glioblastoma cells: preference for hypoxia and high dependency on glycolysis. *J. Biol. Chem.* 286, 32843–32853. doi:10.1074/jbc.M111.260935

Zon, L. I. (2008). Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. *Nature* 453, 306–313. doi:10.1038/nature07038

frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 16 July 2024
DOI 10.3389/fphar.2024.1392241

OPEN ACCESS

EDITED BY
Valentina Audrito,
Università del Piemonte Orientale, Italy

REVIEWED BY
Baharan Fekry,
University of Texas Health Science Center at Houston, United States
Di Ouyang,
Nanjing University of Chinese Medicine, China

*CORRESPONDENCE
Ziwei Tang,
tangziwei_tzw@163.com

RECEIVED 27 February 2024
ACCEPTED 18 June 2024
PUBLISHED 16 July 2024

CITATION
Li X, Zhou L, Xu X, Liu X, Wu W, Feng Q and Tang Z (2024), Metabolic reprogramming in hepatocellular carcinoma: a bibliometric and visualized study from 2011 to 2023.
Front. Pharmacol. 15:1392241.
doi: 10.3389/fphar.2024.1392241

Metabolic reprogramming in hepatocellular carcinoma: a bibliometric and visualized study from 2011 to 2023

Xia Li[1], Liping Zhou[1], Xinyi Xu[1], Xiyang Liu[1], Wenjun Wu[1], Quansheng Feng[1] and Ziwei Tang[2]*

[1]School of Basic Medical Sciences, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]The Beibei Affiliated Hospital of Chongqing Medical University, The Ninth People's Hospital of Chongqing, Chongqing, China

Background and aims: Metabolic reprogramming has been found to be a typical feature of tumors. Hepatocellular carcinoma (HCC), a cancer with high morbidity and mortality, has been extensively studied for its metabolic reprogramming-related mechanisms. Our study aims to identify the hotspots and frontiers of metabolic reprogramming research in HCC and to provide guidance for future scientific research and decision-making in HCC metabolism.

Methods: Relevant studies on the metabolic reprogramming of HCC were derived from the Web of Science Core Collection (WoSCC) database up until November 2023. The bibliometrix tools in R were used for scientometric analysis and visualization.

Results: From 2011 to 2023, a total of 575 publications were obtained from WoSCC that met the established criteria. These publications involved 3,904 researchers and 948 organizations in 37 countries, with an average annual growth rate of 39.11% in research. These studies were published in 233 journals, with *Cancers* (*n* = 29) ranking first, followed by *Frontiers in Oncology* (*n* = 20) and *International Journal of Molecular Sciences* (*n* = 19). The top ten journals accounted for 26% of the 575 studies. The most prolific authors were Wang J (*n* = 14), Li Y (*n* = 12), and Liu J (*n* = 12). The country with the most publications is China, followed by the United States, Italy, and France. Fudan University had the largest percentage of research results with 15.48% (*n* = 89). Ally A's paper in *Cell* has the most citations. A total of 1,204 keywords were analyzed, with the trend themes such as "glycolysis," "tumor microenvironment," "Warburg effect," "mitochondria," "hypoxia ," etc. Co-occurrence network and cluster analysis revealed the relationships between keywords, authors, publications, and journals. Moreover, the close collaboration between countries in this field was elucidated.

Conclusion: This bibliometric and visual analysis delves into studies related to metabolic reprogramming in HCC between 2012 and 2023, elucidating the characteristics of research in this field, which has gradually moved away from single glycolipid metabolism studies to the integration of overall metabolism in the body, pointing out the trend of research topics, and the dynamics of the interaction between the tumor microenvironment and metabolic

reprogramming will be the future direction of research, which provides blueprints and inspirations for HCC prevention and treatment programs to the researchers in this field.

Systematic Review Registration: [https://www.bibliometrix.org].

KEYWORDS

hepatocellular carcinoma, metabolic reprogramming, tumor microenvironment, Warburg effect, bibliometric

1 Introduction

Today, cancer remains the leading cause of death among the world's population. Liver cancer ranks sixth in incidence and third in mortality among cancers in the Global Cancer Database 2020, underscoring its high degree of malignancy (IARC, 2020). More than 90% of liver cancer cases are hepatocellular carcinoma (HCC) (Llovet et al., 2016). Compared to the world ranking, HCC morbidity and mortality rates in Asian countries have increased by one place, respectively (Zhang et al., 2022). It is estimated that around 50% of the global population of patients with HCC are from China (Llovet et al., 2021). This indicates that HCC has become a significant global challenge, with China being particularly affected (Villanueva, 2019). Although hepatitis B and C viruses and alcohol remain the primary causes of HCC, non-alcoholic fatty liver disease (NAFLD) associated with metabolic dysregulation is gradually becoming a crucial risk factor for HCC, and has been receiving more attention in recent years (Estes et al., 2018). Moreover, since NAFLD involves abnormalities in fat and glucose metabolism, often resulting in conditions such as obesity or diabetes, NAFLD-associated HCC has a unique pathogenesis involving metabolic, oxidative stress, pathological inflammatory response, and altered immune function (Anstee et al., 2019). To draw attention to metabolic abnormalities, academics have collectively referred to liver disease associated with obesity, diabetes, and systemic metabolic abnormalities as metabolic dysfunction-associated fatty liver disease (MAFLD), replacing the terminology NAFLD and "non-viral" (Eslam et al., 2020). With lifestyle changes, MAFLD may be a major contributor to liver cancer in the future (Huang et al., 2021). MAFLD-associated HCC is more likely to have metabolic complications (Tan et al., 2022), tends to have larger tumors, and more often than not does not go through the cirrhotic stage, leaving a high proportion of patients with a lack of indications for surveillance (Crane et al., 2023). HCC can be managed through various strategies such as surgery (resection, liver transplantation), local regional (ablation and embolization therapy), and pharmacological treatment. However, the current situation is not very optimistic. The development of metabolically targeted drugs such as 2-Deoxy-d-glucose (noncompetitively inhibit the activity of HK2), TKT inhibitor oxythiamine (thiamine antagonist), and TVB-2640 (fatty acid synthase inhibitor) is still in the early stage and many are still in preclinical studies (Du et al., 2022). A comprehensive understanding of the pathological mechanisms of HCC is necessary to develop targeted therapeutic strategies to improve the poor prognosis of HCC.

Metabolic reprogramming refers to the process of systematic adjustment and transformation of the metabolic pattern of a cell to adapt to changes in the external environment and to meet the needs of its own growth and differentiation under specific physiological and pathological conditions, which involves a fundamental change in the way the cell acquires and uses energy and raw materials for biosynthesis, and involves the regulation of a number of metabolic pathways, including glycolysis, oxidative phosphorylation (OXPHOS), fatty acid metabolism, amino acid metabolism, and so on. Cells rely on metabolism to produce energy for their survival and function, with normal human cells using mitochondrial OXPHOS primarily under aerobic conditions and turning to glycolysis when oxygen is scarce. However, tumor cells differ from normal cells in that they prefer cytoplasmic glycolysis even when oxygen is available. The "Warburg effect" or "aerobic glycolysis" was first identified by Otto Warburg in 1956 (Warburg, 1956), and is attributed to the uncontrolled proliferation of tumors and activation of invasive and metastatic pathways. Tumor cells require large amounts of ATP and biomaterials for high biomass synthesis, which can lead to hypoxia and nutrient deficiencies (Martínez-Reyes and Chandel, 2021). Cancer cells undergo biological changes to fulfill their high energy demands as they evolve. Studies have shown that the catabolism and anabolism of cancer cells are greatly enhanced, with genes related to glycolysis, the pentose phosphate pathway (PPP), nucleotide biosynthesis, the tricarboxylic acid cycle, and oxidative phosphorylation persistently upregulated while genes for xenobiotic, fatty acids, and amino acid metabolism are downregulated (Nwosu et al., 2017). These metabolic alterations in HCC have been consistently associated with the Warburg effect. Another study also revealed that major metabolic changes in HCC include upregulation of glycolysis, gluconeogenesis, and β-oxidation as well as downregulation of the tricarboxylic acid (TCA) cycle (Huang et al., 2013). Glycolytic enzymes such as Glut1, Glut4, and HK2 are often elevated in HCC and have been linked to poor prognosis for patients (Schwartzenberg-Bar-Yoseph et al., 2004; Amann et al., 2009; DeWaal et al., 2018; Chai et al., 2019). Glucose-6-phosphate dehydrogenase (G6PD) is a crucial enzyme that regulates the PPP. Clinical studies have found that the expression level of G6PD in tumor tissues of HCC is significant, in comparison to adjacent normal tissues. Moreover, metastatic HCC tissues exhibited a higher level of G6PD compared to non-metastatic tissues, which is directly correlated with shorter survival (Lu et al., 2018). Increased glutamine metabolism is also a key feature of altered tumor metabolism, which produces higher levels of α-ketoglutarate and citrate to support the mitochondrial TCA cycle. In addition, the aberrant lipid metabolism in HCC also plays an important role in carcinogenesis. Notably, HCC cells typically exhibit higher rates of lipid de novo synthesis and fatty acid β-oxidation (FAO) uptake. The significance of glycolipid metabolic reprogramming in all aspects of hepatocarcinogenesis and development is increasingly supported by a

growing body of evidence. Therefore, this study will focus on recent findings of metabolic reprogramming in HCC.

Bibliometrics is the cross-cutting science that quantitatively analyzes all knowledge carriers using mathematical and statistical methods. This comprehensive field combines mathematics, statistics, and documentation to quantify and analyze relationships between published works based on abstracts, keywords, citations, and other relevant information (Ninkov et al., 2022). Bibliometrics is an important tool that can be used to understand how a particular topic has been researched in the literature, track the research trajectory of the topic, and identify the characteristics of high-impact journal articles. Since the formalization of bibliometric research, more and more researchers have carried out important work using journal impact factors, H-indexes, and visualization methods to identify the structure of knowledge, current developments, and research frontiers in specific fields (Hassan et al., 2021). Bibliometric analysis is a quick way to identify the most representative authors, institutions, countries, and journals in a particular field of study (Wang and Maniruzzaman, 2022). A software package called biblioshiny, which operates in an R environment, is a typical bibliometric research software that allows for a friendly graphical interface to help researchers quickly understand the scientific field. Its interactive visual user interface makes it easy to use (Khan et al., 2021), and it integrates bibliographic analysis and visualization into one package. Biblioshiny offers high convenience and flexibility, providing plotting under multiple analyses and customization of different types of advanced charts. This significantly improves the efficiency of the researchers' work compared to traditional analysis software.

In recent years, the study of cancer metabolism has been gradually emerging thanks to the development of systems biology and extensive research. This has led to an increase in publications on the topic, particularly about metabolic reprogramming-based studies on the pathomechanisms and action targets in cancer. In this context, we conducted a bibliometric analysis of publications on metabolic reprogramming in HCC over the past 12 years using biblioshiny. We aimed to provide insights into the current status of metabolic reprogramming in HCC research and future research trends. We hope that this comprehensive bibliometric analysis will help researchers in this field to conduct more systematic and targeted explorations.

2 Materials and methods

2.1 Literature extraction

This study quantitatively assessed existing scientific results on metabolism related to HCC to characterize the evolution of HCC metabolism research over the last decade. In the current work, we conducted a literature search on November 13, 2023, using the search terms "hepatocellular carcinoma" and "metabolic reprogramming" to retrieve literature published between 2011 and 2023 from the Web of Science Core Collection (WoSCC) Science Citation Index Expanded (SCIE), a mainstream and authoritative high-quality database of all types of materials. We limited the type of publication to "articles" and "reviews," restricted the language to English to ensure the representativeness of the included studies, and exported "fully documented and cited references." Two independent reviewers were involved in the process and excluded duplicate/unrelated documents to ensure the accuracy of the scientometric analysis. Data collected included title, author, institution, country, journal, abstract, keywords, and references. If there was a disagreement between two reviewers, it was resolved through joint negotiation by a third independent reviewer.

2.2 Statistical and visual analysis

We used the R language package Bibliometrix (Aria and Cuccurullo, 2017) for the scientometric analysis and visualization. Bibliometrix provides all the tools for a complete bibliometric analysis following a scientific mapping workflow. The package was built in R, a programming language for statistical computing and graphics. In this study, the basic information obtained about the metabolic research field of HCC was uploaded to Bibliometrix, and analyzed by automatic algorithms and machine intelligence for country, journal, author productivity, and institution. We mainly used two major series of functions of Bibliometrix, adopting bibliometrics-based analysis and extraction techniques for analyzing indicators and mining techniques for literature-related concepts, knowledge, and social structures for correlation analysis. Firstly, we use clean corpus() function to clean the literature data, remove invalid information or incorrectly formatted data, and extract the metadata information of the literature data such as authors, journals, etc., to lay the foundation for the subsequent analysis work, and then use biblioNetwork() function to construct the literature co-citation graph, and use biblioAnalysis() function to conduct the literature research trend analysis, in-depth understanding of the analyzed literature data. In addition, thematic modeling analysis, temporal analysis, network analysis, MCA, and clustering techniques were performed to analyze the realized network matrix and historical network matrix, to discover the hidden patterns and trends in the literature data. Finally, networkPlot(), histPlot(), and conceptualStructure() functions were used for network visualization and conceptual structure visualization.

3 Results

3.1 Basic characteristics of the publication

A total of 575 studies meeting the eligibility criteria were collected at WoSCC, involving 3,904 authors (Figure 1A). Only three documents were single-authored, while the rest contained multiple authors, with an average of 8.89 authors per document, and international collaborations accounting for 22.26% of the documents (Figure 1A). Literature published in this field has been increasing year by year from 2011–2023 with an annual growth rate of 39.11% (Figure 1B). This peaked in 2022 with 114 publications, or 19.8% of the total, with a slight drop-off in 2023, probably related to the fact that the amount of data for the whole year was not counted. All documents have been cited in 36,618 references while the average number of citations per document is 28.07 (Figure 1A). The average citations per year from 2011 to 2023 in these documents is also shown in

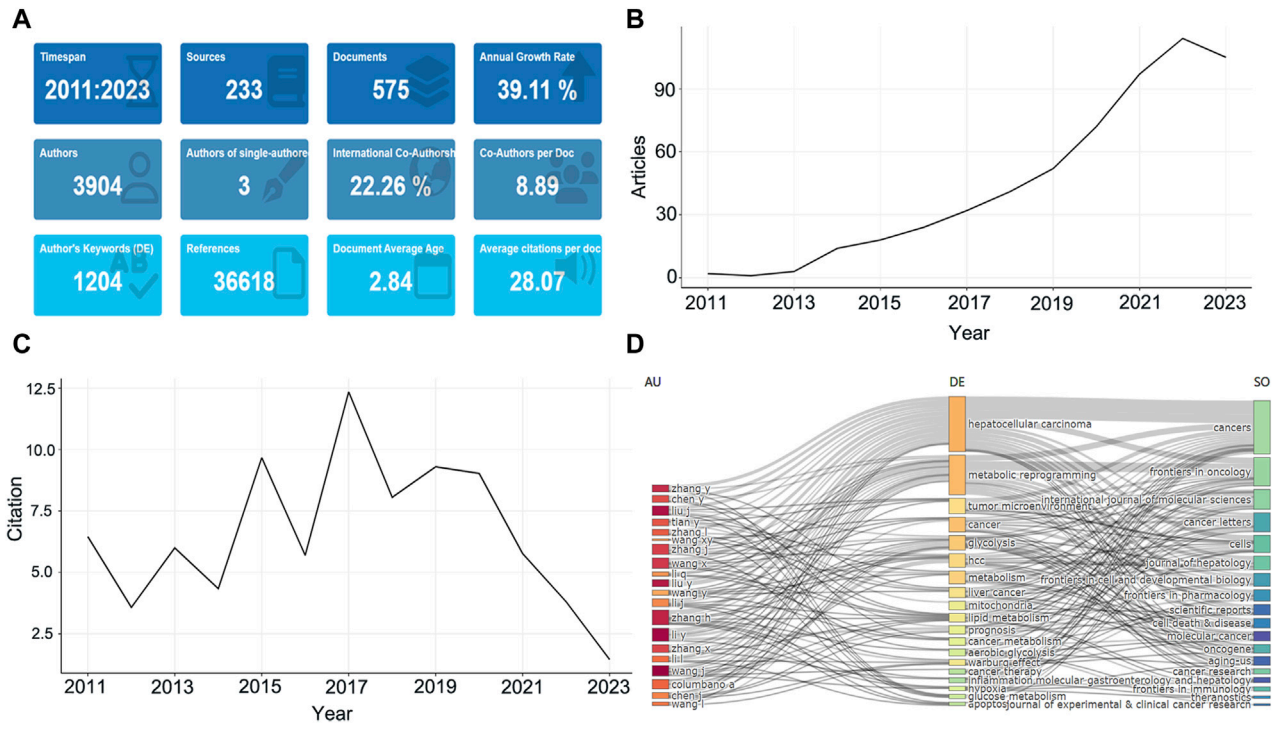


FIGURE 1
The basic features of published studies. **(A)** The main information of publications. **(B)** Annual growth of the documents. **(C)** Average citations per year of documents. **(D)** Three-Fields Plot. The left is the author, the middle is keywords, right is journal sources.

Supplementary Table S1, with articles from 2015 to 2019 being cited the most, indicating that the literature in this period has a greater impact (Figure 1C). Additionally, a three-fields plot of authors, keywords, and publication sources for the top twenty most relevant documents in this research area was developed (Figure 1D).

3.2 Analysis of representative journals

These studies were originated from 233 publications. The most published journal in this research area is *Cancers* with 29 articles, followed by *Frontiers in Oncology* (n = 20), *International Journal of Molecular Sciences* (n = 19) and *Hepatology* (n = 16) (Figure 2A). The top ten journals collectively published about 26% of the articles and were derived by Bradford's Law as the core regional journals in the field (Figure 2B). Among the top ten journals it can be observed that the vast majority are high impact journals with impact factors (IF) ranging from 4.7 to 25.7, and about 70% scored Q1 in the JCR division (Supplementary Table S2). *Journal of Hepatology* and *Hepatology* have high IF, while *Cancers* has the highest total citations (TCs). Moreover, among the most cited local journals, *Cancer Research* (Citations = 1,390), *Hepatology* (Citations = 1,383), and *Cell* (Citations = 1,269) ranked in the top three (Figure 2C), highlighting the high impact. The H-index represents to some extent the number of academic outputs and the level of outputs, whereas the *International Journal of Molecular Sciences* was ranked first in this research area, followed by *Cancers*, *Hepatology* (Supplementary Table S2). Finally, we have observed the cumulative output of the five most productive journals in this research area for the period from 2011 to 2023 (Figure 2D). The first journal to publish research on the topic of HCC metabolism was the *International Journal of Molecular Sciences*, the most represented journal between 2016 and 2019 was *Hepatology*, while after 2020 the production of *Cancers* skyrocketed way ahead of the other journals.

3.3 Analysis of authors

The most prolific author in this field of research is Wang J with 14 articles involved, followed by Li Y, Liu J, and Liu Y (Figure 3A). However, the most cited authors are Saksena G, Liu Y, Fujiwara N, all with more than 35 citations (Figure 3B). To more objectively explore the authors with high academic level in this research field, we further tracked the output of these highly productive top ten authors in recent years and found that Liu J published one article in 2017 with a total citation count of 168.29, and four articles in 2022 with a total citation count of 19.5 (Figure 3C). Similarly, Li Y, Zhang H, Zhang Y, Wang X, Zhang X all published high impact articles between 2020 and 2022 with an average of more than 20 citations per year (Figure 3C). The H-index still showed that Liu J, Li Y, Liu Y et al. are still highly influential, which is consistent with the trend presented by the production and citation numbers in recent years (Table 1). It can be observed that the percentage of authors decreases as the scientific output in the field of study continues to be produced, in accordance with Lotka's Law (Figure 3D).

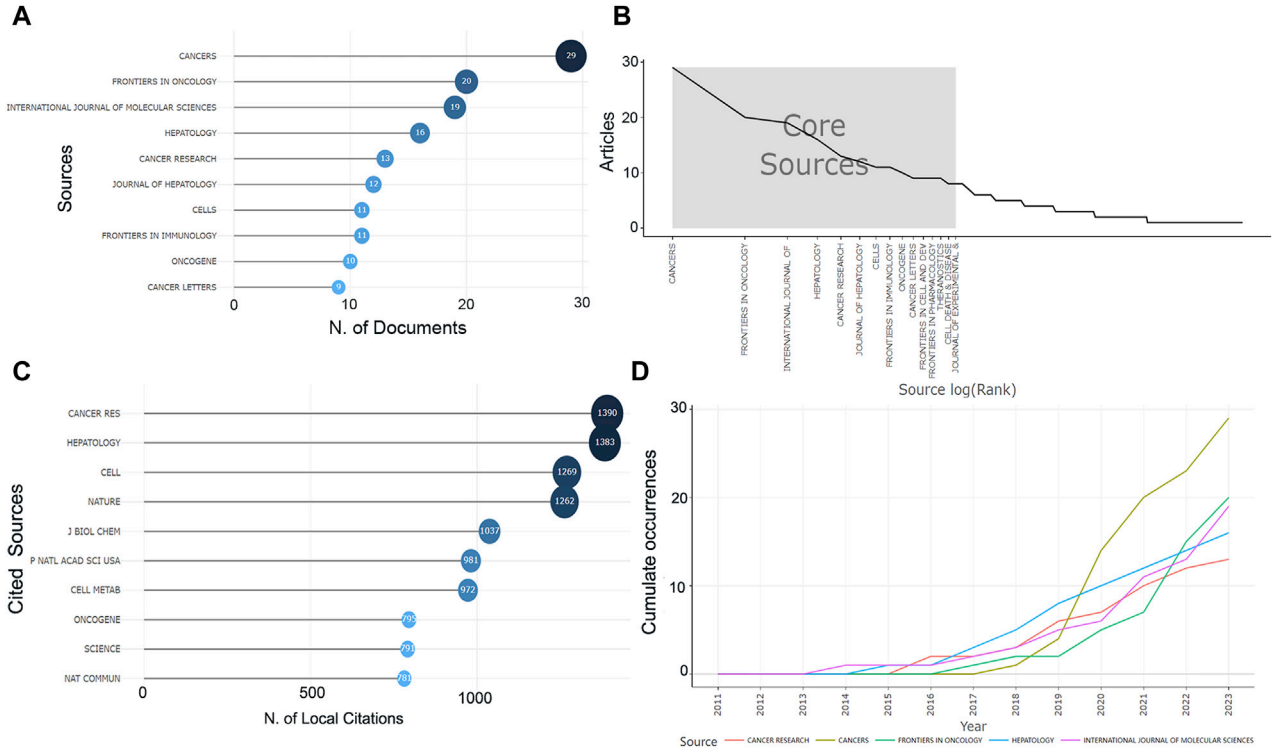


FIGURE 2
Information about the journal. **(A)** The number of publications in the journal. **(B)** The core journals. **(C)** The number of citations of journals. **(D)** The cumulative number of publications in recent years in the top five journals in this field.

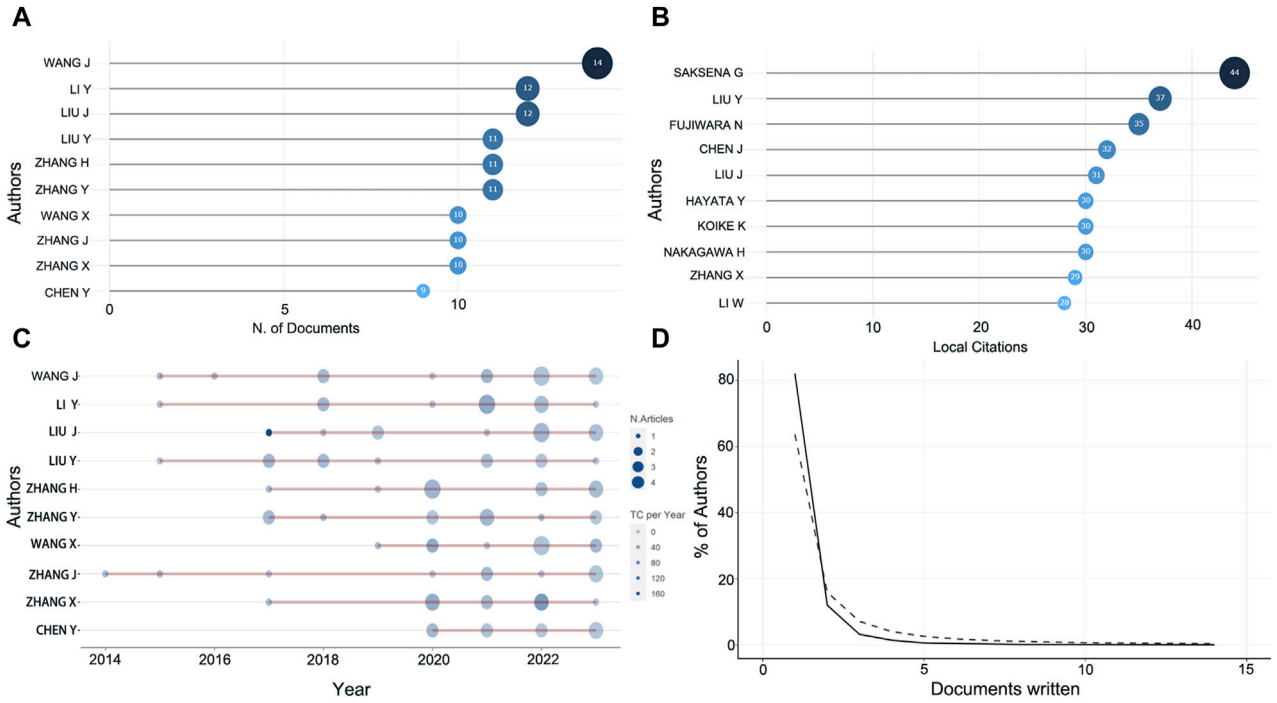


FIGURE 3
Information about the authors. **(A)** The top ten most prolific authors. **(B)** Top ten highly cited authors. **(C)** Number of publications and citations in recent years of prolific authors. Color intensity and bubble size are positively correlated with the total number of annual citations and the number of documents, respectively. **(D)** Lotka's law.

TABLE 1 The top ten authors' impact in this field.

Author	H index	G index	M index	TC	PY start
Liu J	9	12	1.286	1,310	2017
Li Y	8	12	0.889	372	2015
Liu Y	8	11	0.889	373	2015
Zhang H	8	11	1.143	234	2017
Zhang X	8	10	1.143	398	2017
Li L	7	8	1	114	2017
Wang J	7	14	0.778	452	2015
Wang X	7	10	1.4	254	2019
Zhang Y	7	11	1	281	2017
Chen J	6	7	0.75	1,310	2016

TC, total citations; PY, start, the year of the author's first publication.

3.4 Distribution of countries and institutions

Based on the corresponding authors of the manuscripts, it can be determined that a total of 37 countries are involved, with China publishing the most research (n = 335), accounting for 58.3% of the total, significantly more than any other country, followed by the United States (n = 69, 12%), Italy (n = 31, 5.4%), France (n = 18, 3.1%), and Germany (n = 14, 2.4%) (Figures 4A, B; Supplementary Table S3). It can be found that the number of collaborative papers with the same nationality among the countries is predominant, while Iran is the highest in terms of MCP ratio (Supplementary Table S3). The development trend of the top five countries in terms of the number of publications in recent years showed that China has always been ahead of other countries, especially after 2020, when the number of publications showed a remarkable upward trend (Figure 4C). As for the number of paper citations, China is still the highly cited country with 7,791 citations, followed by the United States (TCs = 2,139), Italy (TCs = 1,251) and France (TCs = 825) (Figure 4D). In terms of the average number of paper citations, Japan, Mexico and France, are the highest, with 48.2, 47.2, and 45.8 respectively, indicating their advanced academic level in this research field (Supplementary Table S4).

Figure 5A illustrates the institutions contributing to this field, involving 948 institutions, with the most published research coming from Fudan University (n = 89), the Chinese Academy of Sciences (n = 52), and Sun Yat-sen University (n = 42), all from China. The top five institutions accounted for 43.66% of the 575 research publications. Although the earliest published study was from the Air Force Military Medical University, it showed a slow growth trend after 2017. Fudan University, Chinese Academy of Sciences, and Sun Yat-sen University, on the other hand, have shown a rapid growth trend in the last 3 years (Figure 5B).

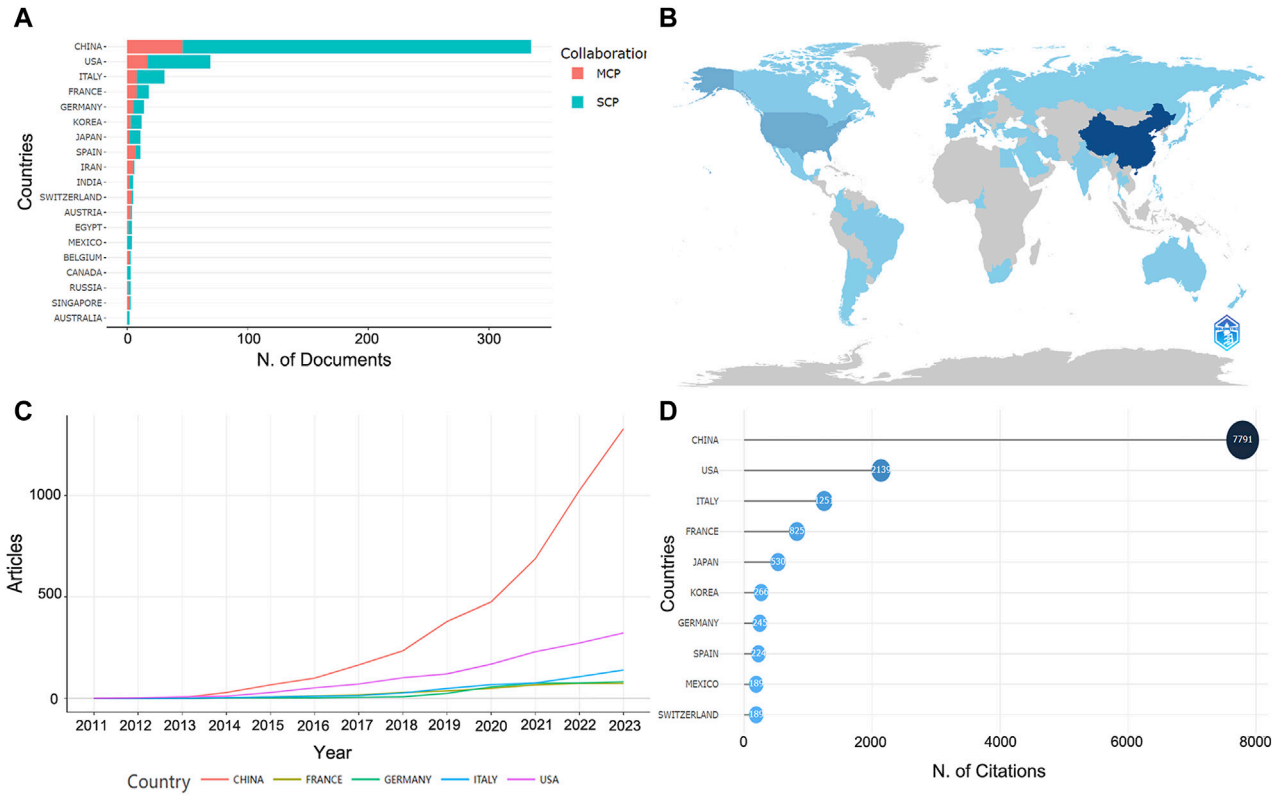


FIGURE 4
The information about countries of publications. **(A)** Corresponding author's countries. MCP, number of papers co-authored with authors from other countries. SCP, number of papers co-authored with authors from the same country. **(B)** The number of papers in each country. The darker the color, the higher the number of papers. **(C)** Trends in the number of papers by the top five high-producing countries over time. **(D)** Highly cited countries and total citations.

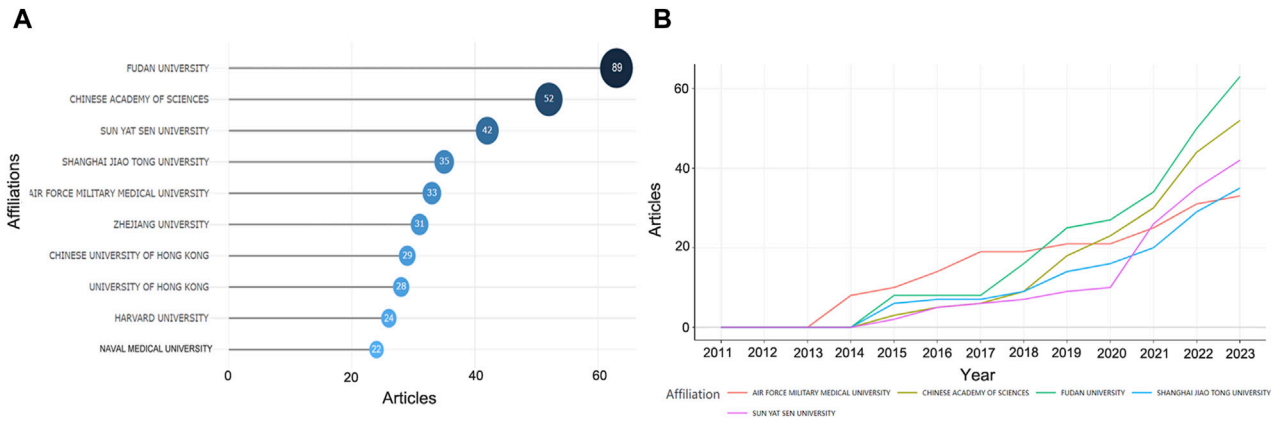


FIGURE 5
The most relevant affiliations. **(A)** The top ten affiliations. **(B)** Trends in the number of papers by the top five high-producing affiliations over time.

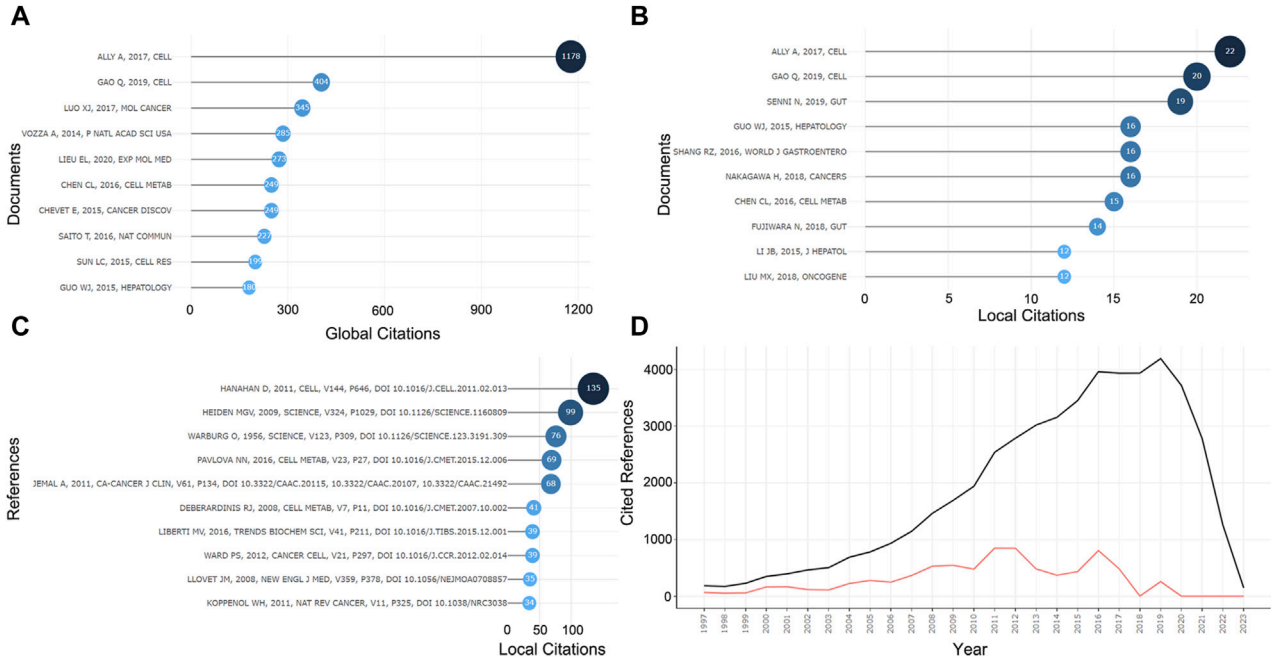


FIGURE 6
Information about cited papers and references. **(A)** Top ten highly cited papers in WOCSS in 575 papers. **(B)** Top ten highly cited papers in the current dataset. **(C)** Top ten highly cited references in the current dataset. **(D)** Number of references per year.

3.5 Analysis of cited references

In the 575 publications, we focused on the highly cited literature. Ally A's paper published in *Cell* in 2017 was the focus of attention, and it ranked first in TCs for both the global WoSCC citations and the local citations, with 1,178 and 22, respectively (Figures 6A, B). Given the high degree of relevance to the current field of research, we focused primarily on the local citations and listed the top ten articles (Table 2). LC/GC ratio could indicate the impact of this article's results in this research area on the broader field of HCC. Among the top ten locally cited documents, Senni N's study published in *Gut* has the greatest impact, accounting for 24.68% (Table 2). Senni et al. (2019) formally proposed PPAR-α as a target involved in the metabolism reprogramming of fatty acid oxidative in β-conjugated protein-causing HCC. In addition, we analyzed the references that were most cited by the current dataset (Figure 6C). Hanahan D's review article (Hanahan and Weinberg, 2011) in *Cell* was the most cited paper in this research area with 135 citations, followed by Heiden MGV's (Vander Heiden et al., 2009) and Warburg O's (Warburg, 1956) articles in *Science*, both of which offered crucial insights into tumor metabolism and are landmark events in the metabolism reprogramming of HCC (Figure 6C). In these cited references, the highest number of documents were from 2016 to 2018, indicating a high level of scholarship in this research area during this period (Figure 6D).

TABLE 2 Most local cited papers.

Document	DOI	Year	Local citations	Global citations	LC/GC ratio (%)	Normalized local citations	Normalized global citations
Ally A, 2017, Cell	10.1016/j.cell.2017.05.046	2017	22	1,178	1.87	8.48	13.63
Gao Q, 2019, Cell	10.1016/j.cell.2019.08.052	2019	20	404	4.95	7.59	8.69
Senni N, 2019, Gut	10.1136/gutjnl-2017-315448	2019	19	77	24.68	7.21	1.66
Guo WJ, 2015, Hepatology	10.1002/hep.27929	2015	16	180	8.89	4.00	2.07
Shang RZ, 2016, World J Gastroenterology	10.3748/wjg.v22.i45.9933	2016	16	74	21.62	5.82	1.62
Nakagawa H, 2018, Cancers	10.3390/cancers10110447	2018	16	84	19.05	5.91	1.74
Chen CL, 2016, Cell Metab	10.1016/j.cmet.2015.12.004	2016	15	249	6.02	5.45	5.46
Fujiwara N, 2018, Gut	10.1136/gutjnl-2017-315193	2018	14	100	14.00	5.17	2.07
Li JB, 2015, J Hepatology	10.1016/j.jhep.2015.07.039	2015	12	140	8.57	3.00	1.61
Liu MX, 2018, Oncogene	10.1038/s41388-017-0070-6	2018	12	102	11.76	4.43	2.11

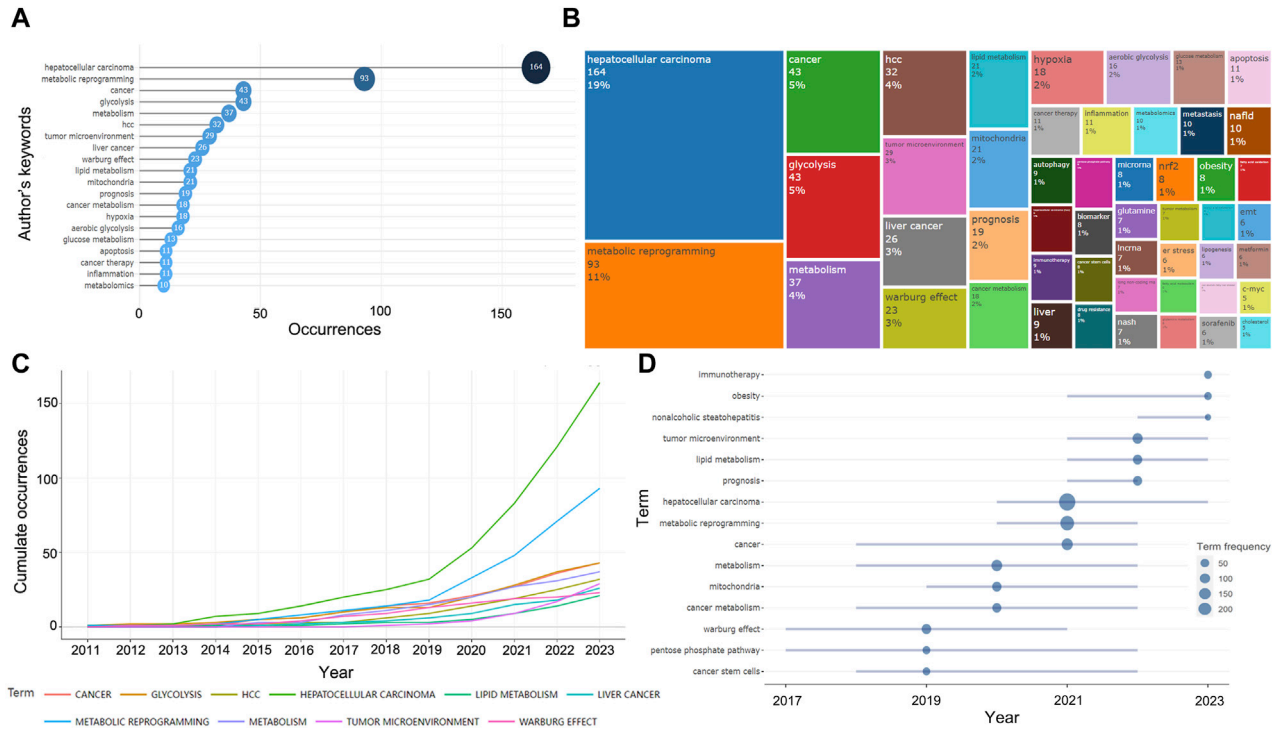


FIGURE 7
Information about keywords and trend topics. **(A)** Top 20 most frequent keywords in 575 papers. **(B)** The treemap of the top 50 keywords. **(C)** Cumulate the occurrence of the top 20 most frequent keywords in recent years. **(D)** Trend topics in the last 3 years.

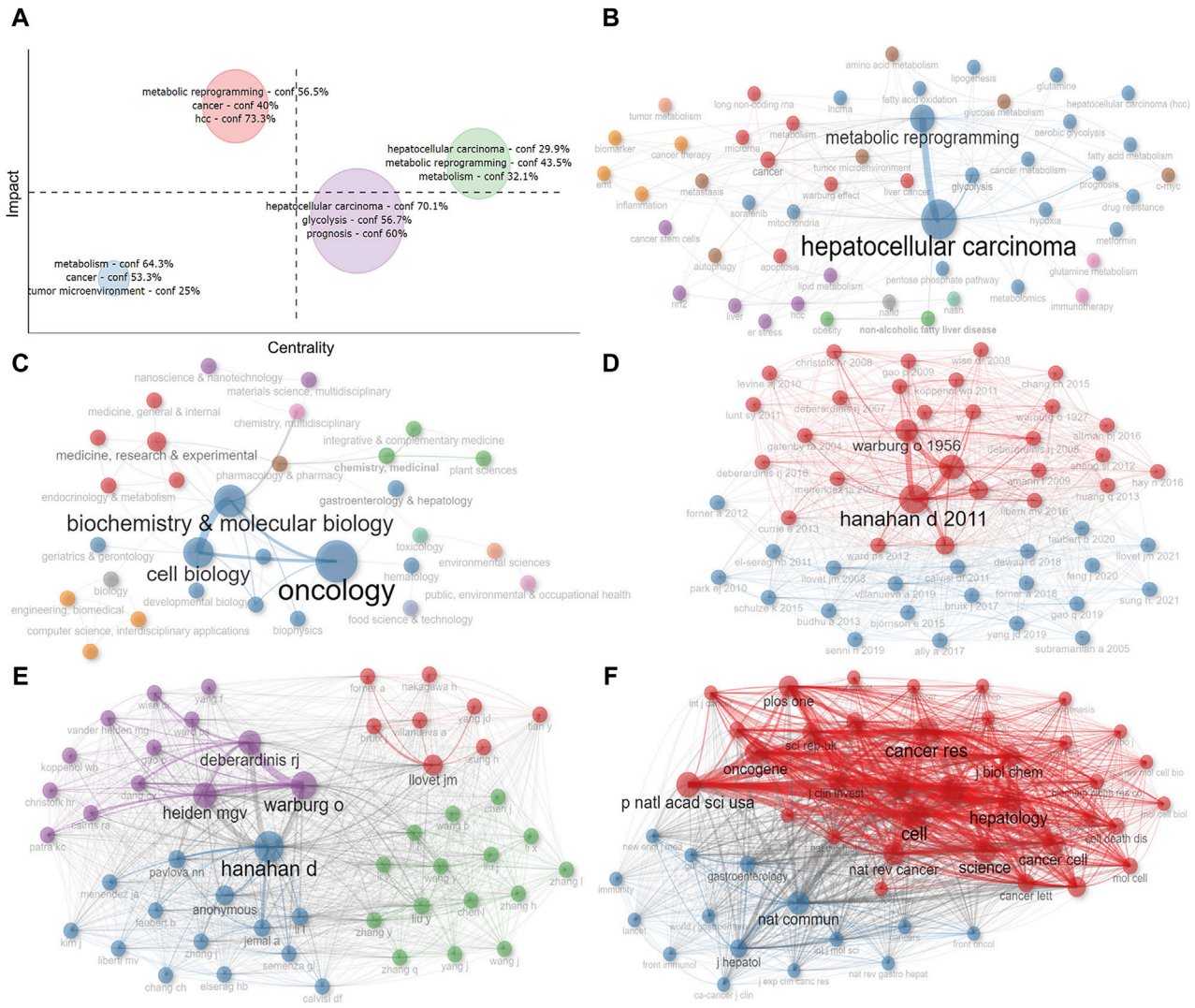


FIGURE 8
Analysis of cluster and co-citation. **(A)** Cluster by coupling keywords. **(B)** Co-occurrence network of keywords. **(C)** Co-occurrence network of disciplines. **(D)** Co-citation network of papers. **(E)** Co-citation network of authors. **(F)** Co-citation network of publications. Dot size and line thickness were positively correlated with the number of articles and citations, respectively.

3.6 Analysis of keywords and trend topics

A total of 1,204 keywords were collected in 575 documents, and the high-frequency keywords could help us quickly grasp the research hotspots in this field. As can be seen in the Figures 7A, B, excluding the keywords we searched for, "glycolysis," "tumor microenvironment," "Warburg effect," "lipid metabolism," "mitochondria," "hypoxia," and "inflammation" appeared with high frequency, representing the main content of the current research on metabolic reprogramming in HCC. The frequency of these keywords has increased gradually from year to year, especially significantly after 2019, suggesting that metabolic abnormalities in HCC are attracting more and more widespread attention (Figure 7C). We then speculated on the hot topics in future metabolic reprogramming research in HCC based on the temporal development of high-frequency words, which might still be mainly focused on "tumor microenvironment," "lipid metabolism," "prognosis," "Warburg effect," and "mitochondria" in the future (Figure 7D).

3.7 Analysis of clustering and collaborative network

To more visually illustrate the linkages between the above key information, we performed clustering and collaboration network analysis. We used the number of global citations as the impact evaluation index and performed cluster analysis based on keywords, and found that there was a total of four major categories involving tumor microenvironment, glycolysis, metabolic reprogramming, and so on, among which glycolysis-related studies were more influential (Figure 8A). Subsequently, we constructed co-occurrence network diagrams between keywords and disciplines, respectively, reflecting the relevance of each topic under this

10.3389/fphar.2024.1392241

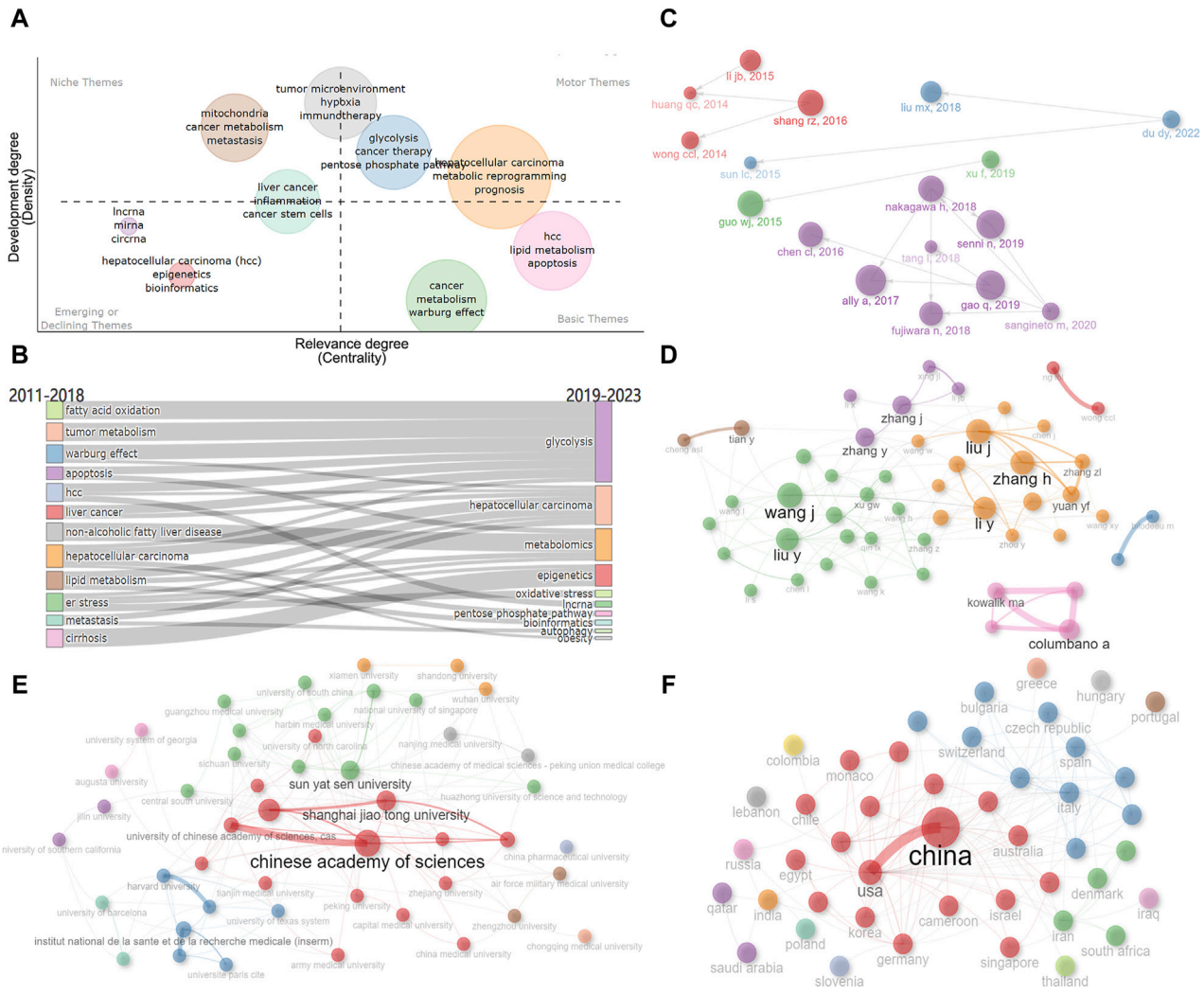
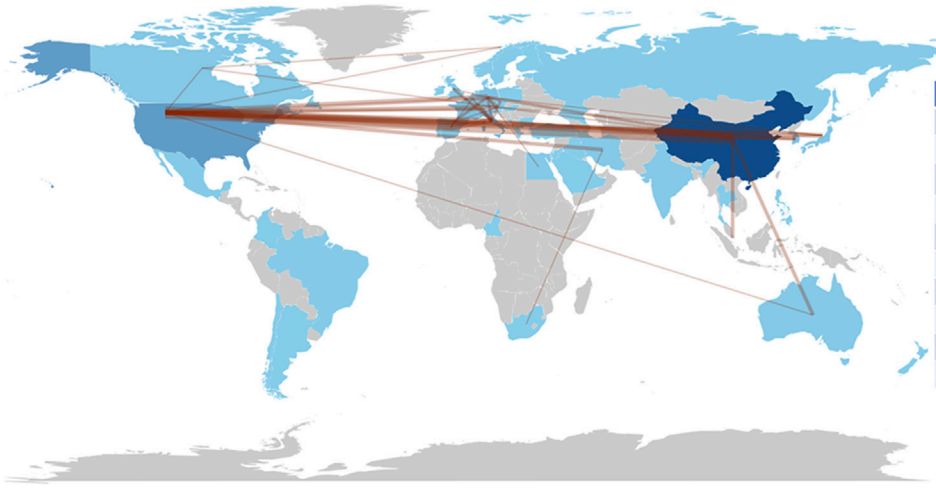


FIGURE 9
Analysis of themes and social structure. **(A)** The thematic map is created by machine algorithms. **(B)** Evolution of research themes using 2018 as a boundary. **(C)** Historiograph of these papers. **(D)** Author's collaborative network diagram. **(E)** Institution's collaborative network diagram. **(F)** Countries' collaborative network diagram. Dot size and line thickness were positively correlated with the number of articles and the degree of cooperation, respectively.

research area (Figures 8B, C). Furthermore, multiple disciplines were also involved, with Oncology, Biochemistry and Molecular Biology, and Cell Biology being the main ones (Figure 8C). Moreover, to discover the co-citation of the 575 studies more visually, we mapped the co-citation network of papers, authors and publications (Figures 8D–F). The results showed that Hanahan D's paper published in 2011 and the journal *Cell* in which it appeared are at the center of the network.

We further created the thematic map by machine algorithms (Figure 9A), with the themes in the first quadrant (top right) representing themes that are both important and well-developed for this research area and are the focus of current research. The second quadrant (top left) represents topics that have been well-developed but are not as relevant to this research area. The third quadrant (bottom left) represents emerging research themes that are not yet well developed. Quadrant IV (bottom right) generally refers to concepts that are fundamental to this research area and have strong relevance to this research area. As a result, we could see that the impact of metabolic reprogramming on the prognosis of HCC patients, glycolysis, and tumor microenvironment are important themes in this research area. From the number of articles published each year, it can be found that the number of articles increased rapidly after 2018, therefore, we took 2018 as the boundary and speculated the evolution of topics before and after 2018 based on the keywords, and Figure 9B showed that fatty acid oxidation, tumor metabolism, and the Warburg effect all gradually evolved into the study of glycolysis, the study of non-alcoholic fatty liver disease and lipid metabolism gradually evolved into the study of overall metabolomics, the study of endoplasmic reticulum stress gradually evolved into the study of oxidative stress, and so on. Both the Historiograph figure and table show the literature with high importance in this dataset, with Ally et al., (2017) publication having a significant impact on several articles (Figure 9C; Supplementary Table S5).



From	To	Frequency
China	USA	39
USA	Germany	8
Italy	Spain	7
USA	France	7
USA	Italy	7
USA	Japan	6
Italy	France	5
USA	Austria	5
USA	Korea	5
USA	Spain	5

FIGURE 10
Map of cooperation in countries around the world and the top 10 closely cooperating countries. The thickness of the connecting lines is positively correlated with cooperation between countries.

In addition, we analyzed the collaboration between authors, institutions, and countries (Figures 9D–F). As shown in Figure 9D, there is a closer cooperation between Zhang H and Liu J, Liu Y and Wang J. There is a strong connection between Fudan University, Shanghai Jiaotong University, and the Chinese Academy of Sciences (Figure 9E). The connection between China and the United States is the most, followed by the United States and Germany, Italy and Spain, the United States and France, Italy, Japan, etc. (Figure 9F). It can be seen that the United States maintains close international cooperation with several countries, which plays an important role in the achievement of its high-level results (Figure 10).

4 Discussion

4.1 General overview

Metabolic reprogramming is a topic that has gained a lot of attention in tumor biology research in recent years. The abnormal changes in the metabolism of glucose, lipids, and amino acids in tumor cells are necessary for their growth, especially in HCC. Studying the mechanisms behind metabolic reprogramming in HCC can help in identifying potential therapeutic targets and developing effective treatment strategies (Ohshima and Morii, 2021). This study used bibliometrics to analyze high-impact publications on metabolic reprogramming in HCC, helping researchers understand the latest trends and research hotspots in this field. The study analyzed 575 openly published articles in WoSCC between 2012 and 2023, identifying key journals, authors, and high-impact papers, as well as analyzing the intellectual structure and social collaborations in this area. This analysis provides valuable insights into the historical development and research frontiers of metabolic reprogramming in HCC.

Our research indicates that there has been a significant increase in the number of publications and total citations on metabolic reprogramming in HCC due to the growing interest in tumor metabolism over the past decade. Researchers have primarily concentrated their work in *Cancers*, *Frontiers in Oncology*, and *International Journal of Molecular Sciences*, which are the core journals in this field. *Cancer Research* and *Hepatology* were the most influential sources of publications based on citation analysis and *International Journal of Molecular Sciences* had the highest H-index. Wang J, Li Y, and Liu J were the most prolific authors, and Saksena G ranked first in terms of citation ranking. In terms of productivity, China and the United States were active countries in this field, with 335 and 69 papers published respectively. Fudan University, Chinese Academy of Sciences, and Sun Yat-sen University were the primary research institutions. Despite having the largest number of publications, China still needs to strengthen its international cooperation. Our study showed that the United States collaborates with most countries, and Iran, Spain, and France have a higher percentage of international collaborations in their total number of publications, suggesting that these countries rely more on international collaborations for research results in this field.

4.2 Metabolic reprogramming

Since the Warburg effect has been formally proposed, metabolic abnormalities in tumors have been gradually emphasized, and metabolic reprogramming has been regarded as a major feature of tumors.

4.2.1 Reasons why metabolic reprogramming has emerged

The activities of life are inextricably linked to a corresponding material and energy base, a truth that extends to tumor cells. The unique characteristics of tumor cells require them to proliferate rapidly, evade immune surveillance, and under specific circumstances metastasize to other parts of the body while resisting drug attacks. Consequently, scholars have dedicated significant attention to the energy and material metabolism of

tumor cells in search of selective control of certain metabolic pathways to impede the corresponding malignant phenotype. Although the field is still in its nascent stages, there is a history of diagnostic and therapeutic tools developed based on the properties of tumor metabolism. In the field of tumor imaging, for example, the 18F-FDG PET/CT method is frequently employed due to the high glucose uptake capacity of tumors (Cho et al., 2015). Another example is the anticancer drugs 5-fluorouracil and cytosine arabinoside, which are a class of compounds that resist DNA metabolism (De Jager et al., 1976). As research into tumor metabolism advances, we can expect the development of more advanced diagnostic and therapeutic tools.

The study of tumor metabolism is a crucial aspect of cancer diagnosis and treatment. Beyond this, it holds significant implications for daily life. While oncogenic mutations can cause normal cells to become cancerous (Zucman-Rossi et al., 2015), the presence of mutations does not necessarily translate to tumor development. The environment, therefore, plays a critical role in the development of tumors. Consequently, the study of metabolic abnormalities in tumors aims to decipher the influence of environmental factors, such as diet, exercise, and other lifestyle habits, in tumor development, treatment, and prognosis. This knowledge is pivotal for cancer prevention and treatment (Zhang et al., 2021).

Metabolic reprogramming in tumors is a complex phenomenon that is closely related to various factors, including epigenetic regulation, which are classical areas of tumor research, and all of them are inevitably associated with metabolic reprogramming (Xu et al., 2023). Studies have shown that almost all epigenetic modification processes require the participation of metabolites, such as lactate, acetyl coenzyme A, nicotinamide adenine dinucleotide, α-ketoglutarate, succinate, etc., which are used as substrates to participate in post-translational modification processes, such as acetylation, methylation, and phosphorylation (Sun et al., 2022). Thus, metabolites play a very broad and important role in tumor epigenetic modification, drawing the attention of scholars to the metabolic patterns of tumor cells.

4.2.2 The implications of milestone studies for future research

Metabolic studies on liver cancer were first published in 2012. However, the results published by Ally A in 2017 in the journal *Cell* (Ally et al., 2017) are considered a landmark in metabolic reprogramming research in HCC. The study comprehensively analyzed and identified mutated genes in HCC through multi-omics testing of a large number of HCC clinical samples, and identified abnormal genes albumin (ALB), apolipoprotein B (APOB), carbamoyl phosphate synthetase 1 (CPS1) that lead to metabolic reprogramming in HCC. The study suggested that combinations of multiple drugs targeting these abnormal genes can achieve the most effective therapeutic effects (Ally et al., 2017), which provides a clear direction for future research. For example, CPS1 is one of the key enzymes in HCC metabolism, and studies have determined that decreased expression of CPS1 in HCC led to an increase in pyrimidine synthesis, which promoted the development of HCC. The network was filtered to exclude any duplicates, as well as duplicates more than three steps away from CPS1 or carbamoyl phosphate synthetase/aspartate transcarbamoylase/dihydroorotase (CAD). Then CPS1-related metabolites and metabolic gene networks were analyzed by the R package, showing the prognostic role of network genes (Dumenci et al., 2020). In addition to alterations in these specific genes in metabolic reprogramming of HCC, Ally A's study also identified other important targets such as Wnt signaling, mesenchymal-epithelial transition (Met), vascular endothelial growth factor A (VEGFA), telomerase reverse transcriptase (TERT), and the immune checkpoint proteins CTLA-4, PD-1, and PD-L1, which were regulated by anticancer agents. Researchers have therefore started studying metabolic reprogramming in HCC beyond glycolipid metabolic patterns to better understand its regulatory mechanisms and investigate the causes of reprogramming from upstream pathways. Growing evidence reported the role of Wnt/β-linker signaling in aerobic glycolysis, fatty acid metabolism, and glutamine catabolic synthesis of HCC (Leung and Lee, 2022). Lactate, which is produced by aerobic glycolysis, acidifies the tumor microenvironment and facilitates tumor-associated macrophage (TAM) polarization (San-Millán and Brooks, 2017; de la Cruz-López et al., 2019). Meanwhile, Wnt/β-catenin signaling activated glycolysis and promoted macrophage polarization, leading to endothelial mesenchymal transition (EMT) development and HCC invasion (Jiang et al., 2021). Furthermore, Wnt/β-catenin could regulate glucose metabolism and promote the EMT process by inducing mitochondrial inhibition and glycolytic activation (Lee et al., 2012). The membrane receptor tyrosine kinase Met plays an important role in HCC EMT and mesenchymal phenotype acquisition. Studies have found that hyperglycemia activated Met, which in turn enhanced HCC cell invasion. However, inhibition of Met kinase activity reverses glycolytic gene expression in HCC cells (Topel et al., 2021). Additionally, metformin has been found to reduce phospho-ERK and Cyclin D1 and c-Myc expression in AKT/c-Met mice and hindered the malignant transformation of hepatocytes in an AKT/c-Met-activated HCC mouse model (Zhang et al., 2019). TERT, which synthesizes telomeric DNA to maintain telomere stability, is commonly altered in HCC (Lee et al., 2018). Aerobic glycolysis could also impact tumor biology through epigenetic regulation of tumor-associated genes. Studies have shown that metabolic reprogramming increased the expression of the TERT oncogene through epigenetic changes such as histone acetylation, thereby promoting tumor cell proliferation (Onizuka et al., 2021). In addition, hypoxia-inducible factors 1α (HIF-1α), are often closely associated with tumor progression, and they could promote the acquisition of a malignant phenotype in HCC by activating glycolysis and the transcription of angiogenic cytokines such as VEGF (Hamaguchi et al., 2008; Rigiracciolo et al., 2015). When T cells are activated, they undergo metabolic reprogramming to meet their differentiation and functional expression requirements (Frauwirth and Thompson, 2004). However, studies have confirmed the activated T cells connected to PD-1 shift their metabolic process from glycolysis to enhanced fatty acid oxidation, which activates CPT1A and promotes lipolysis to maintain long-term survival. On the other hand, CTLA-4 inhibited glycolysis through the expression of the glucose transporter protein Glut1, it did not enhance CPT1A and FAO, playing a role in preserving the metabolic profile of unstimulated cells and maintaining immune quiescence (Patsoukis et al., 2015). These findings mechanistically reveal key targets and mechanisms in metabolic reprogramming of HCC, providing intervention targets for subsequent treatment of HCC, and laying a scientific foundation for future research.

4.2.3 The recent studies in the metabolic reprogramming of HCC

A recent study published in *Cell* looks at increased levels of abnormally metabolized arginine in HCC with decreased expression of arginine synthesis genes, suggesting that arginine undergoes metabolic reprogramming with a reduction in arginine-polyamine conversion, ultimately leading to the accumulation of high levels of arginine. Mechanistically, it also discovered that RBM39-mediated upregulation of asparagine synthesis results in enhanced arginine uptake, which helps to maintain high arginine levels and oncogenic metabolism (Mossmann et al., 2023). This study initially focused on the phenotype of abnormal metabolism in HCC and then searched for relevant regulated target genes to elucidate the mechanism of abnormal metabolism in HCC. Similarly, another study found that reduced metabolism of propionyl-CoA due to the downregulation of ALDH6A1 in metabolic reprogramming is closely associated with HCC development. Mechanistically, Pro-CoA produced by ALDH6A1 inhibited the activity of citrate synthase, impaired mitochondrial respiration, and membrane potential, reduced ATP production, and inhibited HCC proliferation (Sun et al., 2023). This study discovered the key pathway to inhibit the growth of HCC by targeting the abnormal metabolic enzymes in HCC cells. Li et al. (2023) identified TK1 as a key driver of metabolic reprogramming in HCC and verified its role in HCC progression by TK1 inhibition and overexpression. Mechanistically, TK1 could bind PRMT1 to promote HCC glycolysis and enhance the malignant phenotype of HCC. All these researches aim to deeply explore the adaptive regulation of key enzymes in HCC metabolic reprogramming, elucidate their molecular features, identify diagnostic and therapeutic targets for HCC, and provide HCC prevention and treatment strategies by intervening in metabolic reprogramming of HCC cells.

4.3 Tumor microenvironment

According to current research and predicted future trends in the field, tumor microenvironment (TME) is an important factor that cannot be overlooked. In the TME, interactions between immune cells (the main cell population besides tumor cells) have a significant impact on metabolic reprogramming, which is a key determinant of the antitumor immune response. By understanding the metabolism of immune cells and their interactions with tumor cells, we can come up with new ideas for targeting metabolic pathways in antitumor immunotherapy.

4.3.1 Immune cell metabolism in cancer

Complex metabolic patterns similar to those of tumor cells are also present in immune cells. Under normal circumstances, immune cells maintain only basic nutritional intake, the lowest rate of glycolysis, and biosynthesis level to remain in their resting state. However, when the body is stimulated by external substances like inflammation, these immune cells are activated, resulting in increased energy and biosynthesis demand, leading to significant changes in their metabolic pattern (Pearce et al., 2013).

Activated T cells preferentially use aerobic glycolysis over TCA-coupled OXPHOS for ATP production and biosynthesis, while regulatory T cells (Treg) rely on OXPHOS and FAO to support their survival and differentiation (Beier et al., 2015). Similarly, activated neutrophils (Bodac and Meylan, 2021), M1-type macrophages (Netea-Maier et al., 2018), and dendritic cells (Peng et al., 2021) are primarily dependent on glycolysis for energy. This suggests that different metabolic patterns can influence the differentiation of immune cell subpopulations. However, tumor cells consume most of the nutrients and energy in the TME, hindering the function of immune cells. Therefore, immune cells undergo metabolic reprogramming during proliferation, differentiation, and execution of their functions, which ultimately determines the anti-immune response of the tumor (Leone and Powell, 2020). Macrophages, also known as TAM, are the most critical immune cell population in TME. They can secrete various cytokines and chemokines that promote tumor development. M1 cells perform their pro-inflammatory function mainly through glycolysis, PPP, while M2 cells exert their anti-inflammatory function by enhancing OXPHOS and FAO, however, secretion of IL-1β by M2 cells could lead to proliferation, invasion, and spreading and promote HCC metastasis (Zhang et al., 2018). Neutrophils are one of the major immune cells in TME, also known as tumor-associated neutrophils (TAN). Neutrophil metabolism mainly relies on glycolysis and OXPHOS to produce more ATP (Borregaard and Herlin, 1982; Patel et al., 2018). However, in TME, due to an insufficient amount of glucose, TAN usually utilizes mitochondria for fatty acid oxidation (Patel et al., 2018), which promotes growth and invasion of HCC (Granot and Jablonska, 2015). Tumor-derived hypoxia with lactate accumulation leads to inhibition of glycolysis and upregulation of OXPHOS in dendritic cells. Depletion of extracellular amino acids, lactate accumulation, and nutrient deprivation-induced AMPK activation inhibit TCR signaling and its downstream glycolysis in T effector cells, such as CD8 cytotoxic T cells, while activating OXPHOS and FAO. This leads to the differentiation of Tregs, which promotes immune escape and tumor growth by relying on FAO for energy (Biswas, 2015). Tregs also mainly rely on FAO for energy and play an immunosuppressive role in TME (Michalek et al., 2011). Memory T cells, on the other hand, have a rather unique metabolic state with mitochondrial fatty acid oxidation to maintain energy requirements for basic survival (van der Windt et al., 2012).

4.3.2 Tumor-derived metabolites regulate metabolic reprogramming of immune cells

The TME is an intrinsically self-interested environment that develops during tumor growth. Its purpose is to maintain the rapid proliferation of cancer cells by altering cellular functions and related signaling pathways (Odegaard and Chawla, 2011). The TME and tumor cells have an interaction that is one of the key factors contributing to tumor immune escape (Martínez-Reyes and Chandel, 2021). For example, the accumulation of lactic acid produced by aerobic glycolysis in tumor cells, decreased pH, hypoxia, and enhanced reactive oxygen species (ROS) favor the production of TME, leading to tumor progression and immune escape (Watson et al., 2021). In HCC, succinate levels are elevated (Yang et al., 2023), which promoted the inflammatory state of TME by activating the receptor and amplifying toll-like receptor (TLR) signaling, leading to increased IL-1β secretion (Wu et al., 2020).

TME contains not only cancer cells but also various immune cells. The metabolites produced during tumor metabolism affect the differentiation and metabolic pattern of immune cells. For instance, lactate production through glycolysis in early tumor stages promotes TAM polarization via HIF-1α. Hypoxia in the TME promoted TAM polarization to the M2 phenotype, which activated metabolic reprogramming and facilitated tumor cell proliferation and angiogenesis (Chen X. J. et al., 2019). Excessive lactic acid also inhibited the upregulation of nuclear factor of activated T cell (NFAT) signaling in NK cells, impaired IFN-γ secretion, and promoted apoptosis (Brand et al., 2016). Most tumor cells compete with T cells, NK cells, etc. for arginine uptake, inhibiting their metabolic activity and creating an immunosuppressive microenvironment. However, T cells stimulated by arginine supplementation have significantly enhanced anti-tumor immunity and prolonged the survival time of mice (Davel et al., 2002). Immunosuppressive cells can suppress anti-tumor immunity by degrading arginine, such as TAM M2, tolerogenic DCs, and Treg cells (Buck et al., 2016). T cell activation is extremely sensitive to the concentration of tryptophan in the surrounding environment, and tryptophan is heavily utilized by tumor cells, leading to a deficiency that triggers apoptosis of T cells (Cronin et al., 2019). In addition, fatty acids and cholesterol are energy substances essential for immune cell differentiation and functioning. Abnormal accumulation of short-chain and long-chain fatty acids in immunosuppressive cells is involved in the metabolic reprogramming of these cells in TME (Currie et al., 2013).

4.3.3 Mechanisms regulating metabolic reprogramming of immune cells

Studies have demonstrated that macrophage polarization and metabolism are influenced by multiple signals and pathways, such as HIF, PI3K/AKT, PPAR, and AMPK pathways (Wang et al., 2022). Consequently, modifying the macrophage metabolism pathway can directly impact activated TAM polarization and modulate tumor progression. In addition, it was reported that tumor-associated monocytes in HCC showed enhanced glycolysis, increased expression of the key glycolytic enzyme PFKFB3, and activation of the nuclear factor kappa B signaling pathway mediated the increased expression of PD-L1 (Chen D. P. et al., 2019). Ectosomes PKM2 were also found to promote HCC development by affecting metabolic reprogramming of monocytes in the TME, promoting STAT3 phosphorylation in the nucleus, inducing macrophage differentiation, and secreting the related chemokines CCL1 and CCR8 (Hou et al., 2020). Similarly, T cells in cancer prefer aerobic glycolysis for biosynthesis and nutrient uptake (Fox et al., 2005; Vander Heiden et al., 2009; Chang et al., 2013). However, the metabolic needs of different T cell subsets vary depending on their function. $CD4^+$T cells are mainly dependent on glycolysis and fatty acid *de novo* synthesis (Berod et al., 2014), while $CD8^+$T cells mainly rely on upregulation of glycolysis, glutamine catabolism, and FAO to exert potent antitumor cytotoxic activity (Pearce et al., 2009), which is associated with the activation of PI3K/Akt/mTOR-related pathway and c-Myc, glycolytic genes (GLUT1, PDK1, or HK2) (MacIver et al., 2013; Maciolek et al., 2014). Studies have shown that IFN-α improved glucose metabolism in the TME by inhibiting HIF-1α signaling, decreasing glucose consumption, activating mTOR-FOXM1 signaling, promoting the toxic effects of $CD8^+$T cells, and enhancing PL-D-blocked immune responses to achieve anti-HCC (Hu et al., 2022). Moreover, the mTOR and the AMPK signaling pathway are extensively involved in the metabolic regulation of immune cells. mTORC1 promoted the expression of PD-L1, inhibited the infiltration of NK cells and T cells in the tumor immune microenvironment, and allowed tumor cells to evade killing by immune cells (Mafi et al., 2021). Activation of the AMPK signaling pathway is involved in macrophage polarization as well as T lymphocyte differentiation (Keerthana et al., 2023).

Overall, multiple metabolic pathways of immune cells like glycolysis and FAO in TME are designed for anti-tumor immune responses and pro-tumor immune escape, such as the deletion of M1 macrophages, N1 neutrophils, and $CD8^+$T cells, and the activation of M2 macrophages, N2 neutrophils, and Treg cells (Xia Y. J. et al., 2021). Meanwhile, metabolites and cytokines generated during metabolic reprogramming can promote the establishment of TME. For example, accumulation of lactate, low glucose, and a hypoxic state all promote rapid proliferation and metastasis of HCC. Nevertheless, the complex roles and potential mechanisms between the tumor microenvironment and metabolic reprogramming in HCC are not yet fully understood. Further research in this area can enhance our understanding of HCC pathogenesis and inform the development of clinical therapeutic strategies.

4.4 Implications and recommendations for future research

Numerous studies have substantiated that HCC cells undergo significant metabolic reprogramming when compared to normal hepatocytes. It is mainly manifested in the abnormally active glycolysis, fatty acid synthesis, and glutamine metabolism, which is related to the overexpression and activation of the key enzymes or pathways that regulate these processes. Several investigations have demonstrated that targeting these abnormal metabolic enzymes and pathways in HCC cells can significantly impede HCC growth and metastasis. As such, this presents a promising avenue for clinical application in the management of HCC (Foglia et al., 2023). However, there are still some issues with the diagnosis and treatment of tumors marked by metabolic enzymes and pathways that deserve attention and in-depth research. Besides, these metabolites and key enzymes have not been quantified yet. On the one hand, individual metabolic enzymes or products tend to be less effective as diagnostic markers, whereas combining multiple metabolic markers shows better results. On the other hand, most studies are centered around one or a few metabolic enzymes or pathways, which are relatively inefficient for research purposes. Due to the existence of complex metabolic compensation mechanisms in tumor cells, targeting a single metabolic enzyme or pathway often has a very limited inhibitory effect on tumors. The combination of multiple metabolic targets may enhance the killing effect on tumors, which is the most attractive direction for tumor targeted therapy in the future. With the development of proteomics and metabolomics, the joint multi-omics analysis will facilitate researchers to conduct a comprehensive and systematic study of the metabolic reprogramming network of HCC cells more efficiently, which will assist in the search for new and more critical metabolic

enzymes and metabolites of HCC abnormalities. Identification of key enzymes involved in metabolic reprogramming in HCC by visualization and quantification techniques could be a valuable reference for targeting HCC therapy.

There may be a synergistic relationship between metabolic reprogramming and the TME. A deeper understanding and appropriate utilization of the cross-talk between the two has the potential to enhance the efficacy of tumor immunotherapy and ameliorate the low response rate to immunotherapy. Moreover, immune metabolism in tumors presents a new research field, and interfering with the metabolic reprogramming of immune cells can not only affect the function of HCC cells but also change the immunosuppression in the TME, which provides a promising therapeutic strategy for the future (Xia L. et al., 2021). In recent years, the fields of tumor metabolism, immune metabolism, and immunotherapy have experienced significant progress, but studies exploring the association between these fields have been insufficient. The appropriate utilization of these strategies in the complex tumor microenvironment remains uncertain. Although metabolic drugs in combination with immunotherapy have been used in clinical trials, they are still in their infancy. To optimize the combination strategies of metabolic and immune drugs, it is imperative to gain a deeper understanding of the relationship between metabolic reprogramming and immune cells in HCC. This necessitates restoring physiological conditions from clinical to animal experiments, from cellular to molecular levels, clarifying targets, and elucidating mechanisms. However, this process requires extensive clinical trials and animal model validation, which can be costly in terms of time and economics. Nevertheless, the emergence of new biological technologies, such as organoids, stem cells, microfluidics, and nano-drug delivery systems, has ushered in a new era of research. These emerging technologies can provide crucial evidence for studying tumor metabolism and immune interactions, and they represent a key focus for future development.

This scientific study analyzed the metabolic reprogramming in HCC from 2012 to 2023 using the R package software tool. The study used scientific and rigorous methods to evaluate and screen the literature, which enabled a more comprehensive collection of relevant information on metabolic reprogramming in HCC than traditional narrative reviews. The study analyzed the most representative articles, authors, journals, and countries in the field, and also analyzed the co-citations and collaborations among them. Notably, the study visually demonstrated the research development history, current research status, research hotspots, and developmental trends, which provided valuable insights for researchers. According to the study, metabolic reprogramming in HCC is undergoing a rapid development stage, with a primary focus on the regulation of metabolic enzymes and key pathways behind the abnormal glucose, lipid, and glutamine metabolism in HCC cells. This development is linked to the TME immune cell metabolism, which has become a new research hotspot and trend in this field. The study's findings indicate that metabolic reprogramming in HCC is a dynamic and rapidly evolving field with significant potential for future research. It is worth noting that as a systematic review, meta-analysis may not be able to summarize and organize the latest research progress in this field and provide academic insights well, as the field mostly focuses on basic research. Therefore, it is essential to utilize other methods to complement and supplement the findings of analytical studies. Overall, this study's findings provide a valuable overview of the research progress in metabolic reprogramming in HCC, which will be useful for researchers in this field.

4.5 Limitations and strengths

This study comprehensively analyzes the research characteristics and trends of metabolic reprogramming in HCC, which will provide new insights into the diagnosis, prognosis, and identification of therapeutic targets for HCC. However, there are some technical limitations to this study.

Although WoSCC is a highly regarded academic database worldwide, its coverage remains limited, particularly in non-English-speaking countries and regions. Consequently, the study's inclusion of literature from WoSCC may not be comprehensive enough. However, it is essential to note that the WoSCC database provides researchers with high-quality literature content that is thoroughly screened and vetted to ensure credibility and accuracy. It also offers reliable citations of the literature and comprehensive assessments of scholarly achievements. Therefore, despite the fact that only WoSCC literature was included in this study, the results remained largely unaffected. Nevertheless, to ensure a comprehensive assessment and avoid potential impacts, it is advisable to consider the inclusion of several more databases in future studies.

In addition, the statistical analysis of literature is often marred by issues of sample selection bias and endogeneity. The use of the number of citations and H-index to measure the impact of papers is a prime example of such biases. The metrics employed are limited to the output and impact of published papers, ignoring their quality. Moreover, the method does not account for the impact of different stages of the life cycle in which a discipline or field is found. Therefore, to obtain the most representative results in the research field, more paper evaluation indices need inclusion. The choice of measurement methods should depend on the purpose of the study and the characteristics of the data to select appropriate statistical models and analysis methods. The interpretation of the resulting measurements should also account for the inevitable selection bias, thereby ensuring the development of an objective and fair statistical report.

In the context of predicting thematic trends within a research field, it is important to note that the frequency of first-level subject terms may not serve as an accurate indicator for analyzing the specific research direction of the field. Instead, a more comprehensive approach involves extracting the second-level subject terms for analysis. While co-occurrence analysis and citation network analysis can reveal the structure and development of the research field, these methods may not adequately capture rapid changes in emerging fields or trends in non-mainstream research. In addition, predicting future research hotspots and directions can be uncertain in the face of disruptive technologies and swift changes within a specific field. To enhance

the accuracy and depth of analysis, methods and techniques from other disciplines like artificial intelligence and machine learning can be applied in the future. Researchers are actively developing new predictive models and methods to improve their ability to anticipate future research trends.

Finally, it should be noted that despite the strong functionality of the biblioshiny tool we employed, it is not without its limitations. Thus, it is recommended that it be complemented with other literature analysis software to enhance its strengths in the future. A promising area of development that can be utilized for this purpose is Webometrics, which incorporates traditional literature bibliometric methods on web pages. This is particularly valuable in the context of the current network information age, providing comprehensive statistics on the development trends in a particular research direction, which has significant potential for broad applications.

5 Conclusion

In summary, this study utilized bibliometrix and visual analysis to provide a comprehensive summary of the most significant research papers concerning HCC metabolism in the extant literature. This analysis effectively identified crucial contributions that have advanced the discipline and the field over the past decade. In particular, the trend in HCC metabolic research is moving away from isolated studies of glucose and lipid metabolism and towards more comprehensive, holistic investigations of metabolic reprogramming. Moreover, these studies are progressively taking into account the increasingly close relationship between the TME and metabolic reprogramming, making the combination of targeted metabolism and immunotherapy a possible new strategy for HCC control in the future, thus providing new insights and research directions for the field.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

XL: Conceptualization, Investigation, Methodology, Writing–original draft, Writing–review and editing. LZ: Investigation, Writing–review and editing. XX: Investigation, Writing–review and editing. XYL: Writing–review and editing, Validation. WW: Validation, Writing–review and editing. QF: Writing–review and editing, Funding acquisition. ZT: Writing–review and editing, Methodology, Software.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Natural Science Foundation of Sichuan Province (No. 2024NSFSC1827) and the National Natural Science Foundation of China (82074399).

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1392241/full#supplementary-material

References

Ally, A., Balasundaram, M., Carlsen, R., Chuah, E., Clarke, A., Dhalla, N., et al. (2017). Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell* 169, 1327–1341.e23. doi:10.1016/j.cell.2017.05.046

Amann, T., Maegdefrau, U., Hartmann, A., Agaimy, A., Marienhagen, J., Weiss, T. S., et al. (2009). GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. *Am. J. Pathol.* 174, 1544–1552. doi:10.2353/ajpath.2009.080596

Anstee, Q. M., Reeves, H. L., Kotsiliti, E., Govaere, O., and Heikenwalder, M. (2019). From NASH to HCC: current concepts and future challenges. *Nat. Rev. Gastroenterol. Hepatol.* 16, 411–428. doi:10.1038/s41575-019-0145-7

Aria, M., and Cuccurullo, C. (2017). Bibliometrix: an R-tool for comprehensive science mapping analysis. *J. Informetr.* 11, 959–975. doi:10.1016/j.joi.2017.08.007

Beier, U. H., Angelin, A., Akimova, T., Wang, L., Liu, Y., Xiao, H., et al. (2015). Essential role of mitochondrial energy metabolism in Foxp3$^+$ T-regulatory cell function and allograft survival. *FASEB J.* 29, 2315–2326. doi:10.1096/fj.14-268409

Berod, L., Friedrich, C., Nandan, A., Freitag, J., Hagemann, S., Harmrolfs, K., et al. (2014). *De novo* fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat. Med.* 20, 1327–1333. doi:10.1038/nm.3704

Biswas, S. K. (2015). Metabolic reprogramming of immune cells in cancer progression. *Immunity* 43, 435–449. doi:10.1016/j.immuni.2015.09.001

Bodac, A., and Meylan, E. (2021). Neutrophil metabolism in the cancer context. *Semin. Immunol.* 57, 101583. doi:10.1016/j.smim.2021.101583

Borregaard, N., and Herlin, T. (1982). Energy metabolism of human neutrophils during phagocytosis. *J. Clin. Invest.* 70, 550–557. doi:10.1172/jci110647

Brand, A., Singer, K., Koehl, G. E., Kolitzus, M., Schoenhammer, G., Thiel, A., et al. (2016). LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab.* 24, 657–671. doi:10.1016/j.cmet.2016.08.011

Buck, M. D., O'sullivan, D., Klein Geltink, R. I., Curtis, J. D., Chang, C. H., Sanin, D. E., et al. (2016). Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell* 166, 63–76. doi:10.1016/j.cell.2016.05.035

Chai, F., Li, Y., Liu, K. Y., Li, Q., and Sun, H. Z. (2019). Caveolin enhances hepatocellular carcinoma cell metabolism, migration, and invasion *in vitro* via a hexokinase 2-dependent mechanism. *J. Cell. Physiol.* 234, 1937–1946. doi:10.1002/jcp.27074

Chang, C. H., Curtis, J. D., Maggi, L. B., Faubert, B., Villarino, A. V., O'sullivan, D., et al. (2013). Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* 153, 1239–1251. doi:10.1016/j.cell.2013.05.016

Chen, D. P., Ning, W. R., Jiang, Z. Z., Peng, Z. P., Zhu, L. Y., Zhuang, S. M., et al. (2019a). Glycolytic activation of peritumoral monocytes fosters immune privilege via the PFKFB3-PD-L1 axis in human hepatocellular carcinoma. *J. Hepatol.* 71, 333–343. doi:10.1016/j.jhep.2019.04.007

Chen, X. J., Wu, S., Yan, R. M., Fan, L. S., Yu, L., Zhang, Y. M., et al. (2019b). The role of the hypoxia-Nrp-1 axis in the activation of M2-like tumor-associated macrophages in the tumor microenvironment of cervical cancer. *Mol. Carcinog.* 58, 388–397. doi:10.1002/mc.22936

Cho, E., Jun, C. H., Kim, B. S., Son, D. J., Choi, W. S., and Choi, S. K. (2015). 18F-FDG PET CT as a prognostic factor in hepatocellular carcinoma. *Turk. J. Gastroenterol.* 26, 344–350. doi:10.5152/tjg.2015.0152

Crane, H., Gofton, C., Sharma, A., and George, J. (2023). MAFLD: an optimal framework for understanding liver cancer phenotypes. *J. Gastroenterol.* 58, 947–964. doi:10.1007/s00535-023-02021-7

Cronin, S. J. F., Seehus, C., Weidinger, A., Talbot, S., Reissig, S., Seifert, M., et al. (2019). Publisher Correction: the metabolite BH4 controls T cell proliferation in autoimmunity and cancer. *Nature* 572, E18. doi:10.1038/s41586-019-1459-x

Currie, E., Schulze, A., Zechner, R., Walther, T. C., and Farese, R. V., Jr. (2013). Cellular fatty acid metabolism and cancer. *Cell. Metab.* 18, 153–161. doi:10.1016/j.cmet.2013.05.017

Davel, L. E., Jasnis, M. A., De La Torre, E., Gotoh, T., Diament, M., Magenta, G., et al. (2002). Arginine metabolic pathways involved in the modulation of tumor-induced angiogenesis by macrophages. *FEBS Lett.* 532, 216–220. doi:10.1016/s0014-5793(02)03682-7

De Jager, R. L., Magill, G. B., Golbey, R. B., and Krakoff, I. H. (1976). Combination chemotherapy with mitomycin C, 5-fluorouracil, and cytosine arabinoside in gastrointestinal cancer. *Cancer Treat. Rep.* 60, 1373–1375.

De La Cruz-López, K. G., Castro-Muñoz, L. J., Reyes-Hernández, D. O., García-Carrancá, A., and Manzo-Merino, J. (2019). Lactate in the regulation of tumor microenvironment and therapeutic approaches. *Front. Oncol.* 9, 1143. doi:10.3389/fonc.2019.01143

Dewaal, D., Nogueira, V., Terry, A. R., Patra, K. C., Jeon, S. M., Guzman, G., et al. (2018). Hexokinase-2 depletion inhibits glycolysis and induces oxidative phosphorylation in hepatocellular carcinoma and sensitizes to metformin. *Nat. Commun.* 9, 446. doi:10.1038/s41467-017-02733-4

Du, D., Liu, C., Qin, M., Zhang, X., Xi, T., Yuan, S., et al. (2022). Metabolic dysregulation and emerging therapeutical targets for hepatocellular carcinoma. *Acta Pharm. Sin. B* 12, 558–580. doi:10.1016/j.apsb.2021.09.019

Dumenci, O. E., Abellona, M. R. U., Khan, S. A., Holmes, E., and Taylor-Robinson, S. D. (2020). <p>Exploring Metabolic Consequences of CPS1 and CAD Dysregulation in Hepatocellular Carcinoma by Network Reconstruction</p>. *J. Hepatocell. Carcinoma* 7, 1–9. doi:10.2147/JHC.S239039

Eslam, M., Sanyal, A. J., George, J., and International Consensus, P. (2020). MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* 158, 1999–2014.e1. doi:10.1053/j.gastro.2019.11.312

Estes, C., Razavi, H., Loomba, R., Younossi, Z., and Sanyal, A. J. (2018). Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 67, 123–133. doi:10.1002/hep.29466

Foglia, B., Beltra, M., Sutti, S., and Cannito, S. (2023). Metabolic reprogramming of HCC: a new microenvironment for immune responses. *Int. J. Mol. Sci.* 24, 7463. doi:10.3390/ijms24087463

Fox, C. J., Hammerman, P. S., and Thompson, C. B. (2005). Fuel feeds function: energy metabolism and the T-cell response. *Nat. Rev. Immunol.* 5, 844–852. doi:10.1038/nri1710

Frauwirth, K. A., and Thompson, C. B. (2004). Regulation of T lymphocyte metabolism. *J. Immunol.* 172, 4661–4665. doi:10.4049/jimmunol.172.8.4661

Granot, Z., and Jablonska, J. (2015). Distinct functions of neutrophil in cancer and its regulation. *Mediat. Inflamm.* 2015, 701067. doi:10.1155/2015/701067

Hamaguchi, T., Iizuka, N., Tsunedomi, R., Hamamoto, Y., Miyamoto, T., Iida, M., et al. (2008). Glycolysis module activated by hypoxia-inducible factor 1alpha is related to the aggressive phenotype of hepatocellular carcinoma. *Int. J. Oncol.* 33, 725–731. doi:10.3892/ijo_00000058

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi:10.1016/j.cell.2011.02.013

Hassan, W., Zafar, M., Duarte, A. E., Kamdem, J. P., and Da Rocha, J. B. T. (2021). Pharmacological Research: a bibliometric analysis from 1989 to 2019. *Pharmacol. Res.* 169, 105645. doi:10.1016/j.phrs.2021.105645

Hou, P. P., Luo, L. J., Chen, H. Z., Chen, Q. T., Bian, X. L., Wu, S. F., et al. (2020). Ectosomal PKM2 promotes HCC by inducing macrophage differentiation and remodeling the tumor microenvironment. *Mol. Cell* 78, 1192–1206. doi:10.1016/j.molcel.2020.05.004

Hu, B., Yu, M., Ma, X., Sun, J., Liu, C., Wang, C., et al. (2022). IFNα potentiates anti-PD-1 efficacy by remodeling glucose metabolism in the hepatocellular carcinoma microenvironment. *Cancer Discov.* 12, 1718–1741. doi:10.1158/2159-8290.CD-21-1022

Huang, D. Q., El-Serag, H. B., and Loomba, R. (2021). Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 18, 223–238. doi:10.1038/s41575-020-00381-6

Huang, Q., Tan, Y. X., Yin, P. Y., Ye, G. Z., Gao, P., Lu, X., et al. (2013). Metabolic characterization of hepatocellular carcinoma using nontargeted tissue metabolomics. *Cancer Res.* 73, 4992–5002. doi:10.1158/0008-5472.CAN-13-0308

Iarc, W. H. O. (2020). GLOBAL CANCER OBSERVATORY. Available at: https://gco.iarc.fr/(Accessed November 22, 2023).

Jiang, Y., Han, Q. J., Zhao, H. J., and Zhang, J. (2021). Promotion of epithelial-mesenchymal transformation by hepatocellular carcinoma-educated macrophages through Wnt2b/β-catenin/c-Myc signaling and reprogramming glycolysis. *J. Exp. Clin. Cancer Res.* 40, 13. doi:10.1186/s13046-020-01808-3

Keerthana, C. K., Rayginia, T. P., Shifana, S. C., Anto, N. P., Kalimuthu, K., Isakov, N., et al. (2023). The role of AMPK in cancer metabolism and its impact on the immunomodulation of the tumor microenvironment. *Front. Immunol.* 14, 1114582. doi:10.3389/fimmu.2023.1114582

Khan, A. S., Rehman, S. U., Ahmad, S., Almaimouni, Y. K., Alzamil, M. a.S., and Dummer, P. M. H. (2021). Five decades of the international endodontic journal: bibliometric overview 1967-2020. *Int. Endod. J.* 54, 1819–1839. doi:10.1111/iej.13595

Lee, M., Ko, H., and Yun, M. J. (2018). Cancer metabolism as a mechanism of treatment resistance and potential therapeutic target in hepatocellular carcinoma. *Yonsei Med. J.* 59, 1143–1149. doi:10.3349/ymj.2018.59.10.1143

Lee, S. Y., Jeon, H. M., Ju, M. K., Kim, C. H., Yoon, G., Han, S. I., et al. (2012). Wnt/Snail signaling regulates cytochrome C oxidase and glucose metabolism. *Cancer Res.* 72, 3607–3617. doi:10.1158/0008-5472.CAN-12-0006

Leone, R. D., and Powell, J. D. (2020). Metabolism of immune cells in cancer. *Nat. Rev. Cancer* 20, 516–531. doi:10.1038/s41568-020-0273-y

Leung, R. W. H., and Lee, T. K. W. (2022). Wnt/β-Catenin signaling as a driver of stemness and metabolic reprogramming in hepatocellular carcinoma. *Cancers* 14, 5468. doi:10.3390/cancers14215468

Li, Q., Zhang, L., Yang, Q., Li, M., Pan, X., Xu, J., et al. (2023). Thymidine kinase 1 drives hepatocellular carcinoma in enzyme-dependent and -independent manners. *Cell Metab.* 35, 912–927.e7. doi:10.1016/j.cmet.2023.03.017

Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., et al. (2021). Hepatocellular carcinoma. *Nat. Rev. Dis. Prim.* 7, 6. doi:10.1038/s41572-020-00240-3

Llovet, J. M., Zucman-Rossi, J., Pikarsky, E., Sangro, B., Schwartz, M., Sherman, M., et al. (2016). Hepatocellular carcinoma. *Nat. Rev. Dis. Prim.* 2, 16018. doi:10.1038/nrdp.2016.18

Lu, M., Lu, L., Dong, Q. Z., Yu, G. Y., Chen, J. H., Qin, L. X., et al. (2018). Elevated G6PD expression contributes to migration and invasion of hepatocellular carcinoma cells by inducing epithelial-mesenchymal transition. *Acta Biochim. Biophys. Sin.* 50, 370–380. doi:10.1093/abbs/gmy009

Maciolek, J. A., Pasternak, J. A., and Wilson, H. L. (2014). Metabolism of activated T lymphocytes. *Curr. Opin. Immunol.* 27, 60–74. doi:10.1016/j.coi.2014.01.006

Maciver, N. J., Michalek, R. D., and Rathmell, J. C. (2013). Metabolic regulation of T lymphocytes. *Annu. Rev. Immunol.* 31, 259–283. doi:10.1146/annurev-immunol-032712-095956

Mafi, S., Mansoori, B., Taeb, S., Sadeghi, H., Abbasi, R., Cho, W. C., et al. (2021). mTOR-mediated regulation of immune responses in cancer and tumor microenvironment. *Front. Immunol.* 12, 774103. doi:10.3389/fimmu.2021.774103

Martínez-Reyes, I., and Chandel, N. S. (2021). Cancer metabolism: looking forward. *Nat. Rev. Cancer* 21, 669–680. doi:10.1038/s41568-021-00378-6

Michalek, R. D., Gerriets, V. A., Jacobs, S. R., Macintyre, A. N., Maciver, N. J., Mason, E. F., et al. (2011). Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J. Immunol.* 186, 3299–3303. doi:10.4049/jimmunol.1003613

Mossmann, D., Muller, C., Park, S., Ryback, B., Colombi, M., Ritter, N., et al. (2023). Arginine reprograms metabolism in liver cancer via RBM39. *Cell* 186, 5068–5083.e23. doi:10.1016/j.cell.2023.09.011

Netea-Maier, R. T., Smit, J. W. A., and Netea, M. G. (2018). Metabolic changes in tumor cells and tumor-associated macrophages: a mutual relationship. *Cancer Lett.* 413, 102–109. doi:10.1016/j.canlet.2017.10.037

Ninkov, A., Frank, J. R., and Maggio, L. A. (2022). Bibliometrics: methods for studying academic publishing. *Perspect. Med. Educ.* 11, 173–176. doi:10.1007/s40037-021-00695-4

Nwosu, Z. C., Megger, D. A., Hammad, S., Sitek, B., Roessler, S., Ebert, M. P., et al. (2017). Identification of the consistently altered metabolic targets in human hepatocellular carcinoma. *Cell. Mol. Gastroenterol. Hepatol.* 4, 303–323. doi:10.1016/j.jcmgh.2017.05.004

Odegaard, J. I., and Chawla, A. (2011). Alternative macrophage activation and metabolism. *Annu. Rev. Pathol.* 6, 275–297. doi:10.1146/annurev-pathol-011110-130138

Ohshima, K., and Morii, E. (2021). Metabolic reprogramming of cancer cells during tumor progression and metastasis. *Metabolites* 11, 28. doi:10.3390/metabo11010028

Onizuka, H., Masui, K., Amano, K., Kawamata, T., Yamamoto, T., Nagashima, Y., et al. (2021). Metabolic reprogramming drives pituitary tumor growth through epigenetic regulation of TERT. *Acta histochem. cytochem.* 54, 87–96. doi:10.1267/ahc.21-00007

Patel, S., Fu, S., Mastio, J., Dominguez, G. A., Purohit, A., Kossenkov, A., et al. (2018). Unique pattern of neutrophil migration and function during tumor progression. *Nat. Immunol.* 19, 1236–1247. doi:10.1038/s41590-018-0229-5

Patsoukis, N., Bardhan, K., Chatterjee, P., Sari, D., Liu, B. L., Bell, L. N., et al. (2015). PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat. Commun.* 6, 6692. doi:10.1038/ncomms7692

Pearce, E. L., Poffenberger, M. C., Chang, C. H., and Jones, R. G. (2013). Fueling immunity: insights into metabolism and lymphocyte function. *Science* 342, 1242454. doi:10.1126/science.1242454

Pearce, E. L., Walsh, M. C., Cejas, P. J., Harms, G. M., Shen, H., Wang, L. S., et al. (2009). Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* 460, 103–107. doi:10.1038/nature08097

Peng, X., He, Y., Huang, J., Tao, Y., and Liu, S. (2021). Metabolism of dendritic cells in tumor microenvironment: for immunotherapy. *Front. Immunol.* 12, 613492. doi:10.3389/fimmu.2021.613492

Rigiracciolo, D. C., Scarpelli, A., Lappano, R., Pisano, A., Santolla, M. F., De Marco, P., et al. (2015). Copper activates HIF-1α/GPER/VEGF signalling in cancer cells. *Oncotarget* 6, 34158–34177. doi:10.18632/oncotarget.5779

San-Millán, I., and Brooks, G. A. (2017). Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. *Carcinogenesis* 38, 119–133. doi:10.1093/carcin/bgw127

Schwartzenberg-Bar-Yoseph, F., Armoni, M., and Karnieli, E. (2004). The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. *Cancer Res.* 64, 2627–2633. doi:10.1158/0008-5472.can-03-0846

Senni, N., Savall, M., Granados, D. C., Alves-Guerra, M. C., Sartor, C., Lagoutte, I., et al. (2019). β-catenin-activated hepatocellular carcinomas are addicted to fatty acids. *Gut* 68, 322–334. doi:10.1136/gutjnl-2017-315448

Sun, J., Ding, J., Shen, Q., Wang, X., Wang, M., Huang, Y., et al. (2023). Decreased propionyl-CoA metabolism facilitates metabolic reprogramming and promotes hepatocellular carcinoma. *J. Hepatol.* 78, 627–642. doi:10.1016/j.jhep.2022.11.017

Sun, L., Zhang, H., and Gao, P. (2022). Metabolic reprogramming and epigenetic modifications on the path to cancer. *Protein Cell* 13, 877–919. doi:10.1007/s13238-021-00846-7

Tan, D. J. H., Ng, C. H., Lin, S. Y., Pan, X. H., Tay, P., Lim, W. H., et al. (2022). Clinical characteristics, surveillance, treatment allocation, and outcomes of non-alcoholic fatty liver disease-related hepatocellular carcinoma: a systematic review and meta-analysis. *Lancet Oncol.* 23, 521–530. doi:10.1016/S1470-2045(22)00078-X

Topel, H., Bagirsakçi, E., Yilmaz, Y., Günes, A., Bagci, G., Çömez, D., et al. (2021). High glucose induced c-Met activation promotes aggressive phenotype and regulates expression of glucose metabolism genes in HCC cells. *Sci. Rep.* 11, 11376. doi:10.1038/s41598-021-89765-5

Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033. doi:10.1126/science.1160809

Van Der Windt, G. J. W., Everts, B., Chang, C. H., Curtis, J. D., Freitas, T. C., Amiel, E., et al. (2012). Mitochondrial respiratory capacity is a critical regulator of CD8+T cell memory development. *Immunity* 36, 68–78. doi:10.1016/j.immuni.2011.12.007

Villanueva, A. (2019). Hepatocellular carcinoma. *N. Engl. J. Med.* 380, 1450–1462. doi:10.1056/NEJMra1713263

Wang, J. W., and Maniruzzaman, M. (2022). A global bibliometric and visualized analysis of bacteria-mediated cancer therapy. *Drug Discov. Today* 27, 103297. doi:10.1016/j.drudis.2022.05.023

Wang, S. L., Liu, G. H., Li, Y. R., and Pan, Y. B. (2022). Metabolic reprogramming induces macrophage polarization in the tumor microenvironment. *Fron. Immunol.* 13, 840029. doi:10.3389/fimmu.2022.840029

Warburg, O. (1956). On the origin of cancer cells. *Science* 123, 309–314. doi:10.1126/science.123.3191.309

Watson, M. J., Vignali, P. D. A., Mullett, S. J., Overacre-Delgoffe, A. E., Peralta, R. M., Grebinoski, S., et al. (2021). Metabolic support of tumour-infiltrating regulatory T cells by lactic acid. *Nature* 591, 645–651. doi:10.1038/s41586-020-03045-2

Wu, J. Y., Huang, T. W., Hsieh, Y. T., Wang, Y. F., Yen, C. C., Lee, G. L., et al. (2020). Cancer-derived succinate promotes macrophage polarization and cancer metastasis via succinate receptor. *Mol. Cell* 77, 213–227. doi:10.1016/j.molcel.2019.10.023

Xia, L., Oyang, L., Lin, J., Tan, S., Han, Y., Wu, N., et al. (2021). The cancer metabolic reprogramming and immune response. *Mol. Cancer* 20, 28. doi:10.1186/s12943-021-01316-8

Xia, Y. J., Brown, Z. J., Huang, H., and Tsung, A. (2021). Metabolic reprogramming of immune cells: shaping the tumor microenvironment in hepatocellular carcinoma. *Cancer Med.* 10, 6374–6383. doi:10.1002/cam4.4177

Xu, X., Peng, Q., Jiang, X., Tan, S., Yang, Y., Yang, W., et al. (2023). Metabolic reprogramming and epigenetic modifications in cancer: from the impacts and mechanisms to the treatment potential. *Exp. Mol. Med.* 55, 1357–1370. doi:10.1038/s12276-023-01020-1

Yang, Z., Yan, C., Ma, J., Peng, P., Ren, X., Cai, S., et al. (2023). Lactylome analysis suggests lactylation-dependent mechanisms of metabolic adaptation in hepatocellular carcinoma. *Nat. Metab.* 5, 61–79. doi:10.1038/s42255-022-00710-w

Zhang, C., Hu, J. J., Sheng, L., Yuan, M., Wu, Y., Chen, L., et al. (2019). Metformin delays AKT/c-Met-driven hepatocarcinogenesis by regulating signaling pathways for *de novo* lipogenesis and ATP generation. *Toxico. Appl. Pharmacol.* 365, 51–60. doi:10.1016/j.taap.2019.01.004

Zhang, C. H., Cheng, Y. F., Zhang, S., Fan, J., and Gao, Q. (2022). Changing epidemiology of hepatocellular carcinoma in Asia. *Liver Int.* 42, 2029–2041. doi:10.1111/liv.15251

Zhang, Q., Wang, H. R., Mao, C. Y., Sun, M., Dominah, G., Chen, L. Y., et al. (2018). Fatty acid oxidation contributes to IL-1β secretion in M2 macrophages and promotes macrophage-mediated tumor cell migration. *Mol. Immunol.* 94, 27–35. doi:10.1016/j.molimm.2017.12.011

Zhang, X., Coker, O. O., Chu, E. S., Fu, K., Lau, H. C. H., Wang, Y. X., et al. (2021). Dietary cholesterol drives fatty liver-associated liver cancer by modulating gut microbiota and metabolites. *Gut* 70, 761–774. doi:10.1136/gutjnl-2019-319664

Zucman-Rossi, J., Villanueva, A., Nault, J. C., and Llovet, J. M. (2015). Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* 149, 1226–1239. doi:10.1053/j.gastro.2015.05.061

Glossary

HCC	Hepatocellular carcinoma
WoSCC	Web of Science Core Collection
NAFLD	Non-alcoholic fatty liver disease
MAFLD	Metabolic dysfunction-associated fatty liver disease
OXPHOS	Oxidative phosphorylation
PPP	Pentose phosphate pathway
TCA	Tricarboxylic acid
FAO	Fatty acid β-oxidation
SCIE	Science Citation Index Expanded
IF	Impact factor
TC	Total citation
ALB	Albumin
APOB	Apolipoprotein B
CPS1	Carbamoyl phosphate synthetase 1
CAD	Carbamoyl phosphate synthetase/aspartate transcarbamoylase/ dihydroorotase
Met	Mesenchymal-epithelial transition
VEGFA	Vascular endothelial growth factor A
NFAT	Nuclear factor of activated T cell
TAM	Tumor-associated macrophage
EMT	Endothelial mesenchymal transition
TERT	Telomerase reverse transcriptase
TLR	Toll-like receptor
TME	Tumor microenvironment
ROS	Reactive oxygen species
TAN	Tumor-associated neutrophils

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 25 July 2024
DOI 10.3389/fimmu.2024.1353787

OPEN ACCESS

EDITED BY
Elisa Giovannetti,
VU Medical Center, Netherlands

REVIEWED BY
Hongfei Jiang,
Qingdao University, China
Sweta Ghosh,
University of Louisville, United States

*CORRESPONDENCE
Andrea Nicolini
andrea.nicolini@med.unipi.it

†Retired

RECEIVED 11 December 2023
ACCEPTED 04 March 2024
PUBLISHED 25 July 2024

CITATION
Nicolini A and Ferrari P (2024) Involvement of tumor immune microenvironment metabolic reprogramming in colorectal cancer progression, immune escape, and response to immunotherapy. *Front. Immunol.* 15:1353787. doi: 10.3389/fimmu.2024.1353787

Involvement of tumor immune microenvironment metabolic reprogramming in colorectal cancer progression, immune escape, and response to immunotherapy

Andrea Nicolini[1*†] and Paola Ferrari[2]

[1]Department of Oncology, Transplantations and New Technologies in Medicine, University of Pisa, Pisa, Italy, [2]Unit of Oncology, Department of Medical and Oncological Area, Azienda Ospedaliera-Universitaria Pisana, Pisa, Italy

Metabolic reprogramming is a k`ey hallmark of tumors, developed in response to hypoxia and nutrient deficiency during tumor progression. In both cancer and immune cells, there is a metabolic shift from oxidative phosphorylation (OXPHOS) to aerobic glycolysis, also known as the Warburg effect, which then leads to lactate acidification, increased lipid synthesis, and glutaminolysis. This reprogramming facilitates tumor immune evasion and, within the tumor microenvironment (TME), cancer and immune cells collaborate to create a suppressive tumor immune microenvironment (TIME). The growing interest in the metabolic reprogramming of the TME, particularly its significance in colorectal cancer (CRC)—one of the most prevalent cancers—has prompted us to explore this topic. CRC exhibits abnormal glycolysis, glutaminolysis, and increased lipid synthesis. Acidosis in CRC cells hampers the activity of anti-tumor immune cells and inhibits the phagocytosis of tumor-associated macrophages (TAMs), while nutrient deficiency promotes the development of regulatory T cells (Tregs) and M2-like macrophages. In CRC cells, activation of G-protein coupled receptor 81 (GPR81) signaling leads to overexpression of programmed death-ligand 1 (PD-L1) and reduces the antigen presentation capability of dendritic cells. Moreover, the genetic and epigenetic cell phenotype, along with the microbiota, significantly influence CRC metabolic reprogramming. Activating RAS mutations and overexpression of epidermal growth factor receptor (EGFR) occur in approximately 50% and 80% of patients, respectively, stimulating glycolysis and increasing levels of hypoxia-inducible factor 1 alpha (HIF-1α) and MYC proteins. Certain bacteria produce short-chain fatty acids (SCFAs), which activate CD8+ cells and genes involved in antigen processing and presentation, while other mechanisms support pro-tumor activities. The use of immune checkpoint inhibitors (ICIs) in selected CRC patients has shown promise, and the combination of these with drugs that inhibit aerobic glycolysis is currently being intensively researched to enhance the efficacy of immunotherapy.

KEYWORDS

metabolic reprogramming, colorectal cancer, tumor immune microenvironment, tumor immune escape, immunotherapy

Introduction

Preventing cancer's origin and progression, also known as tumor immune surveillance, is among the responsibilities of the human immune system (1, 2). However, many factors and mechanisms, primarily within the tumor microenvironment (TME), influence tumor immune surveillance (3–5), while others facilitate tumor immune escape. Tumor cells rapidly adapt to hypoxia and nutrient deficiencies that occur during tumor progression. This adaptation involves changes in the bioenergetic systems of cancer cells, termed "metabolic reprogramming," which is one of the primary hallmarks of cancer. Metabolic reprogramming impacts cell activity and its ability to differentiate, with numerous factors interacting within the TME to account for various metabolic phenotypes. Alterations in metabolic cells within the TME can also promote tumor immune escape, where cancer and immune cells collaborate to establish a suppressive tumor immune microenvironment (TIME). The recent surge in research interest regarding metabolic TME reprogramming as a mechanism of tumor progression and a potential therapeutic target, especially in colorectal cancer (CRC)—one of the most common cancers—has prompted us to address this issue. The first part of this review highlights the common features of metabolic reprogramming in cancer and immune cells, as well as their roles in facilitating tumor immune escape. Subsequently, we provide a comprehensive overview of the current knowledge on metabolic reprogramming, immune escape, and the use of immunotherapy in CRC.

Metabolic reprogramming in the TME

In the TME, tumors typically exhibit high blood content and oxygen consumption, while exporting lactate to the extracellular space through aerobic glycolysis. This process leads to acidification (6) and hypoxia within the TME (7). Furthermore, the interaction between cancer and immune cells contributes to their metabolic reprogramming and the shaping of the microenvironment.

Metabolic interactions between cancer and immune cells

Within the TME, cancer cells and immune cells compete for the same limited metabolic resources. The proliferation of cancer cells is associated with increased metabolic demands, which also affect the metabolic requirements of immune cells, influencing their function and fate. However, both cancer and immune cells possess a significant capacity for adaptation, allowing them to interact and reshape their metabolism to overcome adverse conditions and utilize the metabolic nutrients made differently available. This reprogramming of cancer cell metabolism creates a hostile environment for immune cells, leading to functional defects, primarily impaired effector cell abilities, which provide an advantage to tumor progression (6). Here, we briefly discuss the main metabolic pathways reprogrammed in the TME that facilitate the growth and survival of cancer cells by increasing energy production, antioxidant defense, and the synthesis of macromolecules (8).

Hypoxia, acidification, and the Warburg effect

The abnormal neovasculature that develops in a growing tumor does not deeply penetrate the tumor tissue (9), resulting in an increased need for oxygen and energy precursors for the synthesis of nucleic acids, lipids, and proteins from the surroundings to the core of the solid tumor (8). Hypoxia-inducible factor 1-alpha (HIF-1α), a transcription factor commonly upregulated in tumors (10), can also be activated by PI3K/Akt/mTOR or MAPK signaling pathways (11) and by oncometabolites such as fumarate and succinate (12). HIF-1α regulates the transcription of various genes, including vascular endothelial growth factor (VEGF), hexokinase 2 (HK2), lactate dehydrogenase (LDH) enzymes, glucose transporters, and carbonic anhydrase IX (CA-IX) for pH regulation (13), among others critical for immune cell function (14). Hypoxia in the TME leads to a metabolic shift in cancer cells from oxidative phosphorylation (OXPHOS) to aerobic glycolysis, known as the Warburg effect (15). This shift increases glucose transport into tumor cells, reducing its availability in the extracellular space while simultaneously increasing lactate and LDH activity (16). Lactate produced by cancer cells is expelled, along with protons, through monocarboxylate transporters (MCTs), inducing TME acidification, further enhanced by CA-IX. CA-IX, with its active site facing the extracellular space, catalyzes CO_2 hydration, contributing to proton production outside cancer cells. Additionally, the proteoglycan-like domain of CA-IX facilitates the non-catalytic export of protons along with lactate from cancer cells (17). TME acidification provides a growth advantage to cancer cells over immune cells by inhibiting T-cell proliferation, affecting the chemotaxis and migration of neutrophils and dendritic cells (DCs), increasing regulatory T cells (Tregs), and facilitating the infiltration of myeloid-derived suppressor cells (MDSCs) and M2 macrophages. Overall, this supports the immunosuppressive effects on T cells (18) and the TME.

Role of lipids in tumor and immune cells

Tumor growth is accompanied by a strong demand for lipids and cholesterol, met by increased uptake of exogenous lipids (19), and lipoproteins through promoting lipolysis in adipocytes (8) or by enhancing endogenous synthesis. This lipid metabolism is driven by the overexpression of enzymes in cancer cells for lipid uptake, such as CD36, and transcription factors for lipid oxidation enzymes or lipid synthesis by the tumor cells themselves. Lipids serve not only as an energy source but also play roles in signal transduction and are integral components of cellular structures (20). Specifically, the biosynthesis of cholesterol and phospholipids in tumor cells ensures their survival by maintaining the structural and functional

integrity of the cell membrane and facilitating adaptation to the TME, while specific lipids mediate interactions with cells within the TME (21). The role of free fatty acids (fFAs) in immune cell function within the TME is actively investigated, with findings indicating that glycolysis and OXPHOS by long-chain fatty acids can modulate CD8+ Tmem cells to differentiate and promote M2 phenotype polarization (22, 23). CD8+ cells with overexpression of carnitine palmitoyltransferase 1a (Cpt1a), an enzyme catalyzing mitochondrial long-chain fatty acid oxidation (FAO), show a metabolic advantage *in vivo* (23, 24). Recent studies also highlight the necessity of fatty acid (FA) import and Cpt1a-dependent lipid oxidation for maintaining tissue-resident CD9+ cells in peripheral tissues (25), while CD36 is necessary for the increase of CD4+ Tregs in the TME (26).

Role of amino acids in tumor and immune cells

In the TME, tumor cells exhibit higher glutamine uptake than infiltrated immune cells (27) leading to a glutamine-deficient TIME. To meet the increased demand for ATP and lipids, tumor proliferating cells enhance the uptake and synthesis of glutamate through glutaminase (GLS), which metabolizes glutamine into glutamate. Tumor cells utilize glutamine to synthesize essential amino acids and produce alpha-ketoglutarate, subsequently generating energy via the tricarboxylic acid (TCA) cycle (28). Receptors for SLC1A5 (ASCT2) and SLC38A5 glutamine transporters are hyper-expressed in some cancers, and their pharmacological inhibition blocks tumor growth (29, 30). The acidic TME activates p53 and increases glucose-6-phosphate dehydrogenase (G6PD) and glutaminase 2 (GLS2) in tumor cells (31). Increased lactate stabilizes HIF-2α, which activates the c-Myc oncogene and leads to the overexpression of the ASCT2 glutamine transporter and glutaminase 1 (GLS1), providing a steady flux of glutamine to the cells (32). Amino acid metabolism homeostasis is also crucial for immune cells, where T-cell activation involves the upregulation of genes encoding amino acid transporters (33). Genetic or pharmacological blockade of GLS inhibits the proliferation and activation of CD4+ and CD8+ T cells, as well as the differentiation of CD4+ Th17 cells (34, 35). GLS overexpression, along with glutamine depletion and elevated glutamate levels, can impair immune cell function (36). The methionine transporter SLC3A2 is hyper-expressed in tumor cells, which compete with CD8+ T cells for methionine import and use (37). Methionine deprivation reduces the methyl donor S-adenosylmethionine (SAM) and then H3K79me2, downregulating STAT5 and impairing the anti-tumor cytotoxicity of CD8+ T cells. Extracellular availability of methionine through one-carbon (1C) metabolism can affect tumor cell growth and T lymphocyte proliferation (38, 39). In T cells, methionine is a crucial amino acid for methylation reactions as the sole origin of the methyl group in SAM (39). Early during T-cell activity, protein synthesis can be affected by alanine levels (40). Tryptophan deprivation counteracts T-cell function through the integrated stress response (41). Upregulation of indoleamine 2,3-dioxygenase (IDO) due to mutations in BIN and KIT oncogenes leads to tryptophan deficiency, inhibiting CD8+ T and NK cells while promoting Tregs and MDSCs, favoring an immunosuppressed TME (42). Cystine uptake and its subsequent intracellular breakdown to cysteine, followed by glutathione synthesis, are critical for activating T cells and for detoxifying reactive oxygen species (ROS) (43). Consequently, a deficiency in cystine hampers T-cell activation and compromises the ROS detoxification process (36). Additionally, the biosynthesis and transport of polyamines are increased in tumor cells, associated with elevated activity of ornithine decarboxylase (ODC), an enzyme essential for carcinogenesis (44, 45). Within the TIME, cancer and immunosuppressive myeloid cells compete with T cells for the uptake and utilization of polyamines. This competition for polyamines can obstruct the proper differentiation of CD4+ T cells and instead facilitate their transformation into immunosuppressive Tregs (14). The primary mechanisms of metabolic reprogramming in tumor cells and the subsequent immune suppression in the TIME are illustrated in Figure 1.

Many mechanisms related to metabolic reprogramming in immune cells facilitate tumor immune escape

Metabolic mechanisms of tumor immune escape in T cells

In the TME, pro-inflammatory T effector (Teff) cells, including Th1, Th2, and Th17 phenotypes, primarily rely on increased glycolysis rather than OXPHOS (46, 47). This aerobic glycolysis regulates Teff functions, including IFN-gamma release. In T cells, lactate dehydrogenase A (LDHA) promotes aerobic glycolysis, IFN-gamma production, or Th1 differentiation through epigenetic mechanisms (48). The accumulation of lactate in the TME suppresses essential activities such as the reduction of oxidized nicotinamide adenine dinucleotide (NAD+) to NADH and serine production, crucial for T-cell proliferation (49), and is associated with reduced LDHA expression. Decreased LDHA in T cells inhibits their IFN-gamma-mediated activities and facilitates their conversion to FoxP3+ Tregs. FoxP3 expression in Tregs enhances NAD+ oxidation and shifts their metabolism towards OXPHOS, inhibiting Myc and glycolysis (50), enabling Tregs to thrive in the highly acidic TME (51). Tregs consume more lactate than Teffs, using it to fuel the TCA cycle or gluconeogenesis, thus reducing their glucose requirements in the TME with limited metabolic resources (50, 52). In the TIME, Tregs overexpress MCT-1 for lactate import (52). Moreover, owing to low glucose transporter 1 (Glut1) and high 5-adenosine monophosphate-activated protein kinase (AMPK) expression, Tregs' immune-regulatory activities depend heavily on FAs or beta-lipid oxidation and OXPHOS, while TME-derived lactate promotes programmed cell death protein 1 (PD-1 or CD279) expression (46, 47). In Tregs, fatty acid binding protein 5 (FABP5) governs OXPHOS and induces immune suppression through IL-10 secretion following type 1 IFN release in a lipid-deficient TME for immune cells (53). FABP5 expression in plasmacytoid dendritic cells (pDCs) induces their

tolerogenic function and promotes Treg production (54). Moreover, CD36 upregulation in Tregs, by increasing FA uptake and OXPHOS, favors their survival and proliferation in a highly acidic TIME (26, 55). Concurrently, CD36 expression in CD8+ cytotoxic T cells enhances the uptake of oxidized lipids/low-density lipoproteins (oxLDLs), leading to lipid peroxidation (LPO) (56). and ferroptosis, which are responsible for their death and contribute to immune suppression (57). LPO, p38 mitogen-activated protein kinase (p38MAPK) activity, and impaired mitochondrial biogenesis also promote CD8+ T-cell dysfunction (56, 58). Death/damage-associated molecular patterns (DAMPs) activate Tregs' Toll-like receptors (TLRs) and FoxP3, which counteract mTORC1 signaling and glucose metabolism, regulating Treg proliferation and immune suppression (59, 60). HIF-1α plays a crucial role in promoting and maintaining glycolysis; its absence in T cells during differentiation redirects them towards a Treg phenotype (47). Tumor-associated Th17 cells with decreased glycolysis are reprogrammed to FoxP3+ Tregs (61). The extracellular accumulation of lactate in the TIME impairs the activation of nuclear factor of activated T-cells (NFAT), IFN gamma production by T and NK cells (62, 63), and the antitumor action of CD4+ and CD8+ cells, also promoting the conversion of CD4+ T cells to Th17 cells (62). Hypoxia, induced by HIF-1α, triggers PDL-1 (CD274) expression in tumor and immune cells, thus promoting immune suppression in the TIME (64). Mitochondrial loss compromises the function of PD-1+ CD8+ T cells in the TIME (65), with a significant correlation observed between the extent of mitochondrial loss and PD-1 expression.

Moreover, the specific nutrient deficiencies in the TIME induce metabolic stress, leading to the failure of Teff cells. Under conditions of hypoxia and mTORC1 signaling inhibition, due to limited glucose availability, there is hypo-expression of antigen-inducing genes, decreased proliferation, and diminished function of CD8+ cell (66, 67), as well as apoptosis of Teff cells triggered by the activation of pro-apoptotic genes/proteins. Additionally, IL-2 signaling-mediated STAT5 activation is impaired in the TME with increased acidity (68), further inhibiting the antitumor function of CD8+ T cells while facilitating the reprogramming of tumor-associated Th17 cells to FoxP3+ Tregs. A glutamine-deficient TIME inhibits the proliferation of infiltrating T cells (69). The lack of glutamine in the TME also reduces cytosolic alpha-ketoglutarate (alpha-KG) in Th1 cells, thereby inducing their differentiation into Tregs (70). PD-1+ CD8+ T cells, owing to enhanced lipolysis of endogenous lipids and FAO, survive longer and promote immune suppression in the TIME. These immune-suppressive CD8+ T cells overexpress markers of lipolysis, including CPT1A, adipose triglyceride lipase (ATGL), and glycerol (71). The primary mechanisms of tumor immune escape through metabolic reprogramming in T cells are illustrated in Figure 2.

Metabolic mechanisms of tumor immune escape involve macrophages, neutrophils, and myeloid-derived suppressor cells significantly

Pro-inflammatory macrophages (M1) often dominate the immune cell population in the TIME, and a scarcity of glucose, glutamine, and FAs promotes the polarization of M1 into immunosuppressive M2 macrophages or tumor-associated macrophages (TAMs). Elevated levels of TGF-beta, IL-4, IL-5, IL-6, and IL-10 in the TME further induce M2 polarization. The IL-4-driven polarization from M1 to M2 is mediated by the mTORC2-interferon regulatory factor 4 (IRF4) signaling axis, which favors OXPHOS (72) and occurs in the absence of nitric oxide (NO)

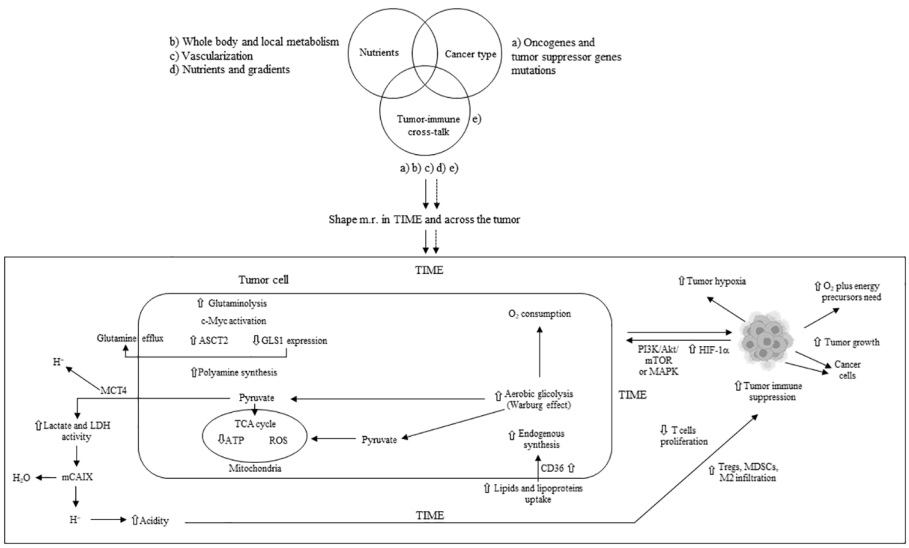


FIGURE 1
Main mechanisms of metabolic tumor cell reprogramming and tumor immune suppression in TIME. m.r.; metabolism reprogramming; TIME: tumor immune microenvironment; ASTC2 = SLC1A5: solute carrier family 1 member A5; GLS1: glutaminase 1; MCT4: monocarboxylate transporter 4; TCA: tricarboxylic acid; LDH: lactate dehydrogenase; mCAIX: membrane carbonic anhydrase; Tregs: T regulatory cells; MDSCs: myeloid-derived suppressor cells; HIF-1α: hypoxia inducible factor 1 alpha; ATP: adenosine triphosphate; ROS: reactive oxygen species; PI3K: phosphoinositide-3 kinase; Akt: protein kinase B; mTOR: mammalian target of rapamycin; MAPK: mitogen-activated protein kinase; M2: macrophage type 2; ⇧ increase; ⇩ decrease.

production due to dysregulated mitochondrial function (73, 74). Interestingly, this IL-4-promoted polarization does not require metabolic reprogramming towards FAO (75). The presence of high lactate, succinate, and other pro-tumor metabolites in the TME further supports the M1-to-M2 polarization through mechanisms such as the yes-1 associated protein (YAP) and NF-kB inhibition via the G protein-coupled receptor 81 (GPR-81)-mediated pathway (76, 77). Overexpression of MCT1 allows macrophages to import lactate, enhancing OXPHOS and FAO, thus favoring the M2 phenotype (78, 79). M2 macrophages secrete immunosuppressive cytokines and chemokines like TGF-beta and IL-10, promoting tumor progression. Tumor cells' secretion of macrophage colony-stimulating factor (M-CSF) induces fatty acid synthase (FASN) in TAMs (80), which, in turn, promotes the production and secretion of immunosuppressive IL-10. Enhanced lipid availability from tumor cells triggers an endoplasmic reticulum (ER) stress response, leading to increased inositol-requiring enzyme 1 (IRE1) availability and M1 to M2 macrophage polarization and survival in the TIME (81). Elevated levels of IRE1 and STAT3 activation induce the M2 phenotype and immune suppression in the TIME (81–83). M2 macrophage polarization is associated with significant stimulation of OXPHOS in TAMs, mitochondrial damage, and high ROS production (84), further inducing hypoxia and inhibiting the antitumor function of Teff cells. Arginase 1 (Arg1) expression in TAMs depletes L-arginine for T cells and attracts Tregs (85). Concurrently, inducible nitric oxide synthase (iNOS) production in the presence of low arginine can increase ROS and reactive nitrogen species (RNS) levels, thereby facilitating tumor immune escape in the TIME (86, 87). The M2 phenotype is also associated with increased glutaminolysis, supporting the TCA cycle (88). IDO upregulation in M2 macrophages leads to local tryptophan depletion and the production of immunosuppressive kynurenine metabolites (89, 90). Additionally, tumor exosomes in the TME can facilitate the M1-to-M2 conversion by activating the NLRP6/NF-kB signaling pathway (91). While N1 neutrophils maintain antitumor functions, in the TME, pro-tumor N2 neutrophils and the conversion of N1 tumor-associated neutrophils (TANs) to N2 TANs are induced by transforming growth factor-beta (TGF-beta) and granulocyte colony-stimulating factor (G-CSF) (92, 93). In the hypoxic TME, HIF-1α promotes neutrophil survival by enhancing glycolysis (94), while HIF-2α supports the survival of tumor-associated neutrophils (TANs) (95). The pentose phosphate pathway (PPP) in neutrophils increases ROS availability, promoting apoptosis of infiltrating T cells and further immune suppression in the TME (96, 97). The PPP also contributes to neutrophil extracellular trap (NET) formation, or NETosis, by supplying NADPH oxidase with NADPH to generate superoxide, which facilitates cancer spread (98). MDSCs, including monocytic-MDSCs (M-MDSCs) and polymorphonuclear-MDSCs (PMN-MDSCs) (99), inherently promote immune suppression. In cancer patients, PMN-MDSCs appear at very early stages and exhibit enhanced spontaneous migration (100, 101). Various chemokines, including CXCL8, CXCR1/2, and CXCR4, attract MDSCs to the TME (102–104), where they secrete cytokines like IL-10 and TGF-beta, contributing to their immunosuppressive function. Hypoxic conditions in the TME drive MDSCs' immune-metabolic reprogramming towards FAO and AMPK activation, enhancing their immunosuppressive activity (105, 106). L-glutamine (L-GLn) provided by the TME is metabolized via AMPK, supporting MDSCs' immunosuppressive activity by fueling the TCA cycle (107). Tumor-associated MDSCs (T-MDSCs) produce their own L-glutamine and, along with

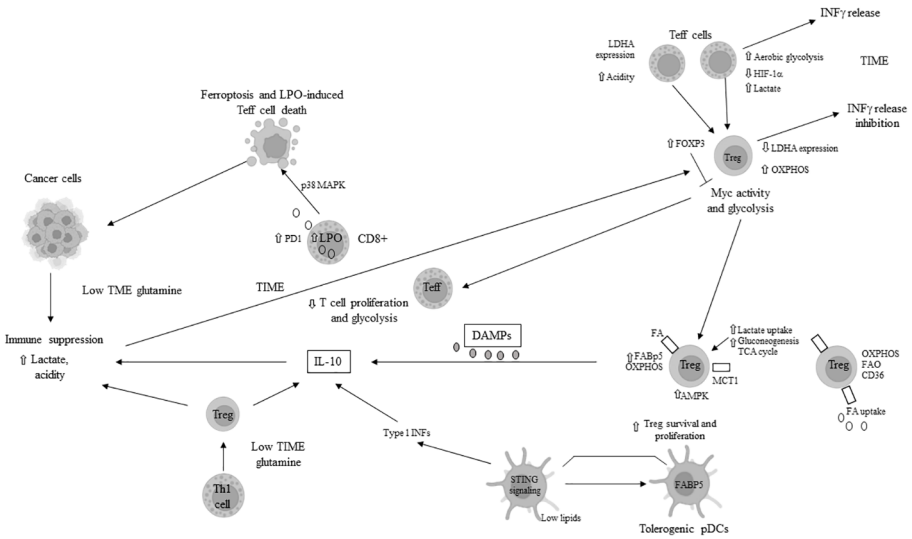


FIGURE 2
Main mechanisms of tumor immune escape in tumor immune microenvironment (TIME) through metabolic reprogramming in T cells. LDH: lactate dehydrogenase; INFγ: interferon gamma; HIF-1α: hypoxia inducible factor 1 alpha; OXPHOS: oxidative phosphorylation; Treg: T regulatory cell; FABP5: fatty acid binding protein 5; AMPK: AMP-activated protein kinase; TCA: tricarboxylic acid; FA: fatty acid; FAO: fatty acid oxydation; MCT1: monocarboxydate transporter1; PD1: programmed death protein 1; pDCs: plasmacytoid dendritic cells; STING: stimulator of interferon genes; Th: T helper; DAMPs: death/damage-associated molecular proteins; LPO: lipid peroxydase; IL: interleukin; p38MAPK: p38 mitogen-activated protein kinase; ⇧ increase; ⇩ decrease; ⟞ inhibited.

upregulated transglutaminase (TGM), enhance their pro-tumor immunosuppressive activity (108, 109). T-MDSCs exhibit increased FAO, OXPHOS, and glycolysis in the TME due to elevated lipid/FA availability through CD36-mediated FA uptake (106). The fatty acid transport protein 2 (FATP2) on PMN-MDSCs, through arachidonic acid (AA) import and prostaglandin E2 (PGE2) production, further enhances their immunosuppressive activity (110, 111). GM-CSF induces FATP2 overexpression in PMN-MDSCs following STAT5 activation. Hypoxia in the TME, alongside high levels of HIF-1α (112, 113), supports the immunosuppressive phenotype of T-MDSCs and induces PD-L1 hyperexpression, inhibiting the cytotoxic and immunological activities of CD8+ and CD4+ T cells (64). Increased lactate in the TME, through the GPR81/mTOR/HIF-1a/STAT3 signaling pathway, supports the survival and proliferation of immunosuppressive MDSCs (114–116), which, in turn, inhibit natural killer cell cytotoxicity (NKCC) (115). The primary mechanisms of immune escape in the TIME through metabolic reprogramming involve macrophages, neutrophils, and MDSCs, as depicted in Figure 3.

Mechanisms of tumor immune escape in dendritic cells and NK cells

In the competitive TIME, AMPK overexpression in tumor-associated dendritic cells (TADCs) enhances FAO and OXPHOS, transforming them into tolerogenic DCs (117, 118). Their essential antigen-presenting function is compromised by an abnormal increase in lipids, imported from the extracellular space through macrophage scavenger receptor 1 (MSR1) (119, 120). The binding of exogenous adenosine monophosphate (AMP) to the adenosine A2b receptor on TADCs elevates their pro-tumor functions, creating an immunosuppressive environment through the secretion of VEGF, TGF-beta, IL-10, and the expression of cyclooxygenase-2 (COX-2) and IDO (121, 122). IDO mediates the metabolic production of kynurenine (123) from tryptophan, while the decrease in tryptophan inhibits T-cell proliferation and encourages the transition of naïve CD4+ T cells to FoxP3+ Tregs (41–126). Kynurenine and other metabolites activate the aryl hydrocarbon receptor (AhR) on T cells, inducing Treg differentiation and promoting an immune-suppressive phenotype in macrophages and DCs (126–129). Wnt5 protein, secreted by tumor cells, activates the peroxisome proliferator-activated receptor (PPAR)-gamma through beta-catenin, inhibiting the immune-metabolic shift to glycolysis and activating FAO by upregulating CPT1A in TADCs. Beta-catenin also enhances vitamin A metabolism in TADCs, with retinoic acid (RA) production further inducing Tregs and supporting an immune-suppressive TIME (130). Innate lymphoid cells (ILCs) include group 1 ILCs, which encompass NK cells. In NK cells, an increased energy demand prompts immune-metabolic reprogramming towards aerobic glycolysis to support their function, though mTORC1 activity also leads to an increase in OXPHOS (131, 132). However, the high demand for glucose is not met in the TIME, where elevated TGF-beta impairs mitochondrial metabolism and OXPHOS, inhibiting NK cell function (133). TGF-

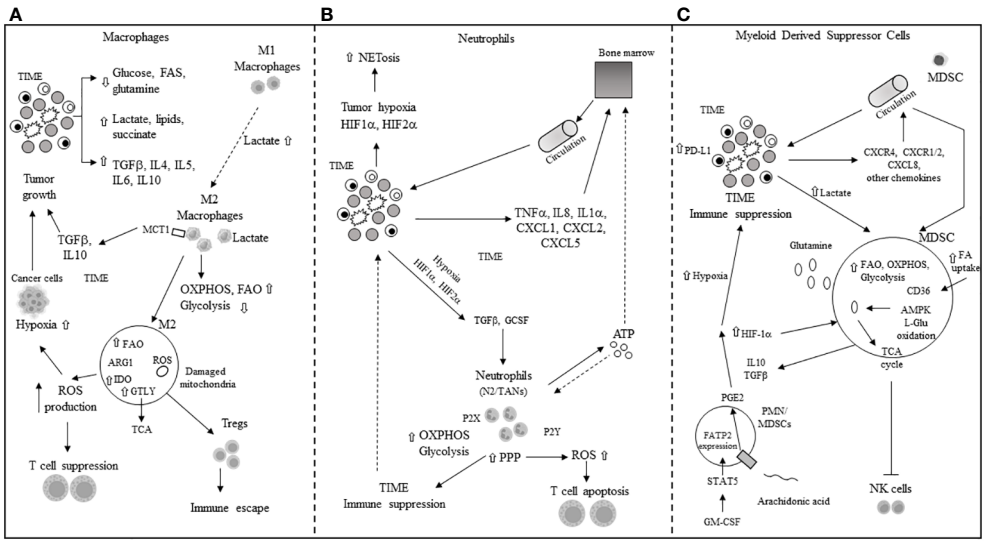


FIGURE 3
Main mechanisms of immune escape in tumor immune microenvironment (TIME) through metabolic reprogramming in macrophages, neutrophils and myeloid derived suppressor cells. **(A)** M1, M2: macrophage type 1 and type 2 phenotype; TGFβ: tumor growth factor beta; IL: interleukin; FAs: fatty acids; MCT1: monocarboxylic transporter 1; OXPHOS: oxidative phosphorylation; FAO: fatty acid oxidation; ROS: reactuve oxygen species; Tregs: T regulatory cells; IDO: indoleamine 2,3 dioxygenase; ARG: arginase; GTLY: glutaminolysis. **(B)** NETosis: neutrophils extracellular transactivation and release; TNFα: tumor necrosis factor alpha; ATP: adenosin triphosphate; CXCL: chemokine ligand; PPP: pentose phosphate pathway; P2X, P2Y: purinergic receptors; N2: neutrophil type 2; TANs: tumor-associated neutrophils; OXPHOS and ROS: see panel A. **(C)** MDSC: myeloid derived suppressor cell; HIF-1α: hypoxia inducible factor 1 alpha; CXCR: chemokine receptor; AMPK: 5' adenosine monophosphate activated protein kinase; L-Glu: L-glutamine; CD36: cluster of differentiation 36; TCA: tricarboxylic acid; PGE2: prostaglandin E2; NK: natural killer; GM-CSF: granulocyte-macrophage colony stimulating factor; STAT-5: signal transducer and activator of transcription 5; FATP2: fatty acid transport protein 2; CXCL: see panel B; FAO: see panel A; ⇧ increase; ⇩ decrease.

beta, through mTOR inhibition, hampers NK cell proliferation and maturation, which are promoted by IL-15 (134), while blocking TGF-beta restores NK cell anti-tumor function (133, 134). High lactate levels in the TME promote mitochondrial dysfunction and increased ROS, inhibiting NK cells' OXPHOS and leading to their apoptosis due to energy deficiency (135). In the presence of TGF-beta, secreted by tumor and suppressive immune cells, NK cells in the TIME can avoid apoptosis and transition to less cytotoxic ILC1s, which survive on lower energy requirements. GM-CSF in the TIME also converts immature NK cells to MDSCs, promoting cancer metastasis and spread (136). TGF-beta in the TME is responsible for the hypo-expression of eomesodermin (EOMES) (137), crucial for NK cell development and cytotoxicity (138, 139), and reprograms anti-tumor ILC3s to pro-tumor regulatory ILC3s (ILCregs) that release IL-10 (140). IL-25, part of the IL-17 cytokine subfamily, converts inflammatory ILC2s (iILC2s) to natural ILC2s (nILC2s), which overexpress IL-25R+ and are responsible for increased secretion of IL-5 and IL-13, contributing to an immunosuppressive TIME (141). In CRC, inhibition of IL-25R decreases tumor growth and triggers an immune response against the tumor in mice (142). IL-33 exerts pro-tumor activity, including PPAR-gamma-mediated delivery of IL-4, IL-13, and IL-15 from ILC2s (143). Elevated levels of IL-33 in the TIME, through binding to its receptor ST2, lead to the transient accumulation of imported FAs in lipid droplets, inducing ILC2's pro-tumor activity (144). The primary mechanisms of immune escape in the TIME through metabolic reprogramming in dendritic and natural killer (NK) cells are depicted in Figure 4.

Metabolic reprogramming in CRC

In CRC, the TME facilitates metabolic intercommunication among various cells, leading to abnormal glycolysis, glutaminolysis, and lipid synthesis (145), largely in line with previous descriptions and partly regulated by genetic and epigenetic phenotypes. Additionally, the intestinal microbiota plays a crucial role in this intercellular metabolic cross-talk. This summary encapsulates the primary findings related to the reconfiguration of metabolic pathways in CRC.

Glycolysis and glutaminolysis

Aerobic glycolysis, also known as the Warburg effect, has been observed in CRC (146). The enzyme pyruvate kinase (PK) is crucial for glycolysis (147), with the altered form PKM2 (PK muscle isozyme 2) (148, 149), increased lactate production, and the upregulation of glucose transporter 1 (GLUT1), Hk2, and other glycolytic enzymes being reported in CRC (150–152). Additionally, mutations in several enzymes related to the TCA cycle are linked to poor outcomes in CRC. Glutamine, transported into the cell cytosol and facilitated by SLC1A5 (also known as ASCT2, an alanine, serine, cysteine transporter 2) (153, 154), is overexpressed in CRC (155), promoting cell survival and proliferation (156). Serine, a non-essential amino acid involved in 1C metabolism, is crucial for the proliferation of CRC cells (157–159). The enzyme IDO, which converts tryptophan to kynurenine, is upregulated in CRC and correlates with tumor growth and patient outcomes (160).

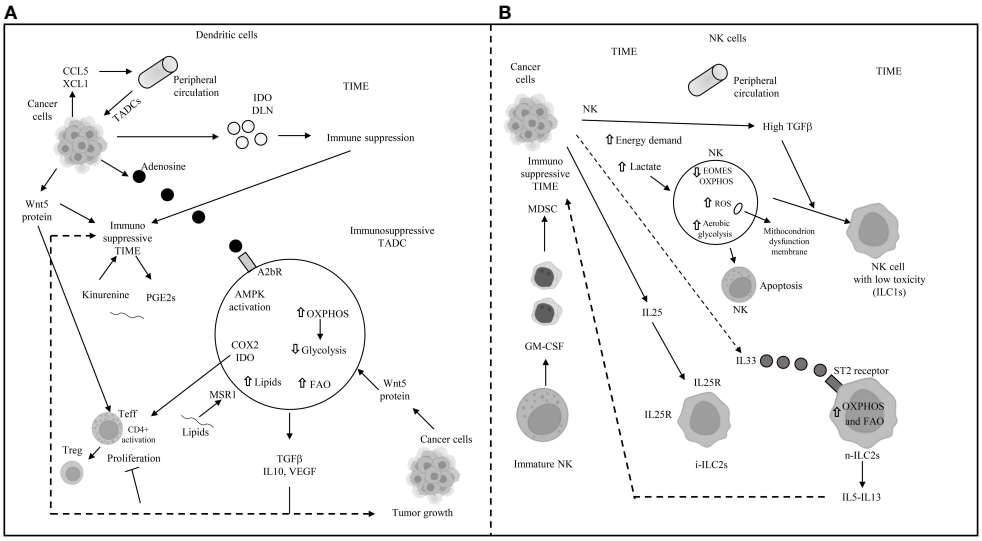


FIGURE 4
Main mechanisms of immune escape in tumor immune microenvironment (TIME) through metabolic reprogramming in dendritic and natural killer cells. **(A)** DLN: draining lymph-nodes; TADC: tumor-associated dendritic cell; TIME: tumor immune microenvironment; AMPK: 5' adenosine monophosphate-activated protein kinase; OXPHOS: oxidative phosphorylation; FAO: fatty acid oxidation; COX2: cyclooxygenase2; IDO: indoleamine pyrrole-2,3-dioxygenase; TGFβ: transforming growth factor beta; IL: interleukin; VEGF: vascular endothelial growth factor; MSR1: macrophages scavenger receptor 1; GCN2: general control non depressing 2; A2bR: adenosine A2B receptor; PGE2: prostaglandin E2; CCL5: chemokine (C-C motif) ligand 5; XCL1: chemokine (C motif) ligand 1. **(B)** NK: natural killer; EOMES: eomesodermin; ROS: reactive oxygen species; ILC1s: innate lymphoid cell group 1; i-ILC2s: inflammatory innate lymphoid cells group 2; n-ILC2s: natural innate lymphoid cells group 2; GM-CSF: granulocyte-macrophage colony stimulating factor; MDSC: myeloid derived suppressor cell; IL: interleukin; IL25R: interleukin 25 receptor; OXPHOS and FAO: see panel A; ⇧ increase; ⇩ decrease; —| inhibited.

1C metabolism, involving the folate and methionine cycles, generates 1C groups necessary for synthesizing vital precursors, such as purines and pyrimidines, and for methylation processes (161). Folate, a form of vitamin B9 obtained from the diet, transfers 1C groups from inside to outside the cells (157). There is an upregulation of folate-dependent 1C metabolic enzymes, including folate receptor-1 (FOLR1), dihydrofolate reductase (DHFR), and serine hydroxymethyltransferase 1 (SHMT1) in CRC cells, unlike in non-transformed cells (162, 163). Increased SHMT2 expression, which becomes stabilized through NAD-dependent deacetylase sirtuin-3 (SIRT3)-mediated deacetylation (164), is associated with a worse prognosis in CRC patients (164). Overexpression of methylenetetrahydrofolate dehydrogenase 1-like (MTHFD1L) has been found in primary CRC patients, and its suppression inhibits colon cancer growth and spread (165). The upregulation of SHMT2, MTHFD2 enzymes, and mitochondrial 10-formyltetrahydrofolate dehydrogenase (ALDH1L2) distinguishes CRC

TABLE 1 Main components/agents targeting glucose, glutamine and lipid metabolism for CRC treatment.

Metabolic target	Metabolic agent/ compound	IT combined	Immune cells	Cancer cells	Mechanism	Ref
Glucose metabolism	PEP, GAPDH	No	Metabolic checkpoint regulation	Indirect action	ACT through T-cell reprogramming	(8)
	Oxamate	No	No	Tumor growth inhibition	LDHA inhibitor; synergistic effect when combined with mTOR inhibitor rapamycin	(167)
	2-DG	No	Tregs depletion, Th2 and Th17 shift to Th1, polarization to M1	Increased local tumor control	Tumor cells sensitized to radiation and CT drugs following ATP and NADPH decrease due to HK inhibition	(8, 168)
	2-DG and 6-AN	No	As above	As above	Increased radiosensitization	(8)
	2-DG, Metformin, Caulerpin	No	FOXO1 decrease; memory T cells and CD8+ increase	Indirect action	AMPK activity increase and negative mTOR regulation (2-DG and Caulerpin); c-myc downregulation (Metformin)	(8, 169–172)
	Quercetin, 3-BP, Lonidamide	No	[a]No	Tumor growth inhibition	MCT1 inhibition	(173–175)
	Indisulam (E7070)	No	No	Tumor growth inhibition	Multiple CAs inhibitor; synergize with capecitabine and irinotecan	(176, 177)
	Bortezomib, Irinotecan, EZN-2208	No	No	Tumor growth inhibition	HIF1 inhibitors	(177)
	Aspirin, Apatinib, Trametinib	With ICB	Teff cells activity increase	Tumor growth inhibition	The combination of these glycolytic inhibitors with ICIs can be helpful in reversing the resistance to single agent ICI	(178–180)
Glutamine metabolism	6-diazo-5-oxo L-norleucine (DOS)	No	No	Tumor growth inhibition	Inhibitor of glutaminolysis and induction of cellular ROS	(181)
	CB839	No	No	Tumor growth inhibition	Inhibitor of glutaminolysis; combination with cetuximab	(181)
Lipid metabolism	Cerulenin	No	No	Tumor growth inhibition	FASN inhibitor; suppression of CRC cell proliferation, apoptosis induction and inhibition of metastasis	(182, 183)
	Luteolin (3,4,5,7-tetrahydroxyflavone)	No	No	Tumor growth inhibition	FASN inhibitor; modulation of IGF-1 and Wnt-beta-catenin oncogenic pathways	(184)
	EGCG (epigallocatechin-3-gallate)	No	No	Tumor growth inhibition	FASN inhibitor; CRC cell proliferation and diffusion inhibition through STAT3 downregulation	(181)
	TOFA	No	No		ACC inhibitor; induction of CRC cell apoptosis	(185)

(Continued)

TABLE 1 Continued

Metabolic target	Metabolic agent/ compound	IT combined	Immune cells	Cancer cells	Mechanism	Ref
				Tumor growth inhibition		
	[b]CD36 inhibitor	Anti PD-1 treatment	Tregs dysfunction	Tumor growth inhibition	Increased efficacy of anti-PD-1 therapy following CD36 inhibition in intratumor Tregs	(26, 57)
	[b]cPLA2-alpha inhibitor	Adaptive T cell transfer therapy	Teffs	Indirect action	Prevent the dysfunction and senescence of Teff cells	(186)
	Bezafibrate	Anti PD-1 treatment	Teffs	Indirect action	FAO increase to prevent cell death due to FAO inhibition of anti-PD-1 therapy	(187–189)
	AZD1208	Anti PD-L1	MDSCs	Indirect action	PIM1 inhibitor, which inhibits FA uptake and FAO in MDSCs to increase MDSCs and recruit Teff cells	(190)

CRC, colorectal cancer; IT, immunotherapy; PEP, phosphoenolpyruvic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ACT, adoptive cell therapy; 2-DG, 2-deoxy-D-glucose; LDHA, lactate dehydrogenase A; Th, T helper cell; M1, macrophage phenotype 1; CT, chemotherapy; ATP, adenosine triphosphate; HK, hexokinase; 6-AN, 6-aminonicotinamide; FOXO1, forkhead box protein 01; AMPK, 5-adenosine monophosphate-activated protein kinase; 3-BP, 3-bromopyruvate; MCT, monocarboxylate transporter; CAs, carbonic anhydrases; HIF, hypoxia-inducible factor; ICB, immune checkpoint blockade; ROS, reactive oxygen species; ICIs, immune checkpoint inhibitors; FASN, fatty acid synthase; IGF1, insulin growth factor 1; STAT-3, signal transducer and activator of transcription 3; ACC, acetyl-CoA carboxylase; CD36, cluster of differentiation 36 also known fatty acid translocase (FAT); PD-1, programmed death-1; FAO, fatty acid oxidation; MDSCs, myeloid-derived suppressor cells; PIM1, proto-oncogene serine/threonine-protein kinase; FA, fatty acid. [a]Recovery of ICB resistance in murine models; [b]not available.

tissues from normal controls (166). Oxamate, 2-DG, and lonidamide, as glycolytic inhibitors, along with 6-diazo-5-oxo-L-norleucine (DON) and CB839 as glutaminolysis inhibitors have been extensively researched for their potential in CRC treatment (Table 1). The role of protein N-homocysteinylation (N-Hcy) in CRC has also been documented (191). Studies involving high-risk CRC patients and animals have shown that a high-fat diet can elevate homocysteine (Hcy) levels (192–194). Progressing CRC patients have been found to have higher N-Hcy plasma levels compared to healthy controls (194). Additionally, there is an observed upregulation of N-Hcy-protein and methionyl-tRNA synthetase (MARS) in CRC tissues versus normal tissues (194), along with changes in protein structure and function due to irregular N-Hcy, particularly affecting DNA damage repair proteins such as ataxia telangiectasia and Rad3-related protein (ATR) (194). These alterations lead to increased microsatellite instability and promote diffusion in cancer cells (194). ATR K-Hcy is associated with increased DNA damage and enhances CRC cell survival and growth (194). Inhibiting the production of Hcy-thiolactone (HTL), which is facilitated by MARS, thereby reducing K-Hcy changes, has been shown to decrease DNA damage and CRC cell proliferation. This suggests that MARS inhibitors could be beneficial in CRC therapy (194).

Lipid synthesis

High levels of lipids and increased lipogenesis have been identified in CRC cases with poorer prognoses (19, 195, 196), with 24 distinct lipids among 36 metabolites showing differential expression in adenocarcinomas compared to non-adenocarcinomas (196). Alterations in FA metabolic pathways have been linked to tumor growth and worse outcomes in CRC (197, 198). The activation of CD36 has been shown to inhibit CRC growth and induce apoptosis (199). Notably, CD36 overexpression is more common in CRC metastases than in primary CRC, indicating a greater dependency on FA uptake in metastatic cells (200–202). Overexpression of FABP5 enhances CRC growth and spread through a PPAR β/δ-independent signaling pathway (203). Within cells, long-chain acyl-CoA synthetases (ACSLs) and very long-chain acetyl-CoA synthetases (ACSVL) activate FAs by coupling them with CoA, while the concurrent upregulation of ACSL1, ACSL4, and SCD1 promotes epithelial-to-mesenchymal transition (EMT) in CRC (204).

Carnitine palmitoyltransferase 1 (CPT1), by interacting with carnitine, converts acyl-CoA into acyl carnitine, which is then transformed back into acyl-CoA by carnitine palmitoyltransferase 2 (CPT2) inside the mitochondria (205). In FAO, acetyl-CoA is the final product of acyl-CoA and then enters the TCA cycle (205), with CPT1A being more expressed in metastatic than in primary CRC (201). Citrate, derived from the TCA cycle, is converted into acetyl-CoA and oxaloacetate by ATP-citrate lyase (ACLY). In the cytoplasm, acetyl-CoA is transformed into malonyl-CoA, which are then condensed by fatty acid synthase (FASN). Wen et al. found that ACLY induces CRC growth inhibition by promoting β-catenin stabilization and its nuclear transport and transcriptional activity (206), although ACLY also induced CRC metastasis *in vivo* (206). Upregulation of FASN enhances CRC growth and spread through the AMPK/mTOR signaling pathway and is associated with secondary lymph node involvement, tumor stage (TNM), and poorer CRC outcomes (207).

The metabolic axis of stearoyl-CoA desaturase (SCD)/ACSLs induces EMT in CRC cells and, in stage II CRC patients, is associated with worse prognosis (204). Inhibiting SCD along

with ACSLs reduced the viability of CRC cells without affecting normal cells (204). Elevated serum cholesterol has been linked to a higher rate of CRC (208, 209). Stage III–IV CRC patients overexpressing 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (209) showed better clinical outcomes. Conversely, the upregulation of ABCA1, an ATP-binding cassette transporter involved in cholesterol and phospholipid homeostasis, was associated with worse CRC outcomes by promoting tumor proliferation and caveolin-1-dependent spread (210). A high-fat diet and the main features of metabolic reprogramming in CRC are illustrated in Figure 5. Lipid metabolism inhibitors such as FASN, ACC, CD36, and cPLA2-alpha have been extensively investigated for CRC treatment.

The genetic and epigenetic phenotype regulate metabolic reprogramming

Mutations in the adenomatous polyposis coli (APC) gene, which are present in over 80% of sporadic CRC cases, lead to increased glycolysis (211, 212). These APC mutations result in the activation of β-catenin/T-cell factor (TCF) transcription, subsequently increasing the expression of cMYC, PKM2, pyruvate dehydrogenase kinase 1 (PDK1), and MCT1 genes (211–214). PDK1, a glycolytic enzyme, inhibits the conversion of pyruvate into acetyl-CoA, thereby reducing OXPHOS in mitochondria and maintaining aerobic glycolysis in cancer cells (215). The APC/β-catenin axis also regulates HIF-1α and MYC transcription factors (214), with aberrant activation of β-catenin via HIF-1α promoting metabolic reprogramming of glucose in CRC (216, 217). Genetic aberrations in the TP53 gene, including inactivating mutations or deletions, occur in 40%–50% of sporadic and 80% of advanced CRC cases (218, 219). p53 modulates glucose metabolism by inhibiting transcription and transportation of GLUT1, GLUT3, and GLUT4, reducing glucose import (220). It also regulates the expression of glycolytic enzymes Hk2 and fructose 2,6-biphosphate (F2,6BP), thereby decreasing glycolysis (221). Additionally, p53 reduces intercellular lactate translocation by downregulating MCT1 (222) and interacts with HIF-1α and MYC (223), promoting the degradation of HIF-1α through parkin overexpression (224, 225) and suppressing c-myc transcription (226). In cancer cells with p53 mutations, the lack of inhibition on HIF-1α and MYC leads to their accumulation. Activating mutations in RAS and hyper-expression of EGFR occur in approximately 50% and 80% of CRC cases, respectively, leading to the continuous activation of the PI3K/Akt/mTORC1 pathway (227, 228), which alters cancer metabolism (229). AKT and mTORC1 enhance glycolysis by increasing glucose import and phosphorylating glycolytic enzymes. PI3K/Akt pathway also promotes MYC transcription and increases HIF-1α and MYC proteins by inhibiting their degradation and facilitating their translation (229, 230). Methylation, both epigenetic and genetic, plays a key role in regulating glycolytic metabolism (231). DNA methylation activates aberrant glycolytic metabolism in cancer (232). For example, hypermethylation of the LDHB promoter increases the LDHA/LDHB ratio, enhancing lactate formation in cancer cells (232). Hypomethylation of the HK2 promoter leads to HK2 overexpression and tumor progression (233). DNA methylation also upregulates HIF-1α, enhancing HIF pathway signaling in cancer (232). In CRC, N6-methyladenosine (m6A) modification of Hk2 and SLC2A1 mRNA by METTL3 stabilizes them, promoting the glycolytic pathway (234). Methylation of glycolytic enzymes LDHA and PKM2 by methyltransferases regulates their function (235, 236), underscoring the significant role of methylation in CRC glycolytic metabolism.

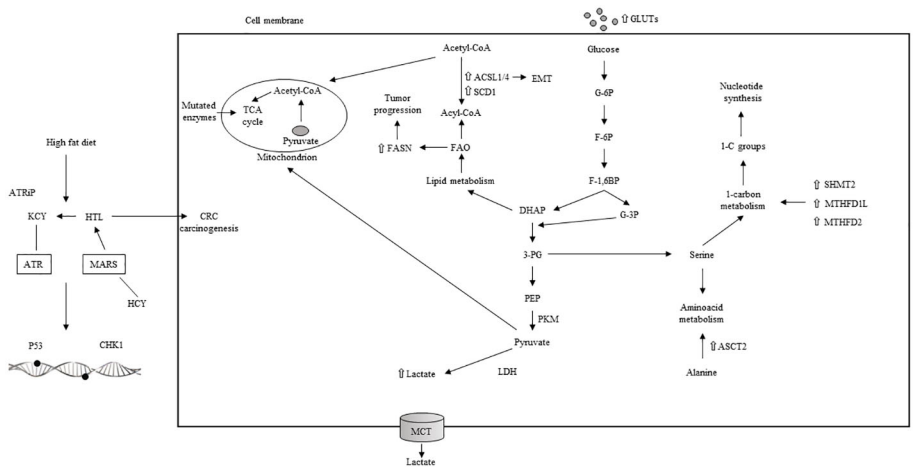


FIGURE 5
High fat diet, aerobic glycolisis and main characteristics of metabolic reprogramming in CRC. TCA: tricarboxilic acid; ACSL: acyl-CoA synthetase; SCD: stearoyl-CoA desaturase; EMT: epithelial to mesenchymal transition; FAO: fatty acid oxydation; DHAP: dihydroxyacetone phosphate; G-6P: glucose 6 phosphate; F-6P: fructose 6 phosphate; F-1,6BP: fructose 1,6 biphophate; G-3P: glucose 3 phosphate: 3-PG: 3 phosphogliceric acid; PEP: phosphoenolpyruvate; PKM: pyruvate kinase muscle isoenzyme; LDH: lactate dehydrogenase; MCT: monocarboxylate transporter; 1-C: one carbon; SHMT: serine hydroxymethyltransferase; MTHFD1L: methylene tetrahydrofolate dehydrogenase 1-like; ASCT: alanine, serine, cysteine transporter; ATRiP: ATR interacting protein; KCY: colonic lysine homocysteinylation; ATR: ataxia-teleangectasia and RAD 3-related protein; HTL: homocysteine thiolactone: MARS: methionyl-tRNA synthetase; CHK1: cheek point kinase 1; HCY: homocysteilation; ⇧ increase; ⇩ decrease.

Genetic and epigenetic regulation of glycolysis in CRC, illustrated in Figure 6A, is often associated with various signaling pathways, including Wnt-beta-catenin, EGFR/RAS/RAF/MAPK, PI3K, VEGF, and p53, which contribute to CRC initiation and progression. Targeted therapies against these pathways, such as cetuximab (anti-EGFR), encorafenib (BRAF inhibitor), and bevacizumab (anti-VEGF), have been successfully administered for treating metastatic CRC patients.

The metabolic immune escape in CRC

In CRC, the competition for nutrients within the TME triggers a reprogramming of key metabolic pathways, which, in turn, promotes immune escape. The metabolites produced by the intestinal microbiota offer an additional explanation for immune tolerance.

Reprogramming of key metabolic pathways

Within the TME, both tumor cells and other cells exhibit increased lactate production due to the Warburg effect, leading to a highly acidic environment. This acidosis, similar to that observed in other cancers, impedes the activity of anti-tumor immune cells in CRC (237). Specifically, lactate diminishes the functionality of CD8+ T cells (63), with studies showing that CD8+ T cells exposed to acidic conditions undergo intracellular acidification via lactate uptake (63), significantly reducing the activity of activated T cells (NFAT), a crucial transcription factor, and the production of IFN-γ, a critical cytokine for immune response (63). In CRC with deficient mismatch repair (dMMR)/microsatellite instability-high (MSI-H), proteomic analyses have revealed an inverse relationship between glycolytic enzyme levels and the presence of infiltrating CD8+ T cells (238). High glycolysis levels in MSI-H CRC samples correlate with fewer infiltrating CD8+ T cells, suggesting that glycolysis may influence the efficacy of immune checkpoint inhibitor (ICI) therapy and could predict the prognosis of CRC patients undergoing immunotherapy (238). The scarcity of nutrients within the TME fosters an immune-suppressive phenotype, characterized by Tregs and M2-like macrophages (239). Furthermore, lactate produced by CRC cells inhibits the phagocytosis of TAMs by activating the Ap-2α/ETS-like-1 protein (Elk-1) axis, which increases levels of signal-regulatory protein alpha (SIRPα), dampening TAMs' anti-tumor activity (240). Conversely, reducing lactate levels can enhance the effectiveness of immune therapy in CRC by diminishing the presence of tumor-infiltrating Tregs and MDSCs (241). Lactate also serves as an agonist for the GPR81, which, when activated in cancer cells, leads to PD-L1 overexpression and facilitates tumor immune escape (242). In DCs, lactate activation of GPR81 inhibits MHC class II presentation on the cell surface and reduces the production of cAMP, IL-6, and IL-12, further contributing to immune evasion (243). An increase in GPR81 signaling not only diminishes the pro-tumor activities of TAMs but also increases the immune-suppressive capabilities of MDSC (77). Recent studies have further highlighted the critical role of lactylation in modulating immune cell activity within the hypoxic and acidic TME (244). Lactate-induced histone lactylation can elevate

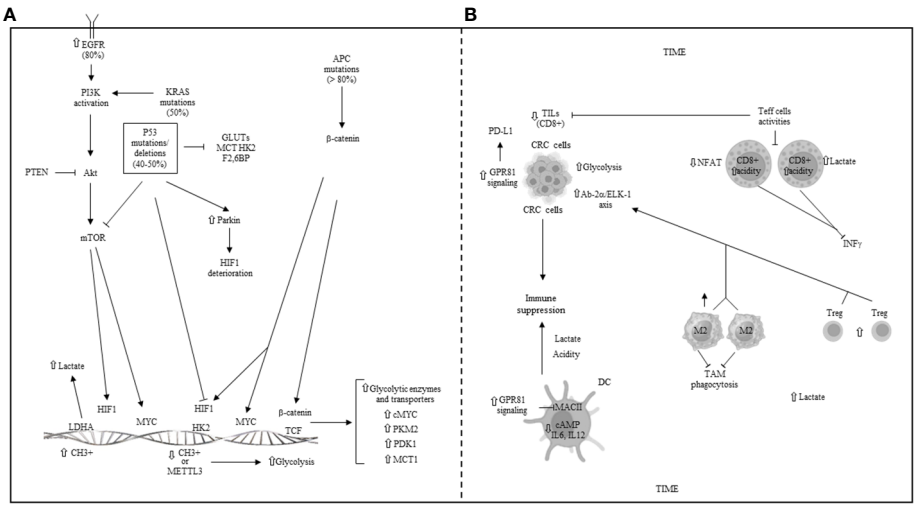


FIGURE 6
(A) Genetic and epigenetic regulation of glycolysis in CRC. EGFR: epidermal growth factor receptor; APC: adenomatous polyposis coli gene; PI3K: phosphoinositide-3-kinase; Akt: protein kinase B; mTOR: mammalian target of rapamycin; PTEN: phosphatase and tensin homolog; KRAS: kirsten and sarcoma virus gene; P53: tumor protein 53; GLUTs: glucose transporters; MCT: monocarboxylate transporter; HK2: hexokinase 2; F2,6BP: fructose 2,6 biphosphate; HIF1: hypoxia inducible factor 1; PKM: pyruvate kinase muscle isoenzyme; PDK1: piruvate dehydrogenase kinase 1; TCF: T cell factor; METTL3: methyl adenosine (m6A) transferase; LDHA/B: lactate dehydrogenase A/B. **(B)** Metabolic reprogramming and some main mechanisms of immune escape in the colorectal tumor immune microenvironment (TIME). TILs: tumor infiltrating lymphocytes; CRC: colorectal cancer; GPR81: G-protein coupled receptor 81; Ap2α: activating protein 2 alpha; Elk-1: ETS-like 1 protein; DC: dendritic cell; NFAT: nuclear factor of activated T cells; M2: macrophage phenotype 2; Treg: T regulatory cells; TAM: tumor-associated macrophage; ⇧ increase; ⇩ decrease; ⟞ inhibited.

METTL3 expression in tumor-infiltrating myeloid cells (TIMs) (245), activating JAK/STAT signaling pathways, thereby fostering an immune-suppressive TME and promoting tumor growth and spread (245). Decreased density of mature DCs has been observed in both human colon cancers and experimental rat colon cancer models, with an even lower DC density in metastases compared to primary colorectal tumors. Additionally, various factors secreted by tumors and on a systemic level contribute to the functional defects of tumor-infiltrating DCs in CRC. Factors such as CCL2, CXCL1, CXCL5, and VEGF are implicated in inhibiting IL-12p70 secretion by DCs, while COX2 impedes DC differentiation and maturation through the mediation of the downstream signal molecule PGE2 and its receptors EP2/EP4 (246). Apoptosis is a mechanism for DC elimination in the TME, with MUC2 mucins increasing the rate of apoptosis in cultures of DCs derived from human monocytes. It has also been reported that the number of CD205-positive intra-tumor DCs decreases in patients with CRC. Furthermore, upregulation of HMGB1, a multifunctional cytokine secreted by cancer cells, is associated with lymph node metastasis, suggesting that nodal DCs are suppressed by HMGB1 produced by colon cancer cells (247). Regarding NK cells, various mechanisms have been identified for escaping NK cell-mediated tumor surveillance. Fucosylation plays significant roles in carcinogenesis and is among the most critical oligosaccharide modifications in cancer (248). Research has shown that mutations in the GDP-mannose-4,6-dehydratase (GMDS) gene, essential for fucosylation, result in resistance to TRAIL-induced apoptosis and subsequent evasion from NK cell-mediated surveillance in human colon cancer. Additionally, a more recent study (249) focused on the long non-coding RNA (lncRNA) ELFN1-AS1, which is aberrantly expressed in multiple tumors and considered an oncogene in cancer development. This study revealed that ELFN1-AS1 increases the ability of CRC cells to evade NK cell surveillance both *in vitro* and *in vivo* by attenuating NK cell activity. It achieves this by downregulating NKG2D and GZMB through the GDF15/JNK pathway. Metabolic reprogramming and several primary mechanisms of immune escape within the colorectal TIME are depicted in Figure 6B.

The dual role of the microbiota

Intestinal cancer cells and other components of the TME interact with the microbiota, which plays a significant role in reshaping the TME (250). Metabolites from the microbiota, such as short-chain fatty acids (SCFAs) and lipopolysaccharide, influence the TME. CRC is characterized by high levels of *Fusobacterium nucleatum* and low levels of *Holdemanella biformis*, which promote anti-tumor activity by enhancing SCFA formation (251, 252). Conversely, dysbiosis is associated with decreased levels of SCFAs and polyamines. SCFAs, including butyrate, acetate, and propionate, are essential for maintaining intestinal homeostasis (251, 253). Butyrate and acetate, in particular, are known to increase the activity of CD8+ effector T cells (Teff) through direct effects (254, 255) and epigenetic mechanisms (256). Polyamines, however, have been shown to create an immunosuppressive TME by reducing IFN-gamma and TNF levels, though contrasting effects of polyamines have also been reported (257). Among polyamines, spermine favors the M2 polarization of TAMs, while spermidine promotes an M1 phenotype (258).

An experimental study on CRC (259) explored how SCFA treatment affects the ability of CRC cells to activate CD8+ T cells. SCFAs, as microbial metabolites, possess immune-regulatory properties within CRC cells that are not fully understood. SCFA-treated CRC cells were found to activate CD8+ T cells more effectively than untreated cells. Additionally, SCFAs activated CD8+ T cells more in CRCs with microsatellite instability (MSI) compared to chromosomally unstable (CIN) CRCs without DNA repair deficiency. This suggests that SCFAs, depending on the genotype of CRC cells, may induce DNA damage that promotes the upregulation of chemokines, MHC class I, and genes involved in antigen processing or presentation. An increase in response is facilitated by a direct feedback loop between SCFA-treated CRC cells and activated CD8+ T cells in the TME. In CRCs, the suppression of histone deacetylation by SCFAs initiates a process that leads to genetic instability and the overexpression of genes likely involved in SCFA signaling and chromatin regulation. This effect is observed in MSI CRC samples, regardless of the quantity of SCFA-producing bacteria in the bowel. These findings indicate that the heightened response of MSI CRCs to microbially delivered SCFAs significantly enhances CD8+ T-cell activity, thus identifying a potential therapeutic target to boost anti-tumor immunity in CIN CRCs (259).

The immunotherapy with ICIs

Recently, immunotherapy using ICIs has emerged as a promising therapeutic option for selected CRC patients. Nivolumab and pembrolizumab, ICIs that have shown success in treating patients with MSI-H (microsatellite instability-high) colorectal tumors, are notable examples (260). However, the immunosuppressive TME in most CRC patients likely impedes the effectiveness of immunotherapy. As mentioned earlier, effector T cells require ample nutrients to sustain their antitumor activity, and glucose deprivation compromises their presence and functionality (261). Furthermore, lactic acid (LA), a metabolic by-product prevalent in the competitive TME, significantly inhibits T cell-mediated lysis of cancer cells (261). Recent studies have shown that an acidic TME enhances the expression of PD-1 and other suppressive molecules in Tregs, while dampening PD-1 expression in effector T cells due to Tregs' preferential utilization of lactate (262). This suggests that PD-1 blockade might inadvertently bolster PD-1+ Treg cell function, thereby facilitating resistance to immunotherapy (262). An inverse relationship between tumor glycolysis and tumor-infiltrating CD8+ T cells has been observed (238), with high serum LDH levels predicting poor responses to pembrolizumab (263). Research in a murine model of mismatch-repair-proficient (pMMR) CRC confirmed the inverse association between the therapeutic efficacy of PD-1 inhibitors and serum LDH levels (263, 264), with patients experiencing enhanced effectiveness of PD-1 blockade when combined with LDHA inhibition (178). Despite the metabolic overlap between cancer and immune cells in

the highly competitive TME, subtle differences exist. Drugs targeting glycolysis can inhibit both pro-tumor and anti-tumor immune cells (265). Specifically, treatment with 2-DG has been shown to significantly suppress key activities in CD4 and CD8 T cells, such as cell proliferation and lactate production (266) and further inhibit the production of IFN-γ, TNF, IL-10, and IL-4 in CD4 T cells (266). Additionally, effector T cells deficient in glucose transporter 1 (Glut1) were unable to augment and promote an inflammatory response (267), unlike Treg cells, which retained their functionality in a Glut1-independent manner (267). This underscores the importance of precisely defining the subtle differences among subpopulations of the same cell type, particularly immune cells, to optimally exploit their vulnerability to metabolic inhibition.

Clinical applications of the metabolic reprogramming and ICIs

Targeting ICIs and/or glucose metabolism

Targeting ICIs and/or glucose metabolism involves identifying specific metabolites, metabolic enzymes/pathways, and genes that are differentially expressed or regulated in tumor and immune cells. The goal is often to enhance the antitumor activity of effector T cells (Teff) while diminishing the immunosuppressive functions of Tregs, without adversely affecting their functionality. Focusing on glucose metabolism, the glycolytic metabolite phosphoenolpyruvic acid (PEP) can influence the activity of tumor-infiltrating lymphocytes (TILs), and enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serve as metabolic checkpoint regulators. Reprogramming T-cell metabolism by altering the levels of these metabolites and enzymes can act as an adoptive cell therapy (ACT) and supplement immunotherapy (8). Inhibiting key enzymes is another primary therapeutic approach. For instance, oxamate, an inhibitor of lactate dehydrogenase A (LDHA), has been shown to be effective in CRC treatment when used in combination with metformin and doxorubicin (167). This combination inhibits Hk, leading to a metabolic arrest with decreased ATP from glycolysis and reduced NADPH from the PPP. 2-DG has been observed to sensitize tumor cells to radiation and chemotherapeutic agents, increasing tumor control while sparing normal tissues (8). Systemic administration of 2-DG with focal tumor irradiation has direct effects on tumor cells, likely through changes in gene expression and phosphorylation of proteins involved in signaling, cell cycle control, DNA repair, and apoptosis (168). This treatment also promotes antitumor immunity in peripheral blood, with increased recruitment of innate and adaptive immune cells, a shift from Th2 and Th17 to Th1, and depletion of Tregs. Additionally, 2-DG combined with tumor irradiation has been shown to shift splenic macrophages towards an M1 phenotype, correlating with improved local tumor control (8). The inhibitor 6-aminonicotinamide (6-AN) targets the PPP by inhibiting G6PD, enhancing radiosensitization when combined with 2-DG (8). Treatments affecting the PI3K/Akt/mTOR and AMPK signaling pathways, such as 2-DG, have been found to increase memory T-cell presence by inhibiting glycolysis, thereby enhancing CD8+ T cell-mediated antitumor effects through increased AMPK activity and negative regulation of mTOR and Foxo1 (169). Metformin treatment also boosts AMPK activation and memory T-cell generation (170), potentially through its impact on mTOR signaling (171) or upregulation of miR33a, which reduces c-Myc expression (172). The secondary metabolite caulerpin has shown anticancer properties by disrupting the glycolytic process via the AMPK pathway (8). In the highly acidic TME, inhibitors of LDHA or MCT1 have been suggested to overcome resistance to ICIs in murine models. HIF-1α signaling, which controls several genes involved in glucose and lactate transport and glycolysis, such as GLUT-1, MCT1, and MCT4, is a key regulatory mechanism in this context (8). Quercetin, a natural compound and nonspecific MCT inhibitor, has shown significant effects in inhibiting proliferation, inducing cell death, reducing glycolytic activity, and enhancing the cytotoxicity of 5-fluorouracil (5-FU) in CRC cells (173). Other metabolic analogs, such as 3-bromopyruvate (3-BP) and lonidamine, target glycolysis by interfering with the activity of Hk. 3-BP also reduces MCT1 expression (174), while lonidamine inhibits it, thereby suppressing tumor progression (175). Carbonic anhydrases (CAs), which facilitate the conversion of water and CO_2 to intracellular bicarbonate and a proton, work alongside MCTs in tumor cells to export excess protons and lactate, maintaining acid–base balance. Indisulam/E7070, a multiple CAs inhibitor (176) showing anti-tumor activity in xenograft CRC models, synergized when given in association with capecitabine or irinotecan in metastatic CRC patients (177). HIF-1 is crucial for sustaining high glycolysis rates in cancer cells. Bortezomib, a proteasome inhibitor that can inhibit HIF-1α transactivation, and topoisomerase I (TOP1) inhibitors like irinotecan/CPT-11 and EZN-2208 (a derivative of SN38, the active metabolite of irinotecan) that suppress HIF-1α/HIF-2α expression and HIF-induced targets are under clinical evaluation. However, in a Phase II trial (NCT00931840) involving patients with advanced CRC, EZN-2208, did not induce objective radiographic responses in KRAS-mutant patients resistant to CPT-11, with similar outcomes observed between cetuximab+EZN-2208 and cetuximab+CPT-11 groups, potentially due to unfavorable pharmacokinetics of EZN-2208 (177). Highly glycolytic tumors can deplete glucose and release large amounts of LA, inducing PD-1 overexpression and increasing the suppressive activity of Treg cells, partially explaining the limited efficacy of PD-1 blockade therapy (262). Treg cells actively uptake LA through MCT1, promoting NFAT1 translocation to the nucleus and enhancing PD-1 expression, while effector T-cell PD-1 expression is reduced. As a result, PD-1 blockade may inadvertently strengthen PD-1-expressing Treg cells, leading to treatment failure. Microsatellite-stable (MSS) CRCs, unlike MSI-H CRCs, exhibit lower responsiveness to ICIs, likely due to the Warburg effect contributing to an immunosuppressive TME and the induction of immune checkpoints. Combining glycolysis inhibitors with ICIs could potentially reverse resistance in patients treated with single-agent ICI (265, 268). In mouse models of CT26 CRCs, a combination of aspirin and anti-PD-1 therapy led to tumor growth reduction and complete response, outperforming monotherapy (178). Additionally, biological drugs

like apatinib (a VEGF inhibitor) and trametinib (a MEK inhibitor) that can inhibit glycolysis, when used in conjunction with ICIs, may enhance therapeutic responses in CRC. Thus, emerging research supports the therapeutic benefits of combining glycolysis inhibitors with ICIs (179, 180).

Targeting ICIs and/or lipid metabolism

Upregulation of lipogenic enzymes is commonly observed in patients with aggressive metastatic CRC, and targeting these enzymes, particularly FASN, a key element in the *de novo* biosynthesis of long-chain fatty acids, presents a viable therapeutic option. Cerulenin, the first FASN inhibitor, was found to decrease proliferation in murine CRC cells, promote apoptosis, and suppress liver metastasis of CRC (182). Moreover, when combined with oxaliplatin, it improved the efficacy of the treatment while reducing side effects (183). Luteolin, a potent FASN inhibitor with anti-tumor activity in CRC, likely operates by interfering with various signaling pathways, including the IGF-1 and Wnt-beta-catenin pathways (184). Epigallocatechin-3-gallate (EGCG), a polyphenol found in green tea, is another FASN inhibitor that has been shown to suppress proliferation and diffusion of CRC cells through STAT3 downregulation, significantly decreasing liver metastasis in SCID mice (181). Additionally, the acetyl-CoA carboxylase inhibitor (ACC) TOFA induced apoptosis in CRC cells (185). Targeting lipid metabolism alongside PD-1 inhibitors represents a novel therapeutic strategy to enhance the effectiveness of ICI blockade. Tregs are among the most significant intratumor immunosuppressive cells, and inhibiting lipid metabolism in Tregs is a focus of ongoing research. However, selectively targeting Tregs in tumors without harming cytotoxic T cells or triggering autoimmune diseases remains a major challenge. CD36, overexpressed on both Tregs and cytotoxic T cells, when deleted, decreased the expression of several immune regulatory receptors but not PD-1. Thus, inhibiting CD36 in intra-tumor Tregs through genetic ablation or using CD36 monoclonal antibodies effectively delayed tumor growth and enhanced the efficacy of anti-PD-1 treatment (26). Further studies on CD36 revealed that its overexpression in intra-tumor effector T cells exposes them to oxidative damage and ferroptosis due to lipid accumulation, and combining anti-PD-1 therapy with CD36 or ferroptosis inhibitors offered greater immunotherapeutic benefits (57). Senescent cytotoxic T cells also contribute to tumor immune evasion. Senescent and dysfunctional T cells exhibit aberrant lipid metabolism and intracellular lipid accumulation, with overexpression of group IVA phospholipase A2 (cPLA2-a) closely related to aging and lipid metabolism reprogramming in T cells via MAPK and STAT signaling pathways. Inhibiting cPLA2a prevented T-cell senescence and enhanced the efficacy of immunotherapies, such as adoptive T-cell transfer therapy (186). Moreover, activators of FAO, like bezafibrate, could potentiate anti-PD-1 therapy by inducing the expression of CXCL9 and CXCL10 from tumor cells and CXCR3 on intra-tumor effector T cells (187, 188). In a recent *in vivo* study (189), the expression of chemokine ligand 10 (CXCL10) was linked to enhanced CD8+ T-cell infiltration, and upregulation of CXCL10 improved the responsiveness of CRC cells to a combined treatment of cetuximab and anti-PD-1, unlike when these treatments were administered individually. The relationship between MDSCs and resistance to ICB appears to hinge on differing gene expressions of CD8+ T cells between sensitive and resistant patients. Inhibiting the function of PIM1, a serine/threonine kinase, through pharmacological means or genetic ablation reduces FA uptake and FAO in MDSCs via a PPARgamma-mediated pathway, leading to a significant decrease in MDSCs, increased recruitment of CD8+ T cells, and improved resistance to ICB (190).

Future directions

"Glycolytic enzymes," traditionally considered "housekeeping" proteins, have been identified as "moonlighting" proteins that perform multiple functions. These roles are influenced not just by their structural peculiarities but also by compartmentalization and the metabolic environment (269). The interplay between cell metabolism and gene transcription provides specific molecular and functional targets for cancer treatment, with many enzymes catalyzing reactions in the glycolytic pathway to lactate production also involved in regulating gene transcription (270). Understanding the dual roles of glycolytic enzymes in gene expression regulation within tumor cells offers a novel approach to cancer treatment, targeting the additional functions of these metabolic enzymes. However, designing pharmaceutical inhibitors that specifically target these moonlighting functions remains challenging, and the potential of such inhibitors in cancer therapy is yet to be fully determined. Ferroptosis, an iron-dependent programmed cell death induced by lipid peroxide accumulation and ROS, represents another emerging area for cancer treatment strategies (271). Experimentation has shown that manipulating ferroptosis in tumor cells can enhance the effectiveness of anti-PD1 therapy (8). AZD3965, an MCT1 inhibitor, is currently being investigated in clinical trial NCT01791595 (262). Moreover, elevated serum ammonia levels have been observed in CRC patients (272), with an ammonia-related gene signature correlating with poorer prognosis and lack of response to ICIs, suggesting that targeting tumor-associated ammonia could enhance the efficacy of immunotherapy, including PD-L1 inhibitors. Nanocarriers is further promising therapeutic strategy for drugs targeting the PD-1/PD-L1 pathway. In fact, nanocarriers represent a rationally conceived intelligent delivery system that can control therapeutic agent delivery and improve tumor targeting ability. Nanocarriers are responsive to tumor acidic microenvironment, high level of GSH and ROS, and specifically upregulated enzymes (internal stimuli) or light, ultrasound, and radiation (external stimuli). Therefore, they can carry out the target immunomodulators at the tumor site, increasing anti-tumor efficacy but reducing off-target toxicity (273). Lastly, abnormal microbial metabolites and microbial dysbiosis significantly impact CRC pathogenesis. Therefore, improving gut microbiota through dietary interventions, probiotic/prebiotic supplementation, or the administration of beneficial microbial metabolites could offer an additional therapeutic strategy for CRC.

Discussion

The Warburg effect, recognized as a key player in the metabolic reprogramming of cancer cells, is among the 10 widely accepted hallmarks of cancer. Nonetheless, emerging evidence suggests that tumor growth may also rely on mitochondrial metabolism, to some extent. In the TME, proliferating tumor cells exhibit shared metabolic reprogramming pathways to meet increased nutrient and oxygen demands. Hypoxia in the TME often leads to a metabolic shift from OXPHOS to aerobic glycolysis, followed by enhanced lipid synthesis and glutaminolysis due to lactate acidification. This acidification provides cancer cells with a growth advantage over immune cells, inhibiting T-cell proliferation, promoting the migration of neutrophils and DCs, and increasing Tregs and the infiltration of MDSCs and TAMs, thereby fostering an immunosuppressive TME. High lactate levels suppress T-cell proliferation and IFN-gamma release, while low LDHA expression in T cells promotes their conversion to FoxP3+ Tregs, further encouraging PD-1 expression. Nutrient deficiency in the TME leads to the polarization of M1 macrophages to immunosuppressive M2 TAMs, while certain chemokines attract MDSCs to the TME, where they secrete immunosuppressive cytokines. Hypoxic conditions promote the immune-metabolic reprogramming of MDSCs towards FAO, with AMPK activation and FAO enhancing their immunosuppressive activity. Overexpression of AMPK in TADCs and increased abnormal lipids convert them into tolerogenic DCs. High levels of TGF-beta, coupled with decreased OXPHOS, inhibit NK cell function, while high lactate levels induce mitochondrial dysfunction, increased ROS and apoptosis in NK cells. In CRC, studies have documented abnormal glycolysis, glutaminolysis, and enhanced lipid synthesis, including high lactate levels and upregulation of GLUT1, Hk2, and enzymes involved in glycolysis, as well as ASCT2 upregulation. High lipid levels and increased lipogenesis, along with altered FA metabolic pathways in CRC, have been linked to tumor growth and poorer outcomes. Acidosis in CRC cells impairs the activity of anti-tumor immune cells, inhibits IFN-γ production by CD8+ T cells, and suppresses TAM phagocytosis. The competitive nature of nutrient availability in the TME promotes the formation of immunosuppressive phenotypes such as Tregs and M2-like macrophages. GPR81 signaling activation by lactate in CRC cells induces PD-L1 overexpression and decreases the pro-tumor capability of TAMs. Lactate can also activate GPR81 in DCs, diminishing their antigen presentation capabilities. The genetic and epigenetic phenotype of cells, along with the microbiota, plays a significant role in the metabolic reprogramming of CRC. High acidity and lactate levels in the TME, through various mechanisms, inhibit T-effector cell activities, while certain bacteria increase SCFA production, enhancing CD8+ T-cell activation and inducing genes involved in antigen processing or presentation. However, other microbiota mechanisms may promote pro-tumor activities. Reports of successful outcomes with ICIs in selected CRC patients have emerged, and the combination of these immune therapies with drugs targeting metabolic pathways, particularly glycolysis and lipolysis, is being extensively investigated to enhance clinical outcomes and expand the population benefiting from immunotherapy.

Author contributions

AN: Writing – original draft, Writing – review & editing. PF: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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.

Publisher's note

References

1. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol.* (2002) 3:991–8. doi: 10.1038/ni1102-991

2. Bui JD, Schreiber RD. Cancer immunosurveillance, immunoediting and inflammation: independent or interdependent processes? *Curr Opin Immunol.* (2007) 19:203–8. doi: 10.1016/j.coi.2007.02.001

3. Nayak DA, Binder RJ. Agents of cancer immunosurveillance: HSPs and dsDNA. *Trends Immunol.* (2022) 43:404–13. doi: 10.1016/j.it.2022.03.004

4. Sedlacek AL, Younker TP, Zhou YJ, Borghesi L, Shcheglova T, Mandoiu II, et al. CD91 on dendritic cells governs immunosurveillance of nascent, emerging tumors. *JCI Insight.* (2019) 4:e127239. doi: 10.1172/jci.insight.127239

5. Binder RJ, Han DK, Srivastava PK. CD91: a receptor for heat shock protein gp96. *Nat Immunol.* (2000) 1:151–5. doi: 10.1038/77835

6. Frades I, Foguet C, Cascante M, Araúzo-Bravo MJ. Genome scale modeling to study the metabolic competition between cells in the tumor microenvironment. *Cancers (Basel).* (2021) 13:4609. doi: 10.3390/cancers13184609

7. Soga T. Cancer metabolism: key players in metabolic reprogramming. *Cancer Sci*. (2013) 104:275–81. doi: 10.1111/cas.12085

8. Gupta S, Roy A, Dwarakanath BS. Metabolic cooperation and competition in the tumor microenvironment: implications for therapy. *Front Oncol*. (2017) 7:68. doi: 10.3389/fonc.2017.00068

9. Siemann DW. The unique characteristics of tumor vasculature and preclinical evidence for its selective disruption by Tumor-Vascular Disrupting Agents. *Cancer Treat Rev*. (2011) 37:63–74. doi: 10.1016/j.ctrv.2010.05.001

10. Jun JC, Rathore A, Younas H, Gilkes D, Polotsky VY. Hypoxia-inducible factors and cancer. *Curr Sleep Med Rep*. (2017) 3:1–10. doi: 10.1007/s40675-017-0062-7

11. Masoud GN, Li W. HIF-1α pathway: role, regulation and intervention for cancer therapy. *Acta Pharm Sin B*. (2015) 5:378–89. doi: 10.1016/j.apsb.2015.05.007

12. Laukka T, Mariani CJ, Ihantola T, Cao JZ, Hokkanen J, Kaelin WG Jr, et al. Fumarate and succinate regulate expression of hypoxia-inducible genes via TET enzymes. *J Biol Chem*. (2016) 291:4256–65. doi: 10.1074/jbc.M115.688762

13. Harris AL. Hypoxia–a key regulatory factor in tumour growth. *Nat Rev Cancer*. (2002) 2:38–47. doi: 10.1038/nrc704

14. Kumar V, Stewart JH4. Immunometabolic reprogramming, another cancer hallmark. *Front Immunol*. (2023) 14:1125874. doi: 10.3389/fimmu.2023.1125874

15. Gillies RJ, Gatenby RA. Metabolism and its sequelae in cancer evolution and therapy. *Cancer J*. (2015) 21:88–96. doi: 10.1097/PPO.0000000000000102

16. Sullivan MR, Danai LV, Lewis CA, Chan SH, Gui DY, Kunchok T, et al. Quantification of microenvironmental metabolites in murine cancers reveals determinants of tumor nutrient availability. *Elife*. (2019) 8:e44235. doi: 10.7554/eLife.44235

17. Ames S, Pastorekova S, Becker HM. The proteoglycan-like domain of carbonic anhydrase IX mediates non-catalytic facilitation of lactate transport in cancer cells. *Oncotarget*. (2018) 9:27940–57. doi: 10.18632/oncotarget.v9i46

18. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol*. (2007) 25:267–96. doi: 10.1146/annurev.immunol.25.022106.141609

19. Beloribi-Djefaflia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. *Oncogenesis*. (2016) 5:e189. doi: 10.1038/oncsis.2015.49

20. Snaebjornsson MT, Janaki-Raman S, Schulze A. Greasing the wheels of the cancer machine: the role of lipid metabolism in cancer. *Cell Metab*. (2020) 31:62–76. doi: 10.1016/j.cmet.2019.11.010

21. Kopecka J, Trouillas P, Gašparović AČ, Gazzano E, Assaraf YG, Riganti C. Phospholipids and cholesterol: Inducers of cancer multidrug resistance and therapeutic targets. *Drug Resist Update*. (2020) 49:100670. doi: 10.1016/j.drup.2019.100670

22. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat Immunol*. (2014) 15:846–55. doi: 10.1038/ni.2956

23. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity*. (2012) 36:68–78. doi: 10.1016/j.immuni.2011.12.007

24. Klein Geltink RI, O'Sullivan D, Corrado M, Bremser A, Buck MD, Buescher JM, et al. Mitochondrial priming by CD28. *Cell*. (2017) 171:385–397.e11.

25. Pan Y, Tian T, Park CO, Lofftus SY, Mei S, Liu X, et al. Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. *Nature*. (2017) 543:252–6. doi: 10.1038/nature21379

26. Wang H, Franco F, Tsui YC, Xie X, Trefny MP, Zappasodi R, et al. CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. *Nat Immunol*. (2020) 21:298–308. doi: 10.1038/s41590-019-0589-5

27. Reinfeld BI, Madden MZ, Wolf MM, Chytil A, Bader JE, Patterson AR, et al. Cell-programmed nutrient partitioning in the tumour microenvironment. *Nature*. (2021) 593:282–8. doi: 10.1038/s41586-021-03442-1

28. Hensley CT, Wasti AT, DeBerardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. *J Clin Invest*. (2013) 123:3678–84. doi: 10.1172/JCI69600

29. Wang Q, Hardie RA, Hoy AJ, van Geldermalsen M, Gao D, Fazli L, et al. Targeting ASCT2-mediated glutamine uptake blocks prostate cancer growth and tumour development. *J Pathol*. (2015) 236:278–89. doi: 10.1002/path.4518

30. van Geldermalsen M, Wang Q, Nagarajah R, Marshall AD, Thoeng A, Gao D, et al. ASCT2/SLC1A5 controls glutamine uptake and tumour growth in triple-negative basal-like breast cancer. *Oncogene*. (2016) 35:3201–8. doi: 10.1038/onc.2015.381

31. Lamonte G, Tang X, Chen JL, Wu J, Ding CK, Keenan MM, et al. Acidosis induces reprogramming of cellular metabolism to mitigate oxidative stress. *Cancer Metab*. (2013) 1:23. doi: 10.1186/2049-3002-1-23

32. Pérez-Escuredo J, Dadhich RK, Dhup S, Cacace A, Van Hée VF, De Saedeleer CJ, et al. Lactate promotes glutamine uptake and metabolism in oxidative cancer cells. *Cell Cycle*. (2016) 15:72–83. doi: 10.1080/15384101.2015.1120930

33. Howden AJM, Hukelmann JL, Brenes A, Spinelli L, Sinclair LV, Lamond AI, et al. Quantitative analysis of T cell proteomes and environmental sensors during T cell differentiation. *Nat Immunol*. (2019) 20:1542–54. doi: 10.1038/s41590-019-0495-x

34. Kaymak I, Williams KS, Cantor JR, Jones RG. Immunometabolic interplay in the tumor microenvironment. *Cancer Cell*. (2021) 39:28–37. doi: 10.1016/j.ccell.2020.09.004

35. Johnson MO, Wolf MM, Madden MZ, Andrejeva G, Sugiura A, Contreras DC, et al. Distinct regulation of th17 and Th1 cell differentiation by glutaminase-dependent metabolism. *Cell*. (2018) 175:1780–1795.e19.

36. Singer K, Cheng WC, Kreutz M, Ho PC, Siska PJ. Immunometabolism in cancer at a glance. *Dis Model Mech*. (2018) 11:dmm034272. doi: 10.1242/dmm.034272

37. Bian Y, Li W, Kremer DM, Sajjakulnukit P, Li S, Crespo J, et al. Cancer SLC43A2 alters T cell methionine metabolism and histone methylation. *Nature*. (2020) 585:277–82. doi: 10.1038/s41586-020-2682-1

38. Gao X, Sanderson SM, Dai Z, Reid MA, Cooper DE, Lu M, et al. Dietary methionine influences therapy in mouse cancer models and alters human metabolism. *Nature*. (2019) 572:397–401. doi: 10.1038/s41586-019-1437-3

39. Roy DG, Chen J, Mamane V, Ma EH, Muhire BM, Sheldon RD, et al. Methionine metabolism shapes T helper cell responses through regulation of epigenetic reprogramming. *Cell Metab*. (2020) 31:250–266.e9. doi: 10.1016/j.cmet.2020.01.006

40. Ron-Harel N, Ghergurovich JM, Notarangelo G, LaFleur MW, Tsubosaka Y, Sharpe AH, et al. T cell activation depends on extracellular alanine. *Cell Rep*. (2019) 28:3011–3021.e4. doi: 10.1016/j.celrep.2019.08.034

41. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity*. (2005) 22:633–42. doi: 10.1016/j.immuni.2005.03.013

42. Domblides C, Lartigue L, Faustin B. Control of the antitumor immune response by cancer metabolism. *Cells*. (2019) 8:104. doi: 10.3390/cells8020104

43. Siska PJ, Kim B, Ji X, Hoeksema MD, Massion PP, Beckermann KE, et al. Fluorescence-based measurement of cystine uptake through xCT shows requirement for ROS detoxification in activated lymphocytes. *J Immunol Methods*. (2016) 438:51–8. doi: 10.1016/j.jim.2016.08.013

44. Weiss TS, Bernhardt G, Buschauer A, Thasler WE, Dolgner D, Zirngibl H, et al. Polyamine levels of human colorectal adenocarcinomas are correlated with tumor stage and grade. *Int J Colorectal Dis*. (2002) 17:381–7. doi: 10.1007/s00384-002-0394-7

45. O'Brien TG, Megosh LC, Gilliard G, Soler AP. Ornithine decarboxylase overexpression is a sufficient condition for tumor promotion in mouse skin. *Cancer Res*. (1997) 57:2630–7.

46. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol*. (2011) 186:3299–303. doi: 10.4049/jimmunol.1003613

47. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med*. (2011) 208:1367–76. doi: 10.1084/jem.20110278

48. Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science*. (2016) 354:481–4. doi: 10.1126/science.aaf6284

49. Quinn WJ3rd, Jiao J, TeSlaa T, Stadanlick J, Wang Z, Wang L, et al. Lactate limits T cell proliferation via the NAD(H) redox state. *Cell Rep*. (2020) 33:108500. doi: 10.1016/j.celrep.2020.108500

50. Angelin A, Gil-de-Gómez L, Dahiya S, Jiao J, Guo L, Levine MH, et al. Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metab*. (2017) 25:1282–1293.e7. doi: 10.1016/j.cmet.2016.12.018

51. Grzes KM, Field CS, Pearce EJ. Treg cells survive and thrive in inhospitable environments. *Cell Metab*. (2017) 25:1213–5. doi: 10.1016/j.cmet.2017.05.012

52. Watson MJ, Vignali PDA, Mullett SJ, Overacre-Delgoffe AE, Peralta RM, Grebinoski S, et al. Metabolic support of tumour-infiltrating regulatory T cells by lactic acid. *Nature*. (2021) 591:645–51. doi: 10.1038/s41586-020-03045-2

53. Field CS, Baixauli F, Kyle RL, Puleston DJ, Cameron AM, Sanin DE, et al. Mitochondrial integrity regulated by lipid metabolism is a cell-intrinsic checkpoint for Treg suppressive function. *Cell Metab*. (2020) 31:422–437.e5. doi: 10.1016/j.cmet.2019.11.021

54. Kobayashi S, Wannakul T, Sekino K, Takahashi Y, Kagawa Y, Miyazaki H, et al. Fatty acid-binding protein 5 limits the generation of Foxp3+ regulatory T cells through regulating plasmacytoid dendritic cell function in the tumor microenvironment. *Int J Cancer*. (2022) 150:152–63. doi: 10.1002/ijc.33777

55. Chen Y, Zhang J, Cui W, Silverstein RL. CD36, a signaling receptor and fatty acid transporter that regulates immune cell metabolism and fate. *J Exp Med*. (2022) 219:e20211314. doi: 10.1084/jem.20211314

56. Xu S, Chaudhary O, Rodríguez-Morales P, Sun X, Chen D, Zappasodi R, et al. Uptake of oxidized lipids by the scavenger receptor CD36 promotes lipid peroxidation and dysfunction in CD8+ T cells in tumors. *Immunity*. (2021) 54:1561–1577.e7. doi: 10.1016/j.immuni.2021.05.003

57. Ma X, Xiao L, Liu L, Ye L, Su P, Bi E, et al. CD36-mediated ferroptosis dampens intratumoral CD8+ T cell effector function and impairs their antitumor ability. *Cell Metab*. (2021) 33:1001–1012.e5. doi: 10.1016/j.cmet.2021.02.015

58. Henson SM, Lanna A, Riddell NE, Franzese O, Macaulay R, Griffiths SJ, et al. p38 signaling inhibits mTORC1-independent autophagy in senescent human CD8$^+$ T cells. *J Clin Invest*. (2014) 124:4004–16. doi: 10.1172/JCI75051

59. Gerriets VA, Kishton RJ, Johnson MO, Cohen S, Siska PJ, Nichols AG, et al. Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat Immunol*. (2016) 17:1459–66. doi: 10.1038/ni.3577

60. Jang GY, Lee JW, Kim YS, Lee SE, Han HD, Hong KJ, et al. Interactions between tumor-derived proteins and Toll-like receptors. *Exp Mol Med*. (2020) 52:1926–35. doi: 10.1038/s12276-020-00540-4

61. Downs-Canner S, Berkey S, Delgoffe GM, Edwards RP, Curiel T, Odunsi K, et al. Suppressive IL-17A+Foxp3+ and ex-Th17 IL-17AnegFoxp3+ Treg cells are a source of tumour-associated Treg cells. *Nat Commun*. (2017) 8:14649. doi: 10.1038/ncomms14649

62. Haas R, Smith J, Rocher-Ros V, Nadkarni S, Montero-Melendez T, D'Acquisto F, et al. Lactate regulates metabolic and pro-inflammatory circuits in control of T cell migration and effector functions. *PloS Biol*. (2015) 13:e1002202. doi: 10.1371/journal.pbio.1002202

63. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, et al. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab*. (2016) 24:657–71. doi: 10.1016/j.cmet.2016.08.011

64. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med*. (2014) 211:781–90. doi: 10.1084/jem.20131916

65. Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, et al. The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity*. (2016) 45:374–88. doi: 10.1016/j.immuni.2016.07.009

66. Cham CM, Driessens G, O'Keefe JP, Gajewski TF. Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+ T cells. *Eur J Immunol*. (2008) 38:2438–50. doi: 10.1002/eji.200838289

67. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol*. (2013) 31:259–83. doi: 10.1146/annurev-immunol-032712-095956

68. Gaggero S, Martinez-Fabregas J, Cozzani A, Fyfe PK, Leprohon M, Yang J, et al. IL-2 is inactivated by the acidic pH environment of tumors enabling engineering of a pH-selective mutein. *Sci Immunol*. (2022) 7:eade5686. doi: 10.1126/sciimmunol.ade5686

69. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity*. (2011) 35:871–82. doi: 10.1016/j.immuni.2011.09.021

70. Klysz D, Tai X, Robert PA, Craveiro M, Cretenet G, Oburoglu L, et al. Glutamine-dependent α-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. *Sci Signal*. (2015) 8:ra97. doi: 10.1126/scisignal.aab2610

71. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun*. (2015) 6:6692. doi: 10.1038/ncomms7692

72. Huang SC, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, et al. Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. *Immunity*. (2016) 45:817–30. doi: 10.1016/j.immuni.2016.09.016

73. O'Neill LA. A metabolic roadblock in inflammatory macrophages. *Cell Rep*. (2016) 17:625–6. doi: 10.1016/j.celrep.2016.09.085

74. Van den Bossche J, Baardman J, Otto NA, van der Velden S, Neele AE, van den Berg SM, et al. Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. *Cell Rep*. (2016) 17:684–96. doi: 10.1016/j.celrep.2016.09.008

75. Namgaladze D, Brüne B. Fatty acid oxidation is dispensable for human macrophage IL-4-induced polarization. *Biochim Biophys Acta*. (2014) 1841:1329–35. doi: 10.1016/j.bbalip.2014.06.007

76. Kes MMG, Van den Bossche J, Griffioen AW, Huijbers EJM. Oncometabolites lactate and succinate drive pro-angiogenic macrophage response in tumors. *Biochim Biophys Acta Rev Cancer*. (2020) 1874:188427. doi: 10.1016/j.bbcan.2020.188427

77. Yang K, Xu J, Fan M, Tu F, Wang X, Ha T, et al. Lactate suppresses macrophage pro-inflammatory response to LPS stimulation by inhibition of YAP and NF-κB activation via GPR81-mediated signaling. *Front Immunol*. (2020) 11:587913. doi: 10.3389/fimmu.2020.587913

78. Sun X, Wang M, Wang M, Yao L, Li X, Dong H, et al. Role of proton-coupled monocarboxylate transporters in cancer: from metabolic crosstalk to therapeutic potential. *Front Cell Dev Biol*. (2020) 8:651. doi: 10.3389/fcell.2020.00651

79. Li B, Yang Q, Li Z, Xu Z, Sun S, Wu Q, et al. Expression of monocarboxylate transporter 1 in immunosuppressive macrophages is associated with the poor prognosis in breast cancer. *Front Oncol*. (2020) 10:574787. doi: 10.3389/fonc.2020.574787

80. Park J, Lee SE, Hur J, Hong EB, Choi JI, Yang JM, et al. M-CSF from cancer cells induces fatty acid synthase and PPARβ/δ Activation in tumor myeloid cells, leading to tumor progression. *Cell Rep*. (2015) 10:1614–25. doi: 10.1016/j.celrep.2015.02.024

81. Di Conza G, Tsai CH, Gallart-Ayala H, Yu YR, Franco F, Zaffalon L, et al. Tumor-induced reshuffling of lipid composition on the endoplasmic reticulum membrane sustains macrophage survival and pro-tumorigenic activity. *Nat Immunol*. (2021) 22:1403–15. doi: 10.1038/s41590-021-01047-4

82. Chen Y, Brandizzi F. IRE1: ER stress sensor and cell fate executor. *Trends Cell Biol*. (2013) 23:547–55. doi: 10.1016/j.tcb.2013.06.005

83. Sriburi R, Jackowski S, Mori K, Brewer JW. XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum. *J Cell Biol*. (2004) 167:35–41. doi: 10.1083/jcb.200406136

84. Kao KC, Vilbois S, Tsai CH, Ho PC. Metabolic communication in the tumour-immune microenvironment. *Nat Cell Biol*. (2022) 24:1574–83. doi: 10.1038/s41556-022-01002-x

85. DeNardo DG, Ruffell B. Macrophages as regulators of tumour immunity and immunotherapy. *Nat Rev Immunol*. (2019) 19:369–82. doi: 10.1038/s41577-019-0127-6

86. Viola A, Bronte V. Metabolic mechanisms of cancer-induced inhibition of immune responses. *Semin Cancer Bio*. (2007) 17:309–16. doi: 10.1016/j.semcancer.2007.06.005

87. Mojsilovic SS, Mojsilovic S, Villar VH, Santibanez JF. The metabolic features of tumor-associated macrophages: opportunities for immunotherapy? *Anal Cell Pathol (Amst)*. (2021) 2021:5523055. doi: 10.1155/2021/5523055

88. Van den Bossche J, Lamers WH, Koehler ES, Geuns JM, Alhonen L, Uimari A, et al. Pivotal Advance: Arginase-1-independent polyamine production stimulates the expression of IL-4-induced alternatively activated macrophage markers while inhibiting LPS-induced expression of inflammatory genes. *J Leukoc Biol*. (2012) 91:685–99. doi: 10.1189/jlb.0911453

89. Meireson A, Devos M, Brochez L. IDO expression in cancer: different compartment, different functionality? *Front Immunol*. (2020) 11:531491. doi: 10.3389/fimmu.2020.531491

90. Zhao Q, Kuang DM, Wu Y, Xiao X, Li XF, Li TJ, et al. Activated CD69+ T cells foster immune privilege by regulating IDO expression in tumor-associated macrophages. *J Immunol*. (2012) 188:1117–24. doi: 10.4049/jimmunol.1100164

91. Rao X, Zhou X, Wang G, Jie X, Xing B, Xu Y, et al. NLRP6 is required for cancer-derived exosome-modified macrophage M2 polarization and promotes metastasis in small cell lung cancer. *Cell Death Dis*. (2022) 13:891. doi: 10.1038/s41419-022-05336-0

92. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. *Nat Rev Cancer*. (2016) 16:431–46. doi: 10.1038/nrc.2016.52

93. Shaul ME, Levy L, Sun J, Mishalian I, Singhal S, Kapoor V, et al. Tumor-associated neutrophils display a distinct N1 profile following TGFβ modulation: A transcriptomics analysis of pro- vs. *antitumor TANs Oncoimmunology*. (2016) 5:e1232221. doi: 10.1080/2162402X.2016.1232221

94. Walmsley SR, Print C, Farahi N, Peyssonnaux C, Johnson RS, Cramer T, et al. Hypoxia-induced neutrophil survival is mediated by HIF-1alpha-dependent NF-kappaB activity. *J Exp Med*. (2005) 201:105–15. doi: 10.1084/jem.20040624

95. Thompson AA, Elks PM, Marriott HM, Eamsamarng S, Higgins KR, Lewis A, et al. Hypoxia-inducible factor 2α regulates key neutrophil functions in humans, mice, and zebrafish. *Blood*. (2014) 123:366–76. doi: 10.1182/blood-2013-05-500207

96. Kumar S, Dikshit M. Metabolic insight of neutrophils in health and disease. *Front Immunol*. (2019) 10:2099. doi: 10.3389/fimmu.2019.02099

97. Britt EC, Lika J, Giese MA, Schoen TJ, Seim GL, Huang Z, et al. Switching to the cyclic pentose phosphate pathway powers the oxidative burst in activated neutrophils. *Nat Metab*. (2022) 4:389–403. doi: 10.1038/s42255-022-00550-8

98. Azevedo EP, Rochael NC, Guimarães-Costa AB, de Souza-Vieira TS, Ganilho J, Saraiva EM, et al. A metabolic shift toward pentose phosphate pathway is necessary for amyloid fibril- and phorbol 12-myristate 13-acetate-induced neutrophil extracellular trap (NET) formation. *J Biol Chem*. (2015) 290:22174–83. doi: 10.1074/jbc.M115.640094

99. Tcyganov E, Mastio J, Chen E, Gabrilovich DI. Plasticity of myeloid-derived suppressor cells in cancer. *Curr Opin Immunol*. (2018) 51:76–82. doi: 10.1016/j.coi.2018.03.009

100. Patel S, Fu S, Mastio J, Dominguez GA, Purohit A, Kossenkov A, et al. Unique pattern of neutrophil migration and function during tumor progression. *Nat Immunol*. (2018) 19:1236–47. doi: 10.1038/s41590-018-0229-5

101. Veglia F, Hashimoto A, Dweep H, Sanseviero E, De Leo A, Tcyganov E, et al. Analysis of classical neutrophils and polymorphonuclear myeloid-derived suppressor cells in cancer patients and tumor-bearing mice. *J Exp Med*. (2021) 218:e20201803. doi: 10.1084/jem.20201803

102. Li BH, Garstka MA, Li ZF. Chemokines and their receptors promoting the recruitment of myeloid-derived suppressor cells into the tumor. *Mol Immunol*. (2020) 117:201–15. doi: 10.1016/j.molimm.2019.11.014

103. Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol*. (2016) 37:208–20. doi: 10.1016/j.it.2016.01.004

104. Liu Y, Lai L, Chen Q, Song Y, Xu S, Ma F, et al. MicroRNA-494 is required for the accumulation and functions of tumor-expanded myeloid-derived suppressor cells via targeting of PTEN. *J Immunol*. (2012) 188:5500–10. doi: 10.4049/jimmunol.1103505

105. Al-Khami AA, Rodriguez PC, Ochoa AC. Metabolic reprogramming of myeloid-derived suppressor cells (MDSC) in cancer. *Oncoimmunology*. (2016) 5:e1200771. doi: 10.1080/2162402X.2016.1200771

106. Hossain F, Al-Khami AA, Wyczechowska D, Hernandez C, Zheng L, Reiss K, et al. Inhibition of fatty acid oxidation modulates immunosuppressive functions of

myeloid-derived suppressor cells and enhances cancer therapies. *Cancer Immunol Res*. (2015) 3:1236–47. doi: 10.1158/2326-6066.CIR-15-0036

107. Hammami I, Chen J, Murschel F, Bronte V, De Crescenzo G, Jolicoeur M. Immunosuppressive activity enhances central carbon metabolism and bioenergetics in myeloid-derived suppressor cells in *vitro* models. *BMC Cell Biol*. (2012) 13:18. doi: 10.1186/1471-2121-13-18

108. Mehta K, Fok J, Miller FR, Koul D, Sahin AA. Prognostic significance of tissue transglutaminase in drug resistant and metastatic breast cancer. *Clin Cancer Res*. (2004) 10:8068–76. doi: 10.1158/1078-0432.CCR-04-1107

109. Hammami I, Chen J, Bronte V, DeCrescenzo G, Jolicoeur M. L-glutamine is a key parameter in the immunosuppression phenomenon. *Biochem Biophys Res Commun*. (2012) 425:724–9. doi: 10.1016/j.bbrc.2012.07.139

110. Veglia F, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, et al. Fatty acid transport protein 2 reprograms neutrophils in cancer. *Nature*. (2019) 569:73–8. doi: 10.1038/s41586-019-1118-2

111. Wellenstein MD, de Visser KE. Fatty acids corrupt neutrophils in cancer. *Cancer Cell*. (2019) 35:827–9. doi: 10.1016/j.ccell.2019.05.007

112. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, et al. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med*. (2010) 207:2439–53. doi: 10.1084/jem.20100587

113. Chang WH, Lai AG. The hypoxic tumour microenvironment: A safe haven for immunosuppressive cells and a therapeutic barrier to overcome. *Cancer Lett*. (2020) 487:34–44. doi: 10.1016/j.canlet.2020.05.011

114. Husain Z, Seth P, Sukhatme VP. Tumor-derived lactate and myeloid-derived suppressor cells: Linking metabolism to cancer immunology. *Oncoimmunology*. (2013) 2:e26383. doi: 10.4161/onci.26383

115. Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *J Immunol*. (2013) 191:1486–95. doi: 10.4049/jimmunol.1202702

116. Yang X, Lu Y, Hang J, Zhang J, Zhang T, Huo Y, et al. Lactate-modulated immunosuppression of myeloid-derived suppressor cells contributes to the radioresistance of pancreatic cancer. *Cancer Immunol Res*. (2020) 8:1440–51. doi: 10.1158/2326-6066.CIR-20-0111

117. O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med*. (2016) 213:15–23. doi: 10.1084/jem.20151570

118. Malinarich F, Duan K, Hamid RA, Bijin A, Lin WX, Poidinger M, et al. High mitochondrial respiration and glycolytic capacity represent a metabolic phenotype of human tolerogenic dendritic cells. *J Immunol*. (2015) 194:5174–86. doi: 10.4049/jimmunol.1303316

119. Herber DL, Cao W, Nefedova Y, Novitskiy SV, Nagaraj S, Tyurin VA, et al. Lipid accumulation and dendritic cell dysfunction in cancer. *Nat Med*. (2010) 16:880–6. doi: 10.1038/nm.2172

120. Zitvogel L, Kroemer G. Targeting dendritic cell metabolism in cancer. *Nat Med*. (2010) 16:858–9. doi: 10.1038/nm0810-858

121. Giovanelli P, Sandoval TA, Cubillos-Ruiz JR. Dendritic cell metabolism and function in tumors. *Trends Immunol*. (2019) 40:699–718. doi: 10.1016/j.it.2019.06.004

122. Kumar V. Adenosine as an endogenous immunoregulator in cancer pathogenesis: where to go? *Purinergic Signal*. (2013) 9:145–65. doi: 10.1007/s11302-012-9349-9

123. Munn DH, Mellor AL. IDO in the tumor microenvironment: inflammation, counter-regulation, and tolerance. *Trends Immunol*. (2016) 37:193–207. doi: 10.1016/j.it.2016.01.002

124. Kumar V. Macrophages: the potent immunoregulatory innate immune cells. In: Bhat KH, editor. *Macrophage activation-Biology and disease*. IntechOpen, Rijeka Ch (2020).

125. Viola A, Munari F, Sánchez-Rodríguez R, Scolaro T, Castegna A. The metabolic signature of macrophage responses. *Front Immunol*. (2019) 10:1462. doi: 10.3389/fimmu.2019.01462

126. Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, et al. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. *J Immunol*. (2006) 176:6752–61. doi: 10.4049/jimmunol.176.11.6752

127. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol*. (2010) 185:3190–8. doi: 10.4049/jimmunol.0903670

128. Manlapat AK, Kahler DJ, Chandler PR, Munn DH, Mellor AL. Cell-autonomous control of interferon type I expression by indoleamine 2,3-dioxygenase in regulatory CD19+ dendritic cells. *Eur J Immunol*. (2007) 37:1064–71. doi: 10.1002/eji.200636690

129. Campesato LF, Budhu S, Tchaicha J, Weng CH, Gigoux M, Cohen IJ, et al. Blockade of the AHR restricts a Treg-macrophage suppressive axis induced by L-Kynurenine. *Nat Commun*. (2020) 11:4011. doi: 10.1038/s41467-020-17750-z

130. Hong Y, Manoharan I, Suryawanshi A, Majumdar T, Angus-Hill ML, Koni PA, et al. β-catenin promotes regulatory T-cell responses in tumors by inducing vitamin A metabolism in dendritic cells. *Cancer Res*. (2015) 75:656–65. doi: 10.1158/0008-5472.CAN-14-2377

131. Marçais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, et al. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. *Nat Immunol*. (2014) 15:749–57. doi: 10.1038/ni.2936

132. Donnelly RP, Loftus RM, Keating SE, Liou KT, Biron CA, Gardiner CM, et al. mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. *J Immunol*. (2014) 193:4477–84. doi: 10.4049/jimmunol.1401558

133. Zaiatz-Bittencourt V, Finlay DK, Gardiner CM. Canonical TGF-β Signaling pathway represses human NK cell metabolism. *J Immunol*. (2018) 200:3934–41. doi: 10.4049/jimmunol.1701461

134. Viel S, Marçais A, Guimaraes FS, Loftus R, Rabilloud J, Grau M, et al. TGF-β inhibits the activation and functions of NK cells by repressing the mTOR pathway. *Sci Signal*. (2016) 9:ra19. doi: 10.1126/scisignal.aad1884

135. Harmon C, Robinson MW, Hand F, Almuaili D, Mentor K, Houlihan DD, et al. Lactate-mediated acidification of tumor microenvironment induces apoptosis of liver-resident NK cells in colorectal liver metastasis. *Cancer Immunol Res*. (2019) 7:335–46. doi: 10.1158/2326-6066.CIR-18-0481

136. Park YJ, Song B, Kim YS, Kim EK, Lee JM, Lee GE, et al. Tumor microenvironmental conversion of natural killer cells into myeloid-derived suppressor cells. *Cancer Res*. (2013) 73:5669–81. doi: 10.1158/0008-5472.CAN-13-0545

137. Gao Y, Souza-Fonseca-Guimaraes F, Bald T, Ng SS, Young A, Ngiow SF, et al. Tumor immunoevasion by the conversion of effector NK cells into type 1 innate lymphoid cells. *Nat Immunol*. (2017) 18:1004–15. doi: 10.1038/ni.3800

138. Simonetta F, Pradier A, Roosnek E. T-bet and eomesodermin in NK cell development, maturation, and function. *Front Immunol*. (2016) 7:241. doi: 10.3389/fimmu.2016.00241

139. Daussy C, Faure F, Mayol K, Viel S, Gasteiger G, Charrier E, et al. T-bet and Eomes instruct the development of two distinct natural killer cell lineages in the liver and in the bone marrow. *J Exp Med*. (2014) 211:563–77. doi: 10.1084/jem.20131560

140. Wang S, Qu Y, Xia P, Chen Y, Zhu X, Zhang J, et al. Transdifferentiation of tumor infiltrating innate lymphoid cells during progression of colorectal cancer. *Cell Res*. (2020) 30:610–22. doi: 10.1038/s41422-020-0312-y

141. Huang Y, Guo L, Qiu J, Chen X, Hu-Li J, Siebenlist U, et al. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells. *Nat Immunol*. (2015) 16:161–9. doi: 10.1038/ni.3078

142. Jou E, Rodriguez-Rodriguez N, Ferreira AF, Jolin HE, Clark PA, Sawmynaden K, et al. An innate IL-25-ILC2-MDSC axis creates a cancer-permissive microenvironment for Apc mutation-driven intestinal tumorigenesis. *Sci Immunol*. (2022) 7:eabn0175. doi: 10.1126/sciimmunol.abn0175

143. Ercolano G, Gomez-Cadena A, Dumauthioz N, Vanoni G, Kreutzfeldt M, Wyss T, et al. PPARγ drives IL-33-dependent ILC2 pro-tumoral functions. *Nat Commun*. (2021) 12:2538. doi: 10.1038/s41467-021-22764-2

144. Karagiannis F, Masouleh SK, Wunderling K, Surendar J, Schmitt V, Kazakov A, et al. Lipid-droplet formation drives pathogenic group 2 innate lymphoid cells in airway inflammation. *Immunity*. (2020) 52:620–634.e6. doi: 10.1016/j.immuni.2020.03.003

145. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab*. (2016) 23:27–47. doi: 10.1016/j.cmet.2015.12.006

146. Li J, Wang R, Zhou X, Wang W, Gao S, Mao Y, et al. Genomic and transcriptomic profiling of carcinogenesis in patients with familial adenomatous polyposis. *Gut*. (2020) 69:1283–93. doi: 10.1136/gutjnl-2019-319438

147. Counihan JL, Grossman EA, Nomura DK. Cancer metabolism: current understanding and therapies. *Chem Rev*. (2018) 118:6893–923. doi: 10.1021/acs.chemrev.7b00775

148. Cortés-Cros M, Hemmerlin C, Ferretti S, Zhang J, Gounarides JS, Yin H, et al. M2 isoform of pyruvate kinase is dispensable for tumor maintenance and growth. *Proc Natl Acad Sci U.S.A*. (2013) 110:489–94. doi: 10.1073/pnas.1212780110

149. Israelsen WJ, Vander Heiden MG. Pyruvate kinase: Function, regulation and role in cancer. *Semin Cell Dev Biol*. (2015) 43:43–51. doi: 10.1016/j.semcdb.2015.08.004

150. Chan EC, Koh PK, Mal M, Cheah PY, Eu KW, Backshall A, et al. Metabolic profiling of human colorectal cancer using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy and gas chromatography mass spectrometry (GC/MS). *J Proteome Res*. (2009) 8:352–61. doi: 10.1021/pr8006232

151. Haber RS, Rathan A, Weiser KR, Pritsker A, Itzkowitz SH, Bodian C, et al. GLUT1 glucose transporter expression in colorectal carcinoma: a marker for poor prognosis. *Cancer*. (1998) 83:34–40. doi: 10.1002/(ISSN)1097-0142

152. Koukourakis MI, Giatromanolaki A, Simopoulos C, Polychronidis A, Sivridis E. Lactate dehydrogenase 5 (LDH5) relates to up-regulated hypoxia inducible factor pathway and metastasis in colorectal cancer. *Clin Exp Metastasis*. (2005) 22:25–30. doi: 10.1007/s10585-005-2343-7

153. Scalise M, Pochini L, Console L, Losso MA, Indiveri C. The human SLC1A5 (ASCT2) amino acid transporter: from function to structure and role in cell biology. *Front Cell Dev Biol*. (2018) 6:96. doi: 10.3389/fcell.2018.00096

154. Kanai Y, Clémençon B, Simonin A, Leuenberger M, Lochner M, Weisstanner M, et al. The SLC1 high-affinity glutamate and neutral amino acid transporter family. *Mol Aspects Med*. (2013) 34:108–20. doi: 10.1016/j.mam.2013.01.001

155. Witte D, Ali N, Carlson N, Younes M. Overexpression of the neutral amino acid transporter ASCT2 in human colorectal adenocarcinoma. *Anticancer Res*. (2002) 22:2555–7.

156. Huang F, Zhao Y, Zhao J, Wu S, Jiang Y, Ma H, et al. Upregulated SLC1A5 promotes cell growth and survival in colorectal cancer. *Int J Clin Exp Pathol*. (2014) 7:6006–14.

157. Shuvalov O, Petukhov A, Daks A, Fedorova O, Vasileva E, Barlev NA. One-carbon metabolism and nucleotide biosynthesis as attractive targets for anticancer therapy. *Oncotarget*. (2017) 8:23955–77. doi: 10.18632/oncotarget.v8i14

158. Maddocks OD, Labuschagne CF, Adams PD, Vousden KH. Serine metabolism supports the methionine cycle and DNA/RNA methylation through *de novo* ATP synthesis in cancer cells. *Mol Cell*. (2016) 61:210–21. doi: 10.1016/j.molcel.2015.12.014

159. Montrose DC, Saha S, Foronda M, McNally EM, Chen J, Zhou XK, et al. Exogenous and endogenous sources of serine contribute to colon cancer metabolism, growth, and resistance to 5-fluorouracil. *Cancer Res*. (2021) 81:2275–88. doi: 10.1158/0008-5472.CAN-20-1541

160. Brandacher G, Perathoner A, Ladurner R, Schneeberger S, Obrist P, Winkler C, et al. Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. *Clin Cancer Res*. (2006) 12:1144–51. doi: 10.1158/1078-0432.CCR-05-1966

161. Newman AC, Maddocks ODK. One-carbon metabolism in cancer. *Br J Cancer*. (2017) 116:1499–504. doi: 10.1038/bjc.2017.118

162. Asante I, Chui D, Pei H, Zhou E, De Giovanni C, Conti D, et al. Alterations in folate-dependent one-carbon metabolism as colon cell transition from normal to cancerous. *J Nutr Biochem*. (2019) 69:1–9. doi: 10.1016/j.jnutbio.2019.02.008

163. Lee GY, Haverty PM, Li L, Kljavin NM, Bourgon R, Lee J, et al. Comparative oncogenomics identifies PSMB4 and SHMT2 as potential cancer driver genes. *Cancer Res*. (2014) 74:3114–26. doi: 10.1158/0008-5472.CAN-13-2683

164. Wei Z, Song J, Wang G, Cui X, Zheng J, Tang Y, et al. Publisher Correction: Deacetylation of serine hydroxymethyl-transferase 2 by SIRT3 promotes colorectal carcinogenesis. *Nat Commun*. (2019) 10:774. doi: 10.1038/s41467-019-08681-5

165. Agarwal S, Behring M, Hale K, Al Diffalha S, Wang K, Manne U, et al. MTHFD1L, A folate cycle enzyme, is involved in progression of colorectal cancer. *Transl Oncol*. (2019) 12:1461–7. doi: 10.1016/j.tranon.2019.07.011

166. Miyo M, Konno M, Colvin H, Nishida N, Koseki J, Kawamoto K, et al. The importance of mitochondrial folate enzymes in human colorectal cancer. *Oncol Rep*. (2017) 37:417–25. doi: 10.3892/or.2016.5264

167. Salgado-García R, Coronel-Hernández J, Delgado-Waldo I, Cantú de León D, García-Castillo V, López-Urrutia E, et al. Negative regulation of ULK1 by microRNA-106a in autophagy induced by a triple drug combination in colorectal cancer cells *in vitro*. *Genes (Basel)*. (2021) 12:245. doi: 10.3390/genes12020245

168. Dwarakanath BS. Cytotoxicity, radiosensitization, and chemosensitization of tumor cells by 2-deoxy-D-glucose in vitro. *J Cancer Res Ther*. (2009) 5:S27–31. doi: 10.4103/0973-1482.55137

169. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest*. (2013) 123:4479–88. doi: 10.1172/JCI69589

170. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature*. (2009) 460:103–7. doi: 10.1038/nature08097

171. Ben Sahra I, Regazzetti C, Robert G, Laurent K, Le Marchand-Brustel Y, Auberger P, et al. Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. *Cancer Res*. (2011) 71:4366–72. doi: 10.1158/0008-5472.CAN-10-1769

172. Blandino G, Valerio M, Cioce M, Mori F, Casadei L, Pulito C, et al. Metformin elicits anticancer effects through the sequential modulation of DICER and c-MYC. *Nat Commun*. (2012) 3:865. doi: 10.1038/ncomms1859

173. Hasan T, Arora R, Bansal AK, Bhattacharya R, Sharma GS, Singh LR. Disturbed homocysteine metabolism is associated with cancer. *Exp Mol Med*. (2019) 51:1–13. doi: 10.1038/s12276-019-0216-4

174. Lis P, Dyląg M, Niedźwiecka K, Ko YH, Pedersen PL, Goffeau A, et al. The HK2 dependent "Warburg effect" and mitochondrial oxidative phosphorylation in cancer: targets for effective therapy with 3-bromopyruvate. *Molecules*. (2016) 21:1730. doi: 10.3390/molecules21121730

175. Di Cosimo S, Ferretti G, Papaldo P, Carlini P, Fabi A, Cognetti F. Lonidamine: efficacy and safety in clinical trials for the treatment of solid tumors. *Drugs Today (Barc)*. (2003) 39:157–74.

176. Thiry A, Dogné JM, Masereel B, Supuran CT. Targeting tumor-associated carbonic anhydrase IX in cancer therapy. *Trends Pharmacol Sci*. (2006) 27:566–73. doi: 10.1016/j.tips.2006.09.002

177. Zhong X, He X, Wang Y, Hu Z, Huang H, Zhao S, et al. Warburg effect in colorectal cancer: the emerging roles in tumor microenvironment and therapeutic implications. *J Hematol Oncol*. (2022) 15:160. doi: 10.1186/s13045-022-01358-5

178. Elenay S, van der Veen AG, Böttcher JP, Snelgrove KJ, Rogers N, Acton SE, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell*. (2015) 162:1257–70. doi: 10.1016/j.cell.2015.08.015

179. Cheng H, Zong L, Kong Y, Wang X, Gu Y, Cang W, et al. Camrelizumab plus apatinib in patients with high-risk chemorefractory or relapsed gestational trophoblastic neoplasia (CAP 01): a single-arm, open-label, phase 2 trial. *Lancet Oncol*. (2021) 22:1609–17. doi: 10.1016/S1470-2045(21)00460-5

180. Dummer R, Lebbé C, Atkinson V, Mandalà M, Nathan P, Arance A, et al. Combined PD-1, BRAF and MEK inhibition in advanced BRAF-mutant melanoma: safety run-in and biomarker cohorts of COMBI-i. *Nat Med*. (2020) 26:1557–63. doi: 10.1038/s41591-020-1082-2

181. Nenkov M, Ma Y, Gaßler N, Chen Y. Metabolic reprogramming of colorectal cancer cells and the microenvironment: implication for therapy. *Int J Mol Sci*. (2021) 22:6262. doi: 10.3390/ijms22126262

182. Murata S, Yanagisawa K, Fukunaga K, Oda T, Kobayashi A, Sasaki R, et al. Fatty acid synthase inhibitor cerulenin suppresses liver metastasis of colon cancer in mice. *Cancer Sci*. (2010) 101:1861–5. doi: 10.1111/j.1349-7006.2010.01596.x

183. Shiragami R, Murata S, Kosugi C, Tezuka T, Yamazaki M, Hirano A, et al. Enhanced antitumor activity of cerulenin combined with oxaliplatin in human colon cancer cells. *Int J Oncol*. (2013) 43:431–8. doi: 10.3892/ijo.2013.1978

184. Pandurangan AK, Esa NM. Luteolin, a bioflavonoid inhibits colorectal cancer through modulation of multiple signaling pathways: a review. *Asian Pac J Cancer Prev*. (2014) 15:5501–8. doi: 10.7314/APJCP.2014.15.14.5501

185. Wang C, Xu C, Sun M, Luo D, Liao DF, Cao D. Acetyl-CoA carboxylase-alpha inhibitor TOFA induces human cancer cell apoptosis. *Biochem Biophys Res Commun*. (2009) 385:302–6. doi: 10.1016/j.bbrc.2009.05.045

186. Liu X, Hartman CL, Li L, Albert CJ, Si F, Gao A, et al. Reprogramming lipid metabolism prevents effector T cell senescence and enhances tumor immunotherapy. *Sci Transl Med*. (2021) 13:eaaz6314. doi: 10.1126/scitranslmed.aaz6314

187. Wan H, Xu B, Zhu N, Ren B. PGC-1α activator-induced fatty acid oxidation in tumor-infiltrating CTLs enhances effects of PD-1 blockade therapy in lung cancer. *Tumori*. (2020) 106:55–63. doi: 10.1177/0300891619868287

188. Chowdhury PS, Chamoto K, Kumar A, Honjo T. PPAR-induced fatty acid oxidation in T cells increases the number of tumor-reactive CD8+ T cells and facilitates anti-PD-1 therapy. *Cancer Immunol Res*. (2018) 6:1375–87. doi: 10.1158/2326-6066.CIR-18-0095

189. Yan W, Qiu L, Yang M, Xu A, Ma M, Yuan Q, et al. CXCL10 mediates CD8+ T cells to facilitate vessel normalization and improve the efficacy of cetuximab combined with PD-1 checkpoint inhibitors in colorectal cancer. *Cancer Lett*. (2023) 567:216263. doi: 10.1016/j.canlet.2023.216263

190. Xin G, Chen Y, Topchyan P, Kasmani MY, Burns R, Volberding PJ, et al. Targeting PIM1-mediated metabolism in myeloid suppressor cells to treat cancer. *Cancer Immunol Res*. (2021) 9:454–69. doi: 10.1158/2326-6066.CIR-20-0433

191. Perła-Kaján J, Jakubowski H. Dysregulation of epigenetic mechanisms of gene expression in the pathologies of hyperhomocysteinemia. *Int J Mol Sci*. (2019) 20:3140. doi: 10.3390/ijms20133140

192. Berstad P, Konstantinova SV, Refsum H, Nurk E, Vollset SE, Tell GS, et al. Dietary fat and plasma total homocysteine concentrations in 2 adult age groups: the Hordaland Homocysteine Study. *Am J Clin Nutr*. (2007) 85:1598–605. doi: 10.1093/ajcn/85.6.1598

193. Fonseca V, Dicker-Brown A, Ranganathan S, Song W, Barnard RJ, Fink L, et al. Effects of a high-fat-sucrose diet on enzymes in homocysteine metabolism in the rat. *Metabolism*. (2000) 49:736–41. doi: 10.1053/meta.2000.6256

194. Wang D, Zhao R, Qu YY, Mei XY, Zhang X, Zhou Q, et al. Colonic lysine homocysteinylation induced by high-fat diet suppresses DNA damage repair. *Cell Rep*. (2018) 25:398–412.e6. doi: 10.1016/j.celrep.2018.09.022

195. Liesenfeld DB, Grapov D, Fahrmann JF, Salou M, Scherer D, Toth R, et al. Metabolomics and transcriptomics identify pathway differences between visceral and subcutaneous adipose tissue in colorectal cancer patients: the ColoCare study. *Am J Clin Nutr*. (2015) 102:433–43. doi: 10.3945/ajcn.114.103804

196. Long Z, Zhou J, Xie K, Wu Z, Yin H, Daria V, et al. Metabolomic markers of colorectal tumor with different clinicopathological features. *Front Oncol*. (2020) 10:981. doi: 10.3389/fonc.2020.00981

197. Carvalho B, Sillars-Hardebol AH, Postma C, Mongera S, Terhaar Sive Droste J, Obulkasim A, et al. Colorectal adenoma to carcinoma progression is accompanied by changes in gene expression associated with ageing, chromosomal instability, and fatty acid metabolism. *Cell Oncol (Dordr)*. (2012) 35:53–63. doi: 10.1007/s13402-011-0065-1

198. Zaytseva Y. Lipid metabolism as a targetable metabolic vulnerability in colorectal cancer. *Cancers (Basel)*. (2021) 13:301. doi: 10.3390/cancers13020301

199. Zhang X, Yao J, Shi H, Gao B, Zhang L. LncRNA TINCR/microRNA-107/CD36 regulates cell proliferation and apoptosis in colorectal cancer via PPAR signaling pathway based on bioinformatics analysis. *Biol Chem*. (2019) 400:663–75. doi: 10.1515/hsz-2018-0236

200. Nath A, Chan C. Genetic alterations in fatty acid transport and metabolism genes are associated with metastatic progression and poor prognosis of human cancers. *Sci Rep*. (2016) 6:18669. doi: 10.1038/srep18669

201. Wang YN, Zeng ZL, Lu J, Wang Y, Liu ZX, He MM, et al. CPT1A-mediated fatty acid oxidation promotes colorectal cancer cell metastasis by inhibiting anoikis. *Oncogene*. (2018) 37:6025–40. doi: 10.1038/s41388-018-0384-z

202. Drury J, Rychahou PG, He D, Jafari N, Wang C, Lee EY, et al. Inhibition of fatty acid synthase upregulates expression of CD36 to sustain proliferation of colorectal cancer cells. *Front Oncol*. (2020) 10:1185. doi: 10.3389/fonc.2020.01185

203. Kawaguchi K, Senga S, Kubota C, Kawamura Y, Ke Y, Fujii H. High expression of Fatty Acid-Binding Protein 5 promotes cell growth and metastatic potential of colorectal cancer cells. *FEBS Open Bio*. (2016) 6:190–9. doi: 10.1002/2211-5463.12031

204. Sánchez-Martínez R, Cruz-Gil S, Gómez de Cedrón M, Álvarez-Fernández M, Vargas T, Molina S, et al. and epithelial-mesenchymal transition provides a target for colon cancer therapy. *Oncotarget*. (2015) 6:38719–36. doi: 10.18632/oncotarget.v6i36

205. Matsushita Y, Nakagawa H, Koike K. Lipid metabolism in oncology: why it matters, how to research, and how to treat. *Cancers (Basel)*. (2021) 13:474. doi: 10.3390/cancers13030474

206. Wen J, Min X, Shen M, Hua Q, Han Y, Zhao L, et al. ACLY facilitates colon cancer cell metastasis by CTNNB1. *J Exp Clin Cancer Res*. (2019) 38:401. doi: 10.1186/s13046-019-1391-9

207. Lu T, Sun L, Wang Z, Zhang Y, He Z, Xu C. Fatty acid synthase enhances colorectal cancer cell proliferation and metastasis via regulating AMPK/mTOR pathway. *Onco Targets Ther*. (2019) 12:3339–47. doi: 10.2147/OTT

208. Murai T. Cholesterol lowering: role in cancer prevention and treatment. *Biol Chem*. (2015) 396:1–11. doi: 10.1515/hsz-2014-0194

209. Ding X, Zhang W, Li S, Yang H. The role of cholesterol metabolism in cancer. *Am J Cancer Res*. (2019) 9:219–27.

210. Aguirre-Portolés C, Feliu J, Reglero G, Ramírez de Molina A. ABCA1 overexpression worsens colorectal cancer prognosis by facilitating tumour growth and caveolin-1-dependent invasiveness, and these effects can be ameliorated using the BET inhibitor apabetalone. *Mol Oncol*. (2018) 12:1735–52.

211. Zhang L, Shay J. Multiple roles of APC and its Therapeutic implications in colorectal cancer. *J Nat Cancer Instit*. (2017) 109. doi: 10.1093/jnci/djw332

212. Cha P, Hwang J, Kwak D, Koh E, Kim K, Choi K. APC loss induces Warburg effect via increased PKM2 transcription in colorectal cancer. *Br J Cancer*. (2021) 124:634–44. doi: 10.1038/s41416-020-01118-7

213. Pate K, Stringari C, Sprowl-Tanio S, Wang K, TeSlaa T, Hoverter N, et al. Wnt signaling directs a metabolic program of glycolysis and angiogenesis in colon cancer. *EMBO J*. (2014) 33:1454–73. doi: 10.15252/embj.201488598

214. He T, Sparks A, Rago C, Hermeking H, Zawel L, da Costa L, et al. Identification of c-MYC as a target of the APC pathway. *Science*. (1998) 281:1509–12. doi: 10.1126/science.281.5382.1509

215. Roche T, Baker J, Yan X, Hiromasa Y, Gong X, Peng T, et al. Distinct regulatory properties of pyruvate dehydrogenase kinase and phosphatase isoforms. *Prog Nucleic Acid Res Mol Biol*. (2001) 70:33–75. doi: 10.1016/S0079-6603(01)70013-X

216. Kaidi A, Williams AC, Paraskeva C. Interaction between beta-catenin and HIF-1 promotes cellular adaptation to hypoxia. *Nat Cell Biol*. (2007) 9:210–7. doi: 10.1038/ncb1534

217. Dong S, Liang S, Cheng Z, Zhang X, Luo L, Li L, et al. ROS/PI3K/Akt and Wnt/β-catenin signalings activate HIF-1α-induced metabolic reprogramming to impart 5-fluorouracil resistance in colorectal cancer. *J Exp Clin Cancer Res CR*. (2022) 41:15. doi: 10.1186/s13046-021-02229-6

218. Li X, Zhou J, Chen Z, Chng W. P53 mutations in colorectal cancer– molecular pathogenesis and pharmacological reactivation. *World J Gastroenterol*. (2015) 21:84–93. doi: 10.3748/wjg.v21.i1.84

219. Brannon A, Vakiani E, Sylvester B, Scott S, McDermott G, Shah R, et al. Comparative sequencing analysis reveals high genomic concordance between matched primary and metastatic colorectal cancer lesions. *Genome Biol*. (2014) 15:454. doi: 10.1186/PREACCEPT-1207406452128377

220. Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. *Can Res*. (2004) 64:2627–33. doi: 10.1158/0008-5472.CAN-03-0846

221. Liu J, Zhang C, Hu W, Feng Z. Tumor suppressor p53 and metabolism. *J Mol Cell Biol*. (2019) 11:284–92. doi: 10.1093/jmcb/mjy070

222. Boidot R, Végran F, Meulle A, Le Breton A, Dessy C, Sonveaux P, et al. Regulation of monocarboxylate transporter MCT1 expression by p53 mediates inward and outward lactate fluxes in tumors. *Can Res*. (2012) 72:939–48. doi: 10.1158/0008-5472.CAN-11-2474

223. Gomes AS, Ramos H, Soares J, Saraiva L. p53 and glucose metabolism: an orchestra to be directed in cancer therapy. *Pharmacol Res*. (2018) 131:75–86. doi: 10.1016/j.phrs.2018.03.015

224. Zhang C, Lin M, Wu R, Wang X, Yang B, Levine AJ, et al. Parkin, a p53 target gene, mediates the role of p53 in glucose metabolism and the Warburg effect. *Proc Natl Acad Sci USA*. (2011) 108:16259–64. doi: 10.1073/pnas.1113884108

225. Liu J, Zhang C, Zhao Y, Yue X, Wu H, Huang S, et al. Parkin targets HIF-1α for ubiquitination and degradation to inhibit breast tumor progression. *Nat Commun*. (2017) 8:1823. doi: 10.1038/s41467-017-01947-w

226. Ho JSL, Ma W, Mao DYL, Benchimol S. p53-Dependent transcriptional repression of c-myc is required for G1 cell cycle arrest. *Mol Cell Biol*. (2005) 25:7423–31. doi: 10.1128/MCB.25.17.7423-7431.2005

227. Li J, Ma X, Chakravarti D, Shalapour S, DePinho R. Genetic and biological hallmarks of colorectal cancer. *Genes Dev*. (2021) 35:787–820. doi: 10.1101/gad.348226.120

228. Spano J, Lagorce C, Atlan D, Milano G, Domont J, Benamouzig R, et al. Impact of EGFR expression on colorectal cancer patient prognosis and survival. *Ann Oncol Off J Eur Soc Med Oncol*. (2005) 16:102–8. doi: 10.1093/annonc/mdi006

229. Hoxhaj G, Manning BD. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. *Nat Rev Cancer*. (2020) 20:74–88. doi: 10.1038/s41568-019-0216-7

230. Szwed A, Kim E, Jacinto E. Regulation and metabolic functions of mTORC1 and mTORC2. *Physiol Rev*. (2021) 101:1371–426. doi: 10.1152/physrev.00026.2020

231. Sun L, Zhang H, Gao P. Metabolic reprogramming and epigenetic modifications on the path to cancer. *Protein Cell*. (2022) 13:877–919. doi: 10.1007/s13238-021-00846-7

232. Zhu X, Xuan Z, Chen J, Li Z, Zheng S, Song P. How DNA methylation affects the Warburg effect. *Int J Biol Sci*. (2020) 16:2029–41. doi: 10.7150/ijbs.45420

233. Thakur C, Chen F. Connections between metabolism and epigenetics in cancers. *Semin Cancer Biol*. (2019) 57:52–8. doi: 10.1016/j.semcancer.2019.06.006

234. Shen C, Xuan B, Yan T, Ma Y, Xu P, Tian X, et al. mA-dependent glycolysis enhances colorectal cancer progression. *Mol Cancer*. (2020) 19:72. doi: 10.1186/s12943-020-01190-w

235. Liu F, Ma F, Wang Y, Hao L, Zeng H, Jia C, et al. PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis. *Nat Cell Biol*. (2017) 19:1358–70. doi: 10.1038/ncb3630

236. Lei Y, Han P, Chen Y, Wang H, Wang S, Wang M, et al. Protein arginine methyltransferase 3 promotes glycolysis and hepatocellular carcinoma growth by enhancing arginine methylation of lactate dehydrogenase A. *Clin Transl Med*. (2022) 12:e686. doi: 10.1002/ctm2.686

237. Ippolito L, Morandi A, Giannoni E, Chiarugi P. Lactate: a metabolic driver in the tumour landscape. *Trends Biochem Sci*. (2019) 44:153–66. doi: 10.1016/j.tibs.2018.10.011

238. Vasaikar S, Huang C, Wang X, Petyuk VA, Savage SR, Wen B, et al. Proteogenomic analysis of human colon cancer reveals new therapeutic opportunities. *Cell*. (2019) 177.

239. Certo M, Tsai C, Pucino V, Ho P, Mauro C. Lactate modulation of immune responses in inflammatory versus tumour microenvironments. *Nat Rev Immunol*. (2021) 21:151–61. doi: 10.1038/s41577-020-0406-2

240. Wang X, Luo X, Chen C, Tang Y, Li L, Mo B, et al. The Ap-2α/Elk-1 axis regulates Sirpα-dependent tumor phagocytosis by tumor-associated macrophages in colorectal cancer. *Signal Transduct Target Ther*. (2020) 5:35. doi: 10.1038/s41392-020-0124-z

241. Li N, Kang Y, Wang L, Huff S, Tang R, Hui H, et al. ALKBH5 regulates anti-PD-1 therapy response by modulating lactate and suppressive immune cell accumulation in tumor microenvironment. *Proc Natl Acad Sci USA*. (2020) 117:20159–70. doi: 10.1073/pnas.1918986117

242. Feng J, Yang H, Zhang Y, Wei H, Zhu Z, Zhu B, et al. Tumor cell-derived lactate induces TAZ-dependent upregulation of PD-L1 through GPR81 in human lung cancer cells. *Oncogene*. (2017) 36:5829–39. doi: 10.1038/onc.2017.188

243. Brown T, Bhattacharjee P, Ramachandran S, Sivaprakasam S, Ristic B, Sikder M, et al. The lactate receptor GPR81 promotes breast cancer growth via a paracrine mechanism involving antigen-presenting cells in the tumor microenvironment. *Oncogene*. (2020) 39:3292–304. doi: 10.1038/s41388-020-1216-5

244. Dai X, Lv X, Thompson E, Ostrikov K. Histone lactylation: epigenetic mark of glycolytic switch. *Trends Genet TIG*. (2022) 38:124–7. doi: 10.1016/j.tig.2021.09.009

245. Xiong J, He J, Zhu J, Pan J, Liao W, Ye H, et al. Lactylation-driven METTL3-mediated RNA mA modification promotes immunosuppression of tumor-infiltrating myeloid cells. *Mol Cell*. (2022). doi: 10.1016/j.molcel.2022.02.033

246. Legitimo A, Consolini R, Failli A, Orsini G, Spisni R. Dendritic cell defects in the colorectal cancer. *Hum Vaccin Immunother*. (2014) 10:3224–35. doi: 10.4161/hv.29857

247. Kusume A, Sasahira T, Luo Y, Isobe M, Nakagawa N, Tatsumoto N, et al. Suppression of dendritic cells by HMGB1 is associated with lymph node metastasis of human colon cancer. *Pathobiology*. (2009) 76:155–62. doi: 10.1159/000218331

248. Moriwaki K, Noda K, Furukawa Y, Ohshima K, Uchiyama A, Nakagawa T, et al. Deficiency of GMDS leads to escape from NK cell-mediated tumor surveillance through modulation of TRAIL signaling. *Gastroenterology*. (2009) 137:188–98, 198.e1-2.

249. Han B, He J, Chen Q, Yuan M, Zeng X, Li Y, et al. ELFN1-AS1 promotes GDF15-mediated immune escape of colorectal cancer from NK cells by facilitating GCN5 and SND1 association. *Discovery Oncol*. (2023) 14:56. doi: 10.1007/s12672-023-00675-6

250. Erdman SE, Poutahidis T. The microbiome modulates the tumor macroenvironment. *OncoImmunology*. (2014) 3:e28271. doi: 10.4161/onci.28271

251. Hanus M, Parada-Venegas D, Landskron G, Wielandt AM, Hurtado C, Alvarez K, et al. Immune system, microbiota, and microbial metabolites: the unresolved triad in colorectal cancer microenvironment. *Front Immunol*. (2021) 12:612826. doi: 10.3389/fimmu.2021.612826

252. Zagato E, Pozzi C, Bertocchi A, Schioppa T, Saccheri F, Guglietta S, et al. Endogenous murine microbiota member Faecalibaculum rodentium and its human homologue protect from intestinal tumour growth. *Nat Microbiol*. (2020) 5:511–24. doi: 10.1038/s41564-019-0649-5

253. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de Los Reyes-Gavilán CG, Salazar N. Intestinal short chain fatty acids and their link with diet and human health. *Front Microbiol*. (2016) 7:185. doi: 10.3389/fmicb.2016.00185

254. Bachem A, Makhlouf C, Binger KJ, de Souza DP, Tull D, Hochheiser K, et al. Microbiota-derived short-chain fatty acids promote the memory potential of antigen-

activated CD8+ T cells. *Immunity*. (2019) 51:285.e5–97.e5. doi: 10.1016/j.immuni.2019.06.002

255. Balmer ML, Ma EH, Bantug GR, Grählert J, Pfister S, Glatter T, et al. CD8 + T cells require increased concentrations of acetate induced by stress for optimal function. *Immunity*. (2016) 44:1312–24. doi: 10.1016/j.immuni.2016.03.016

256. Qiu J, Villa M, Sanin DE, Buck MD, O'Sullivan D, Ching R, et al. Acetate promotes T cell effector function during glucose restriction. *Cell Rep*. (2019) 27:2063–2074.e5. doi: 10.1016/j.celrep.2019.04.022

257. Soda K. The mechanisms by which polyamines accelerate tumor spread. *J Exp Clin Cancer Res*. (2011) 30:95. doi: 10.1186/1756-9966-30-95

258. Latour Y, Gobert AP, Wilson KT. The role of polyamines in the regulation of macrophage polarization and function. *Amino Acids*. (2019) 52:151–60. doi: 10.1007/s00726-019-02719-0

259. Mowat C, Dhatt J, Bhatti I, Hamie A, Baker K. Short chain fatty acids prime colorectal cancer cells to activate antitumor immunity. *Front Immunol*. (2023) 14:1190810. doi: 10.3389/fimmu.2023.1190810

260. Dekker E, Tanis P, Vleugels J, Kasi P, Wallace M. Colorectal cancer. *Lancet*. (2019) 394:1467–80. doi: 10.1016/S0140-6736(19)32319-0

261. Sahin IH, Akce M, Alese O, Shaib W, Lesinski GB, El-Rayes B, et al. Immune checkpoint inhibitors for the treatment of MSI-H/MMR-D colorectal cancer and a perspective on resistance mechanisms. *Br J Cancer*. (2019) 121:809–18. doi: 10.1038/s41416-019-0599-y

262. Kumagai S, Koyama S, Itahashi K, Tanegashima T, Lin Y, Togashi Y, et al. Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumor microenvironments. *Cancer Cell*. (2022) 40:201–18.e9. doi: 10.1016/j.ccell.2022.01.001

263. Awada G, Jansen Y, Schwarze J, Tijtgat J, Hellinckx L, Gondry O, et al. A comprehensive analysis of baseline clinical characteristics and biomarkers associated with outcome in advanced melanoma patients treated with pembrolizumab. *Cancers (Basel)*. (2021) 13:168. doi: 10.3390/cancers13020168

264. Zhang Y, Li J, Wang B, Chen T, Chen Y, Ma W. LDH-A negatively regulates dMMR in colorectal cancer. *Cancer Sci*. (2021) 112:3050–63. doi: 10.1111/cas.15020

265. Reinfeld B, Rathmell W, Kim T, Rathmell J. The therapeutic implications of immunosuppressive tumor aerobic glycolysis. *Cell Mol Immunol*. (2022) 19:46–58. doi: 10.1038/s41423-021-00727-3

266. Renner K, Geiselhöringer A, Fante M, Bruss C, Färber S, Schönhammer G, et al. Metabolic plasticity of human T cells: Preserved cytokine production under glucose deprivation or mitochondrial restriction, but 2-deoxy-glucose affects effector functions. *Eur J Immunol*. (2015) 45:2504–16. doi: 10.1002/eji.201545473

267. Macintyre A, Gerriets V, Nichols A, Michalek R, Rudolph M, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab*. (2014) 20:61–72. doi: 10.1016/j.cmet.2014.05.004

268. Leone R, Powell J. Metabolism of immune cells in cancer. *Nat Rev Cancer*. (2020) 20:516–31. doi: 10.1038/s41568-020-0273-y

269. Shegay PV, Shatova OP, Zabolotneva AA, Shestopalov AV, Kaprin AD. Moonlight functions of glycolytic enzymes in cancer. *Front Mol Biosci*. (2023) 10:1076138. doi: 10.3389/fmolb.2023.1076138

270. Bian X, Jiang H, Meng Y, Li YP, Fang J, Lu Z. Regulation of gene expression by glycolytic and gluconeogenic enzymes. *Trends Cell Biol*. (2022) 32:786–99. doi: 10.1016/j.tcb.2022.02.003

271. Badgley MA, Kremer DM, Maurer HC, DelGiorno KE, Lee HJ, Purohit V, et al. Cysteine depletion induces pancreatic tumor ferroptosis in mice. *Science*. (2020) 368:85–9. doi: 10.1126/science.aaw9872

272. Bell HN, Huber AK, Singhal R, Korimerla N, Rebernick RJ, Kumar R, et al. Microenvironmental ammonia enhances T cell exhaustion in colorectal cancer. *Cell Metab*. (2023) 35:134–149.e6. doi: 10.1016/j.cmet.2022.11.013

273. Liu N, Zhang R, Shi Q, Jiang H, Zhou Q. Intelligent delivery system targeting PD-1/PD-L1 pathway for cancer immunotherapy. *Bioorg Chem*. (2023) 136:106550. doi: 10.1016/j.bioorg.2023.106550

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 08 August 2024
DOI 10.3389/fphar.2024.1422281

OPEN ACCESS

EDITED BY
Valentina Audrito,
Università del Piemonte Orientale, Italy

REVIEWED BY
Joanna Kopecka,
University of Turin, Italy
Alessandra Fiore,
University of Verona, Italy
Huakan Zhao,
Chongqing University Cancer Hospital, China

*CORRESPONDENCE
S. Peppicelli,
silvia.peppicelli@unifi.it
F. Bianchini,
francesca.bianchini@unifi.it

RECEIVED 23 April 2024
ACCEPTED 25 July 2024
PUBLISHED 08 August 2024

CITATION
Peppicelli S, Kersikla T, Menegazzi G, Andreucci E, Ruzzolini J, Nediani C, Bianchini F and Calorini L (2024) The critical role of glutamine and fatty acids in the metabolic reprogramming of *anoikis*-resistant melanoma cells.
Front. Pharmacol. 15:1422281.
doi: 10.3389/fphar.2024.1422281

The critical role of glutamine and fatty acids in the metabolic reprogramming of *anoikis*-resistant melanoma cells

S. Peppicelli*, T. Kersikla, G. Menegazzi, E. Andreucci, J. Ruzzolini, C. Nediani, F. Bianchini* and L. Calorini

Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy

Introduction: Circulating tumor cells (CTCs) represent the sub-population of cells shed into the vasculature and able to survive in the bloodstream, adhere to target vascular endothelial cells, and re-growth into the distant organ. CTCs have been found in the blood of most solid tumor-bearing patients and are used as a diagnostic marker. Although a complex genotypic and phenotypic signature characterizes CTCs, the ability to survive in suspension constitutes the most critical property, known as resistance to *anoikis*, e.g., the ability to resist apoptosis resulting from a loss of substrate adhesion. Here, we selected melanoma cells resistant to *anoikis*, and we studied their metabolic reprogramming, with the aim of identifying new metabolic targets of CTCs.

Methods: Subpopulations of melanoma cells expressing a high *anoikis*-resistant phenotype were selected by three consecutive rocking exposures in suspension and studied for their phenotypic and metabolic characteristics. Moreover, we tested the efficacy of different metabolic inhibitors targeting glycolysis (2DG), LDHA (LDHA-in-3), the mitochondrial electron transport chain complex I (rotenone), glutaminase (BPTES), fatty acid transporter (SSO), fatty acid synthase (denifanstat), CPT1 (etomoxir), to inhibit cell survival and colony formation ability after 24 h of rocking condition.

Results: *Anoikis*-resistant cells displayed higher ability to grow in suspension on agarose-covered dishes respect to control cells, and higher cell viability and colony formation capability after a further step in rocking condition. They showed also an epithelial-to-mesenchymal transition associated with high invasiveness and a stemness-like phenotype. *Anoikis*-resistant melanoma cells in suspension showed a metabolic reprogramming from a characteristic glycolytic metabolism toward a more oxidative metabolism based on the use of glutamine and fatty acids, while re-adhesion on the dishes reversed the metabolism to glycolysis. The treatment with metabolic inhibitors highlighted the effectiveness of rotenone, BPTES, SSO, and etomoxir in reducing the viability and the colony formation ability of cells capable of surviving in suspension, confirming the dependence of their metabolism on oxidative phosphorylation, using glutamine and fatty acids as the most important fuels.

Discussion: This finding opens up new therapeutic strategies based on metabolic inhibitors of glutaminase and fatty acid oxidation for the treatment of CTCs and melanoma metastases.

KEYWORDS

***anoikis* resistance, cell metabolism, circulating tumor cells, melanoma, therapy**

1 Introduction

Metastasis, the major cause of cancer mortality, represents the end of a multi-step process that includes detachment of cancer cells from the primary tumor, survival in the body's vascular system, arrest, and proliferation in distant organs. Once tumor cells reach the circulatory system (circulating tumor cells, CTCs) they are exposed to several stresses and, in order to survive and metastasize, they need to overcome the loss of adhesion, which typically induces a type of programmed cell death known as *anoikis*. *Anoikis*, from the Greek word "homelessness," is an important mechanism for maintaining tissue homeostasis, preventing adherent-independent cell growth or attachment to an inappropriate matrix (Taddei et al., 2012). It follows that tumor cells, which need to survive after detaching themselves from the primary tumor and gain lymphatic or blood circulation, must necessarily acquire the ability to resist *anoikis*. Only tumor cells that acquire this anchorage-independent survival mechanism, can complete the metastatic cascade; thus, resistance to *anoikis* can be considered a requirement for metastasizing CTCs (Paoli et al., 2013; Khan et al., 2022). *Anoikis* resistance is under the control of many different factors, such as cell adhesion molecules, growth factors, oxidative stress, signaling pathways, and biochemical and molecular alterations within the cell milieu (Adeshakin et al., 2021). Several studies have highlighted the link between *anoikis* resistance and the epithelial-to-mesenchymal transition (EMT) (Cao et al., 2016), a process in which epithelial cells remodel the cytoskeleton, detach from neighboring cells, and become mobile, invasive, and resistant to apoptotic stimuli (Brabletz et al., 2018). EMT plays a critical role in tumor metastasis and is the most investigated phenotypic characteristic of CTCs (Lawrence et al., 2023). In recent years, accumulating evidence supports the correlation between the metabolic adaptation that cancer cells undergo after ECM detachment and their ECM-independent survival (Mason et al., 2017; Hawk and Schafer, 2018; Endo et al., 2020), thus making the metabolic regulation of *anoikis* a topic of great interest in cancer research.

Metabolic reprogramming is a hallmark of cancer essential for cancer cells to adapt to the dynamic nutritional conditions of the tumor microenvironment during cancer progression (Faubert et al., 2020). Since the first disclosure in the 1920s by the physiologist Otto Warburg, who observed that cancer cells prefer glycolysis rather than mitochondrial oxidative phosphorylation (OXPHOS) even under sufficient oxygen supply (Warburg effect), several recent studies have demonstrated that malignant cells exhibit metabolic plasticity switching between glycolysis and OXPHOS, and the opposite, depending on microenvironmental conditions (Jose et al., 2011; Shuvalov et al., 2021). As this metabolic conversion depends on the bioenergetic and biosynthetic demands and on the redox homeostasis required to sustain specific behavior of tumor cells (such as proliferation, invasiveness, and survival) (DeBerardinis and Chandel, 2016), it is expected that viable CTCs could be characterized by a metabolic phenotype different from that of proliferating tumor cells of the primary tumor mass.

Although some recent studies have highlighted the importance of metabolic deregulation to allow CTC to bypass *anoikis*, it is still unclear the metabolic adaptation on which surviving detached tumor cells depend.

Our study aims to disclose the metabolic reprogramming of *anoikis*-resistant melanoma cells to identify novel therapeutic approaches to eradicate CTCs and prevent metastasis.

Here, we demonstrate that *anoikis*-resistant melanoma subpopulations, obtained after three consecutive rocking exposures in suspension, show a mesenchymal phenotype with high invasive ability, associated with a metabolic reprogramming from a characteristic glycolytic metabolism toward a more oxidative metabolism based on the use of glutamine and fatty acids. Our results also showed that the inhibition of glutaminase, fatty acid transporters, or beta-oxidation during the rocking condition in suspension was effective in reducing the viability and colony-efficiency of our *anoikis*-resistant melanoma cells, opening up new possibilities for the treatment of CTCs and to counteract melanoma dissemination.

2 Materials and methods

2.1 Cell lines and culture conditions

In this study, we used the melanoma cell lines A375M6, isolated in our laboratory as described in our previous work (Ruzzolini et al., 2017), SKMel2 obtained from ATCC, SKMel28 kindly provided by Dr Laura Poliseno (CNR, Pisa, Italy).

In some experiments, we also used the adenocarcinomic human alveolar basal epithelial A549 and the pancreatic cancer cells PANC1 (kindly provided by Dr. Anna Laurenzana, University of Florence, Italy). Melanoma cells were cultivated in Dulbecco's Modified Eagle Medium high glucose (DMEM 4500, EuroClone, MI, Italy) supplemented with 10% foetal bovine serum (FBS, Boehringer Mannheim, Germany), at 37°C in a humidified atmosphere containing 90% air and 5% CO_2. A549 and PANC1 cells were cultivated in RPMI-1640 medium (EuroClone) supplemented with 10% FBS, and 2 mM glutamine (EuroClone), at 37°C in a humidified atmosphere containing 90% air and 10% CO_2. Cells were harvested from subconfluent cultures by incubation with a trypsin-EDTA solution (EuroClone) and propagated every 3 days. The viability of the cells was determined by Trypan blue exclusion test. Cultures were periodically monitored for mycoplasma contamination.

To select melanoma cells with a well-consolidated *anoikis*-resistant phenotype, A375M6, SkMel28, and SkMel2 melanoma

cells were exposed several times to a loss of adherence condition, as shown in Supplementary Figure 1. In particular, 5×10^4 melanoma cells suspended in a growth factor-free Dulbecco's D-MEM Nutrient mix F12 medium (DME/F12-HEPES EuroClone) were placed in sterile non-adherent 50 mL tubes, that were shacked on a Mini rocker platform shaker (20°angle) (Biosan, Riga, Latvia) for 24 h, at 37°C (Peppicelli et al., 2019). Cells were recovered and placed on an adherent plastic dish to grow until reaching an adequate number to be used to proceed with further rocking exposure. At the end of the third rocking exposure (p2-Suspension, p2-S), recovered cells were grown in an adherent plastic dish to obtain the p3-Adhesion population.

2.2 *Anoikis* assay

To simulate anchorage-independent growth conditions, we cultured melanoma cells in tubes in rocking conditions (Peppicelli et al., 2019). 5×10^4 cells were left rocking in tubes on the Mini Rocker Shaker (Biosan, Riga, Latvia), at room temperature in Dulbecco's D-MEM Nutrient mix F12 (DME/F12-HEPES EuroClone) for 24 h. In some experiments, during the rocking period, cells were treated with 2-Deoxy-D-glucose (2DG), a glucose analog that inhibits glycolysis (D8375, Merk); LDHA-IN-3, a potent lactate dehydrogenase A inhibitor (HY-139319, MedchemExpress, Stockholm, Sweden); rotenone, a mitochondrial electron transport chain complex I inhibitor (557,368, Sigma-Aldrich, Milan, Italy); BPTES, a selective glutaminase inhibitor (HY-12683, MedchemExpress), Denifanstat, a Fatty Acid Synthase (FASN) inhibitor (HY-112829, MedchemExpress); Etomoxir, an irreversible inhibitor of carnitine palmitoyltransferase 1a (CPT-1a), inhibiting fatty acid oxidation (FAO) (HY-50202, MedchemExpress); Sulfo-N-succinimidyl Oleate, a long chain fatty acid that inhibits fatty acid transport into cells (SML2148, Sigma-Aldrich). After 24 h of rocking, cells were used for Western blot analysis, invasion assay, live and dead assay, or colony formation assay.

To test *anoikis* resistance melanoma cells were cultured in dishes coated with agarose. Culture dishes were coated with 1.5% agarose (Promega, San Luis Obispo, California). 5×10^4 cells were plated in agarose-coated petri dishes in complete medium and after 3 or 7 days, the cells were photographed.

2.3 Annexin V/PI flow cytometer analysis

Apoptosis was measured by flow cytometry, using the APC-conjugated Annexin V staining. Cells incubated in rocking condition for 24 h were collected, washed with PBS, and resuspended in 100 µL of 1x Annexin-binding buffer (100 mM HEPES, 140 mM NaCl, 25 mM CaCl2, pH 7.4), with 3 µL of Annexin V APC-conjugated (ImmunoTools, Friesoythe, Germany) and 1 µL of 100 µg/mL propidium iodide (PI, P4864, Sigma-Aldrich) working solution. After 15 min of incubation at 4°C in the dark condition 400 µL of 1X Annexin Binding Buffer was added to each sample and cells were analyzed by flow cytometry (BD-FACS Canto) to find out the viability (annexin V-and PI $^-$, Q3), early apoptosis (annexin V $^+$ and PI $^-$, Q4), or late apoptosis (annexin V $^+$ and PI $^+$, Q2). A minimum of 5,000 events were collected.

2.4 Colony formation assay

After 24 h in rocking conditions, 5×10^3 cells were transferred to a culture dish in fresh medium and incubated for 10 days at 37°C. The colonies were washed with PBS, fixed in cold methanol, and stained using a Diff Quik kit (BD Biosciences, distributed by DBA, Milan, Italy). The stained colonies were photographed with a digital camera and the number of colonies in each well was counted.

2.5 Western blotting analysis

Cells were washed with ice-cold PBS containing 1 mM Na4VO3 and lysed in 100 μL of cell RIPA lysis buffer (Merck Millipore, Vimodrone, MI, Italy) containing PMSF (Sigma-Aldrich), sodium orthovanadate (Sigma-Aldrich), and protease inhibitor cocktail (Merck Millipore).

The protein concentration was measured using Bradford reagent (Merck Millipore), and aliquots of supernatants containing equal amounts of protein were separated in Laemmli buffer on Bolt Bis-Tris Plus gels 4%–12% precast polyacrylamide gels (Life Technologies, Monza, Italy). Fractionated proteins were transferred from the gel to a polyvinylidene difluoride (PVDF) membrane using the iBlot 2 System (Thermo Fischer Scientific, Milan, Italy). Blots were blocked for 5 min, at room temperature, with the EveryBlot Blocking Buffer (BioRad). Subsequently, the membrane was probed at 4°C overnight with primary antibodies diluted in a solution of 1:1 Immobilon® Block – FL (Merck Millipore)/T-PBS buffer. The primary antibodies were as follows: rabbit anti-GLUT-3, rabbit anti-HK2, rabbit anti-PKM2, rabbit anti-FASN, rabbit anti-ACSL, mouse anti-vinculin (1:1,000 Cell Signalling Technology, Danvers, MA, United States), mouse anti-N-Cadherin (1:1,000 DAKO Agilent, Milan, Italy), rabbit anti-IKB alpha (1:1,000 Abcam, Cambridge, United Kingdom), rabbit anti-MCT1, mouse anti-LDHB (Santa Cruz Biotechnology), mouse anti-tubulin antibody (1:2,000, GeneTex, Alton Pkwy Irvine, CA, United States). The membrane was washed in T-PBS buffer, incubated for 1 h with goat anti-rabbit IgG Alexa Fluor 750 antibody or with goat anti-mouse IgG Alexa Fluor 680 antibody (Invitrogen, Monza, Italy), and then visualized by an Odyssey Infrared Imaging System (LI-COR Bioscience). Mouse anti-tubulin or mouse anti-vinculin antibodies were used to assess an equal amount of protein loaded in each lane.

2.6 Quantitative real-time PCR

Total RNA was extracted from cells using Tri Reagent (Cat. No. T9424, Sigma-Aldrich), agarose gel checked for integrity, and 1 µg of total RNA was reverse transcribed with iScript cDNA Synthesis Kit (Cat. No. 1708891, BioRad) according to the manufacturer's instructions. Quantitative real-time PCR (qPCR) was performed using the Sso Advanced Universal SYBR Green

TABLE 1 List of primers used for quantitative real-time PCR.

Gene	Primer FW	Primer REV
18s	5′-cgccgctagaggtgaaattct-3′	5′-cgaacctccga ctttcgttct-3′
ACC1	5′-ttcactccaccttgtcagcgga-3′	5′-gtcagagaagcagcccatcact-3′
ACC2	5′-gacgagctgatctccatcctca-3′	5′-atggactccacctggttatgcc-3′
ALDH1A1	5′-ggtggctggcaagatcgt-3′	5′-ccaaggcgggcaaagag-3′
ASCT2	5′- ggtggctggcaagatcgt -3′	5′- ccaaggcgggcaaagag -3′
β-actin	5′-tcgagccataaaaggcaact-3′	5′-cttcctcaatctcgctctcg-3′
CytC	5′-ttgcacttacaccggtacttaagc-3′	5′-acgtccccactctctaagtccaa-3′
COX5B	5′-tgcgctccatggcatct-3′	5′-cccagtcgcctgctcttc-3′
CPT1	5′-gatcctggacaatacctcggag-3′	5′-ctccacagcatcaagagactgc-3′
CPT2	5′-gcagatgatggttgagtgctcc-3′	5′-agatgccgcagagcaaacaagtg-3′
FATP1	5′-tgacagtcgtcctccgcaagaa-3′	5′-cttcagcaggtagcggcagatc-3′
FATP5	5′-ggaagtctacggctccacagaa-3′	5′-gtcgaactgcaccagctcaaag-3′
GLUT1	5′-cgggccaagagtgtg ctaaa-3′	5′-tgacgataccggagccaatg-3′
GLUT3	5′-cgaacttcctagtcggattg-3′	5′-aggaggcacgacttagacat-3′
GLS1	5′-tgctacctgtctccatggctt-3′	5′- cttagatggcacctcctttgg -3′
GLS2	5′-tgcctatagtggcgatgtctca-3′	5′-gttccatatccaggctgacaa-3′
HK2	5′-caaagtgacagtgggtgtgg-3′	5′-gccaggtccttcactgtctc-3′
KFL4	5′-gcagccacctggcgagtctg-3′	5′-ccgccagcggttattcgggg-3′
LDHA	5′-agcccgattccgttacct-3′	5′-caccagcaacattcattcca-3′
LDHB	5′-ctagatttcgctaccttat-3′	5′-tcattgtcagttcccatt-3′
MCT1	5′-ccaagacctcgtgttgagacc-3′	5′-aatacagctcaggtctccttgg-3′
N-Caderina	5′-cactgctcaggacccagat-3′	5′-taagccgagtgatggtcc-3′
OCT3/4	5′-ttttggtaccccaggctatg-3′	5′-gcaggcacctcagtttgaat-3′
PKM2	5′-cagaggctgccatctaccac-3′	5′-ccagacttggtgaggacgat-3′
Snail1	5′-cccagtgcctcgaccactat-3′	5′-ccagatgagcattggcag-3′
SOX2	5′-gagctttgcaggaagtttgc-3′	5′-gcaagaagcctctccttgaa-3′
Vimentina	5′-tgtccaaatcgatgtggatgtttc-3′	5′-ttgtaccattcttctgcctcctg-3′
Zeb1	5′-cgccgctagaggtgaaattct-3′	5′-cgaacctccga ctttcgttct-3′

Supermix (Cat. No. 1725274, BioRad). The qPCR analysis was carried out in triplicate with a CFX96 Real-Time PCR System (BioRad) with the default PCR setting: 40 cycles of 95°for 10 s and 60°C for 30 s. The fold change was determined by the comparative Ct method using 18S and β-actin as reference genes. Primer sequences are reported in Table 1.

2.7 Invasion assay

Invasiveness of melanoma cells was determined *in vitro* on Matrigel (BD Biosciences) precoated polycarbonate filters, with 8 μm pore size, 6.5 mm diameter, 12.5 μg Matrigel/filter, mounted in Boyden's chambers. 1.5×10^5 cells (200 μL) were seeded in their growth medium in the upper compartment and incubated for 6 h at 37°C in 10% CO_2 in air. In the lower chamber, a complete medium was added as a chemoattractant. After incubation, the inserts were removed and the non-invading cells on the upper surface were wiped off mechanically with a cotton swab and the membranes were fixed overnight in ice-cold methanol. Cells on the lower side of the membranes were then stained using the Diff-Quick kit (BD Biosciences) and photographs of randomly chosen fields were taken.

2.8 Live and dead assay

The LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit (L34964, Invitrogen) was used to determine the viability of cells incubated for 24 h in rocking conditions or treated with inhibitors. Briefly, 0.5 µL of diluted stain was added to 0.5 mL PBS in a flow cytometer tube containing 1×10^5 cells. Cells and stains were mixed and incubated for 30 min at room temperature. Cells were washed and analyzed on a flow cytometer.

2.9 Cell cycle analysis

Cell cycle distribution was analyzed by the DNA content using PI staining method. Cells, incubated for 24 h in rocking condition, were washed 2 times in PBS by centrifugation and stained with a mixture of 50 µg/mL propidium iodide (PI, P4864, Sigma-Aldrich), 20 µg/mL RNase A, 1 mg/mL trisodium citrate and 0.3% (v/v) Triton X-100 in the dark at room temperature for 30 min. The stained cells were analyzed by flow cytometry (BD-FACS Canto) using red propidium-DNA fluorescence.

2.10 Extracellular lactate measurement

For extracellular L-lactate determination, the supernatants of cells grown in adhesion or suspension were collected and centrifuged at 10,000 rpm for 5 min to remove insoluble particles. All sample preparation steps were performed at 4°C and samples were stored at −80°C until analysis. L-lactate concentrations were measured using the Lactate assay kit (colorimetric) (MET-5012, Cell Biolabs, Inc., San Diego, CA, United States) following the manufacturer's instructions. The plate has been read with a spectrophotometric microplate reader at 540 nm.

2.11 Seahorse metabolic flux analyses

Seahorse analysis has been performed as previously described (Ruzzolini et al., 2020) using the Seahorse XF96 Extracellular Flux Analyzer (Seahorse Bioscience, Billerica, MA, United States). 3×10^4 cells were seeded in XF96 Seahorse® microplates precoated with poly-D-lysine (ThermoFisher Scientific, Waltham, MA, United States) in XF Assay Medium (Agilent Technologies, Santa Clara, CA, United States) supplemented with 2 mM glutamine. Mitochondrial stress tests (kit from Agilent Technologies), were performed to

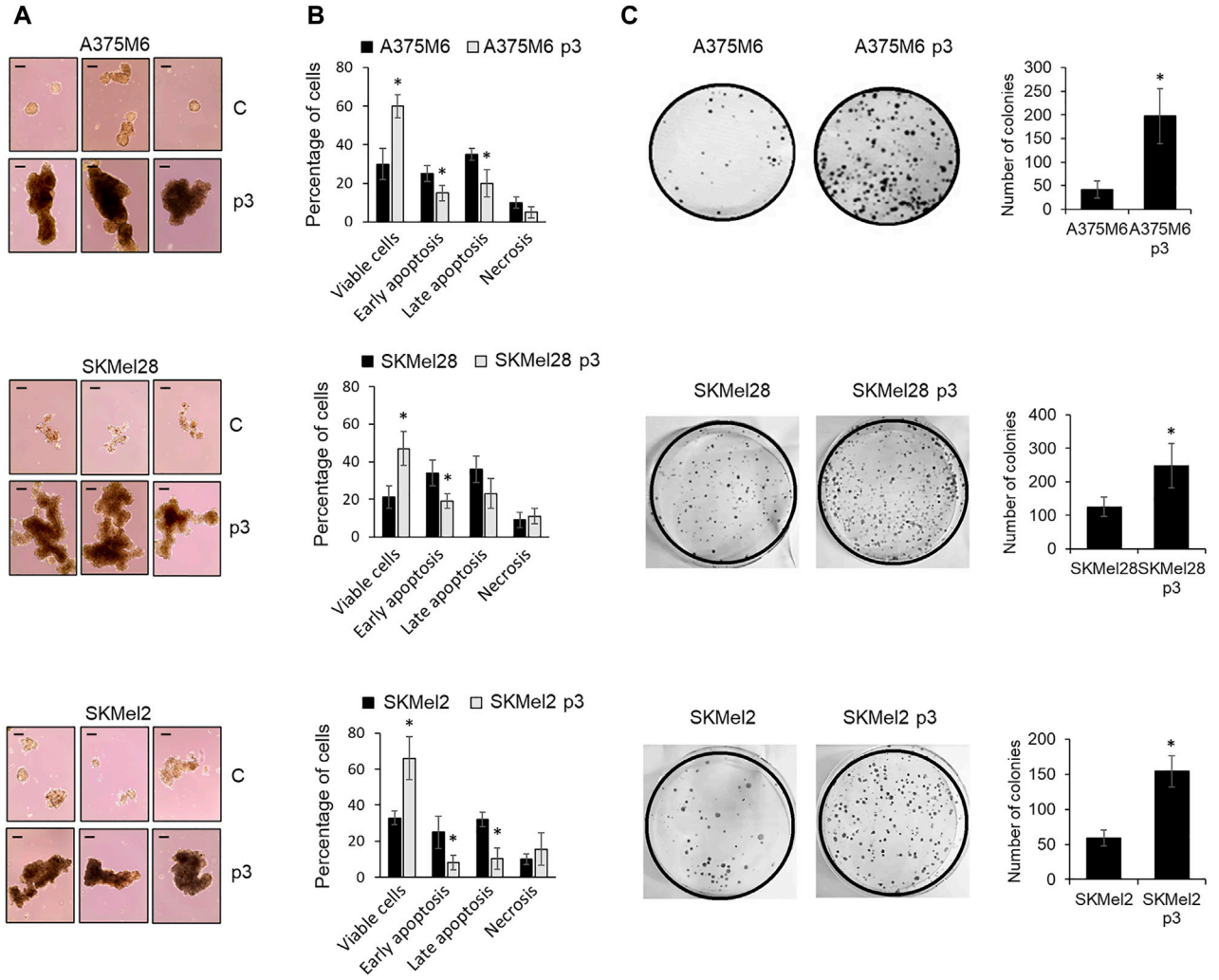


FIGURE 1
Evaluation of the *anoikis* resistance of p3 melanoma cells. **(A)** Three representative images of A375M6, SkMel28, SkMel2 melanoma cells (control vs. p3) allowed to growth for 7 days on agarose-coated dishes. Scale bar: 200 μm. **(B)** Flow cytometry analysis of cells apoptosis after staining with annexin V-APC/propidium iodide (PI), and **(C)** representative images of clonogenic efficiency (left) and their respective quantification expressed as the number of colonies (right) of melanoma cells (control vs. p3) after exposure to 24 h of rocking condition. *$P < 0.05$ compared with control cells.

determine real-time Oxygen Consumption Rate (OCR), while glycolytic rate assay tests (kit from Agilent Technologies) were performed to determine proton efflux rate in melanoma cells grown in adhesion or p3 cells after 24 h in rocking conditions. For the mitochondrial stress test inhibitors were used at the final concentrations of 1 μM oligomycin, 0.5 μM FCCP, and 0.5 μM rotenone/antimycin A. For the glycolytic rate test, Rotenon/antimycin A were used at 0.5 μM, 2DG at 50 mM. Substrate oxidation stress tests were performed to determine the specific substrates oxidated by cells grown in adherent conditions or by p3 cells after 24 h in rocking conditions. We considered the three primary substrates that fuel the mitochondria: long-chain fatty acids, glucose/pyruvate, and/or glutamine. The final concentrations of inhibitors were 3 μM BPTES, 4 μM etomoxir, and 2 μM UK5099. Normalization to protein content was performed after each experiment. The Seahorse XF Report Generator automatically calculated the parameters from Wave data that have been exported to Excel.

2.12 Statistic analysis

Densitometric data are expressed as means ± standard errors of the mean (SEM) depicted by vertical bars from representative experiment of at least three independent experiments.

Statistical analysis of the data was performed by Student's t test or two-way ANOVA (when more than two samples were compared). Values of $p \leq 0.05$ were considered statistically significant.

3 Results

3.1 Metabolic reprogramming of *anoikis*-resistant melanoma cells

To select melanoma cells with a well-consolidated *anoikis*-resistant phenotype, A375M6, SkMel28, and SkMel2 melanoma

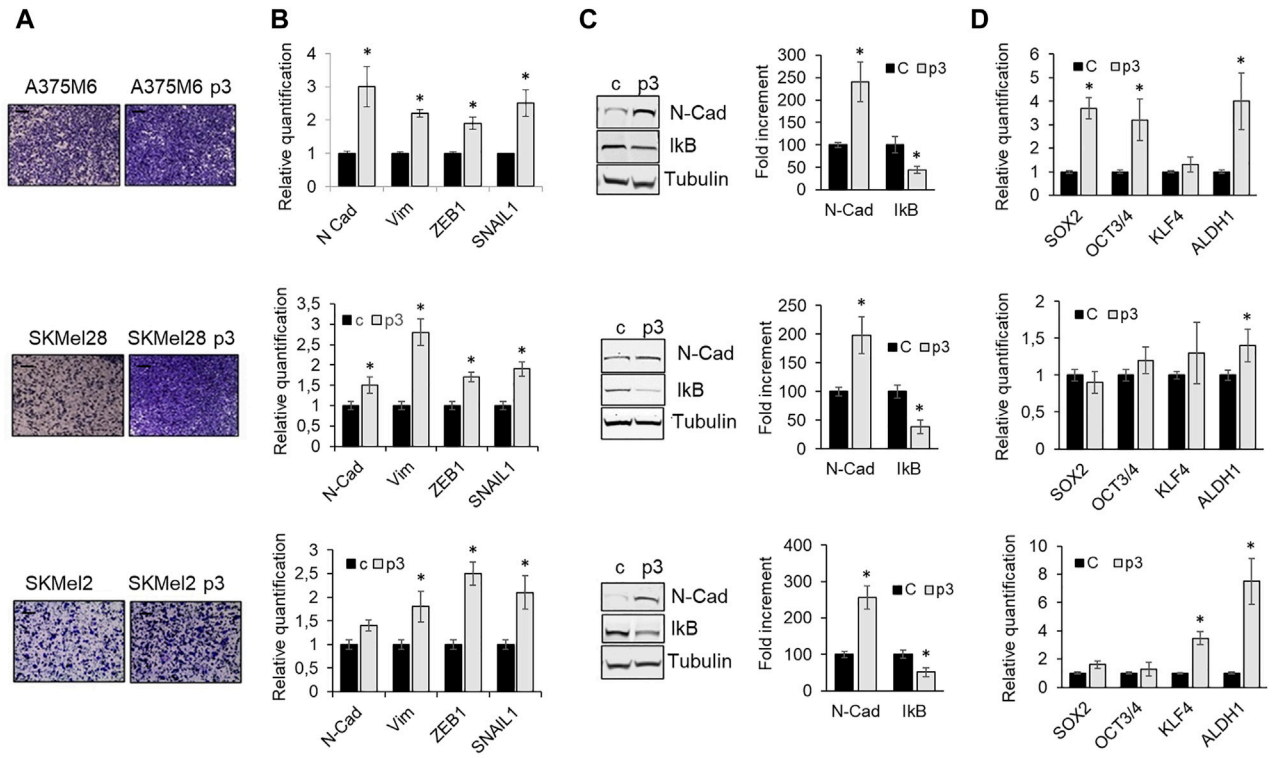


FIGURE 2
Phenotype of p3 melanoma cells. A375M6, SkMel28, SkMel2 melanoma cells were grown in standard conditions or selected by three consecutive rocking exposures in suspension (A375M6 p3, SkMel28 p3, SkMel2 p3). **(A)** Representative images of invasiveness through Matrigel-coated filters of A375M6, SkMel28, SkMel2 melanoma cells (control vs. p3). Scale bar: 200 μm. **(B)** mRNA expression evaluated by quantitative real-time PCR of the EMT markers N-cadherin, Vimentin, ZEB1 and SNAIL1 of A375M6, SkMel28, SkMel2 melanoma cells (control vs. p3). **(C)** Western blot analysis of N-Cadherin and IkB of A375M6, SkMel28, SkMel2 melanoma cells (control vs. p3). Levels of N-Cadherin and IkB were quantified by densitometric analysis and a corresponding histogram was constructed as relative to α-tubulin. Representative Western blot panels on the left. **(D)** mRNA expression evaluated by quantitative real-time PCR of the stemness markers SOX2, OCT3/4, KFL4 and ALDH1A1. Values presented are mean ± SEM of three independent experiments *$P < 0.05$ compared with control cells.

cells were exposed three times to a loss of adherence condition, as described in the "Material and Methods" section and shown in Supplementary Figure 1. At the end of the third rocking exposure (p2-Suspension, p2-S), recovered cells were grown in an adherent plastic dish (p3-Adhesion) and then tested for *anoikis* resistance, culturing cells on agarose-coated dishes. Agarose was used as a support to prevent cell adhesion, to mimic cell growth in suspension. Control cells or p3-Adh population distributed on agarose-coated dishes were grown for 7 days in a complete medium and the number of tumor cell aggregates was evaluated after 7 days. Figure 1A shows the higher ability of the p3 population respect to control cells to give rise to cell aggregates with a bigger diameter. To further prove the greater ability to resist *anoikis* acquired by the p3 population compared to the starting population, we also placed the cells again in a rocking condition for 24 h and then we evaluated cell viability, apoptosis induction, and colony-forming ability. The selected populations of melanoma cells (A375M6 p3, SKMel28 p3, SKMel2 p3) showed higher cell viability evaluated by live/dead cell staining (Supplementary Figure 2), a lower percentage of apoptotic cells (Figure 1B; representative Annexin V/PI plots are shown in Supplementary Figure S3) and higher colony efficiency respect to control cells (Figure 1C).

Since *anoikis* evasion is often associated with the acquisition of invasive and metastatic properties driven by EMT (Cao et al., 2016), we evaluated cell invasiveness and the expression of some EMT markers, including the expression of IkB, the inhibitor of the nuclear factor-κB (NF-κB), a transcription factor required for the induction of the EMT and involved in regulating anoikis (Lin et al., 2011). p3 anoikis-resistant melanoma cells showed a higher level of the EMT markers N-Cadherin, Vimentin, Zeb1, and SNAIL1 (Figure 2B), associated with high invasive ability (Figure 2A) and low IKB expression (Figure 2C), which indicates NF-kB activation. Moreover, since a stem-like phenotype sustains cancer cell survival after detachment from ECM, we evaluated the expression of SOX2, OCT3/4, KFL4 and ALDH1 stemness markers and we found that p3 anoikis-resistant cells show a tendency to increase the expression of these markers, especially ALDH1 (Figure 2D). This finding highlights that melanoma cell resistance in a detached condition needs a transition to a mesenchymal phenotype with some aspect of stemness.

The primary methods for detecting CTCs are based on their physical and/or epithelial characteristics. However, the use of functional markers, such as altered metabolism, remains a subject of ongoing debate. Thus, we investigated the metabolic profile of *anoikis*-resistant cells as our CTC model, using dynamic and

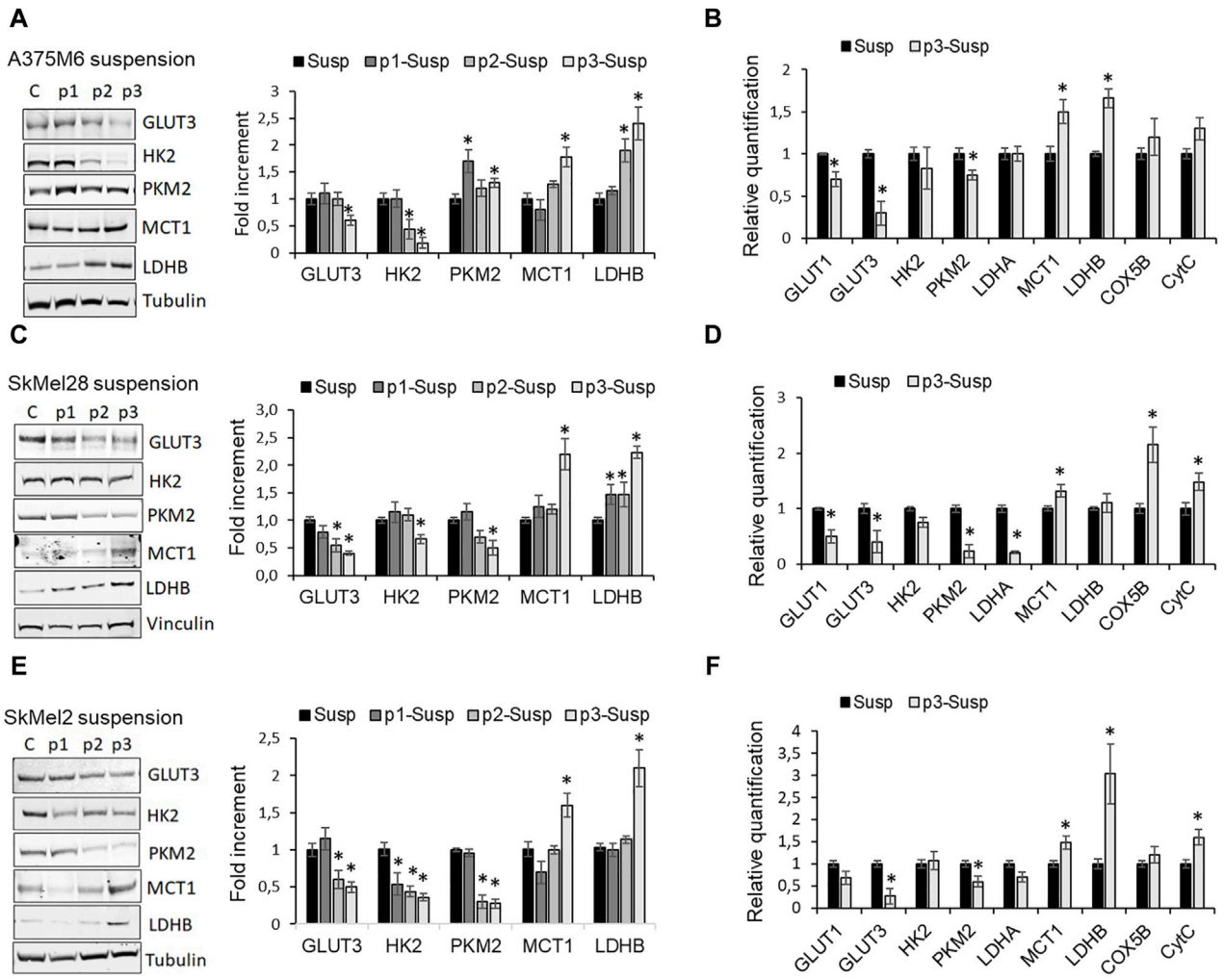


FIGURE 3
Metabolic profile of p3 melanoma cells: evaluation of the expression of glycolytic and oxidative markers. Western blot analysis of GLUT3, HK2, PKM2, MCT1, LDHB of A375M6 **(A)**, SkMel28 **(C)**, SkMel2 **(E)** melanoma cells in suspension after 1-4 consecutive rocking exposures (susp, p1 susp, p2 susp, p3 susp). Representative Western blot panels on the left. Protein levels were quantified by densitometric analysis and a corresponding histogram was constructed relative to α-tubulin. mRNA expression evaluated by quantitative real-time PCR of the glycolytic markers GLUT1, GLUT3, HK2, PKM2, LDHA, and of the oxidative markers MCT1, LDHB, COX5B, CytC of A375M6 **(B)**, SkMel28 **(D)**, SkMel2 **(F)** melanoma cells in suspension after 1 (Susp) or 4 (p3-susp) rocking exposures. Values presented are mean ± SEM of three independent experiments *$P < 0.05$ compared with control cells.

molecular approaches. To perform this aim, the p3 cells placed again in suspension in rocking condition for 24 h (p3-S) were compared for protein expression of glycolytic and oxidative markers with cells recovered at the end of each previous rocking step (S, p1-S, p2-S), before the re-attachment in culture dishes. We found that in all melanoma cell lines, the protein expression of the glycolytic markers glucose transporter (GLUT) 3, mediating glucose uptake from the extracellular environment, hexokinase (HK) 2, a critical enzyme catalyzing the first rate-limiting step of glycolysis and pyruvate kinase M2 (PKM2), gradually decreases as the steps in rocking condition progress from S to p3-S (Figures 3A, C, E). We also compared mRNA expression of glycolytic markers in p3-S melanoma cells respect to S melanoma cells and we confirmed that in *anoikis*-resistant cells the expression of GLUT1, GLUT3, HK2, PKM2, and LDHA significantly reduced (Figures 3B, D, F).

Conversely, the protein expression of the oxidative markers monocarboxylate transporter (MCT) 1, which mediates lactate uptake and lactate dehydrogenase (LDH) B, which catalyzes the conversion of lactate to pyruvate, progressively increases from S to p3-S (Figures 3A, C, E), as mRNA expression of MCT1, LDHB, Cytochrome C Oxidase Subunit 5B (COX5B) and Cytochrome C (CytC) (Figures 3B, D, F).

A further source of carbon and nitrogen for both catabolic and anabolic demands is glutamine. The importance of glutamine lies in its being converted to glutamate and then to α-ketoglutarate (α-KG), a catabolic process known as glutaminolysis. α-KG enters the tricarboxylic acid (TCA) cycle, referred to as anaplerosis, not only for the generation of ATP via oxidative phosphorylation, but also for the production of acetyl-coA as a critical precursor for lipid and nucleotide synthesis. Many malignant tumor cells always display glutamine addiction. Alanine, serine, cysteine-preferring transporter 2 (ASCT2; SLC1A5) mediates the uptake of glutamine, while glutaminase (GLS), identified in two forms, GLS1 and GLS2, is the metabolic enzyme that catalyzes the first step

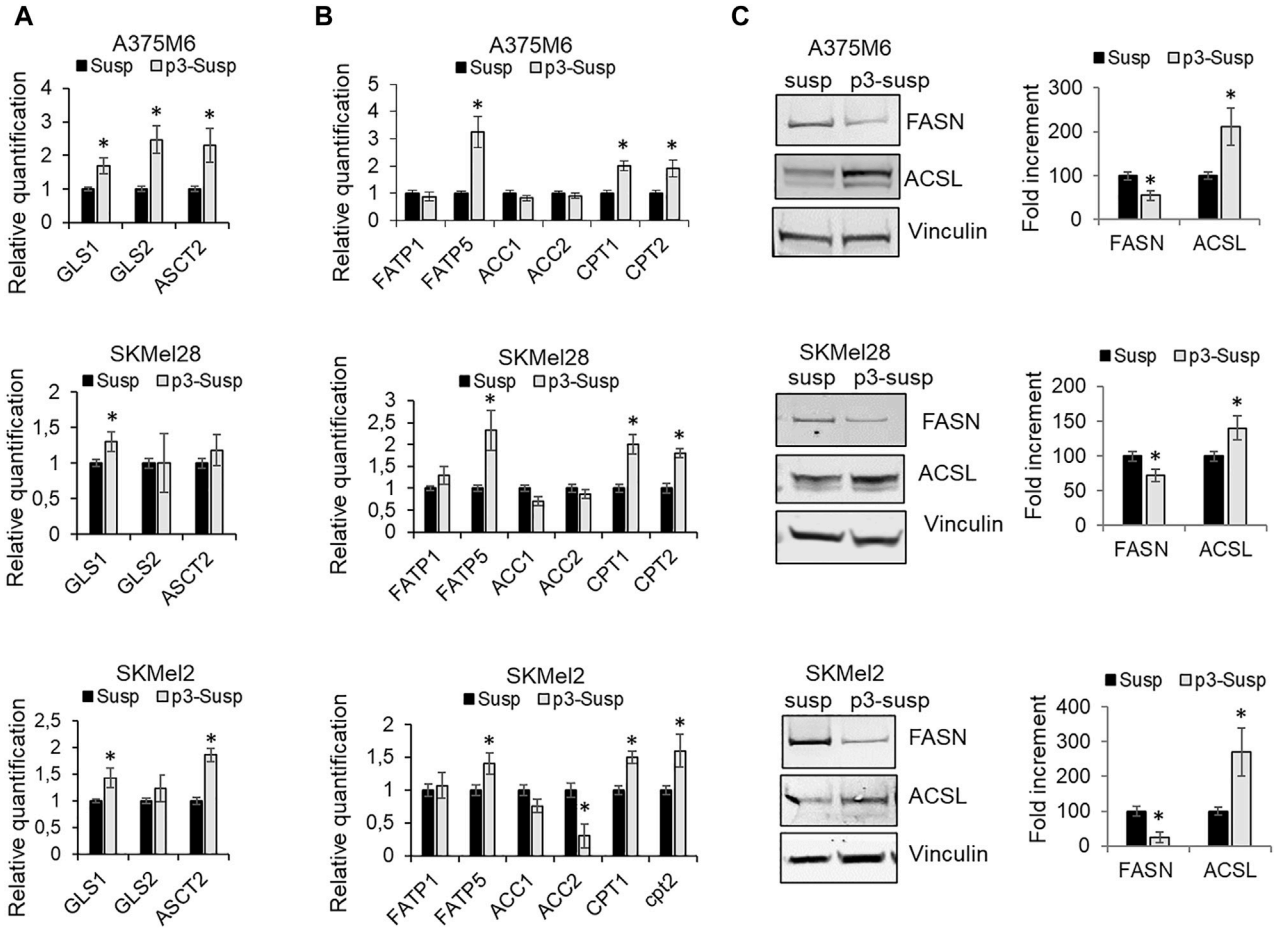


FIGURE 4
Metabolic profile of p3 melanoma cells: evaluation of markers of glutamine and fatty acid metabolism. mRNA expression evaluated by quantitative real-time PCR of **(A)** some markers of glutamine metabolism, GLS1, GLS2 ASCT2 and **(B)** markers of fatty acid metabolism, such as FATP1, FATP5, ACC1, ACC2, CPT1, CPT2 of A375M6, SkMel28, SkMel2 melanoma cells in suspension after 1 (Susp) or 4 (p3-susp) rocking exposures. **(C)** Western blot analysis of FASN and ACSL of A375M6, SkMel28, SkMel2 melanoma cells in suspension after 1 (Susp) or 4 (p3-susp) rocking exposures. Representative Western blot panels on the left. Protein levels were quantified by densitometric analysis and a corresponding histogram was constructed relative to vinculin. Values presented are mean ± SEM of three independent experiments *$P < 0.05$ compared with control cells.

in mitochondrial glutaminolysis. We observed that GLS mRNA expression, in particular the GLS1 isoform, and ASCT2 expression were increased in p3-S respect to S cells (Figure 4A).

However, to maintain a malignant phenotype, cancer cells exposed to an unfavorable tumor microenvironment need additional bioenergetic pathways, such as the mitochondrial fatty acid β-oxidation (FAO) (Qu et al., 2016). mRNA expression analysis of lipid metabolic markers revealed that p3-S melanoma cells express higher levels of fatty acid transporters FATP1 and FATP5 and comparable (or lower) levels of acetyl-CoA carboxylases 1 and 2 (ACC1, ACC2) when compared with S populations. ACCs are enzymes that catalyze the carboxylation of acetyl-CoA to produce malonyl-CoA. ACC1 localizes in the cytosol and acts as the first rate-limiting enzyme in the *de novo* fatty acid synthesis pathway, ACC2 localizes on the outer membrane of mitochondria and produces malonyl-CoA to inhibit carnitine palmitoyl-transferase 1 (CPT1) involved in the β-oxidation of fatty acids. P3-S melanoma cells express higher levels with respect to S melanoma cells of carnitine palmitoyl-transferases CPT1 and CPT2, which are mitochondrial enzymes responsible for the formation of acylcarnitines by catalyzing the transfer of the acyl group of a long-chain fatty acyl-CoA from coenzyme A to l-carnitine (Figure 4B). We also evaluated by Western blot analysis the protein expression of the multi-enzyme protein Fatty acid synthase (FASN), which catalyzes the *de novo* synthesis of palmitate, and of the intrinsic membrane proteins Long-chain fatty acyl-CoA synthetases (ACSLs), which catalyze the conversion of fatty acids to their corresponding fatty acyl-CoAs (Figure 4C). ACSL1 has a specific function in directing the metabolic partitioning of FAs toward β-oxidation in adipocytes (Ellis et al., 2010). In agreement with our previously described results, we found that p3-S cells show low FASN expression and high ACSL levels. On the whole, our results suggest that *anoikis*-resistant melanoma sub-populations increase glutamine and fatty acid uptake and fatty acid beta-oxidation revealing a transition from a glycolytic to a more oxidative phenotype based on glutamine and fatty acid substrates.

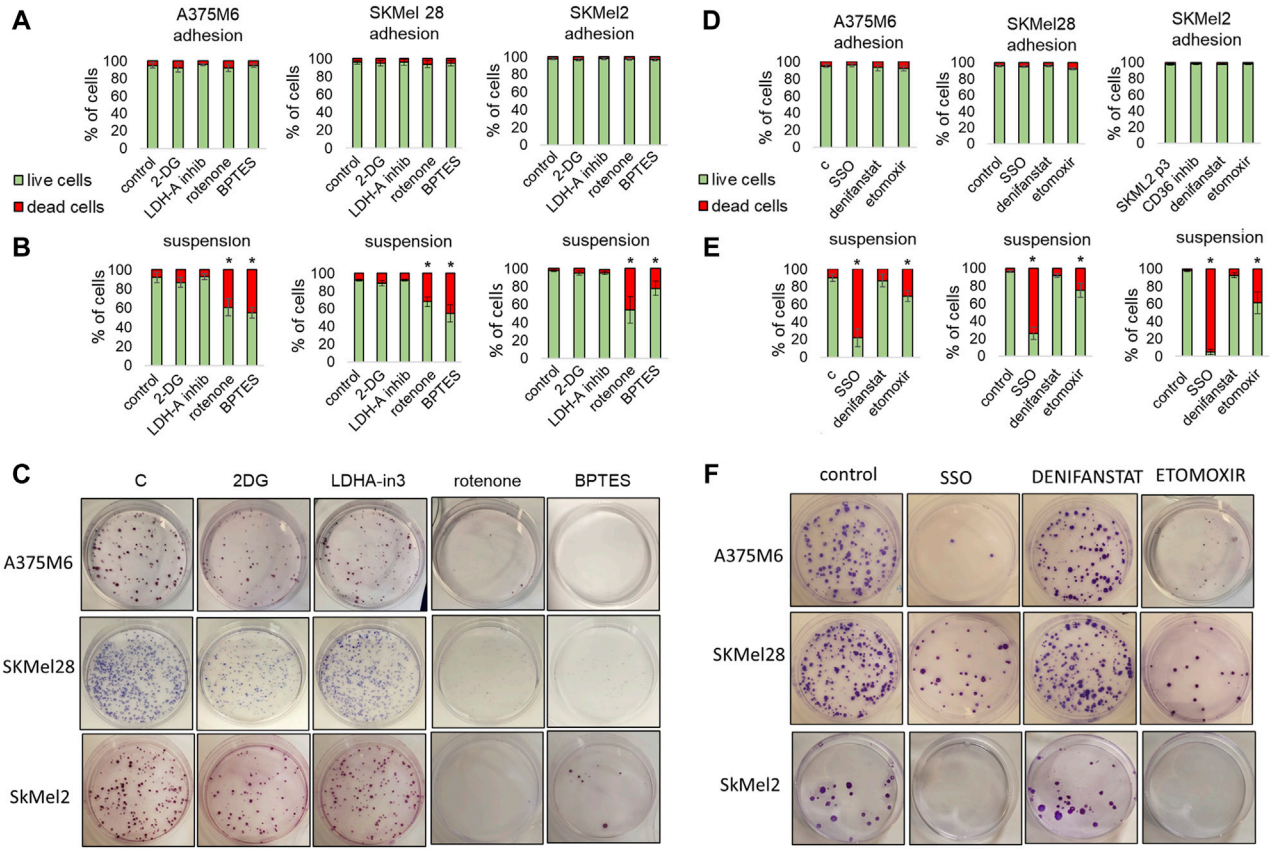


FIGURE 5
Effect of metabolic inhibitors on melanoma cell survival under non-adherent condition. A375M6, SkMel28, SkMel2 p3 melanoma cells under adherent (adhesion) **(A,D)** or rocking conditions (Suspension) **(B,C,E,F)** were treated for 24 h with 2DG (20 mM), LDH-in-3 (50 μM), rotenone (1 μM), BPTES (10 μM) **(A–C)**, SSO (50 μM), denifanstat (10 μM) or etomoxir (50 μM) **(D,E,F)**. After the treatment cells were analyzed for cell viability, evaluated by LIVE/DEAD Fixable Violet Dead Cell Staining **(A,B,D,E)** or for clonogenic efficiency **(C,F)**. Data are expressed as mean ± SEM of at least three independent experiments; images are representative of experiments performed in triplicate. *$P < 0.05$ compared with control cells.

3.2 Effect of metabolic drugs on *anoikis*-resistant melanoma cells

We treated p3 melanoma cells with different metabolic inhibitors during their incubation in rocking conditions to understand whether *anoikis*-resistant melanoma cells could be targetable with specific metabolic drugs. We first used glycolytic and oxidative inhibitors such as 2-Deoxy-D-Glucose (2DG), a glucose analog inhibiting HK2; LDH-IN-3, a potent noncompetitive LDHA inhibitor; rotenone, a mitochondrial respiratory chain complex I inhibitor; BPTES, a glutaminase inhibitor. At the end of the incubation in rocking condition, we analyzed the viability of the cells by flow cytometry and the cloning efficiency by colony formation assay. We found that while none of the treatments had any effects on the viability of cells grown in adhesion (viability always above 92%) (Figure 5A; cell viability was significantly affected by specific drugs when the treatment was carried out during the rocking condition (Figure 5B; representative flow cytometric plots in Supplementary Figures S4–S6). In particular rotenone and BPTES caused more than 25% cell death. The colony formation assay corroborated the results previously described, albeit with a more pronounced effect. Cells treated with 2DG showed a weak reduction in the number of colonies, which was significant only in SKMel28, while LDHA-IN-3 showed no significant effect in all three cell lines used. Rotenone and BPTES almost completely blocked colony formation in all the different cell lines (Figure 5C). Finally, we tested the effect of fatty acid metabolism inhibitors: sulfosuccinimidyl oleate (SSO), a long-chain fatty acid that inhibits fatty acid transport into cells; denifanstat, a potent Fatty Acid Synthase (FASN) inhibitor; etomoxir, which blocks fatty acid oxidation through CPT-1a inhibition. 24-h treatment with these drugs did not affect the viability of adherent cells (Figure 5D). When these drugs were used on suspended cells, we observed that SSO caused a mortality of more than 80%, etomoxir mortality of more than 25%, while the cells treated with denifanstat showed a mortality comparable to control cells (about 2%–5%) (Figure 5E; representative flow cytometric plots in Supplementary Figures S4–S6). When we analyzed the colony formation ability, we found that etomoxir and sulfosuccinimidyl oleate significantly reduced colony number, while denifanstat showed no effect (Figure 5F).

These findings confirm that the *anoikis*-resistant melanoma cells require glutamine and fatty acid metabolic rewiring.

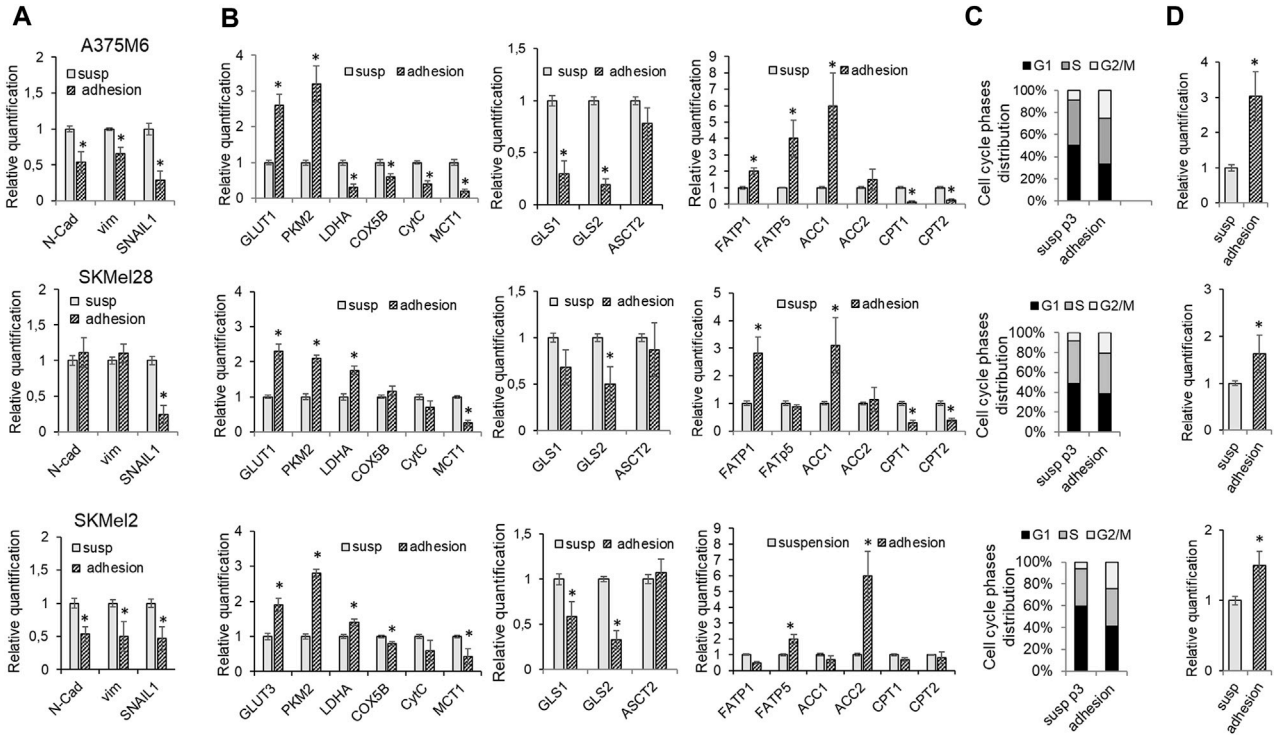


FIGURE 6
Effect of the adhesive re-attachment of *anoikis*-resistant melanoma cells on their metabolic rewiring. A375M6, SkMel28, SkMel2 p3 melanoma cells were subjected to a rocking exposure in suspension (susp) and then allowed to re-grow in adherent plastic dishes (adhesion). mRNA expression evaluated by quantitative real-time PCR of: **(A)** the EMT markers N-cadherin, Vimentin and SNAIL1, **(B)** the glycolytic markers GLUT1, PKM2, LDHA; the oxidative markers COX5B, CytC, MCT1; the markers of glutamine metabolism GLS1, GLS2, ASCT2 and of fatty acid metabolism FATP1, FATP5, ACC1, ACC2, CPT1, CPT2. **(C)** Cell cycle analysis and **(D)** mRNA expression evaluated by quantitative real-time PCR of Cyclin **(D)**. Values presented are mean ± SEM of three independent experiments $*P < 0.05$ compared with control cells.

3.3 Effect of the adhesive reattachment of anoikis-resistant melanoma cells on their metabolic rewiring

When p3-S melanoma cells were allowed to re-grow in adherent plastic dishes, cells reconverted their EMT to MET, as indicated by the reduction in the expression of EMT markers N-Cadherin, Vimentin, and SNAIL1 shown in Figure 6A. We found that these cells also reconvert their metabolic attitude to aerobic glycolysis. Indeed, the expression of the glycolytic markers GLUT1, PKM2, and LDHA was increased, while the expression of the oxidative markers COX5B, CytC, and MCT1 was reduced, compared to parental suspended cells (Figure 6B). These changes are accompanied by a reduction in GLS1 and GLS2 expression (Figure 6B). Moreover, in this setting, melanoma cells express high levels of ACC1 and ACC2, as does the expression of FATP1 and FATP5, while CPT1 and CPT2 are reduced (Figure 6B), justifying a lipid synthesis to be used for proliferation or energy storage in lipid droplets. We performed the cell cycle analysis of *anoikis*-resistant melanoma cells and we found a reduction in the percentage of cells in the G0-G1 phase with an increase in cells in the G2/M phase in all three cell lines, suggesting a strong aid to enter the cell cycle and to initiate cell division (Figure 6C; Flow Cytometry representative histograms in Supplementary Figure S7). The increased expression in re-adapted cells of cyclin D (Figure 6D), a mitogenic sensor able to integrate extracellular signals and cell cycle progression, underlines the association, in these cells, of a proliferative with the regained glycolytic phenotype. These findings further indicate how *anoikis*-resistant melanoma cells, despite three consecutive selections in rocking conditions, are readily able to reconvert their phenotype, indicating a maintained plasticity. This ability may represent a sign of particular importance when CTCs undergo MET and colonize the new organ microenvironment.

3.4 Comparison of bioenergetic phenotype between adherent cells and anoikis-resistant tumor cell subpopulations

To confirm the different energy pathways used by adherent cells and *anoikis*-resistant cells, we first analyzed the extracellular lactate concentrations in media conditioned by control cells, p3 cells grown in rocking conditions, and p3 cells after re-adhesion. The measures of extracellular lactate levels confirmed the glycolytic metabolic profile of adherent cells (both control cells and p3 after re-adhesion), able to produce a high quantity of lactate, while p3 cells in suspension showed a lower level of extracellular lactate, in any cell lines used (Figure 7A).

The real-time measurement of cellular metabolism using the Seahorse XF96 Extracellular Flux Analyzer allowed us to analyze the

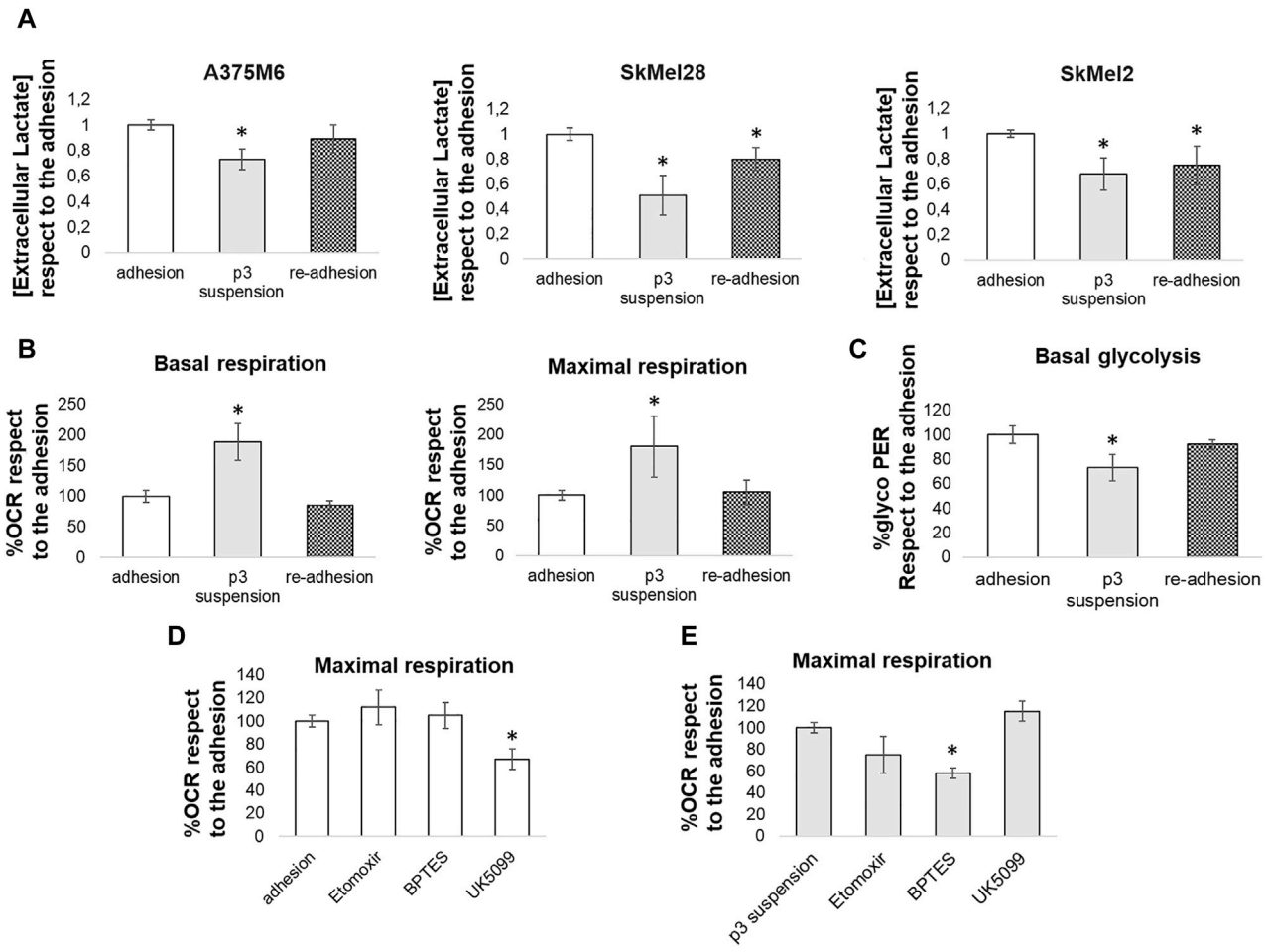


FIGURE 7
Comparison between the energy metabolism of adherent cells and *anoikis*-resistant melanoma cells. **(A)** Extracellular lactate levels were compared between melanoma cells (A375M6, SkMel28, SkMel2) grown in adherent conditions (adhesion), p3 cells subjected to a rocking exposure (p3 suspension) or p3 cells allowed to re-grow in adherent plastic dishes (re-adhesion). Adherent A375M6 cells, p3 A375M6 cells subjected to a rocking exposure or p3 A375M6 cells allowed to re-grow in adherent conditions were subjected to the Seahorse XF Mitochondrial stress test or to the glycolytic rate assay to evaluate oxygen consumption rate (OCR) and glycolytic proton efflux rate (glycoPER) and to determine basal and maximal respiration **(B)** and basal glycolysis **(C)**. The Seahorse XF substrate oxidation stress test was performed to determine the specific substrates oxidated by adherent A375M6 cells **(D)** and p3 A375M6 cells subjected to a rocking exposure **(E)**. Changes in OCR were evaluated after the injection of the indicated inhibitors: UK5099 (glucose oxidation inhibitor); etomoxir (fatty acid oxidation inhibitor); BPTES (glutamine oxidation inhibitor). Values presented are mean ± SEM of three independent experiments $^*P < 0.05$ compared with control cells.

Oxygen Consumption Rate (OCR) and the Proton Efflux Rate of A375M6 control cells (grown in adhesion), p3 cells grown in rocking conditions, and p3 cells after re-adhesion. The p3 population in suspension showed a significant increase in both basal and maximal respiration levels compared to adherent cells (Figure 7B; representative time-course traces of OCR in Supplementary Figure S8A) and a significant reduction in basal glycolysis (Figure 7C; representative time-course traces of Proton Efflux Rate in Supplementary Figure S8B). Control A375M6 melanoma cells showed higher levels of glycolysis and lower levels of OCR (Figures 7B, C).

To determine the specific substrates oxidated by cells grown in adherent conditions or p3 cells after 24 h in rocking conditions, we performed the substrate oxidation stress tests using the inhibitors specific for the three primary substrates that fuel the mitochondria: long-chain fatty acids (etomoxir), glucose/pyruvate (UK5099), and glutamine (BPTES). We found that the OCR of control adherent cells was significantly inhibited by UK5099 (Figure 7D; representative time-course traces of OCR in Supplementary Figure S8C), which blocks the use of glucose/pyruvate for respiration by inhibiting the mitochondrial pyruvate carrier. On the contrary, the OCR of p3 cells in suspension did not change after UK5099 treatment but was significantly inhibited by BPTES, a GLS inhibitor, and partially reduced by etomoxir, a CPT inhibitor (Figure 7E; representative time-course traces of OCR in Supplementary Figure S8C).

These results confirmed that cells in non-adherent conditions undergo a metabolic reprogramming changing the substrate used for respiration from glucose to fatty acids and glutamine.

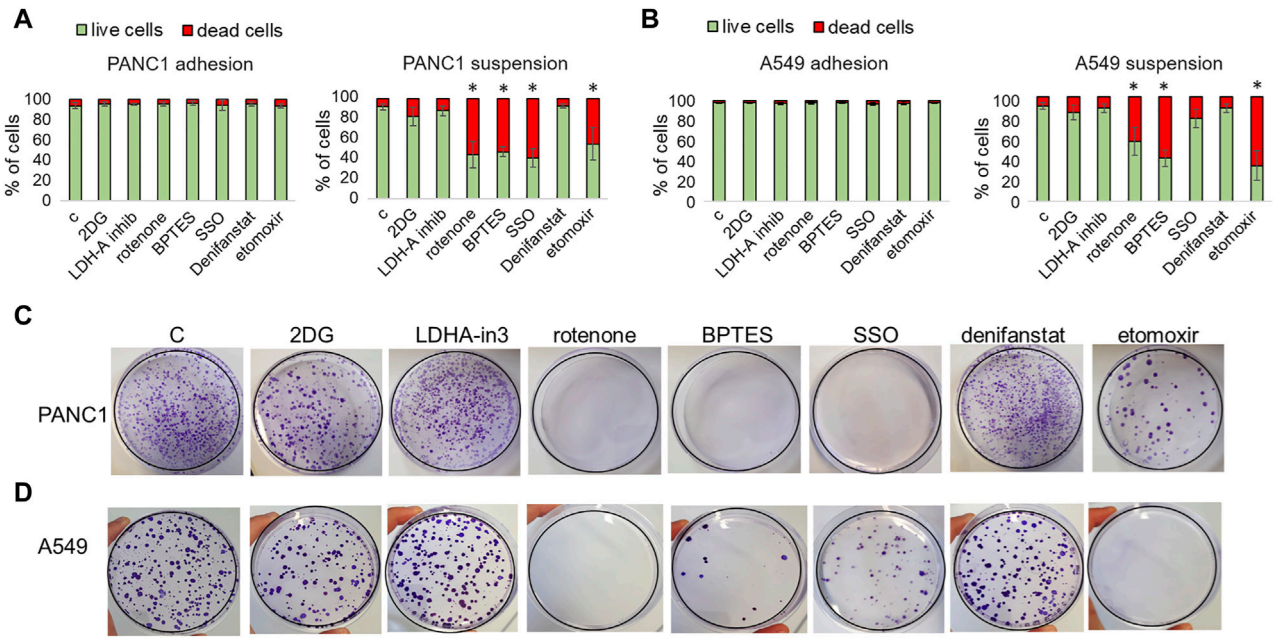


FIGURE 8
Effect of metabolic drugs on non-melanoma cells exposed to a non-adherent condition. PANC1 **(A,C)** and A549 **(B,D)** cells under adherent (adhesion) or rocking conditions (suspension) were treated for 24 h with 2DG (20 mM), LDH-in-3 (50 μM), rotenone (1 μM), BPTES (10 μM), SSO (50 μM), denifanstat (10 μM) or etomoxir (50 μM). After the treatment cells were analyzed for cell viability, evaluated by LIVE/DEAD Fixable Violet Dead Cell Staining **(A,B)** or for clonogenic efficiency **(C,D)**. Data are expressed as mean ± SEM of at least three independent experiments; images are representative of experiments performed in triplicate. *$P < 0.05$ compared with control cells.

3.5 Effect of metabolic drugs on non-melanoma cells exposed to a non-adherent condition

We extended our investigation to tumor cells of a different histotype. We tested some of the metabolic drugs on PANC1 (a human pancreatic cancer cell line) and A549 cells (an adenocarcinoma human alveolar cell line) exposed for 24 h to a rocking condition. At the end of the incubation in rocking condition, we analyzed cell viability by flow cytometry and the cloning efficiency by colony formation assay. We found that none of the treatments had any effects on the viability of cells grown in adhesion (viability always above 94%), while viability was significantly affected by the inhibitors of glutaminase (BPTES), fatty acid uptake (SSO), beta-oxidation (etomoxir) when the treatment was carried out during the rocking condition (Figures 8A, B; representative flow cytometric plots in Supplementary Figures S9, S10). BPTES, SSO, etomoxir, and Rotenone resulted able to suppress colony efficiency (Figures 8C, D), as it has been previously observed using the p3 anoikis-resistant melanoma cells. It is possible that a rapid reconversion to an oxidative metabolism using some available fuels, such as glutamine and fatty acids, represents a general transition of tumor metabolism during the loss of adhesiveness.

4 Discussion

Understanding tumor metabolism and its reprogramming when tumor cells adapt to a changing microenvironment is fundamental for developing innovative therapies. Indeed, cell energetic metabolism is deeply interconnected with and influenced by the surrounding environment. Tumor cells need to adapt their metabolism in response to changes in oxygen levels, such as moving from aerobic to anaerobic glycolysis or compensating for glucose deprivation by utilizing alternative nutrients, thereby switching from glycolysis to oxidative metabolism (Boroughs and DeBerardinis, 2015). Metabolic reprogramming is now considered a hallmark of cancer (Hanahan and Weinberg, 2011). Particularly, tumor cells need to adapt their metabolic phenotype during metastatic dissemination in the bloodstream, when are deprived of matrix attachment and subjected to various physical and physiological stresses including nutrient deprivation. This special condition deserves a transition of tumor cells to a less proliferative state and metabolic reprogramming to overcome the programmed cell death, known as "*anoikis*." Consequently, the metabolic reprogramming of *anoikis*-resistant tumor cells has emerged as a focal point in understanding tumor cell dissemination (Adeshakin et al., 2021).

We investigated the metabolic reprogramming of some selected high *anoikis*-resistant melanoma cells, e.g., A375M6, SKMel2, and SKMel28 cells. Previous investigations addressed the major mechanisms of the *anoikis* resistance of epithelial-derived tumor cells, whereas less attention is devoted to melanoma cells, highly migratory cells of neuroectodermal origin characterized by a high inter- and intra-tumor heterogeneity. This heterogeneity leads to different plastic phenotypes that play an important role in resistance. Our study's model is represented by melanoma cells selected for a

high *anoikis* resistance, obtained through three consecutive exposures to a 24-h rocking condition, resulting in the selected subpopulation, referred to as the p3 cells. The procedure for the high *anoikis*-resistant p3 cell selection represents our attempt to mimic the rapid selection operated by the bloodstream on tumor cells that leave the primary tumor and enter into the circulation. It is known that most tumor cells that enter the bloodstream disappear in 24 h (Fidler, 1975; Kowalik et al., 2017). We confirmed that the p3-selected melanoma cells when transferred to agarose-coated dishes, a further condition where the adhesion is prevented, show a high colony formation ability. Furthermore, when the p3-selected cells were once again exposed to a rocking condition, they exhibited higher viability compared to parental cells and a significantly enhanced ability to form colonies. These results reveal the ability of p3-selected cells to sustain a certain level of *anoikis* resistance and generate new colonies under adhesive conditions. Although p3-selected melanoma cells maintain a certain heterogeneity, most cells show a transition phenotype toward a more mesenchymal-like type, with high *in vitro* invasiveness. Indeed, the p3-selected melanoma cells express the most characteristic EMT markers (see Chiarugi and Giannoni, 2008) and a high ALDH1 among the stem cell markers. ALDH1 is a detoxifying enzyme expressed by some epithelial cancer stem cells able to metabolize a wide variety of intracellular aldehydes providing resistance to alkylating chemicals as cyclophosphamide (Deng et al., 2010; Luo et al., 2012). These findings reveal that the p3-selected subpopulations, obtained for three successive exposures to a non-adhesive condition, express important features of resistance, invasiveness, and stemness associated with the ability to survive in suspension. The significance of EMT markers and a partial stem cell-like phenotype of p3-selected melanoma cells may render these findings a possible resource for CTC diagnosis. *Anoikis* represents the major challenge met by cells of a solid tumor during their diffusion into the circulation. The ability to resist *anoikis* also requires a metabolic flexibility able to sustain survival even during a nutrient limitation in a stress condition, such as blood turbulence, which might require a high energy expenditure: *anoikis*-resistance and the metabolic adaptation of tumor cells as two sides of the same coin. We studied the metabolic profile of p3-selected melanoma cells placed again in suspension, to prevent any possible influence of mechanisms involved in extracellular anchorage. Indeed, at the secondary site, when metastatic cancer cells recover the attachment to a new extracellular substrate and find abundant nutrients, they may switch to a proliferative phenotype linked to a further metabolic change. We found that p3-selected melanoma cells still in suspension show a reduction of glycolytic markers from p1 to p3 subpopulations, at a mRNA, and protein level evaluation confirmed these changes. In parallel, the same cells express higher levels of oxidative phosphorylation markers suggesting a change of p3-selected cells from classical glycolytic phenotype to an oxidative metabolism. Glutamine is one of the most abundant amino acids in plasma and is important for ATP generation and "anaplerosis," which is the process of supplying nitrogen sources for molecular building block synthesis (Kodama et al., 2020). Markers of glutamine metabolism expressed by p3-selected melanoma cells indicate a promoted uptake and catabolism of glutamine via GLS1, required for ATP synthesis. This finding suggests that p3-selected melanoma cells force the use of glutamine as a metabolic fuel instead of glucose. However, under stressed conditions, cancer cells may also adapt to the use of fatty acid metabolism, and fatty acid oxidation (β oxidation) provides more than twice as much ATP as carbohydrates and reduces reactive oxygen species producing NADH and FADH2 (Carracedo et al., 2013). Fatty acids may be acquired from the bloodstream as free fatty acids or within lipoproteins or can be mobilized from cytoplasmic lipid droplets. Malonyl-CoA synthesized by the activity of ACC is the key metabolite at the crossroads of biosynthesis and degradation of fatty acids and CPT is the rate-limiting step in FA oxidation. Markers of fatty acid metabolism of p3-selected melanoma cells indicate an enhanced uptake, activation, and catabolism of fatty acids in the presence of a reduced synthesis. Thus, p3 *anoikis*-resistant melanoma cells seem to switch to an energy-generating fatty acid oxidation metabolism and inhibition of fatty acid synthesis to save energy. The use of several metabolic inhibitors of glucose, glutamine, and fatty acid metabolism confirmed that p3-selected melanoma cells are addicted to the use of oxidative metabolism of glutamine and fatty acids. It is known that FAO induced in breast cancer cells promotes *anoikis* resistance and ALDH 1 activity (De Francesco EM et al., 2017; Bapat and Bitler, 2021), whereas targeting FAO stimulates *anoikis* of ovarian cancer cells (Sawyer et al., 2020).

Of particular interest, p3-selected melanoma cells when re-adapted to growth in an adhesive condition in culture dishes, switched back to a proliferative glycolytic phenotype and fatty acid synthesis, suggesting that although the p3 melanoma subpopulation enriched in *anoikis*-resistant cells express an enhanced oxidative metabolism when in suspension, they retain the ability to promptly reconvert to a glycolytic phenotype, appropriate for cell proliferation and mass expansion in a secondary organ.

This dynamic cell metabolic reprogramming was confirmed by the measurement of lactate production and through metabolic analysis performed using the Seahorse XF96 Extracellular Flux Analyzer, comparing adherent cells, p3 melanoma subpopulation and p3 cells re-adapted to growth in adherent conditions. p3 melanoma subpopulation showed high oxygen consumption rate levels, low proton efflux rate, accompanied with reduced levels of extracellular lactate, all results in agreement with the low expression of glycolytic markers shown by these cells. All these parameters go back to levels comparable to control adherent cells, when p3 cells re-adapt to growth in adherent conditions, confirming the ability of *anoikis*-resistant cells to reconvert to a glycolytic phenotype, under specific scenarios.

The Seahorse XF analysis of the specific substrates oxidated by p3 subpopulation or adherent cells confirmed that, while cells in adhesion exploit glucose, for both glycolysis and respiration, p3 cells mainly use glutamine and fatty acids.

After all, we found that the metabolic changes in the direction of the use of oxidative metabolism of glutamine and fatty acids also characterize some *anoikis*-resistant human pancreatic and alveolar cancer cells.

On the whole, our data suggest that melanoma cells able to survive in blood circulation may reprogram their glycolytic

phenotype to oxidative phosphorylation of glutamine and fatty acids. These findings open up to organizing a complementary therapy for metastatic melanoma based on specific metabolic inhibitors.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

SP: Writing–review and editing, Writing–original draft, Visualization, Supervision, Investigation, Formal Analysis, Data curation, Conceptualization. TK: Writing–review and editing, Visualization, Methodology, Investigation, Formal Analysis. GM: Writing–review and editing, Visualization, Methodology, Formal Analysis. EA: Writing–review and editing, Validation, Formal Analysis. JR: Writing–review and editing, Validation, Formal Analysis. CN: Writing–review and editing, Validation, Funding acquisition, Formal Analysis. FB: Writing–review and editing, Visualization, Resources, Conceptualization. LC: Writing–original draft, Supervision, Resources, Project administration, Funding acquisition.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the University of Florence, Italy (RICATEN23); SP was supported by Fondazione Umberto Veronesi. EA was supported by Fondazione Associazione Italiana per la Ricerca sul Cancro (AIRC) "Fellowship for Italy."

Acknowledgments

The authors thank Anna Laurenzana (University of Florence, Italy) and Laura Poliseno (CNR, Pisa, Italy) for providing us with A549, PANC1 and SkMel28 cell lines.

Conflict of interest

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1422281/full#supplementary-material

References

Adeshakin, F. O., Adeshakin, A. O., Afolabi, L. O., Yan, D., Zhang, G., and Wan, X. (2021). Mechanisms for modulating anoikis resistance in cancer and the relevance of metabolic reprogramming. *Front. Oncol.* 11, 626577. doi:10.3389/fonc.2021.626577

Bapat, J., and Bitler, B. G. (2021). "The intersection of anoikis resistance and fatty acid metabolism in cancer," in *Anoikis*. Editor S. M. Frisch (Cham: Springer). doi:10.1007/978-3-030-73856-3_3

Boroughs, L. K., and DeBerardinis, R. J. (2015). Metabolic pathways promoting cancer cell survival and growth. *Nat. Cell Biol.* 17, 351–359. doi:10.1038/ncb3124

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

Cao, Z., Livas, T., and Kyprianou, N. (2016). Anoikis and EMT: lethal "liaisons" during cancer progression. *Crit. Rev. Oncog.* 21, 155–168. doi:10.1615/CritRevOncog.2016016955

Carracedo, A., Cantley, L. C., and Pandolfi, P. P. (2013). Cancer metabolism: fatty acid oxidation in the limelight. *Nat. Rev. Cancer* 13, 227–232. doi:10.1038/nrc3483

Chiarugi, P., and Giannoni, E. (2008). Anoikis: a necessary death program for anchorage-dependent cells. *Biochem. Pharmacol.* 76 (11), 1352–1364. doi:10.1016/j.bcp.2008.07.023

DeBerardinis, R. J., and Chandel, N. S. (2016). Fundamentals of cancer metabolism. *Sci. Adv.* 2, e1600200. doi:10.1126/sciadv.1600200

de Francesco, E. M., Maggiolini, M., Tanowitz, H. B., Sotgia, F., and Lisanti, M. P. (2017). Targeting hypoxic cancer stem cells (CSCs) with doxycycline: implications for optimizing anti-angiogenic therapy. *Oncotarget* 8, 56126–56142. doi:10.18632/oncotarget.18445

Deng, S., Yang, X., Lassus, H., Liang, S., Kaur, S., Ye, Q., et al. (2010). Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. *PLoS One* 5, e10277. doi:10.1371/journal.pone.0010277

Ellis, J. M., Li, L. O., Wu, P. C., Koves, T. R., Ilkayeva, O., Stevens, R. D., et al. (2010). Adipose acyl-CoA synthetase-1 directs fatty acids toward beta-oxidation and is required for cold thermogenesis. *Cell Metab.* 12, 53–64. doi:10.1016/j.cmet.2010.05.012

Endo, H., Owada, S., Inagaki, Y., Shida, Y., and Tatemichi, M. (2020). Metabolic reprogramming sustains cancer cell survival following extracellular matrix detachment. *Redox Biol.* 36, 101643. doi:10.1016/j.redox.2020.101643

Faubert, B., Solmonson, A., and DeBerardinis, R. J. (2020). Metabolic reprogramming and cancer progression. *Science* 368, eaaw5473. doi:10.1126/science.aaw5473

Fidler, I. J. (1975). Biological behavior of malignant melanoma cells correlated to their survival *in vivo*. *Cancer Res.* 35 (1), 218–224.

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi:10.1016/j.cell.2011.02.013

Hawk, M. A., and Schafer, Z. T. (2018). Mechanisms of redox metabolism and cancer cell survival during extracellular matrix detachment. *J. Biol. Chem.* 293, 7531–7537. doi:10.1074/jbc.TM117.000260

Jose, C., Bellance, N., and Rossignol, R. (2011). Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? *Biochim. Biophys. Acta Bba Bioenerg.* 1807, 552–561. doi:10.1016/j.bbabio.2010.10.012

Khan, S. U., Fatima, K., and Malik, F. (2022). Understanding the cell survival mechanism of anoikis-resistant cancer cells during different steps of metastasis. *Clin. Exp. Metastasis* 39, 715–726. doi:10.1007/s10585-022-10172-9

Kodama, M., Oshikawa, K., Shimizu, H., Yoshioka, S., Takahashi, M., Izumi, Y., et al. (2020). A shift in glutamine nitrogen metabolism contributes to the malignant progression of cancer. *Nat. Commun.* 11, 1320. doi:10.1038/s41467-020-15136-9

Kowalik, A., Kowalewska, M., and Gozdz, S. (2017). Current approaches for avoiding the limitations of circulating tumor cells detection methods-implications for diagnosis and treatment of patients with solid tumors. *Transl. Res.* 185, 58–84. doi:10.1016/j.trsl.2017.04.002

Lawrence, R., Watters, M., Davies, C. R., Pantel, K., and Lu, Y. J. (2023). Circulating tumour cells for early detection of clinically relevant cancer. *Nat. Rev. Clin. Oncol.* 20, 487–500. doi:10.1038/s41571-023-00781-y

Lin, D. C., Zhang, Y., Pan, Q. J., Yang, H., Shi, Z. Z., Xie, Z. H., et al. (2011). PLK1 Is transcriptionally activated by NF-κB during cell detachment and enhances anoikis resistance through inhibiting β-catenin degradation in esophageal squamous cell carcinoma. *Clin. Cancer Res.* 17, 4285–4295. doi:10.1158/1078-0432.CCR-10-3236

Luo, Y., Dallaglio, K., Chen, Y., Robinson, W. A., Robinson, S. E., McCarter, M. D., et al. (2012). ALDH1A isozymes are markers of human melanoma stem cells and potential therapeutic targets. *Stem Cells* 30 (10), 2100–2113. doi:10.1002/stem.1193

Mason, J. A., Hagel, K. R., Hawk, M. A., and Schafer, Z. T. (2017). Metabolism during ECM detachment: achilles heel of cancer cells? *Trends Cancer* 3, 475–481. doi:10.1016/j.trecan.2017.04.009

Paoli, P., Giannoni, E., and Chiarugi, P. (2013). Anoikis molecular pathways and its role in cancer progression. *Biochim. Biophys. Acta* 1833, 3481–3498. doi:10.1016/j.bbamcr.2013.06.026

Peppicelli, S., Ruzzolini, J., Bianchini, F., Andreucci, E., Nediani, C., Laurenzana, A., et al. (2019). Anoikis resistance as a further trait of acidic-adapted melanoma cells. *J. Oncol.* 2019, 8340926. doi:10.1155/2019/8340926

Qu, Q., Zeng, F., Liu, X., Wang, Q. J., and Deng, F. (2016). Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer. *Cell Death Dis.* 7, e2226. doi:10.1038/cddis.2016.132

Ruzzolini, J., Peppicelli, S., Andreucci, E., Bianchini, F., Margheri, F., Laurenzana, A., et al. (2017). Everolimus selectively targets vemurafenib resistant BRAFV600E melanoma cells adapted to low pH. *Cancer Lett.* 408, 43–54. doi:10.1016/j.canlet.2017.08.010

Ruzzolini, J., Peppicelli, S., Bianchini, F., Andreucci, E., Urciuoli, S., Romani, A., et al. (2020). Cancer glycolytic dependence as a new target of olive leaf extract. *Cancers (Basel)* 12, 317. doi:10.3390/cancers12020317

Sawyer, B. T., Qamar, L., Yamamoto, T. M., McMellen, A., Watson, Z. L., Richer, J. K., et al. (2020). Targeting fatty acid oxidation to promote anoikis and inhibit ovarian cancer progression. *Mol. Cancer Res.* 18, 1088–1098. doi:10.1158/1541-7786.MCR-19-1057

Shuvalov, O., Daks, A., Fedorova, O., Petukhov, A., and Barlev, N. (2021). Linking metabolic reprogramming, plasticity and tumor progression. *Cancers (Basel)* 13, 762. doi:10.3390/cancers13040762

Taddei, M. L., Giannoni, E., Fiaschi, T., and Chiarugi, P. (2012). Anoikis: an emerging hallmark in health and diseases. *J. Pathol.* 226, 380–393. doi:10.1002/path.3000

frontiers | Frontiers in Pharmacology

TYPE Mini Review
PUBLISHED 17 October 2024
DOI 10.3389/fphar.2024.1450441

OPEN ACCESS

EDITED BY
Elisa Giovannetti,
VU Medical Center, Netherlands

REVIEWED BY
Chiara Papulino,
University of Campania Luigi Vanvitelli, Italy

*CORRESPONDENCE
Xingxing Yuan,
yuanxingxing@hljucm.edu.cn

RECEIVED 17 June 2024
ACCEPTED 04 October 2024
PUBLISHED 17 October 2024

CITATION
Gao T, Yang L, Zhang Y, Bajinka O and Yuan X (2024) Cancer metabolic reprogramming and precision medicine-current perspective. *Front. Pharmacol.* 15:1450441. doi: 10.3389/fphar.2024.1450441

Cancer metabolic reprogramming and precision medicine-current perspective

Tingting Gao[1], Liuxin Yang[2], Yali Zhang[1], Ousman Bajinka[3] and Xingxing Yuan[1,2]*

[1]Department of Gastroenterology, Heilongjiang Academy of Traditional Chinese Medicine, Harbin, China, [2]First Clinical Medical College, Heilongjiang University of Chinese Medicine, Harbin, China, [3]School of Medicine and Allied Health Sciences, University of The Gambia, Banjul, Gambia

Despite the advanced technologies and global attention on cancer treatment strategies, cancer continues to claim lives and adversely affects socio-economic development. Although combination therapies were anticipated to eradicate this disease, the resilient and restorative nature of cancers allows them to proliferate at the expense of host immune cells energetically. This proliferation is driven by metabolic profiles specific to the cancer type and the patient. An emerging field is exploring the metabolic reprogramming (MR) of cancers to predict effective treatments. This mini-review discusses the recent advancements in cancer MR that have contributed to predictive, preventive, and precision medicine. Current perspectives on the mechanisms of various cancer types and prospects for MR and personalized cancer medicine are essential for optimizing metabolic outputs necessary for personalized treatments.

KEYWORDS

metabolic reprogramming, precision medicine, treatment strategies, immune cell, mechanism

Introduction

Cancer is a multifaceted genetic disease that arises from elaborate changes to the genome. Metabolic reprogramming (MR) is considered to be one of the emerging hallmarkers of cancer, which promotes cell survival and infinite proliferation of malignant cells through changes in the characteristics of metabolic enzymes, upstream regulatory molecules and downstream metabolites (Ciardiello et al., 2022). Owing to the high metabolic plasticity, tumor cells exhibit complex metabolic patterns, with glucose metabolism, amino acid metabolism and lipid metabolism being dominant (Xu X. et al., 2023). Glucose metabolism includes glycolysis and glucose oxidative phosphorylation (OXPHOS), lipid metabolism mainly consists of fatty acid oxidation, fatty acid synthesis and cholesterol esterification, and amino acid metabolism includes pentose phosphate pathway (PPP) and serine/glycine pathway (An et al., 2024; Liu X. et al., 2024; Yang K. et al., 2023). Figure 1. In addition, there is metabolic crosstalk among glucose, lipid and amino acid metabolism. Furthermore, MR also elucidates the inherent fragility of cancer therapeutics, and the foundation of precision medicine for cancers based on MR requires detailed insights into both tumorigenesis and progression.

The challenges of drug resistance and immune evasion remain major, persistent, unsolved issues in conventional cancer treatments, and are closely related to MR and epigenetics factors (Sun et al., 2022). Epigenetic modifications play a crucial role in maintaining normal physiological functions of the body, including covalent modification of bases in DNA, post-translational modification of the terminal amino acids of histones, chromatin remodeling, and

modification and regulation of non-coding RNA. A large number of studies have shown that abnormal epigenetic modifications can affect the occurrence and development of cancer by regulating cancer MR (Wang et al., 2022; Yang J. et al., 2023; Yu et al., 2018). The complex interactions between genes and the environment as epigenetic factors make it impossible to adopt one-size-fits-all approach, thus necessitating precision medicine interventions for cancer (Rulten et al., 2023). MR and dysregulation are fundamental processes that result in phenotypic alterations through molecular mechanisms, which are critical for precision therapeutic interventions for cancer. Therefore, targeting cancer metabolic pathways is a promising approach to combat drug resistance. Moreover, metabolic, epigenetic, and transcriptional regulation involving immune cell plasticity in cancers are crucial for personalized treatment (Yuan et al., 2024). Considering the diverse MR, immune evasion strategies, and drug resistance mechanisms exhibited by different cancers, it is crucial to identify a range of cancer-specific genes for biomarker screening (Wamsley et al., 2023). Therefore, this mini-review collects evidence on energy rewiring mechanisms and the prospects of personalized medicine for various cancers.

Metabolic reprogramming and tumor microenvironment

The secondary objective of cancer MR is to reconfigure the tumor microenvironment (TME), which is primarily characterized by insufficient nutrient supply, hypoxia, and acidosis (Safi et al., 2023). The TME comprises intense interactions among tumor cells, stroma, and immune cells, which are crucial in tumorigenesis, metastasis, and drug resistance. The heterogeneity of TME not only provides survival and adaptability advantages for tumor cells, but also endows tumor cells with different reactivity and resistance to chemotherapy and targeted therapy. Immune cells also undergo MR in TME to produce metabolic adaptations associated with tolerance phenotypes, thereby exerting anti-tumor and gaining the ability to evade immune surveillance (Liu C. et al., 2024). Immunoediting intricately shapes the immunogenic profile of tumor cells and effectively undermines host anti-tumor responses throughout the process of tumorigenesis. Although intrinsic factors and metabolites regulate these processes within the TME, the interaction of immune cells, metabolites, and their niche is essential for effective treatment regimens (Buck et al., 2017). Recent findings have correlated deregulated bioenergetics programs with immunoediting, creating a counterbalance between infiltrating T cells and tumor in the TME (Tsai et al., 2023). Given the wide heterogeneity and adaptability of tumors, the TME also provide potential mechanistic targets, including oncometabolites and epigenetic modifiers (Das et al., 2024).

Various metabolic mechanisms on cancer precision medicine

The dissemination and metastasis of cancer are driven by epithelial-to-mesenchymal transition (EMT) through invasion and migration. This process is intimately linked to MR arising from the rewiring of cellular states and signaling pathways for survival in the context of dietary deprivation. The binding of activating transcription factor 4 (ATF4) to enhancers of mesenchymal factors and amino acid deprivation-responsive genes promotes the loss of epithelial characteristics and the acquisition of transforming growth factor β (TGF-β)-signaling-associated mesenchymal traits, further enhancing lung cancer cell metastasis (Lin et al., 2024). Hence, the specific epithelial enhancers regulation of EMT through MR represents a promising intervention for precision medicine. Hypoxia profoundly impacts the TME, leading to therapy resistance. It is practicable to employ hypoxia biomarkers as predictive and prognostic indicators for solid tumors treatment (Bigos et al., 2024). Lipid metabolism holds potential in targeting isocitrate dehydrogenase 1 (IDH1) and IDH2 for metabolic interventions. Distinct fatty acid metabolism is detected between IDH1 and IDH2, making these mutated genes a potential focus for precision medicine (Thomas et al., 2023). In the targeted therapy of cancer, regulators such as hexokinase 2 (HK2), pyruvate kinase M2 (PKM2), enolase 1 (ENO1), and lactate dehydrogenase A (LDHA) are crucial for the glycolytic pathway. At the transcriptional level, glycolysis is regulated by p53, c-Myc, hypoxia-inducible factor 1 (HIF1), and sine oculis homeobox homolog 1 (SIX1). Additionally, post-translational modifications such as methylation, phosphorylation, ubiquitination, and acetylation also exert significant roles in signal transduction and MR within the glycolytic pathway (Ni et al., 2024).

The response of individual patients to cisplatin treatment have revealed metabolic changes as factors influencing sensitivity and resistance, highlighting the need for personalized medicine for cisplatin resistance and other cancer resistant therapies (Yu et al., 2023). Combination therapy is essential for glioblastoma, and epidermal growth factor receptor (EGFR)-activated MR holds great potential. Temozolomide's antitumor effects, targeting the mevalonate and EGFR/AKT pathways, demonstrate significant clinical management of glioblastoma (Cui et al., 2023). In glioblastoma multiforme, the uptake of phospholipids and fatty acid synthesis is increased, while glycerolipid and glycerophospholipid metabolism is aberrant, disclosing its distinctive metabolic characteristics (Miao et al., 2023). A significant breakthrough has been achieved in the application of predictive medicine for gliomas, where features related to mitochondrial genome composition can predict its sensitivity to chemotherapy drugs, thereby exerting a positive impact on the prognosis of patients (Wu J. et al., 2023).

Luminal B breast cancer (LBBC) has a complex molecular landscape, and a comprehensive muti-platform analysis offers valuable targets and signaling pathways for the examination of differences between the two subtypes to attain more precise treatment of LBBC (Wang H. et al., 2023). Phosphoinositide 3-kinase (PI3K) inhibitors combined with autophagy have a strong rationale for breast cancer treatment. For instance, the metabolism of breast cancer is influenced by mitochondrial translation dysregulation and loss of core binding factor subunit β (CBFB) function (Malik et al., 2023). A metabolic switch involving triple-negative breast cancer (TNBC) shifts from glycolysis to fatty acid β-oxidation (FAO) through the inhibition of PKM2. Impaired histone methyltransferase, enhancer of zeste homolog 2 (EZH2), can be recruited to solute carrier family 16 member 9 (SLC16A9), a carnitine transporter, coordinated by the direct interaction of PKM2, thus epigenetically influencing tumor progression (Zhang Y. et al., 2024). In cervical squamous cell carcinoma (CESC), lipid metabolism-related genes (LMRGs) signature plays a significant role in advancing precision medicine strategies for the management of patients with cervical cancer by enhancing CESC prognostication (Wang and Zhang, 2024). The depletion of aconitate decarboxylase 1 (ACOD1) has been

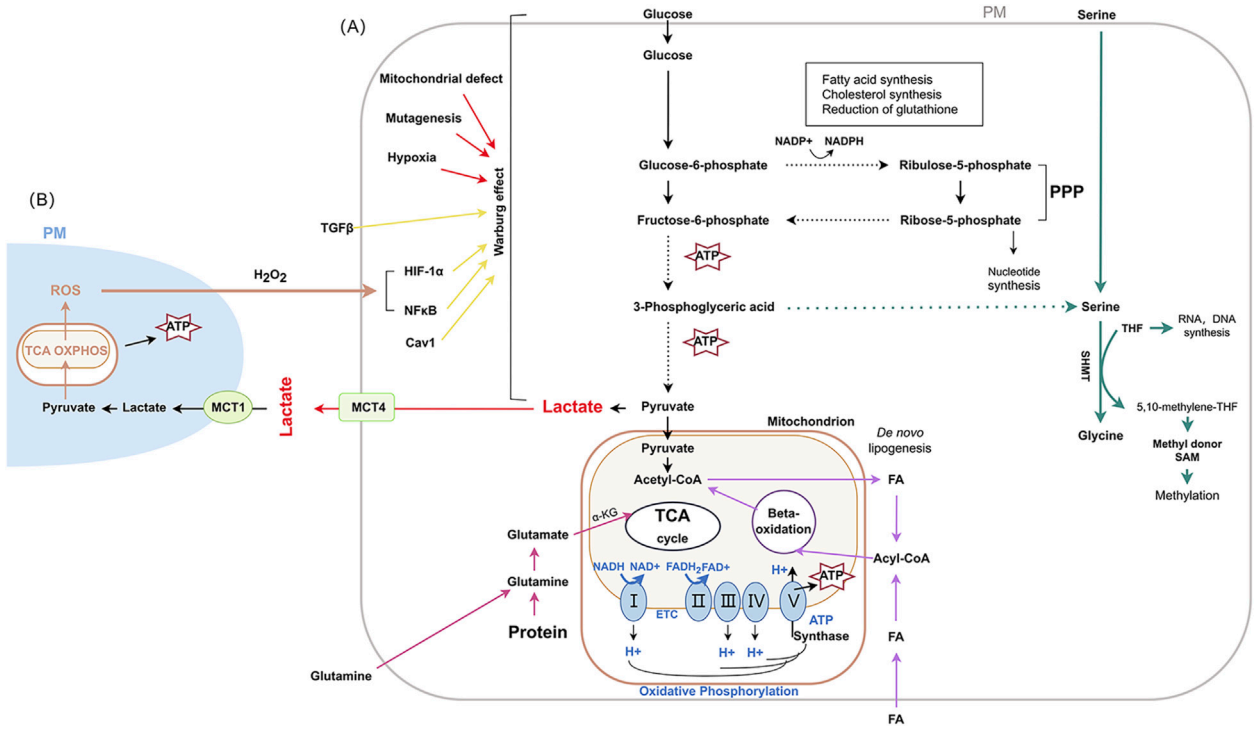


FIGURE 1
The main metabolic pathways and changes in cancer Abbreviation used: PM: plasma membrane; ROS: reactive oxygen species; TCA: tricarboxylic acid; OXPHOS: oxidative phosphorylation; ATP: adenosine triphosphate; MCT1: monocarboxylate transporter-1; TGF-β:Transforming growth factor-β; MCT4: monocarboxylate transporters 4; HIF-1α: hypoxia-inducible factor-1 α; NF-κB: nuclear factor κB; Cav1: Caveolin-1; PPP: pentose phosphate pathway; SHMT: serine hydroxy methyltransferase; THF: tetrahydrofolate; SAM: S-adenosylmethionine; α-KG: α-ketoglutarate; NADH: nicotinamide adenine dinucleotide; FA: fatty acid. FAD: flavin adenine dinucleotide; FADH2: reduced flavin adenine dinucleotide; I: NADH-coenzyme Q reductase; II: succinate coenzyme Q reductase; III: coenzyme Q cytochrome c reductase; IV: cytochrome c oxidase; V: ATP synthase.

shown to reduce the levels of the immune metabolite itaconate, while simultaneously driving macrophages to polarize strongly and persistently towards a pro-inflammatory state, demonstrating an enhanced tumour-inhibiting ability and thereby improving ovarian cancer (OC) survival (Wang X. et al., 2023).

Overexpression of mucin 1 (MUC1) can promote cancer cell proliferation by regulating cell metabolism, and tumor-related MUC1 exhibiting loss of apical localization and aberrant glycosylation in kidney cancer, especially in renal cell carcinoma (RCC) (Milella et al., 2024). Fatty acid metabolism presents the potential clinical application value of PD-1/PD-L1 in the TME of clear cell renal cell carcinoma (ccRCC), thereby providing a predictive treatment response for personalized medicine (Lin et al., 2023). Regulating glycolytic metabolism through targeting the c-Myc oncoprotein is demonstrated in the ubiquitin specific peptidase 43 (USP43) enzyme in bladder cancer. The degradation of c-Myc is achieved through interference with f-box and WD repeat domain containing 7 (FBXW7) by USP43 upregulation (Li M. et al., 2024).

In liver cancer, the identification and characterization of novel invasive cancer types will enhance the understanding of invasion and metastasis, leading to the development of novel precision therapies (Wu L. et al., 2023). In hepatocellular carcinoma (HCC), solute carrier family 25 member 15 (SLC25A15) is hypoxia-responsive with low glutamine reprogramming, facilitating anti-PD-L1 therapy (Zhang Q. et al., 2024). The loss of NADPH oxidase 4 (NOX4) in HCC induces MR in a nuclear factor erythroid 2-related factor 2 (Nrf2)/Myc-dependent manner, promoting tumor progression and making this tumor suppressor function a targeted therapy (Penuelas-Haro et al., 2023). Recently, nanoparticles-mediated co-delivery of cofilin 1 (CFL1) silencing with sorafenib, a chemotherapeutic agent, showed elevated inhibitory properties for HCC tumor growth without exhibiting significant toxicity (Li S. et al., 2023).

For Lung cancer, solute carrier family 3 member 2 (SLC3A2) acts as a metabolic switch factor in tumor-associated macrophages (TAM), suggesting that lung adenocarcinoma (LUAD) phenotyping reprogramming occurs via arachidonic acid (Li Z. et al., 2023). Oncogenic mutations are likely to facilitate MR within cancer cells for sustaining their energy and biomass demands. Results obtained from non-small cell lung cancer (NSCLC) cells with varying EGFR and kristen rat sarcoma (KRAS) statuses indicated that NSCLC cell lines possess a heterogeneous metabolic profile, which could facilitate metabolically targeted therapy for NSCLC patients through identification and stratification (Mendes et al., 2023).

Acute myeloid leukemia (AML) demonstrates a resistance to immunosurveillance and chemotherapy in leukemia stem cells (LSCs). For instance, inhibition of the Src homology region 2 (SH-2) domain-containing phosphatase 1 (Shp1) can alter the energy mechanisms of LSCs to confer sensitivity to chemotherapeutic drugs and presenting a promising prospect for surmounting resistance in AML (Xu et al., 2024). Through the AKT-β-catenin pathway, the expression of phosphofructokinase platelet (PFKP) is upregulated, which increased the metabolic activities and

promote the degradation of Myc, thereby reducing the abilities of LSCs to evade the immune system and enhance the sensitivity to chemotherapy. Despite the emergence of drug resistance, precision treatment with a combined synergistic effect of mTOR and fibroblast growth factor receptor 1 (FGFR1) inhibitors is promising for T-cell acute lymphoblastic leukemia (T-ALL). This is practically achievable through MR, leading to the reversal of FGFR1 inhibitor resistance (Zhang et al., 2023). In osteosarcoma, clustering of lactic acid metabolism could identify the NADH dehydrogenase (ubiquinone) complex I assembly factor 6 (NDUFAF6, also known as C8ORF38) gene, which is a lactic acid metabolism-related gene and a prognostic marker, thus making this gene a functional target for individuals with this cancer type (Wang et al., 2024a).

While promising for metabolism-based therapies, advanced feature analysis through single-cell, multi-omics and spatial technologies, as well as accurate tracking of dynamic changes in metabolic adaptation, will promote the application of precision metabolic therapy in cancer treatment (Wu Z. et al., 2023). Trans-omics, as part of the quest for multiple biomarkers, provides reliability and accuracy for cancer diagnosis. With growing interest in lung cancer and MR, specific metabolites such as lipids can offer a comprehensive analysis of lung cancer. To some extent, precision therapy for lung cancer depends on this novel trans-omics network approach (Yan et al., 2024). Considering the impact of MR on inter-patient heterogeneity, a model pipeline has been developed for ensemble learning, providing insights for the treatment of LUAD (Sun et al., 2024). Formyl peptide receptor 3 (FPR3) can hinder the nuclear translocation of the nuclear factor of activated T cells 1 (NFATc1) by blocking cytoplasmic calcium influx and deactivating the NFATc1-binding neurogenic locus notch homolog protein 3 (NOTCH3) promoter. This process leads to the downregulation of glycolysis through the blocking of the AKT/mTORC1 signaling pathway and NOTCH3 expression. Gastric cancer precision therapy has proven the role of FPR3 in a calcium-dependent manner, thus providing insights into other precision therapy options (Wang et al., 2024b). Cancer-associated fibroblasts (CAF) have metabotropic subtypes with promising precision therapy applications, especially in liver metastases of colorectal cancer (CRC). The interaction of different immune cells with various communication strategies determines the sensitivity of chemotherapeutic drugs (Wu C. et al., 2023). Phosphoglycerate dehydrogenase (PHGDH) can modulate aryl hydrocarbon receptor (AhR) signaling and the redox-dependent autophagy pathway in CRC. Consequently, combination therapy that includes inhibition of both AhR and PHGDH is promising for this type of cancer (Li HM. et al., 2024). Additionally, a notable correlation exists between the expression level of PD-L1 and the individualized treatment regimen, as demonstrated by the mitochondrial pyruvate carrier 3 (MPC3) energy type in the treatment of esophageal squamous cell carcinoma (ESCC) (Wang Z. et al., 2024). Figure 2.

Prospects on metabolic reprogramming and cancer personalized medicine

A model derived from machine learning could potentially identify the metabolic landscape of gastric cancer for early detection (Chen et al., 2024). Another promising approach involves ultrasound-mediated cancer diagnosis using noninvasive mass spectrometry imaging (MSI), particularly effective for breast cancers, offering detailed "fingerprints" of elasticity and histopathology metabolism (Zhou et al., 2024). MR can impact T cell exhaustion via R-loop score patterns present tailored treatment avenues, crucial as R-loop regulators drive tumor progression (Zhang S. et al., 2024). Moreover, MR modulates tumor cell survival under hypoxia, influencing tumor proliferation and genomic stability (Abou Khouzam et al., 2023). The hypoxic TME also enhances chimeric antigen receptor (CAR)-T cell efficacy as a durable antitumor strategy (Zhu et al., 2024).

The risk of cancer, notably metabolic cancers linked to obesity, affects the phenotype and metabolism of immune cells. MR studies focusing on metabolic disorder-induced cancers may uncover biomarkers crucial for immunotherapy (Rosario et al., 2023). Oxidative stress and metabolism-related genes serve as reliable predictors for personalized medicine, aiding in targeted drug therapies and early diagnosis of patients at high risk for stomach adenocarcinoma (STAD) (Dong et al., 2023). In melanoma, targeted therapies exploiting metabolic phenotypes have shown promise with immune checkpoint inhibitors (ICI) (Carlino et al., 2021). Additionally, the study of platelet-derived microparticles in chronic lymphocytic leukemia (CLL) has revealed potential for precision medicine by highlighting immunologically dysfunctional B-lymphocytes (Gharib et al., 2023). A347D mutant p53 variant is implicated in cancer susceptibility among individuals with Li-Fraumeni syndrome (Choe et al., 2023). Mitochondrial OXPHOS adversely impacts antitumor immunity by reducing tumor-infiltrating T cells (Zhao et al., 2022). Future research should explore tumor heterogeneity within the TME and its implications for immune responses in precision medicine (Xu W. et al., 2023).

Improving cancer treatment outcomes and enhancing precision and predictive medicine require MR to reveal treatment vulnerabilities. As one of the newest approaches, some metabolic molecules have already progressed from the preclinical stage to the later stage of clinical trials. For example, AG-120 (ivosidenib), an inhibitor of the IDH1 mutant enzyme, has an acceptable safety profile and clinical activity, according to preliminary data from a Phase I clinical trial recruiting cancer patients with the IDH1 mutation (Popovici-Muller et al., 2018). Inhibition of fatty acid synthase (FASN) revealed a distinct tumor response, which were exacerbated by proliferative potential and mitochondrial respiration. The differences in expression patterns exhibited by FASN in pancreatic ductal adenocarcinoma (PDAC) illuminate the hallmarks of lipid metabolism (Chianese et al., 2023). TVB-2640, a FASN inhibitor, was found to be a well-tolerated oral agent in a Phase II study of recurrent high-grade astrocytoma and could be safely combined with bevacizumab to improve progression-free survival (PFS) (Kelly et al., 2023). Preclinical and clinical trial data suggest that the combination of CB-839, a glutaminase inhibitor, and capecitabine can be an effective treatment for PIK3CA-mutated CRC (Grkovski et al., 2020). CPI-613 is a novel anticancer agent that selectively targets altered forms of mitochondrial energy metabolism in tumor cells, causing changes in mitochondrial enzyme activity and REDOX status, which lead to apoptosis, necrosis, and autophagy in tumor cells. The use of CPI-613 in combination with modified FOLFIRINOX in

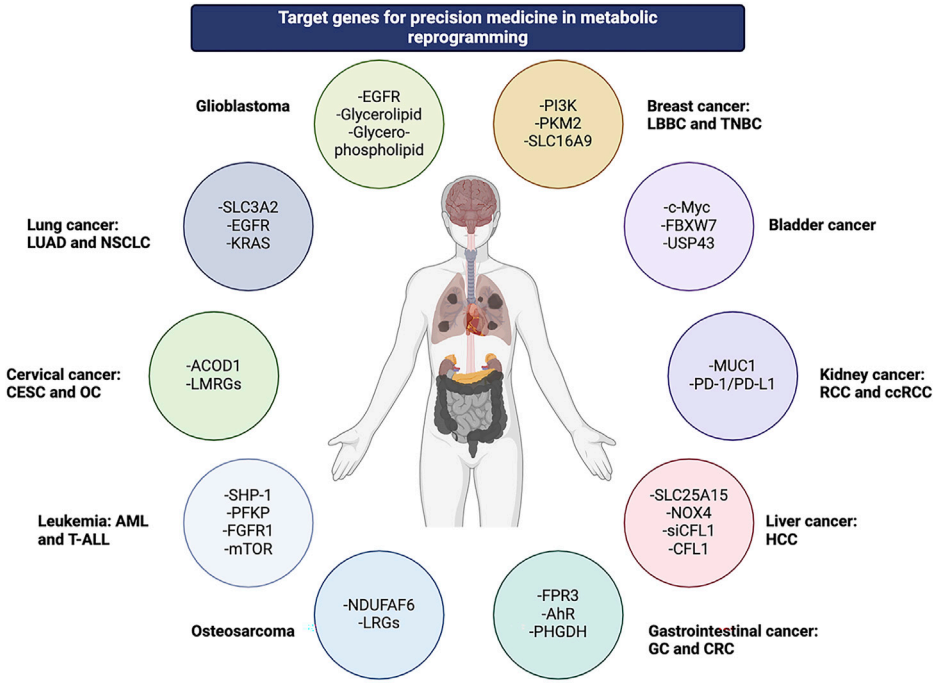


FIGURE 2
Various genes across multiple cancer types are involved in metabolic reprogramming as potential targets for precision medicine.

patients with metastatic pancreatic cancer requires validation in a phase 2 trial (Alistar et al., 2017). However, despite the outstanding results of basic research in tumor MR, many metabolic enzymes have been targeted as tumor therapy, but the vulnerability of specific tumor types to specific inhibitors remains to be further investigated, whether it is a single drug or combination chemotherapy, radiotherapy, targeted therapy or immunotherapy.

In summary, the concept of modifying cancer cell metabolism to slow disease progression while enhancing immune cell function represents a groundbreaking approach in personalized MR intervention. Essential to this endeavor are enzyme inhibitors, metabolic enzyme modifications, pathway interactions, MR drug delivery targets, and methodical study designs (Wang Q. et al., 2024). The efficacy of MR is contingent upon a comprehensive understanding of the diverse metabolic environments present within cancers, which influence DNA repair mechanisms and therapeutic resistance, thus elucidating the broader metabolic landscape in cancer (Das et al., 2023). A novel therapeutic strategy that targets the interplay between cancer epigenetics, metabolism, and DNA repair pathways holds promise (Xing et al., 2023). Recent advances in molecular subtyping, including proteomic, genomic, transcriptomic, and phosphoproteomic profiling, along with assessments of microenvironment dysregulation, genetic alterations, and kinase-substrate regulatory networks, are poised to yield distinct therapeutic responses.

Conclusion

Personalized medicine in cancer therapy is patient-dependent and largely influenced by gene profile. Cancer cells survive within the TME through mechanisms of energy reprogramming. Given the genetic predisposition to various metabolic disorders, research into personalized pharmacotherapy will enhance the long-term efficacy of anti-cancer agents and mitigate drug resistance. Cancer precision medicine based on MR to suppress cancer growth while enhancing immunity is part of the hallmark of cancer research. While this mini review gives some examples of the forms of cancer with distinct energy requirements, obstructing the sources of these energies to rewire metabolic output will give essential requirements for personalized medicine.

Author contributions

TG: Writing–original draft, Conceptualization. LY: Writing–review and editing. YZ: Writing–review and editing. OB: Writing–review and editing. XY: Visualization, Writing–original draft.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. Excellent Youth Project of Natural Science Foundation of Heilongjiang Province (No: YQ2022H015).

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.

Publisher's note

References

Abou Khouzam, R., Sharda, M., Rao, S. P., Kyerewah-Kersi, S. M., Zeinelabdin, N. A., Mahmood, A. S., et al. (2023). Chronic hypoxia is associated with transcriptomic reprogramming and increased genomic instability in cancer cells. *Front. Cell Dev. Biol.* 11, 1095419. doi:10.3389/fcell.2023.1095419

Alistar, A., Morris, B. B., Desnoyer, R., Klepin, H. D., Hosseinzadeh, K., Clark, C., et al. (2017). Safety and tolerability of the first-in-class agent CPI-613 in combination with modified FOLFIRINOX in patients with metastatic pancreatic cancer: a single-centre, open-label, dose-escalation, phase 1 trial. *Lancet Oncol.* 18 (6), 770–778. doi:10.1016/S1470-2045(17)30314-5

An, F., Chang, W., Song, J., Zhang, J., Li, Z., Gao, P., et al. (2024). Reprogramming of glucose metabolism: metabolic alterations in the progression of osteosarcoma. *J. Bone Oncol.* 44, 100521. doi:10.1016/j.jbo.2024.100521

Bigos, K. J., Quiles, C. G., Lunj, S., Smith, D. J., Krause, M., Troost, E. G., et al. (2024). Tumour response to hypoxia: understanding the hypoxic tumour microenvironment to improve treatment outcome in solid tumours. *Front. Oncol.* 14, 1331355. doi:10.3389/fonc.2024.1331355

Buck, M. D., Sowell, R. T., Kaech, S. M., and Pearce, E. L. (2017). Metabolic instruction of immunity. *Cell* 169 (4), 570–586. doi:10.1016/j.cell.2017.04.004

Carlino, M. S., Larkin, J., and Long, G. V. (2021). Immune checkpoint inhibitors in melanoma. *Lancet* 398 (10304), 1002–1014. doi:10.1016/S0140-6736(21)01206-X

Chen, Y., Wang, B., Zhao, Y., Shao, X., Wang, M., Ma, F., et al. (2024). Metabolomic machine learning predictor for diagnosis and prognosis of gastric cancer. *Nat. Commun.* 15 (1), 1657. doi:10.1038/s41467-024-46043-y

Chianese, U., Papulino, C., Ali, A., Ciardiello, F., Cappabianca, S., Altucci, L., et al. (2023). FASN multi-omic characterization reveals metabolic heterogeneity in pancreatic and prostate adenocarcinoma. *J. Transl. Med.* 21 (1), 32. doi:10.1186/s12967-023-03874-5

Choe, J. H., Kawase, T., Xu, A., Guzman, A., Obradovic, A. Z., Low-Calle, A. M., et al. (2023). Li-fraumeni syndrome-associated dimer-forming mutant p53 promotes transactivation-independent mitochondrial cell death. *Cancer Discov.* 13 (5), 1250–1273. doi:10.1158/2159-8290.CD-22-0882

Ciardiello, F., Ciardiello, D., Martini, G., Napolitano, S., Tabernero, J., and Cervantes, A. (2022). Clinical management of metastatic colorectal cancer in the era of precision medicine. *CA Cancer J. Clin.* 72 (4), 372–401. doi:10.3322/caac.21728

Cui, X., Zhao, J., Li, G., Yang, C., Yang, S., Zhan, Q., et al. (2023). Blockage of EGFR/AKT and mevalonate pathways synergize the antitumor effect of temozolomide by reprogramming energy metabolism in glioblastoma. *Cancer Commun. (Lond)* 43 (12), 1326–1353. doi:10.1002/cac2.12502

Das, C., Adhikari, S., Bhattacharya, A., Chakraborty, S., Mondal, P., Yadav, S. S., et al. (2023). Epigenetic-metabolic interplay in the DNA damage response and therapeutic resistance of breast cancer. *Cancer Res.* 83 (5), 657–666. doi:10.1158/0008-5472.CAN-22-3015

Das, C., Bhattacharya, A., Adhikari, S., Mondal, A., Mondal, P., Adhikary, S., et al. (2024). A prismatic view of the epigenetic-metabolic regulatory axis in breast cancer therapy resistance. *Oncogene* 43 (23), 1727–1741. doi:10.1038/s41388-024-03054-9

Dong, Y., Yuan, Q., Ren, J., Li, H., Guo, H., Guan, H., et al. (2023). Identification and characterization of a novel molecular classification incorporating oxidative stress and metabolism-related genes for stomach adenocarcinoma in the framework of predictive, preventive, and personalized medicine. *Front. Endocrinol. (Lausanne)* 14, 1090906. doi:10.3389/fendo.2023.1090906

Gharib, E., Veilleux, V., Boudreau, L. H., Pichaud, N., and Robichaud, G. A. (2023). Platelet-derived microparticles provoke chronic lymphocytic leukemia malignancy through metabolic reprogramming. *Front. Immunol.* 14, 1207631. doi:10.3389/fimmu.2023.1207631

Grkovski, M., Goel, R., Krebs, S., Staton, K. D., Harding, J. J., Mellinghoff, I. K., et al. (2020). Pharmacokinetic assessment of (18)F-(2S,4R)-4-Fluoroglutamine in patients with cancer. *J. Nucl. Med.* 61 (3), 357–366. doi:10.2967/jnumed.119.229740

Kelly, W., Diaz Duque, A. E., Michalek, J., Konkel, B., Caflisch, L., Chen, Y., et al. (2023). Phase II investigation of TVB-2640 (denifanstat) with bevacizumab in patients with first relapse high-grade astrocytoma. *Clin. Cancer Res.* 29 (13), 2419–2425. doi:10.1158/1078-0432.CCR-22-2807

Li, H. M., Li, X., Xia, R., Zhang, X., Jin, T. Z., and Zhang, H. S. (2024b). PHGDH knockdown increases sensitivity to SR1, an aryl hydrocarbon receptor antagonist, in colorectal cancer by activating the autophagy pathway. *FEBS J.* 291 (8), 1780–1794. doi:10.1111/febs.17080

Li, M., Yu, J., Ju, L., Wang, Y., Jin, W., Zhang, R., et al. (2024a). USP43 stabilizes c-Myc to promote glycolysis and metastasis in bladder cancer. *Cell Death Dis.* 15 (1), 44. doi:10.1038/s41419-024-06446-7

Li, S., Xu, L., Wu, G., Huang, Z., Huang, L., Zhang, F., et al. (2023a). Remodeling serine synthesis and metabolism via nanoparticles (NPs)-Mediated CFL1 silencing to enhance the sensitivity of hepatocellular carcinoma to sorafenib. *Adv. Sci. (Weinh)* 10 (19), e2207118. doi:10.1002/advs.202207118

Li, Z., Chen, S., He, X., Gong, S., Sun, L., and Weng, L. (2023b). SLC3A2 promotes tumor-associated macrophage polarization through metabolic reprogramming in lung cancer. *Cancer Sci.* 114 (6), 2306–2317. doi:10.1111/cas.15760

Lin, H., Fu, L., Li, P., Zhu, J., Xu, Q., Wang, Y., et al. (2023). Fatty acids metabolism affects the therapeutic effect of anti-PD-1/PD-L1 in tumor immune microenvironment in clear cell renal cell carcinoma. *J. Transl. Med.* 21 (1), 343. doi:10.1186/s12967-023-04161-z

Lin, J., Hou, L., Zhao, X., Zhong, J., Lv, Y., Jiang, X., et al. (2024). Switch of ELF3 and ATF4 transcriptional axis programs the amino acid insufficiency-linked epithelial-to-mesenchymal transition. *Mol. Ther.* 32 (6), 1956–1969. doi:10.1016/j.ymthe.2024.04.025

Liu, C., Yang, L., Gao, T., Yuan, X., Bajinka, O., and Wang, K. (2024b). A mini-review-cancer energy reprogramming on drug resistance and immune response. *Transl. Oncol.* 49, 102099. doi:10.1016/j.tranon.2024.102099

Liu, X., Ren, B., Ren, J., Gu, M., You, L., and Zhao, Y. (2024a). The significant role of amino acid metabolic reprogramming in cancer. *Cell Commun. Signal* 22 (1), 380. doi:10.1186/s12964-024-01760-1

Malik, N., Kim, Y. I., Yan, H., Tseng, Y. C., du Bois, W., Ayaz, G., et al. (2023). Dysregulation of mitochondrial translation caused by CBFB deficiency cooperates with mutant PIK3CA and is a vulnerability in breast cancer. *Cancer Res.* 83 (8), 1280–1298. doi:10.1158/0008-5472.CAN-22-2525

Mendes, C., Lemos, I., Francisco, I., Almodovar, T., Cunha, F., Albuquerque, C., et al. (2023). NSCLC presents metabolic heterogeneity, and there is still some leeway for EGF stimuli in EGFR-mutated NSCLC. *Lung Cancer* 182, 107283. doi:10.1016/j.lungcan.2023.107283

Miao, Y. Z., Wang, J., Hao, S. Y., Deng, Y. X., Zhang, Z., Jin, Z. P., et al. (2023). The inhibition of Aurora A kinase regulates phospholipid remodeling by upregulating LPCAT1 in glioblastoma. *Neoplasma* 70 (2), 260–271. doi:10.4149/neo_2023_221126N1140

Milella, M., Rutigliano, M., Lasorsa, F., Ferro, M., Bianchi, R., Fallara, G., et al. (2024). The role of MUC1 in renal cell carcinoma. *Biomolecules* 14 (3), 315. doi:10.3390/biom14030315

Ni, X., Lu, C. P., Xu, G. Q., and Ma, J. J. (2024). Transcriptional regulation and post-translational modifications in the glycolytic pathway for targeted cancer therapy. *Acta Pharmacol. Sin.* 45, 1533–1555. doi:10.1038/s41401-024-01264-1

Penuelas-Haro, I., Espinosa-Sotelo, R., Crosas-Molist, E., Herranz-Iturbide, M., Caballero-Diaz, D., Alay, A., et al. (2023). The NADPH oxidase NOX4 regulates redox and metabolic homeostasis preventing HCC progression. *Hepatology* 78 (2), 416–433. doi:10.1002/hep.32702

Popovici-Muller, J., Lemieux, R. M., Artin, E., Saunders, J. O., Salituro, F. G., Travins, J., et al. (2018). Discovery of AG-120 (ivosidenib): a first-in-class mutant IDH1 inhibitor for the treatment of IDH1 mutant cancers. *ACS Med. Chem. Lett.* 9 (4), 300–305. doi:10.1021/acsmedchemlett.7b00421

Rosario, S. R., Dong, B., Zhang, Y., Hsiao, H. H., Isenhart, E., Wang, J., et al. (2023). Metabolic dysregulation explains the diverse impacts of obesity in males and females with gastrointestinal cancers. *Int. J. Mol. Sci.* 24 (13), 10847. doi:10.3390/ijms241310847

Rulten, S. L., Grose, R. P., Gatz, S. A., Jones, J. L., and Cameron, A. J. M. (2023). The future of precision oncology. *Int. J. Mol. Sci.* 24 (16), 12613. doi:10.3390/ijms241612613

Safi, A., Saberiyan, M., Sanaei, M. J., Adelian, S., Davarani Asl, F., Zeinaly, M., et al. (2023). The role of noncoding RNAs in metabolic reprogramming of cancer cells. *Cell Mol. Biol. Lett.* 28 (1), 37. doi:10.1186/s11658-023-00447-8

Sun, L., Zhang, H., and Gao, P. (2022). Metabolic reprogramming and epigenetic modifications on the path to cancer. *Protein Cell* 13 (12), 877–919. doi:10.1007/s13238-021-00846-7

Sun, X., Nong, M., Meng, F., Sun, X., Jiang, L., Li, Z., et al. (2024). Architecting the metabolic reprogramming survival risk framework in LUAD through single-cell landscape analysis: three-stage ensemble learning with genetic algorithm optimization. *J. Transl. Med.* 22 (1), 353. doi:10.1186/s12967-024-05138-2

Thomas, D., Wu, M., Nakauchi, Y., Zheng, M., Thompson-Peach, C. A. L., Lim, K., et al. (2023). Dysregulated lipid synthesis by oncogenic IDH1 mutation is a targetable synthetic lethal vulnerability. *Cancer Discov.* 13 (2), 496–515. doi:10.1158/2159-8290.CD-21-0218

Tsai, C. H., Chuang, Y. M., Li, X., Yu, Y. R., Tzeng, S. F., Teoh, S. T., et al. (2023). Immunoediting instructs tumor metabolic reprogramming to support immune evasion. *Cell Metab.* 35 (1), 118–133.e7. doi:10.1016/j.cmet.2022.12.003

Wamsley, N. T., Wilkerson, E. M., Guan, L., LaPak, K. M., Schrank, T. P., Holmes, B. J., et al. (2023). Targeted proteomic quantitation of NRF2 signaling and predictive biomarkers in HNSCC. *Mol. Cell Proteomics* 22 (11), 100647. doi:10.1016/j.mcpro.2023.100647

Wang, H., Liu, B., Long, J., Yu, J., Ji, X., Li, J., et al. (2023a). Integrative analysis identifies two molecular and clinical subsets in Luminal B breast cancer. *iScience* 26 (9), 107466. doi:10.1016/j.isci.2023.107466

Wang, L., Dou, X., Xie, L., Zhou, X., Liu, Y., Liu, J., et al. (2024a). Metabolic landscape of osteosarcoma: reprogramming of lactic acid metabolism and metabolic communication. *Front. Biosci. Landmark Ed.* 29 (2), 83. doi:10.31083/j.fbl2902083

Wang, L., Mao, X., Yu, X., Su, J., Li, Z., Chen, Z., et al. (2024b). FPR3 reprograms glycolytic metabolism and stemness in gastric cancer via calcium-NFATc1 pathway. *Cancer Lett.* 593, 216841. doi:10.1016/j.canlet.2024.216841

Wang, N., Wang, W., Wang, X., Mang, G., Chen, J., Yan, X., et al. (2022). Histone lactylation boosts reparative gene activation post-myocardial infarction. *Circ. Res.* 131 (11), 893–908. doi:10.1161/CIRCRESAHA.122.320488

Wang, Q., Liu, J., Chen, Z., Zheng, J., Wang, Y., and Dong, J. (2024d). Targeting metabolic reprogramming in hepatocellular carcinoma to overcome therapeutic resistance: a comprehensive review. *Biomed. Pharmacother.* 170, 116021. doi:10.1016/j.biopha.2023.116021

Wang, S., and Zhang, S. (2024). A novel eight-gene signature for lipid metabolism predicts the progression of cervical squamous cell carcinoma and endocervical adenocarcinoma. *Reprod. Sci.* 31 (2), 514–531. doi:10.1007/s43032-023-01364-z

Wang, X., Su, S., Zhu, Y., Cheng, X., Cheng, C., Chen, L., et al. (2023b). Metabolic Reprogramming via ACOD1 depletion enhances function of human induced pluripotent stem cell-derived CAR-macrophages in solid tumors. *Nat. Commun.* 14 (1), 5778. doi:10.1038/s41467-023-41470-9

Wang, Z., Zhang, Y., Yang, X., Zhang, T., Li, Z., Zhong, Y., et al. (2024c). Genetic and molecular characterization of metabolic pathway-based clusters in esophageal squamous cell carcinoma. *Sci. Rep.* 14 (1), 6200. doi:10.1038/s41598-024-56391-w

Wu, C., Yu, S., Wang, Y., Gao, Y., Xie, X., and Zhang, J. (2023d). Metabolic-suppressed cancer-associated fibroblasts limit the immune environment and survival in colorectal cancer with liver metastasis. *Front. Pharmacol.* 14, 1212420. doi:10.3389/fphar.2023.1212420

Wu, J., Zhou, J., Chai, Y., Qin, C., Cai, Y., Xu, D., et al. (2023a). Novel prognostic features and personalized treatment strategies for mitochondria-related genes in glioma patients. *Front. Endocrinol. (Lausanne)* 14, 1172182. doi:10.3389/fendo.2023.1172182

Wu, L., Yan, J., Bai, Y., Chen, F., Zou, X., Xu, J., et al. (2023b). An invasive zone in human liver cancer identified by Stereo-seq promotes hepatocyte-tumor cell crosstalk, local immunosuppression and tumor progression. *Cell Res.* 33 (8), 585–603. doi:10.1038/s41422-023-00831-1

Wu, Z., Li, X., Gu, Z., Xia, X., and Yang, J. (2023c). Pyrimidine metabolism regulator-mediated molecular subtypes display tumor microenvironmental hallmarks and assist precision treatment in bladder cancer. *Front. Oncol.* 13, 1102518. doi:10.3389/fonc.2023.1102518

Xing, X., Hu, E., Ouyang, J., Zhong, X., Wang, F., Liu, K., et al. (2023). Integrated omics landscape of hepatocellular carcinoma suggests proteomic subtypes for precision therapy. *Cell Rep. Med.* 4 (12), 101315. doi:10.1016/j.xcrm.2023.101315

Xu, W., Lu, J., Liu, W. R., Anwaier, A., Wu, Y., Tian, X., et al. (2023b). Heterogeneity in tertiary lymphoid structures predicts distinct prognosis and immune microenvironment characterizations of clear cell renal cell carcinoma. *J. Immunother. Cancer* 11 (12), e006667. doi:10.1136/jitc-2023-006667

Xu, X., Peng, Q., Jiang, X., Tan, S., Yang, Y., Yang, W., et al. (2023a). Metabolic reprogramming and epigenetic modifications in cancer: from the impacts and mechanisms to the treatment potential. *Exp. Mol. Med.* 55 (7), 1357–1370. doi:10.1038/s12276-023-01020-1

Xu, X., Yu, Y., Zhang, W., Ma, W., He, C., Qiu, G., et al. (2024). SHP-1 inhibition targets leukaemia stem cells to restore immunosurveillance and enhance chemosensitivity by metabolic reprogramming. *Nat. Cell Biol.* 26 (3), 464–477. doi:10.1038/s41556-024-01349-3

Yan, F., Liu, C., Song, D., Zeng, Y., Zhan, Y., Zhuang, X., et al. (2024). Integration of clinical phenoms and metabolomics facilitates precision medicine for lung cancer. *Cell Biol. Toxicol.* 40 (1), 25. doi:10.1007/s10565-024-09861-w

Yang, J., Ren, B., Ren, J., Yang, G., Fang, Y., Wang, X., et al. (2023b). Epigenetic reprogramming-induced guanidinoacetic acid synthesis promotes pancreatic cancer metastasis and transcription-activating histone modifications. *J. Exp. Clin. Cancer Res.* 42 (1), 155. doi:10.1186/s13046-023-02698-x

Yang, K., Wang, X., Song, C., He, Z., Wang, R., Xu, Y., et al. (2023a). The role of lipid metabolic reprogramming in tumor microenvironment. *Theranostics* 13 (6), 1774–1808. doi:10.7150/thno.82920

Yu, W., Chen, Y., Putluri, N., Osman, A., Coarfa, C., Putluri, V., et al. (2023). Evolution of cisplatin resistance through coordinated metabolic reprogramming of the cellular reductive state. *Br. J. Cancer* 128 (11), 2013–2024. doi:10.1038/s41416-023-02253-7

Yu, X., Ma, R., Wu, Y., Zhai, Y., and Li, S. (2018). Reciprocal regulation of metabolic reprogramming and epigenetic modifications in cancer. *Front. Genet.* 9, 394. doi:10.3389/fgene.2018.00394

Yuan, X., Ouedraogo, S. Y., Trawally, M., Tan, Y., and Bajinka, O. (2024). Cancer energy reprogramming and the immune responses. *Cytokine* 177, 156561. doi:10.1016/j.cyto.2024.156561

Zhang, Q., Wei, T., Jin, W., Yan, L., Shi, L., Zhu, S., et al. (2024b). Deficiency in SLC25A15, a hypoxia-responsive gene, promotes hepatocellular carcinoma by reprogramming glutamine metabolism. *J. Hepatol.* 80 (2), 293–308. doi:10.1016/j.jhep.2023.10.024

Zhang, S., Liu, Y., Sun, Y., Liu, Q., Gu, Y., Huang, Y., et al. (2024c). Aberrant R-loop-mediated immune evasion, cellular communication, and metabolic reprogramming affect cancer progression: a single-cell analysis. *Mol. Cancer* 23 (1), 11. doi:10.1186/s12943-023-01924-6

Zhang, Y., Wu, M. J., Lu, W. C., Li, Y. C., Chang, C. J., and Yang, J. Y. (2024a). Metabolic switch regulates lineage plasticity and induces synthetic lethality in triple-negative breast cancer. *Cell Metab.* 36 (1), 193–208.e8. doi:10.1016/j.cmet.2023.12.003

Zhang, Z. J., Wu, Q. F., Ren, A. Q., Chen, Q., Shi, J. Z., Li, J. P., et al. (2023). ATF4 renders human T-cell acute lymphoblastic leukemia cell resistance to FGFR1 inhibitors through amino acid metabolic reprogramming. *Acta Pharmacol. Sin.* 44 (11), 2282–2295. doi:10.1038/s41401-023-01108-4

Zhao, Z., Mei, Y., Wang, Z., and He, W. (2022). The effect of oxidative phosphorylation on cancer drug resistance. *Cancers (Basel)* 15 (1), 62. doi:10.3390/cancers15010062

Zhou, P., Xiao, Y., Zhou, X., Fang, J., Zhang, J., Liu, J., et al. (2024). Mapping spatiotemporal heterogeneity in multifocal breast tumor progression by noninvasive ultrasound elastography-guided mass spectrometry imaging strategy. *JACS Au* 4 (2), 465–475. doi:10.1021/jacsau.3c00589

Zhu, X., Chen, J., Li, W., Xu, Y., Shan, J., Hong, J., et al. (2024). Hypoxia-responsive CAR-T cells exhibit reduced exhaustion and enhanced efficacy in solid tumors. *Cancer Res.* 84 (1), 84–100. doi:10.1158/0008-5472.CAN-23-1038

Glossary

ACOD1	Aconitate decarboxylase 1
AhR	Aryl hydrocarbon receptor
AML	Acute myeloid leukemia
ATF4	Activating transcription factor 4
CAF	Cancer-associated fibroblasts
CAR	Chimeric antigen receptor
CBFB	Core binding factor subunit β
ccRCC	Clear cell renal cell carcinoma
CESC	Cervical squamous cell carcinoma
CFL1	Co-delivery of cofilin 1
CLL	Chronic lymphocytic leukemia
CRC	Colorectal cancer
EGFR	Epidermal growth factor receptor
EMT	Epithelial-to-mesenchymal transition
ENO1	Enolase 1
ESCC	Esophageal squamous cell carcinoma
EZH2	Enhancer of zeste homolog 2
FASN	Fatty acid synthase
FAO	Fatty acid β-oxidation
FBXW7	F-box and WD repeat domain containing 7
FGFR1	Fibroblast growth factor receptor 1
FPR3	Formyl peptide receptor 3
HCC	Hepatocellular carcinoma
HIF1	Hypoxia-inducible factor 1
HK2	Hexokinase 2
IDH1	Isocitrate dehydrogenase 1
KRAS	Kristen rat sarcoma
LBBC	Luminal B breast cancer
LDHA	Lactate dehydrogenase A
LMRGs	Lipid metabolism-related genes
LSCs	Leukemia stem cells
LUAD	Lung adenocarcinoma
MPC3	Mitochondrial pyruvate carrier 3
MR	Metabolic reprogramming
MSI	Mass spectrometry imaging
MUC1	Mucin 1
NFATc1	Nuclear factor of activated T cells 1
NOX4	NADPH oxidase 4
NOTCH3	Notch homolog protein 3
NDUFAF6	NADH dehydrogenase (ubiquinone) complex I assembly factor 6
Nrf2	Nuclear factor erythroid 2-related factor 2
NSCLC	Non-small cell lung cancer
OXPHOS	Oxidative phosphorylation
OC	Ovarian cancer
PDAC	Pancreatic ductal adenocarcinoma
PHGDH	Phosphoglycerate dehydrogenase
PKM2	Pyruvate kinase M2
PPP	Pentose phosphate pathway
PI3K	Phosphoinositide 3-kinase
PFS	Progression-free survival
PFKP	Phosphofructokinase platelet
RCC	Renal cell carcinoma
SH-2	Src homology region 2
Shp1	Phosphatase 1
SIX1	Sine oculis homeobox homolog 1
SLC3A2	Solute carrier family 3 member 2
SLC25A15	Solute carrier family 25 member 15
SLC16A9	Solute carrier family 16 member 9
STAD	Stomach adenocarcinoma
TAM	Tumor-associated macrophages
T-ALL	T-cell acute lymphoblastic leukemia
TGF-β	Transforming growth factor β
TME	Tumor microenvironment
TNBC	Triple-negative breast cancer
MPC3	Mitochondrial pyruvate carrier 3
USP43	Ubiquitin specific peptidase 43

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 20 February 2025
DOI 10.3389/fphar.2025.1509568

OPEN ACCESS

EDITED BY
Elisa Giovannetti,
VU Medical Center, Netherlands

REVIEWED BY
Parmanand Malvi,
University of Alabama at Birmingham, United States
Chao Ma,
Shanghai University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Liyan Chen,
lychen@ybu.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 11 October 2024
ACCEPTED 31 January 2025
PUBLISHED 20 February 2025

CITATION
Du P, Li Y, Han A, Wang M, Liu J, Piao Y and Chen L (2025) β-lapachone suppresses carcinogenesis of cervical cancer via interaction with AKT1.
Front. Pharmacol. 16:1509568.
doi: 10.3389/fphar.2025.1509568

β-lapachone suppresses carcinogenesis of cervical cancer via interaction with AKT1

Pan Du[1†], Yue Li[2†], Anna Han[1], Mengying Wang[1], Jiajing Liu[1], Yingshi Piao[1,3] and Liyan Chen[1,3*]

[1]Key Laboratory of Pathobiology (Yanbian University), State Ethnic Affairs Commission, Yanji, China, [2]Changchun Center for Disease Control and Prevention, Changchun, China, [3]Cancer Research Center, Yanbian University, Yanji, China

Introduction: Cervical cancer is one of the most prevalent malignant tumors affecting women worldwide, and affected patients often face a poor prognosis due to its high drug resistance and recurrence rates. β-lapachone, a quinone compound originally extracted from natural plants, is an antitumor agent that specifically targets NQO1.

Methods: CC cells were treated with varying concentrations of β-lapachone to examine its effects on glucose metabolism, proliferation, metastasis, angiogenesis, and EMT *in vitro*. The targets and action pathways of β-lapachone were identified using network pharmacology and molecular docking, with KEGG pathway enrichment analysis. Its effects and toxicity were verified *in vivo* using a nude mouse xenograft model.

Results: β-lapachone significantly inhibited the proliferation and metastasis of cervical cancer cells by regulating glucose metabolism, reducing tumor angiogenesis, and suppressing epithelial-mesenchymal transition (EMT) in cells with high NQO1 expression. Furthermore, we identified the inactivation of the PI3K/AKT/mTOR pathway as the key mechanism underlying these effects. AKT1 was identified as a potential target of β-lapachone in modulating glucose metabolism and EMT in cervical cancer cells.

Conclusion: These findings suggest that β-lapachone inhibits the malignant progression of cervical cancer by targeting AKT1 to regulate glucose metabolism in NQO1-overexpressing cells, providing a theoretical basis for developing novel therapeutic strategies for cervical cancer.

KEYWORDS

β-lapachone, glucose metabolism, AKT1, EMT, cervical cancer

Introduction

Cervical cancer (CC) remains one of the leading causes of cancer-related mortality among women worldwide, particularly in young and middle-aged populations (Siegel et al., 2024). Recent data indicate that by 2050, the World Health Organization (WHO) predicts CC will result in over 1 million deaths annually (Feng et al., 2019). While radiotherapy is a commonly used treatment, it often lacks specificity and is associated with significant side effects, leading to poor prognosis and high recurrence rates (Woo and Kim, 2022; Vaccarella et al., 2013). Therefore, it is crucial to identify novel targeted drugs with minimal side effects to treat CC.

β-lapachone, a 1,2-naphthoquinone compound first isolated from the lapacho tree (*Tabebuia avellanedae*) (Yu et al., 2014), has demonstrated diverse biological activities, including anti-inflammatory, antiviral, and antipsoriatic effects (Sanajou et al., 2021; Kee et al., 2017). In recent years, β-lapachone has gained attention for its selective antitumor activity in various cancers, including hepatocellular carcinoma, gastric carcinoma, and breast cancer (Zhou et al., 2021; Zheng et al., 2021a; Yang et al., 2017). Its effects are closely tied to NAD(P)H: quinone oxidoreductase 1 (NQO1), an enzyme that catalyzes the conversion of β-lapachone into unstable hydroquinone. This compound undergoes rapid autoxidation, producing large amounts of reactive oxygen species (ROS) that cause oxidative DNA damage. This, in turn, hyperactivates poly (ADP-ribose) polymerase-1 (PARP1), leading to depletion of NAD^+/ATP and ultimately inducing cell death (Huang et al., 2016; Lamberti et al., 2018). Despite the established importance of β-lapachone–NQO1 interactions in various cancers, its therapeutic potential in CC remains unclear.

Reprogramming of glucose metabolism serves as a distinguishing feature of tumor cells, which provides sustained energy for tumor growth and metastasis (DeBerardinis et al., 2008; Dong et al., 2022). To achieve sustained proliferation, tumor cells usually adjust their metabolism and nutrient acquisition. Tumor cells are usually dependent on an environment in which aerobic glycolysis produces large amounts of ATP and lactate even when oxygen is sufficient. This cellular switch from the normal respiratory pathway to aerobic glycolysis is known as the Warburg effect (Wu et al., 2016; Gu et al., 2017; Christofk et al., 2008). Moreover, in addition to serving as an energy source for cancer cells, aerobic glycolysis promotes local tumor invasion and metastasis and facilitates tumor immune escape (Tamada et al., 2012; Kitamura et al., 2011). Therefore, the development of antitumor drugs targeting the glucose metabolism pathway has the potential to provide an effective approach to cancer treatment.

In this study, we demonstrated that β-lapachone exhibits significant antitumor effects against NQO1-overexpressing CC cells *in vitro* and *in vivo*. Mechanistic studies revealed that β-lapachone inhibits cell proliferation, metastasis, glucose metabolism, tumor angiogenesis, and EMT by targeting AKT1 to inactivate the PI3K/AKT/mTOR pathway.

Materials and methods

Chemical compounds and cell culture

β-lapachone was purchased from Medchem Express (HY-13555, United States) and was dissolved in dimethyl sulfoxide (DMSO, D8371, Solarbio, China) to prepare a stock solution of 10 mM and stored at −20°C. 740 Y-P was purchased from Medchem Express (HY-P0175, United States). Human cervical cancer cell lines SiHa, HeLa, C33a and HcerEpic were provided by the Oncology Research Center of Yanbian University, China. HeLa, C33a, HcerEpic, and SiHa cells were cultured in Dulbecco's modified Eagle Medium (DMEM) supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS). The cells were kept at a steady temperature of 37°C and exposed to 5% CO_2.

MTT assay

Cells were inoculated in 96-well plates and treated with different concentrations of β-lapachone for 24, 48 and 72 h. Subsequently, 100 μL of MTT (1 μg/mL) was introduced to each well and left to incubate away from light for 4 h. Finally, 100 μL of DMSO was introduced to each well, and the cells were incubated away from light for 10 min. The absorbance measurement at 490 nm was used to determine the OD value.

Colony-formation assay

The cells were placed in 6-well plates at a concentration of 1,000 cells per well for the purpose of colony formation analysis. Subsequent to subjecting the cells to diverse concentrations of β-lapachone for a duration of 24 h on the subsequent day, the culture was sustained for a period of 10–14 days by substituting the drug-free DMEM medium. After the culture was finished, the cells were put in a 4% paraformaldehyde solution for 15 min, while Giemsa was stained for 30 min. Ultimately, a count of all the colonies was made, and pictures were acquired. We conducted three replications of each experiment.

Wound healing assay

After seeding the cells into 6-well plates, we scratched the middle of each well with a 200 μL pipette tip the next day, once the cells had fully fused. Following PBS washing and incubation in DMEM medium containing varying β-lapachone concentrations, measurements of the wound's distance were made and photos were taken at predetermined intervals.

Transwell invasion and migration assays

For migration assay, the cells were routinely digested to prepare a cell suspension, and a serum-free DMEM culture with a cell density of 1×10^5 was added to 200 μL in the upper chamber. The corresponding lower section was incubated with 800 μL of DMEM medium containing 10% FBS in a 37°C, 5% CO_2 incubator. On the following day, the serum-free DMEM medium containing different concentrations of β-lapachone was added to the upper chamber, the liquid in the lower chamber was discarded, and 800 μL of DMEM medium containing 10% FBS was re-added to continue the culture. The upper chamber was fixed with cold 4% paraformaldehyde at room temperature for 20 min, followed by staining with hematoxylin for 10 min. Remove the non-migrating cells from the bottom of the upper chamber with a cotton swab. The stained cells were observed under a light microscope and processed for blocking, and the number of stained cells was measured and analyzed using ImageJ software. In the invasion assay, the bottom of the transwell was coated with matrigel (matrigel and DMEM culture solution in a 1:6 ratio) 1 day in advance. The remaining experimental procedures were the same as for the migration experiments.

ATP, lactate and glucosen level assays

Cells were plated in 6-well plates and then treated with different doses of β-lapafenone for 24 h. The cells and supernatants were collected separately, and then ATP, lactate, and glucose levels were measured according to the requirements related to the Glucose assay kit (Applygen, E1011), ATP assay kit and Lactate assay kit (Njjcbio, A095/A019-2, China).

Tube formation assay

The 96-well plate was pre-chilled with 25 μL of matrigel in a 1: 1 ratio and incubated at 37°C. Siha and C33a cells were treated with different concentrations of β-lapachone, and the supernatant was extracted and set aside after 6 h of incubation. Prepare HUVEC cell suspension with a density of 3×10^4, 50 μL/well. The extracted 150 μL of supernatant and HUVEC cell suspension were mixed and added to a 96-well plate and incubated at 37°C in a 5% CO_2 incubator. The tube formation was imaged at 30 min intervals with a microscope (BX71, Olympus, Japan), and the resulting pictures were evaluated using ImageJ's Angiogenesis Analyzer plugin.

Plasmids and transfection

The siRNA molecules targeting AKT1 were synthesized, purified and purchased by Hippobio (zhejiang, China). All plasmids were transfected with Lipofectamine™ 3000 transfection reagent (#L3000015, ThermoFisher, shanghai, China) following the manufacturer's instructions. The siRNA sequences used were as follows: siAKT1#1: 5′-GGA CAA GGA CGG GCA CAU UAA TT-3'; siAKT1#2: 5′-CUA UGG CGC UGA GAU UGU GUC TT-3'; siAKT1#3: 5′-CGC CUC ACC AUG AAC GAG UUU TT-3'.

Western blot analysis

Cells were treated with different concentrations of β-lapachone for 24 h. The RIPA lysate, protease inhibitor, and phosphatase inhibitor were combined with the entire cell lysate in a ratio of 100:1: 1, and the BCA kit (Beyotime, China) was used to measure the protein concentration. Through the utilization of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), an equal proportion of the overall 40 μg protein was extracted, isolated, and subsequently transferred onto PVDF membranes (Millipore, United States). Following 1 h of blocking with 5% skimmed milk, a primary antibody was subjected to an overnight incubation at 4°C with the PVDF membrane. The primary antibodies used in this study were as follows: anti-AKT (CST, #4060, 1:1000), anti-E-cadherin (Proteintech, #20874, 1:1000), anti-G6PC (Proteintech, #22169, 1:1000), anti-GADPH (CST, #5174, 1:1000), anti-mTOR (CST, #2983, 1:1000), anti-NQO1 (CST, #3187, 1:1000), anti-PKM2 (Proteintech, #15822, 1:1000), anti-Snail (Proteintech, #13099, 1: 1000), anti-Vimentin (Proteintech, #10366, 1:1000), anti-VEGF (Proteintech, #19003, 1:1000), anti-β-actin (Cwbio, #CW0264, 1: 1000). The membranes were treated with a secondary antibody for one hour at room temperature the following day after being rinsed with TBST. Protein bands were examined using electrochemiluminescence (ECL) imaging equipment (Amersham imager 600, United States), and the bands were analyzed using Image Lab software.

Xenegraft model

The Experimental Animal Center of Yanbian University provided us with female BALB/C nude mice that were 4 weeks old. The Animal Research Ethics Committee granted approval to all experiments, and all creatures were kept in a sterile atmosphere (Registration Number: YD20231120001; Approval Date: 20 November 2023). For the *in vivo* xenograft model, SiHa cells (5×10^6) and matrigel were combined proportionately and subcutaneously into the lower dorsal side of naked mice. Following the injection, the mice were allocated into two sets of six in a random manner. During the intraperitoneal injection of β-lapachone (5 mg/kg) every other day for a duration of 14 days, measurements were taken for body weight and tumor size. After the experiment, the mice were euthanized, all tumor samples were collected, recorded, and further examined.

Rescue experiments

In accordance with the instructions provided by the manufacturer, the PI3K activator 740 Y-P was dissolved in DMSO until it reached a final concentration of 5 μM. Siha cells underwent a 24 h treatment with β-lapachone at a concentration of 4 μM, while C33a cells were subjected to a 24 h treatment at a concentration of 6 μM prior to receiving 740 Y-P for the same duration. A western blot was employed to ascertain the expression of AKT, p-AKT, mTOR, p-mTOR, and EMT-related proteins.

Online database extraction

The GEPIA online database (http://gepia.cancer-pku.cn/) was utilized to analyze the expression of NQO1 in cervical cancer patients (Tang et al., 2017). Through the utilization of the websites Pharm Mapper and Gene Cards, it is possible to ascertain the target genes for β-lapachone in cervical cancer (https://www.lilab-ecust.cn/pharmmapper/, https://www.genecards. org/). Gene Ontology enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted on the target genes for β-lapachone using the KOBAS tool (Bu et al., 2021).

Molecular docking

The 3D structure of AKT1 protein (PDB ID:1UNQ) was first obtained by searching from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB, http://www.rcsb.org/pdb/). The SDF format of β-lapachone was obtained from pubchem database (https://pubchem.ncbi.nlm.nih.gov) and

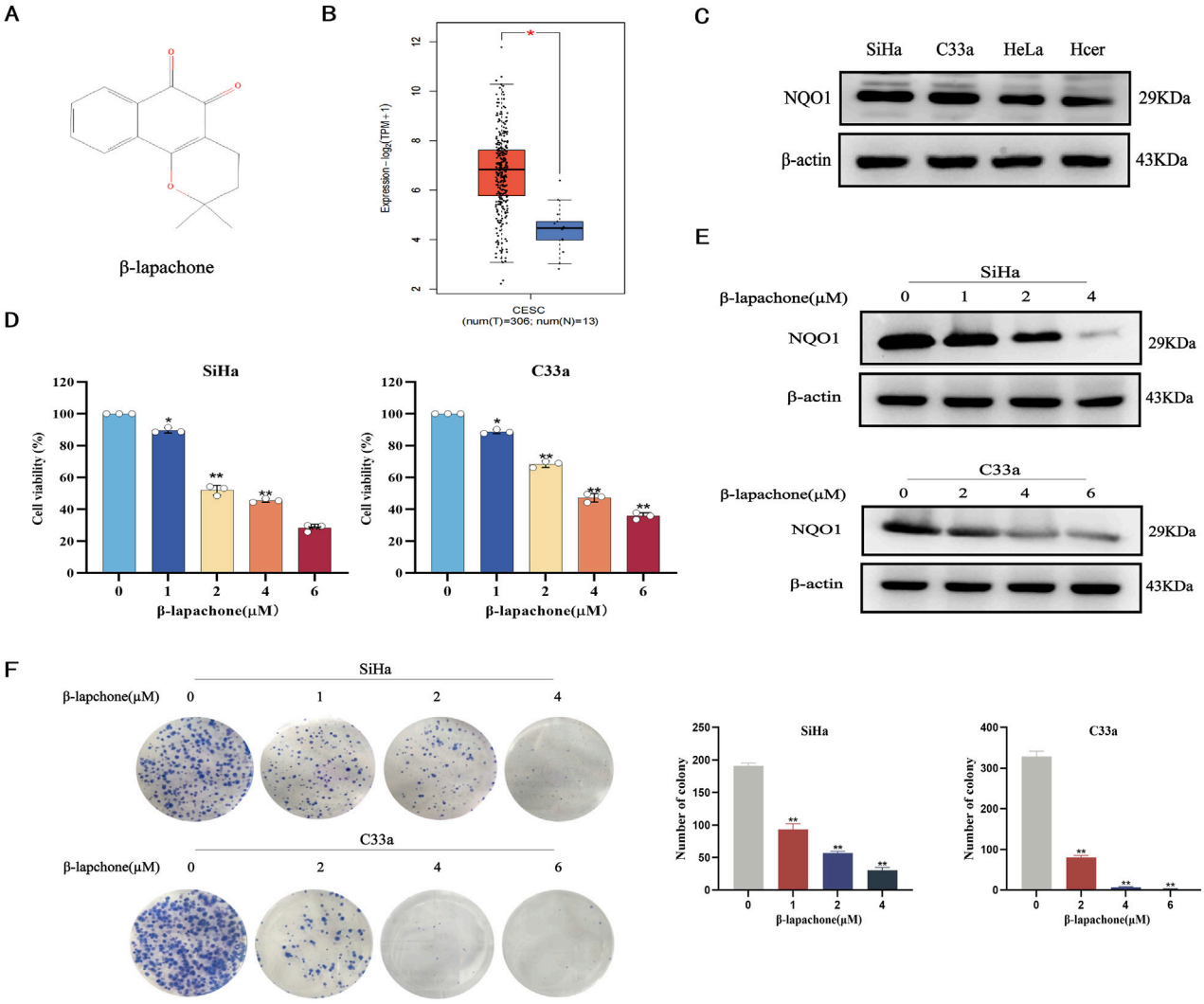


FIGURE 1
β-Lapachone inhibits the proliferation of cervical cancer cells. **(A)** Chemical structures of β-lapachone. **(B)** Expression of NQO1 in cervical cancer and normal tissues were analyzed by GEPIA database. **(C)** NQO1 protein levels in cancer cell lines and normal cells were detected by Western blot. **(D)** Cell viability was detected by MTT assay after treatment of cervical cancer cells with different concentrations of β-lapachone. **(E)** The expression of NQO1 protein in SiHa and C33a cells treated with β-lapachone for 24 h. **(F)** The effect of β-lapachone on cell proliferation was detected by colony formation assay. Error bars represent the means ± SD of three independent experiments. *$P < 0.05$, **$P < 0.01$ vs. 0 μM β-lapachone group.

converted to PDB format by Open Babel. Proteins were dehydrogenated, hydrogenated to calculate charge and converted to pdbqt format using AutoDocktools 1.5.7 software, ligands were hydrogenated, torsional forces were determined and converted to pdbqt format. Docking box coordinates were determined and molecular docking operations were performed using Autodock vina software. And PyMOL 2.1.0 was used to visualize the presentation and obtain the 3D analytical maps; Discovery studio software was used to visualize the interactions between the tested compounds and key residues.

Statistical analysis

Data from the experiment were statistically analyzed using Graphpad Prism 10.0. The standard deviation is used to ± express the data as means. The data was compared among different groups using either a one-way ANOVA or a paired student t-test. All results were performed in at least three independent experiments. $P < 0.05$ showed that the difference was statistically significant.

Results

β-lapachone inhibits the proliferation of cervical cancer cells with high NQO1 expression

To investigate the effects of β-lapachone (Figure 1A) on CC, we first analyzed NQO1 expression in CC tissues using the GEPIA online database. The results indicated that NQO1 expression is

significantly higher in CC tissues compared to normal tissues (Figure 1B). Western blotting further confirmed elevated NQO1 levels in cervical CC lines (SiHa and C33a) compared to normal cervical epithelial cells (HcerEpic). Among these, SiHa and C33a cells, which exhibited the highest NQO1 expression, were selected for subsequent experiments in which they were treated with varying concentrations of β-lapachone (Figure 1C). MTT assays revealed that β-lapachone significantly inhibited the survival of SiHa and C33a cells (Figure 1D). Then, we screened the optimal concentration of β-lapachone for drug action on CC cells, revealing that treatment with β-lapachone also reduced NQO1 expression levels in these cells (Figure 1E). Furthermore, colony formation assays demonstrated that β-lapachone dose-dependently suppressed the colony-forming ability of CC cells compared to the control group (Figure 1F). Collectively, these findings highlight the inhibitory effects of β-lapachone on CC cell proliferation.

β-lapachone attenuates cervical cancer cell migration, invasion, EMT, and angiogenesis

Tumor cell proliferation and metastasis are critical processes in cancer progression. We evaluated the effects of β-lapachone on CC cell migration and invasion using wound healing and Transwell assays. Wound healing assays showed that β-lapachone significantly reduced the wound closure rates for SiHa and C33a cells after 12 h (Figures 2A, B). Similarly, Transwell assays assessing longitudinal migration revealed that β-lapachone markedly decreased the number of migratory cells (Figures 2C, D). Consistent with this, β-lapachone treatment led to a notable reduction in the quantity of invasive cells. Therefore, these findings suggest that β-lapachone can attenuate the migration and invasion of CC cells.

The epithelial-mesenchymal transition (EMT) is essential for the development of tumors. To delve deeper into the potential involvement of the EMT process in the impact of β-lapachone on CC cells, we analyzed the expression of EMT-related markers via Western blotting. The results showed that the expression level of the epithelial marker E-cadherin was significantly elevated in CC cells after β-lapachone treatment. In contrast, there was a marked decrease in the expression of the mesenchymal markers Vimentin and Snail (Figures 2E, F). To further elucidate the mechanisms by which β-lapachone inhibits CC metastasis, supernatants from β-lapachone-treated CC cells were collected and used to culture HUVECs. Microscopic observation of tube formation assay results revealed that β-lapachone significantly inhibited the angiogenic capacity of HUVECs in a concentration-dependent manner (Figure 2G). Additionally, Western blotting showed that β-lapachone substantially suppressed the expression of the angiogenesis-related protein VEGF in treated cells (Figure 2H). These findings collectively suggest that β-lapachone inhibits CC metastasis and progression by suppressing angiogenesis and EMT induction.

β-lapachone regulates glucose metabolism reprogramming in cervical cancer cells

Metabolic reprogramming is a hallmark of cancer, sustaining tumor cell growth, metastasis, survival, and treatment resistance. To evaluate the effect of β-lapachone on glucose metabolism in CC cells, we measured ATP, lactate, and glucose levels after treatment. The results showed a significant increase in intracellular glucose levels and a reduction in ATP and lactate dehydrogenase (LDH) levels following β-lapachone treatment (Figures 3A, B). To further investigate the molecular mechanisms underlying the effects of β-lapachone, we analyzed the expression of the glucose metabolism-associated proteins G6PC and PKM2 in CC cells. Western blotting revealed a significant reduction in G6PC and PKM2 expression levels in β-lapachone-treated SiHa and C33a cells, with more pronounced effects observed in C33a cells (Figures 3C, D). These findings suggest that β-lapachone disrupts glucose metabolism, thereby preventing CC progression.

β-lapachone inhibits cervical cancer progression and metastasis via the PI3K/AKT/mTOR signaling pathway

Using the GeneCards and PharmMapper databases, we identified 94 potential common targets of β-lapachone and CC (Figure 4A). KEGG pathway enrichment analysis of these targets suggested that the PI3K/AKT/mTOR signaling pathway plays a pivotal role in the inhibitory effects of β-lapachone on CC (Figure 4B). To validate this prediction, we analyzed the expression levels of proteins related to the PI3K/AKT/mTOR signaling pathway in β-lapachone-treated CC cells. Western blotting revealed a significant reduction in p-mTOR/mTOR and p-AKT/AKT levels following β-lapachone treatment (Figure 4C).

To confirm the involvement of the PI3K/AKT/mTOR pathway in the tumor-suppressive effects of β-lapachone, we conducted rescue experiments using the PI3K pathway activator 740Y-P. Treatment with 740Y-P reversed the β-lapachone-induced suppression of p-AKT and p-mTOR expression. Additionally, 740Y-P restored β-lapachone-induced changes in EMT markers, including E-cadherin, Snail, and Vimentin (Figure 4D). Collectively, these results indicate that β-lapachone suppresses CC progression and metastasis by inactivating the PI3K/AKT/mTOR signaling pathway.

β-lapachone inhibits glucose metabolism and EMT induction in cervical cancer cells by targeting AKT1

To identify the specific candidate target proteins of β-lapachone involved in inhibiting CC progression, we combined target prediction results with pathway analysis and hypothesized that AKT1, a key protein in the PI3K/AKT/mTOR pathway, is involved in β-lapachone's regulation of CC cells. Molecular docking using AutoDock Vina revealed strong binding between β-lapachone and AKT1, with a binding free energy of −6.7 kcal/mol and the formation of two hydrogen bonds at residues LYS39 and LEU52 (Figure 5A). To validate the role of AKT1, we knocked down AKT1 expression in CC cells (Figure 5B). AKT1 knockdown significantly attenuated the inhibitory effects of β-lapachone on the glucose metabolism

10.3389/fphar.2025.1509568

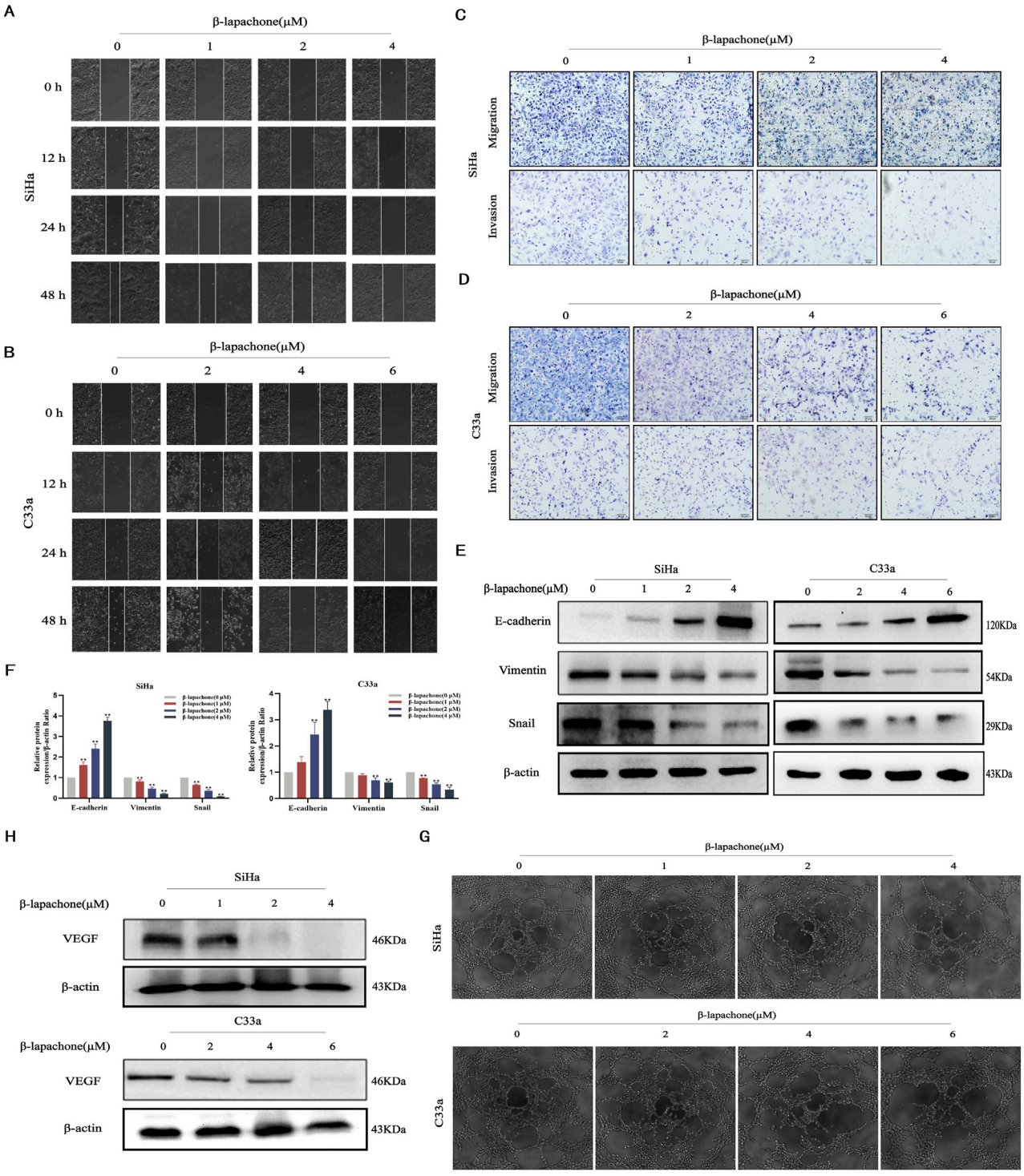


FIGURE 2
β-Lapachone attenuated cervical cancer cell migration, invasion, EMT and angiogenesis. **(A, B)** Wound healing assay was used to reflect the migration ability of SiHa and C33a cells after treatment with the indicated concentrations (0–6 μM) of β-lapachone. **(C, D)** Transwell assay was used to detect the effect of the indicated concentrations of β-lapachone treatment on the migration and invasion ability of CC cells. **(E, F)** Cells were treated with β-lapachone (0–6 μM) for 24 h, and protein levels of E-cadherin, Vimentin, and Snail were analyzed by Western blot. **(G)** The supernatant from cells treated with β-lapachone (0–6 μM) for 24 h was collected for the tube formation assay. **(H)** After treating cells with β-lapachone (0–6 μM), VEGF protein expression was detected by Western blot. Error bars represent the means ± SD of three independent experiments. *$P < 0.05$, **$P < 0.01$ vs. 0 μM β-lapachone group.

10.3389/fphar.2025.1509568

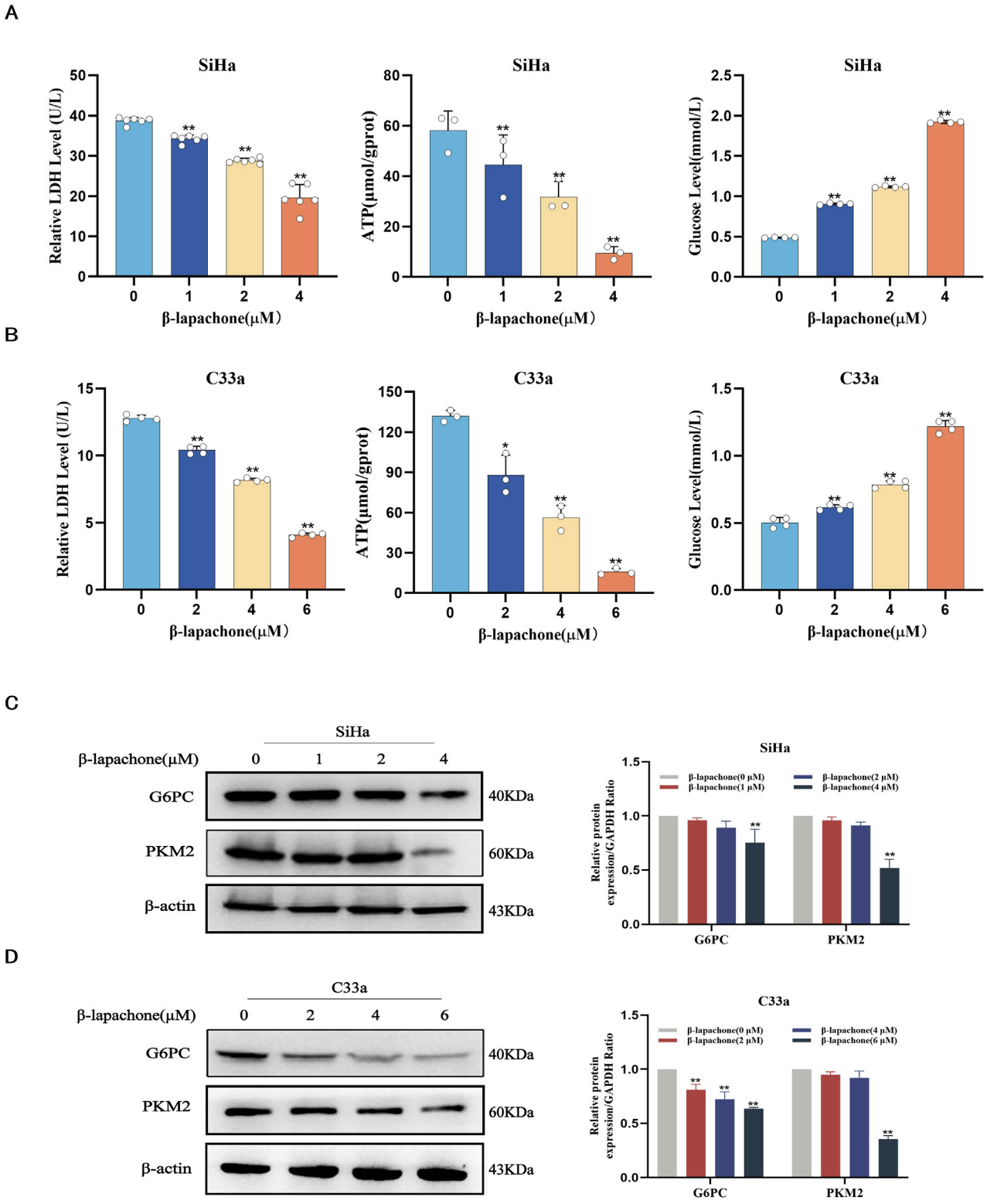


FIGURE 3
β-lapachone regulates glucose metabolism reprogramming in cervical cancer cells. **(A, B)** SiHa and C33a cells were treated with the indicated concentrations of β-lapachone (0–6 μM) for 24 h. Relative levels of ATP, Lactate and Glucose were determined. **(C, D)** After treating cells with β-lapachone (0–6 μM), PKM2 and G6PC protein expression was detected by Western blot. Error bars represent the means ± SD of three independent experiments. *$P < 0.05$, **$P < 0.01$ vs. 0 μM β-lapachone group.

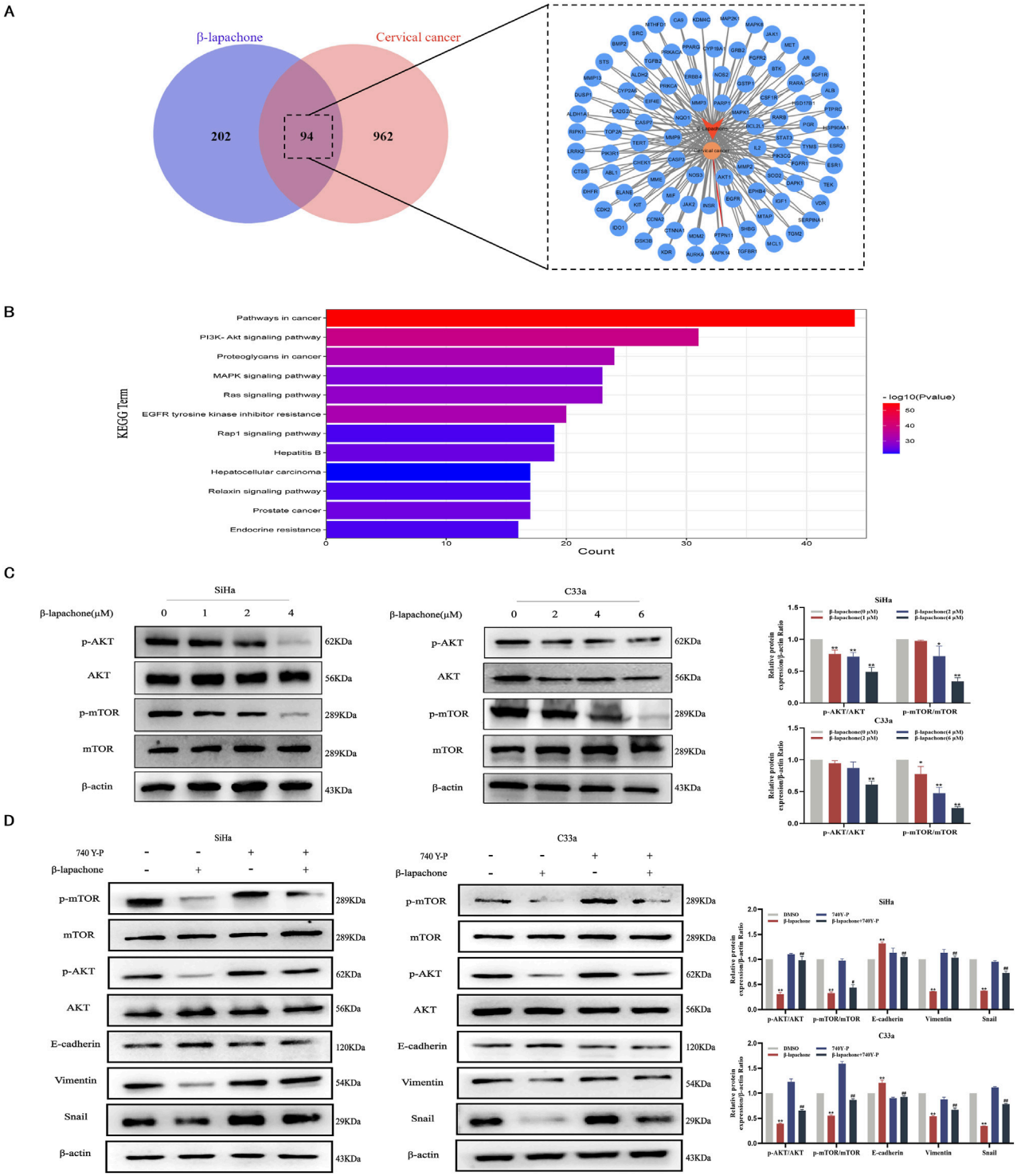


FIGURE 4
β-lapachone inhibits cervical cancer progression and metastasis via the PI3K/AKT/mTOR signaling pathway. **(A)** Prediction of β-lapachone related target genes in cervical cancer. **(B)** KEGG pathway enrichment analysis. **(C)** After treating cells with β-lapachone (0–6 μM), p-AKT/t-AKT, p-mTOR/t-mTOR protein expression was detected by Western blot. **(D)** The expression of proteins related to the PI3K/AKT/mTOR pathway and EMT process in CC cells was detected by Western blot after treating with β-lapachone and the pathway activator 740 Y-P. Error bars represent the means ± SD of three independent experiments. *$P < 0.05$, **$P < 0.01$ vs. DMSO group, #$P < 0.05$, ##$P < 0.01$ vs. β-lapachone group.

markers PKM2 and G6PC. Similarly, the regulation of the EMT-related proteins E-cadherin, Snail, and Vimentin by β-lapachone was also abrogated by AKT1 knockdown (Figures 5C, D). These findings thus suggest that β-lapachone inhibits glucose metabolism and the EMT process in CC cells by targeting AKT1.

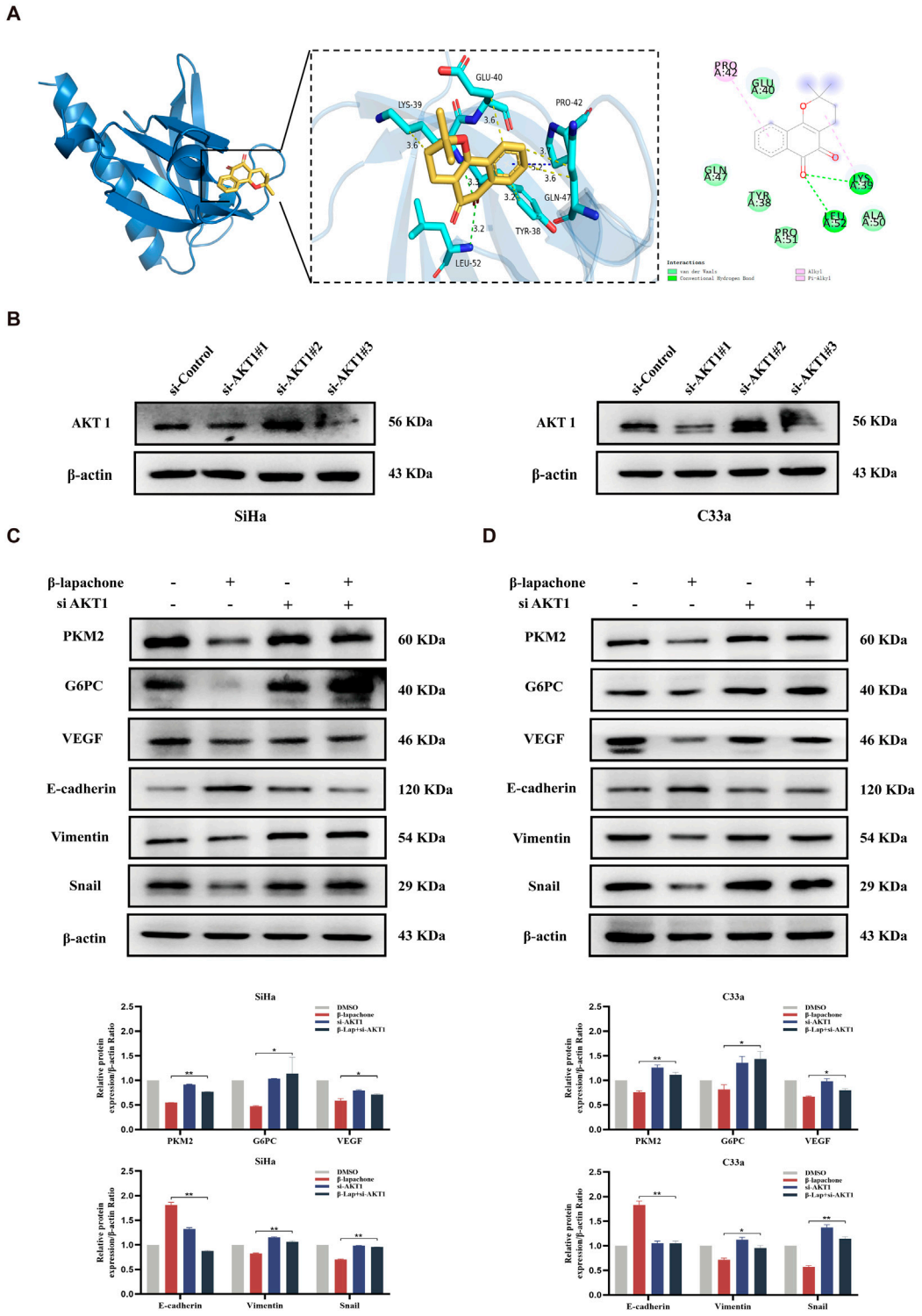


FIGURE 5
β-lapachone inhibits glucose metabolism and EMT process in CC cells by targeting AKT1. **(A)** Docking analysis for predicting the binding mode of β-lapachone to AKT1. **(B)** Western blot analysis of AKT1 in CC cells transfected with siRNA-control, siRNA-AKT1-1、siRNA-AKT1-2 and siRNA-AKT1-3. **(C, D)** CC cells with AKT1 knockdown were treated with β-lapachone for 24 h, and protein expression levels were analyzed by Western blot. *$P < 0.05$, **$P < 0.01$ vs. β-lapachone group.

β-lapachone inhibits the progression of cervical cancer *in vivo*

Finally, a nude mouse xenograft tumor model was established to examine the inhibitory effect of β-lapachone on CC *in vivo*. β-lapachone (5 mg/kg) was injected intraperitoneally every other day. The mice were euthanized 2 weeks after initiating treatment. Compared with those in the control group, the tumor weights and volumes in the treatment groups were significantly reduced (Figures 6A–C). In addition, no morphological changes were observed in the kidneys, lungs, and livers of mice in the β-lapachone treatment group. Immunohistochemical analysis of the tumor tissues from these nude mice revealed that the expression of the proliferation-associated antigen Ki67 was reduced in the β-lapachone-treated group relative to the control group (Figure 6D). Together, these findings demonstrate that β-lapachone exhibits a substantial inhibitory effect on the proliferation of CC tumors *in vivo* with no obvious toxicity.

Discussion

β-lapachone, as a natural product, has attracted attention for its therapeutic potential in a variety of tumors (Gomes et al., 2021; Gong et al., 2021). Recent studies have shown that β-lapachone exerts its antitumor effects by inducing NQO1-mediated redox cycling, forming peroxides that damage DNA and lead to the overactivation of PARP, which in turn leads to ATP depletion. Tumors with high NQO1 expression, such as hepatocellular carcinoma, pancreatic cancer, and breast cancer, are thus particularly sensitive to β-lapachone (Yang et al., 2017; Li et al., 2011; Zhao et al., 2021; Qadir et al., 2022). In this study, we explored the potential anti-tumor effects of β-lapachone in CC *in vitro* and *in vivo*. The GEPIA database analysis revealed that NQO1 is highly expressed in CC, a finding further confirmed by the significantly higher expression of NQO1 in CC cells compared to normal cervical epithelial cells. Further investigation demonstrated that β-lapachone effectively inhibited CC cells with high NQO1 expression, exhibiting selective targeting properties.

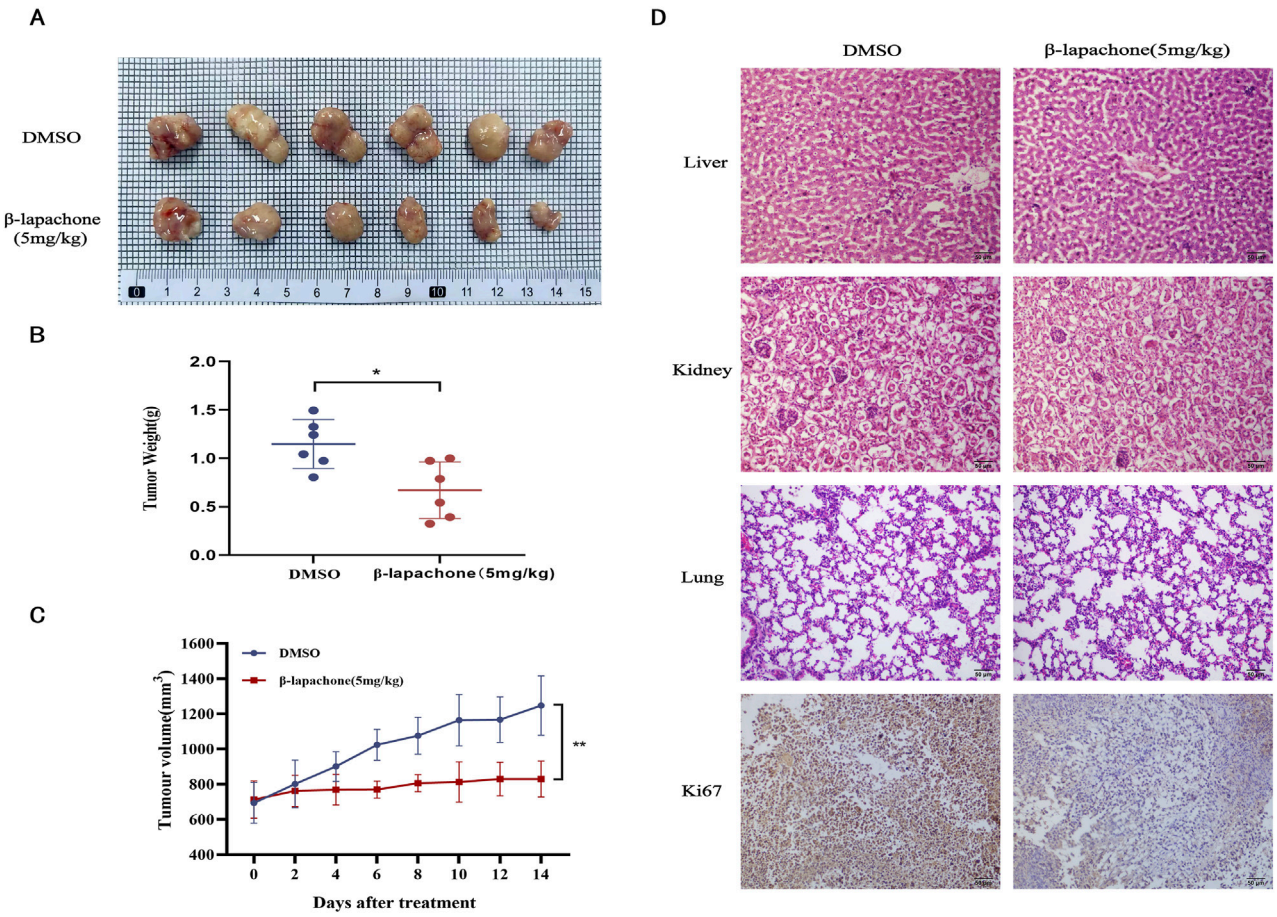


FIGURE 6
β-Lapachone inhibits tumor growth *in vivo*. **(A)** Images of subcutaneous xenograft tumors in the treatment group β-lapachone and control group. **(B)** Weight statistics of subcutaneous xenograft tumors in the treatment and control groups. **(C)** Growth volume statistics of subcutaneous xenograft tumors in the treatment and control groups during treatment with β-lapachone. **(D)** Representative immunohistochemical staining images of Ki67 expression in grafted tumor tissues from various groups of mice; Representative H&E stained images of liver, kidney and lung tissues from each group of mice. Scale bar: 50 μm $^{*}P < 0.05$, $^{**}P < 0.01$.

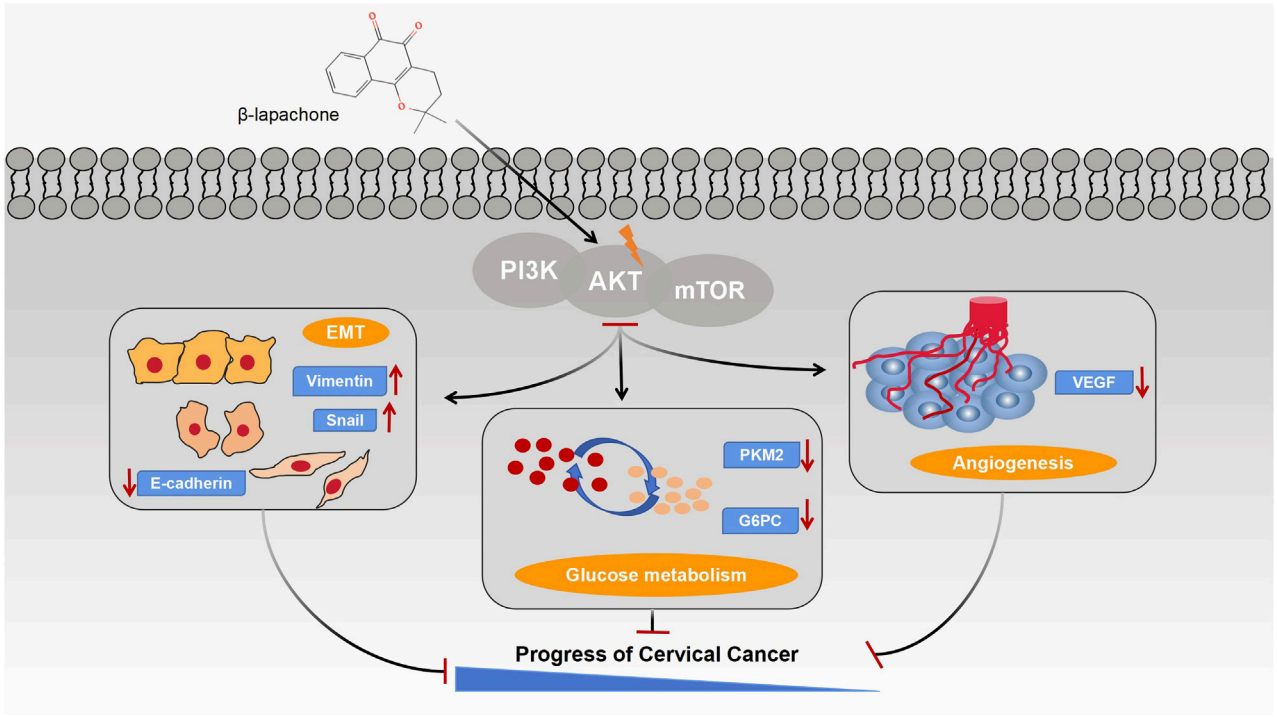


FIGURE 7
Schematic illustration of the mechanisms by which β-lapachone regulates glucose metabolism, EMT process and angiogenesis in cervical cancer through the PI3K/AKT/mTOR signaling pathway.

Additionally, β-lapachone showed low toxicity to HcerEpic cells (Supplementary Figure S1). Significant reductions in HcerEpic cell viability were observed only at high β-lapachone concentrations, highlighting the importance of selecting appropriate therapeutic doses to maximize anticancer efficacy. β-lapachone treatment resulted in a dose-dependent reduction in CC cell viability and colony-forming ability, particularly in cells with higher levels of NQO1 expression. Consistent with these findings, a xenograft model demonstrated that β-lapachone significantly reduced tumor weight and volume without apparent toxicity, aligning with the *in vitro* results. We hypothesize that the primarily cytostatic, rather than cytotoxic, effects of β-lapachone may stem from differences in NQO1 expression between normal and tumor tissues and that β-lapachone exerts its oncostatic effects mainly through the generation of peroxides via NQO1-mediated redox cycling, which then affects the DNA damage repair mechanism rather than inducing cell death directly.

Aberrant cellular metabolism is a hallmark of cancer, with tumor cells requiring large amounts of glucose to produce lactic acid via aerobic glycolysis. This unique metabolic process supports cancer cell energy needs and promotes survival (Huang et al., 2021; Bose and Le, 2018). Research has shown increased glycogen levels in various cancers, such as breast and ovarian cancers, with glycogen levels being inversely correlated with replication rates (Yang et al., 2016). In our study, we observed significantly elevated glucose levels in CC cells treated with β-lapachone, accompanied by marked reductions in ATP and lactate levels. These findings suggest that β-lapachone disrupts the aerobic glycolytic pathway critical for CC cell survival. PKM2, a key enzyme in the Warburg effect, catalyzes the final stage of aerobic glycolysis and often exists as a low-activity dimer in tumor cells (Sun et al., 2011; Liu et al., 2017). PKM2 facilitates cancer cell growth by enhancing macromolecular synthesis through the pentose phosphate pathway (Zhu et al., 2022). In addition, G6PC, a key enzyme in glucose homeostasis, plays a crucial role in gluconeogenesis and glycogenolysis, with its inhibition significantly slowing CC growth (Zhu et al., 2021; Singh et al., 2018). β-lapachone produces ROS, which are converted to hydrogen peroxide, leading to excessive DNA damage (Ross and Siegel, 2017; Hong et al., 2019). This overactivates PARP1, resulting in the depletion of NAD^+ and ATP, ultimately impairing glycolysis, redox balance, and downstream metabolic processes (Silvers et al., 2017). Previous studies have also shown that β-lapachone regulates glucose metabolism in tumor types such as lung, pancreatic, and colorectal cancers (Huang et al., 2016; Mahar et al., 2021). Consistent with these findings, we observed the downregulation of PKM2 and G6PC expression in CC cells treated with β-lapachone. Collectively, our results confirm that β-lapachone reduces ATP and LDH levels by modulating PKM2 and G6PC expression, thereby preventing CC progression.

EMT induction is a biological process wherein epithelial cells lose their characteristics and acquire mesenchymal traits, enhancing metastatic potential and promoting cancer progression (Li et al., 2019; Hisano and Hla, 2019). Western blotting in this study demonstrated that β-lapachone downregulated mesenchymal markers Vimentin and Snail while upregulating the epithelial marker E-cadherin. This suggests that β-lapachone inhibits CC cell migration and invasion by suppressing EMT activity.

Angiogenesis, another critical factor in tumor growth, facilitates metastasis by promoting endothelial cell proliferation and new tumor vessel formation (Lugano et al., 2020; Plate et al., 2012; Shibuya, 2011). VEGF and its receptor VEGFR play a pivotal role in angiogenesis, making them key targets for anti-angiogenic cancer therapies (Sharma et al., 2017). Consistent with these observations, our study revealed that β-lapachone significantly suppressed VEGF protein expression in CC cells, reducing microtubule formation in HUVECs. These findings suggest that β-lapachone inhibits CC progression by targeting both angiogenesis and EMT induction.

The PI3K/AKT/mTOR signaling pathway regulates cell proliferation, survival, and metabolism, with its dysregulation frequently observed in tumors (Reddy et al., 2020; Zheng et al., 2021b). This pathway is an attractive therapeutic target in various cancers, including CC (Yang et al., 2019). In this study, KEGG pathway enrichment analysis indicated that β-lapachone exerts its effects on CC cells through the PI3K/AKT/mTOR pathway. We verified the predicted results via Western blotting, revealing a significant reduction in p-AKT and p-mTOR levels in CC cells. Subsequently, we performed rescue experiments using the pathway activator 740Y-P and found that the expression of proteins related to the PI3K/AKT/mTOR signaling pathway and EMT process was significantly reversed by β-lapachone treatment in CC cells. More importantly, network pharmacology and molecular docking studies identified AKT1 as a potential β-lapachone target, and AKT1 knockdown significantly attenuated the inhibitory effects of β-lapachone on glucose metabolism and EMT in CC cells. These findings suggest that β-lapachone inhibits CC progression by targeting the PI3K/AKT/mTOR signaling pathway.

In conclusion, our study demonstrates that β-lapachone, an antitumor therapeutic agent targeting high NQO1 expression, modulates glucose metabolism, suppresses angiogenesis, and inhibits the EMT process by targeting AKT1. These effects collectively prevent the malignant progression of cervical cancer (Figure 7). Together, this work provides a theoretical foundation for developing β-lapachone as a therapeutic agent for cervical cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used. The animal study was approved by Yanbian University Experimental Animal Welfare Ethics Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

PD: Conceptualization, Data curation, Formal Analysis, Methodology, Resources, Writing–original draft, Writing–review and editing. YL: Conceptualization, Data curation, Formal Analysis, Software, Writing–original draft. AH: Data curation, Formal Analysis, Software, Writing–review and editing. MW: Methodology, Software, Writing–review and editing. JL: Data curation, Formal Analysis, Software, Writing–review and editing. YP: Conceptualization, Data curation, Formal Analysis, Writing–review and editing. LC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Resources, Software, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was supported by the National Natural Science Foundation of China (No. 82160695), the Projects of Science and Technology Department of Jilin Province (No. 20210101422JC).

Acknowledgments

We thank LetPub (www.letpub.com.cn) for its linguistic assistance during the preparation of this manuscript.

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.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1509568/full#supplementary-material

References

Bose, S., and Le, A. (2018). Glucose metabolism in cancer. *Adv. Exp. Med. Biol.* 1063, 3–12. doi:10.1007/978-3-319-77736-8_1

Bu, D., Luo, H., Huo, P., Wang, Z., Zhang, S., He, Z., et al. (2021). KOBAS-i: intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Res.* 49, W317–W325. doi:10.1093/nar/gkab447

Christofk, H. R., Vander Heiden, M. G., Wu, N., Asara, J. M., and Cantley, L. C. (2008). Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* 452, 181–186. doi:10.1038/nature06667

DeBerardinis, R. J., Lum, J. J., Hatzivassiliou, G., and Thompson, C. B. (2008). The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab.* 7, 11–20. doi:10.1016/j.cmet.2007.10.002

Dong, S., Li, W., Li, X., Wang, Z., Chen, Z., Shi, H., et al. (2022). Glucose metabolism and tumour microenvironment in pancreatic cancer: a key link in cancer progression. *Front. Immunol.* 13, 1038650. doi:10.3389/fimmu.2022.1038650

Feng, R. M., Zong, Y. N., Cao, S. M., and Xu, R. H. (2019). Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? *Cancer Commun. (Lond)* 39 (1), 22. doi:10.1186/s40880-019-0368-6

Gomes, C. L., de Albuquerque Wanderley Sales, V., Gomes de Melo, C., Ferreira, da S. R. M., Vicente Nishimura, R. H., Rolim, L. A., et al. (2021). Beta-lapachone: natural occurrence, physicochemical properties, biological activities, toxicity and synthesis. *Phytochemistry* 186, 112713. doi:10.1016/j.phytochem.2021.112713

Gong, Q., Hu, J., Wang, P., Li, X., and Zhang, X. (2021). A comprehensive review on β-lapachone: mechanisms, structural modifications, and therapeutic potentials. *Eur. J. Med. Chem.* 210, 112962. doi:10.1016/j.ejmech.2020.112962

Gu, Z., Xia, J., Xu, H., Frech, I., Tricot, G., and Zhan, F. (2017). NEK2 promotes aerobic glycolysis in multiple myeloma through regulating splicing of pyruvate kinase. *J. Hematol. Oncol.* 10, 17. doi:10.1186/s13045-017-0392-4

Hisano, Y., and Hla, T. (2019). Bioactive lysolipids in cancer and angiogenesis. *Pharmacol. Ther.* 193, 91–98. doi:10.1016/j.pharmthera.2018.07.006

Hong, S. M., Hwang, S. W., Wang, T., Park, C. W., Ryu, Y. M., Jung, J. H., et al. (2019). Increased nicotinamide adenine dinucleotide pool promotes colon cancer progression by suppressing reactive oxygen species level. *Cancer Sci.* 110, 629–638. doi:10.1111/cas.13886

Huang, X., Hou, Y., Weng, X., Pang, W., Hou, L., Liang, Y., et al. (2021). Diethyldithiocarbamate-copper complex (CuET) inhibits colorectal cancer progression via miR-16-5p and 15b-5p/ALDH1A3/PKM2 axis-mediated aerobic glycolysis pathway. *Oncogenesis* 10, 4. doi:10.1038/s41389-020-00295-7

Huang, X., Motea, E. A., Moore, Z. R., Yao, J., Dong, Y., Chakrabarti, G., et al. (2016). Leveraging an NQO1 bioactivatable drug for tumor-selective use of poly(ADP-ribose) polymerase inhibitors. *Cancer Cell* 30, 940–952. doi:10.1016/j.ccell.2016.11.006

Kee, J. Y., Han, Y. H., Park, J., Kim, D. S., Mun, J. G., Ahn, K. S., et al. (2017). β-Lapachone inhibits lung metastasis of colorectal cancer by inducing apoptosis of CT26 cells. *Integr. Cancer Ther.* 16, 585–596. doi:10.1177/1534735416681638

Kitamura, K., Hatano, E., Higashi, T., Narita, M., Seo, S., Nakamoto, Y., et al. (2011). Proliferative activity in hepatocellular carcinoma is closely correlated with glucose metabolism but not angiogenesis. *J. Hepatol.* 55, 846–857. doi:10.1016/j.jhep.2011.01.038

Lamberti, M. J., Morales Vasconsuelo, A. B., Chiaramello, M., Ferreira, V. F., Macedo Oliveira, M., Baptista Ferreira, S., et al. (2018). NQO1 induction mediated by photodynamic therapy synergizes with β-Lapachone-halogenated derivative against melanoma. *Biomed. Pharmacother.* 108, 1553–1564. doi:10.1016/j.biopha.2018.09.159

Li, L. S., Bey, E. A., Dong, Y., Meng, J., Patra, B., Yan, J., et al. (2011). Modulating endogenous NQO1 levels identifies key regulatory mechanisms of action of β-lapachone for pancreatic cancer therapy. *Clin. Cancer Res.* 17, 275–285. doi:10.1158/1078-0432.CCR-10-1983

Li, S., Cong, X., Gao, H., Lan, X., Li, Z., Wang, W., et al. (2019). Tumor-associated neutrophils induce EMT by IL-17a to promote migration and invasion in gastric cancer cells. *J. Exp. Clin. Cancer Res.* 38, 6. doi:10.1186/s13046-018-1003-0

Liu, F., Ma, F., Wang, Y., Hao, L., Zeng, H., Jia, C., et al. (2017). PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis. *Nat. Cell Biol.* 19, 1358–1370. doi:10.1038/ncb3630

Lugano, R., Ramachandran, M., and Dimberg, A. (2020). Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol. Life Sci.* 77, 1745–1770. doi:10.1007/s00018-019-03351-7

Mahar, R., Chang, M. C., and Merritt, M. E. (2021). Measuring NQO1 bioactivation using [2H7]Glucose. *Cancers (Basel)* 13, 4165. doi:10.3390/cancers13164165

Plate, K. H., Scholz, A., and Dumont, D. J. (2012). Tumor angiogenesis and anti-angiogenic therapy in malignant gliomas revisited. *Acta Neuropathol.* 124, 763–775. doi:10.1007/s00401-012-1066-5

Qadir, M. I., Iqbal, M. S., and Khan, R. (2022). β-Lapachone: a promising anticancer agent with a unique NQO1 specific apoptosis in pancreatic cancer. *Curr. Cancer Drug Targets* 22, 537–540. doi:10.2174/1568009622666220427121127

Reddy, D., Ghosh, P., and Kumavath, R. (2020). Strophanthidin attenuates MAPK, PI3K/AKT/mTOR, and wnt/β-catenin signaling pathways in human cancers. *Front. Oncol.* 9, 1469. doi:10.3389/fonc.2019.01469

Ross, D., and Siegel, D. (2017). Functions of NQO1 in cellular protection and CoQ10 metabolism and its potential role as a redox sensitive molecular switch. *Front. Physiol.* 8, 595. doi:10.3389/fphys.2017.00595

Sanajou, D., Bahrambeigi, S., and Aslani, S. (2021). β-LAPachone is renoprotective in streptozotocin-induced diabetic mice via regulating the PI3K/Akt/mTOR signaling pathway. *Iran. J. Basic Med. Sci.* 24, 650–656. doi:10.22038/ijbms.2021.55565.12422

Sharma, V. R., Gupta, G. K., Sharma, A. K., Batra, N., Sharma, D. K., Joshi, A., et al. (2017). PI3K/Akt/mTOR intracellular pathway and breast cancer: factors, mechanism and regulation. *Curr. Pharm. Des.* 23, 1633–1638. doi:10.2174/1381612823666161116125218

Shibuya, M. (2011). Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. *Genes Cancer* 2, 1097–1105. doi:10.1177/1947601911423031

Siegel, R. L., Giaquinto, A. N., and Jemal, A. (2024). Cancer statistics, 2024. *CA Cancer J. Clin.* 74 (1), 12–49. doi:10.3322/caac.21820

Silvers, M. A., Deja, S., Singh, N., Egnatchik, R. A., Sudderth, J., Luo, X., et al. (2017). The NQO1 bioactivatable drug, β-lapachone, alters the redox state of NQO1+ pancreatic cancer cells, causing perturbation in central carbon metabolism. *J. Biol. Chem.* 292, 18203–18216. doi:10.1074/jbc.M117.813923

Singh, M., Yelle, N., Venugopal, C., and Singh, S. K. (2018). EMT: mechanisms and therapeutic implications. *Pharmacol. Ther.* 182, 80–94. doi:10.1016/j.pharmthera.2017.08.009

Sun, Q., Chen, X., Ma, J., Peng, H., Wang, F., Zha, X., et al. (2011). Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. *Proc. Natl. Acad. Sci. U. S. A.* 108, 4129–4134. doi:10.1073/pnas.1014769108

Tamada, M., Suematsu, M., and Saya, H. (2012). Pyruvate kinase M2: multiple faces for conferring benefits on cancer cells. *Clin. Cancer Res.* 18, 5554–5561. doi:10.1158/1078-0432.CCR-12-0859

Tang, Z., Li, C., Kang, B., Gao, G., Li, C., and Zhang, Z. (2017). GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* 45 (W1), W98–W102. doi:10.1093/nar/gkx247

Vaccarella, S., Lortet-Tieulent, J., Plummer, M., Franceschi, S., and Bray, F. (2013). Worldwide trends in cervical cancer incidence: impact of screening against changes in disease risk factors. *Eur. J. Cancer* 49, 3262–3273. doi:10.1016/j.ejca.2013.04.024

Woo, H. Y., and Kim, H. S. (2022). Local and metastatic relapses in a young woman with papillary squamous cell carcinoma of the uterine cervix. *Diagn. (Basel)* 12, 599. doi:10.3390/diagnostics12030599

Wu, H., Yang, P., Hu, W., Wang, Y., Lu, Y., Zhang, L., et al. (2016). Overexpression of PKM2 promotes mitochondrial fusion through attenuated p53 stability. *Oncotarget* 7, 78069–78082. doi:10.18632/oncotarget.12942

Yang, J., Nie, J., Ma, X., Wei, Y., Peng, Y., and Wei, X. (2019). Targeting PI3K in cancer: mechanisms and advances in clinical trials. *Mol. Cancer* 18, 26. doi:10.1186/s12943-019-0954-x

Yang, Y., Zhou, X., Xu, M., Piao, J., Zhang, Y., Lin, Z., et al. (2017). β-lapachone suppresses tumour progression by inhibiting epithelial-to-mesenchymal transition in NQO1-positive breast cancers. *Sci. Rep.* 7, 2681. doi:10.1038/s41598-017-02937-0

Yang, Y. C., Cheng, T. Y., Huang, S. M., Su, C. Y., Yang, P. W., Lee, J. M., et al. (2016). Cytosolic PKM2 stabilizes mutant EGFR protein expression through regulating HSP90-EGFR association. *Oncogene* 35, 3387–3398. doi:10.1038/onc.2015.397

Yu, H. Y., Kim, S. O., Jin, C. Y., Kim, G. Y., Kim, W. J., Yoo, Y. H., et al. (2014). β-lapachone-Induced apoptosis of human gastric carcinoma AGS cells is caspase-dependent and regulated by the PI3K/akt pathway. *Biomol. Ther. Seoul.* 22, 184–192. doi:10.4062/biomolther.2014.026

Zhao, W., Jiang, L., Fang, T., Fang, F., Liu, Y., Zhao, Y., et al. (2021). β-Lapachone selectively kills hepatocellular carcinoma cells by targeting NQO1 to induce extensive DNA damage and PARP1 hyperactivation. *Front. Oncol.* 11, 747282. doi:10.3389/fonc.2021.747282

Zheng, Y., Xie, L., Xu, S., Yan, W., Zhang, H., Meng, Y., et al. (2021b). Effects of miR-202-5p silencing PIK3CA gene expression on proliferation, invasion, and epithelial-mesenchymal transition of cervical cancer SiHa cells through inhibiting PI3K/Akt/mTOR signaling pathway activation. *Mol. Cell Biochem.* 476, 4031–4044. doi:10.1007/s11010-021-04211-4

Zheng, Y., Zhang, H., Guo, Y., Chen, Y., Chen, H., and Liu, Y. (2021a). X-ray repair cross-complementing protein 1 (XRCC1) loss promotes β-lapachone -induced apoptosis in pancreatic cancer cells. *BMC Cancer* 21, 1234. doi:10.1186/s12885-021-08979-y

Zhou, Y., Yan, H., Zhou, Q., Feng, R., Wang, P., Yang, F., et al. (2021). Beta-lapachone attenuates BMSC-mediated neuroblastoma malignant transformation by inhibiting gal-3/gal-3BP/IL6 Axis. *Front. Pharmacol.* 12, 766909. doi:10.3389/fphar.2021.766909

Zhu, K., Deng, C., Du, P., Liu, T., Piao, J., Piao, Y., et al. (2022). G6PC indicated poor prognosis in cervical cancer and promoted cervical carcinogenesis *in vitro* and *in vivo*. *Reprod. Biol. Endocrinol.* 20, 50. doi:10.1186/s12958-022-00921-6

Zhu, P., Lu, J., Zhi, X., Zhou, Y., Wang, X., Wang, C., et al. (2021). tRNA-derived fragment tRFLys-CTT-010 promotes triple-negative breast cancer progression by regulating glucose metabolism via G6PC. *Carcinogenesis* 42, 1196–1207. doi:10.1093/carcin/bgab058

Frontiers in Pharmacology

Explores the interactions between chemicals and living beings

The most cited journal in its field, which advances access to pharmacological discoveries to prevent and treat human disease.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact