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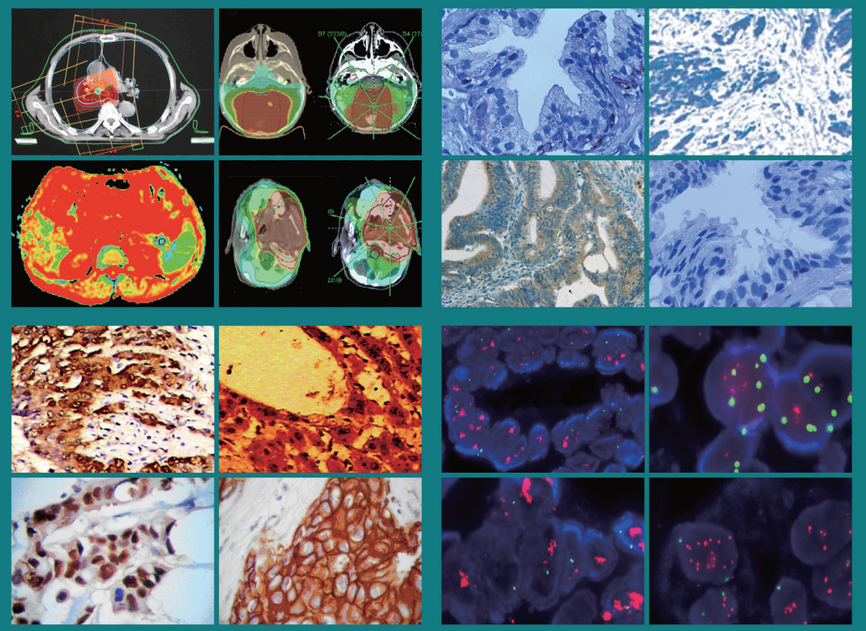

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REVIEW ARTICLE

Role of chemokines in the hepatocellular carcinoma microenvironment and their translational value in immunotherapy*

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Abstract

The difficulty of early diagnosis, high tumor heterogeneity, and high recurrence and metastasis rates lead to an unsatisfactory treatment status for hepatocellular carcinoma (HCC). HCC is a typical inflammation-driven tumor. Chronic inflammation allows nascent tumors to escape immunosurveillance. Chemokines are small, soluble, secreted proteins that can regulate the activation and trafficking of immune cells during inflammation. Several studies have shown that various chemokines with overarching functions disrupt the immune microenvironment during the initiation and progression of HCC. The dysregulated chemokine network in HCC contributes to multiple malignant processes, including angiogenesis, tumor proliferation, migration, invasion, tumor low response, and resistance to immune therapy. Here, we summarize the current studies focusing on the role of chemokines and their receptors in the HCC immune microenvironment, highlighting potential translational therapeutic uses for modulating the chemokine system in HCC.

Key words: hepatocellular carcinoma; chemokine; chemokine receptor; tumor microenvironment; immune therapy; therapeutic target

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Primary liver cancer is the sixth most common malignant tumor and the third leading cause of cancer-related mortality, with approximately 906,000 new cases and 830,000 deaths worldwide in 2020, according to latest data from the World Health Organization [1]. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for 75%-85% of cases [1]. HCC remains a global health challenge. Most patients are not diagnosed until the middle or late stages and therefore miss the optimal window for liver resection and transplantation. Hence, the importance of systemic therapies for HCC, including tyrosine kinase inhibitors (TKIs) and immune-checkpoint inhibitors (ICIs), cannot be overemphasized. However, despite the recent remarkable shift in the HCC treatment landscape, both TKIs and ICIs have limitations of limited drug response rates and development of drug resistance [2, 3].

The blood flow slows in the liver sinusoids, facilitating the execution of the immune response by increasing the detection and capture of circulating pathogens by liver-resident cells [4]. Multiple innate and adaptive immune cells are involved in this process, particularly Kupffer cells (KCs), natural killer (NK) cells, natural killer T (NKT) cells, $CD4^+$ T cells, and $CD8^+$ T cells. Tumor cells can alter the local immune tumor microenvironment (TME) and gain the ability to proliferate and migrate

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while resisting destruction by the host immune system. An increasing number of studies have reported the importance of chemokine signaling in this process [4]. Cancer cells and various stromal cells in the TME interact through chemokine networks to jointly shape an immunosuppressive TME and assist immune cells in evading immune surveillance. M2 tumor-associated macrophages (TAMs), regulatory T (Treg) cells, and myeloid-derived suppressor cells (MDSCs) are significant contributors to the immunosuppressive microenvironment. Further, multiple chemokines have shown dual roles in HCC development, including through a direct impact on tumor cells and through indirect remodeling of the TME. This review summarizes the role of chemokine signaling in different component cells in HCC and reviews the current treatments targeting chemokines or their receptors.

Chemokines and chemokine receptors in HCC

The chemokine system includes 48 chemokine ligands, 20 chemokine receptors, and 4 atypical chemokine receptors. This system participates in multiple tumor-related pathological processes, including angiogenesis, metastasis, vascularization, and distortion of the TME [5]. Chemokines are small, soluble, secreted proteins that regulate the activation and trafficking of immune cells during inflammation [5]. As the largest subfamily of cytokines, chemokines are classified into four main subtypes based on the number and location of N-terminal cysteine (C) residues in their protein sequence, as follows: CC chemokines, CXC chemokines, C chemokines, and CX3C chemokines [6]. Most chemokines, other than CX3CL1 and CXCL16, are secreted proteins. Tumor cells and stromal cells, including immune cells, secrete chemokines. Autocrine and paracrine chemokines are secreted and act on themselves or adjacent cells by binding to specific receptors [5]. CX3CL1 and CXCL16 can remain on the cell surface via a transmembrane mucin-like stalk [7].

The deregulation of chemokines and their receptors is closely associated with HCC pathogenesis (Figs. 1 and 2). Here, we will discuss typical dysregulated chemokine signaling in HCC and its correlation with clinical outcomes and the value of chemokines as prognostic and predictive markers. The detailed role of various chemokines in immune cells will be discussed in the next section.

CCL2 (also known as monocyte chemoattractant protein 1, MCP1) functions mainly in HCC through binding to CCR2 (CD192). CCL2 is a potent chemoattractant for monocytes, lymphocytes, NK cells, dendritic cells, and many other cell types. Therefore, the CCL2-CCR2 signaling pathway performs various

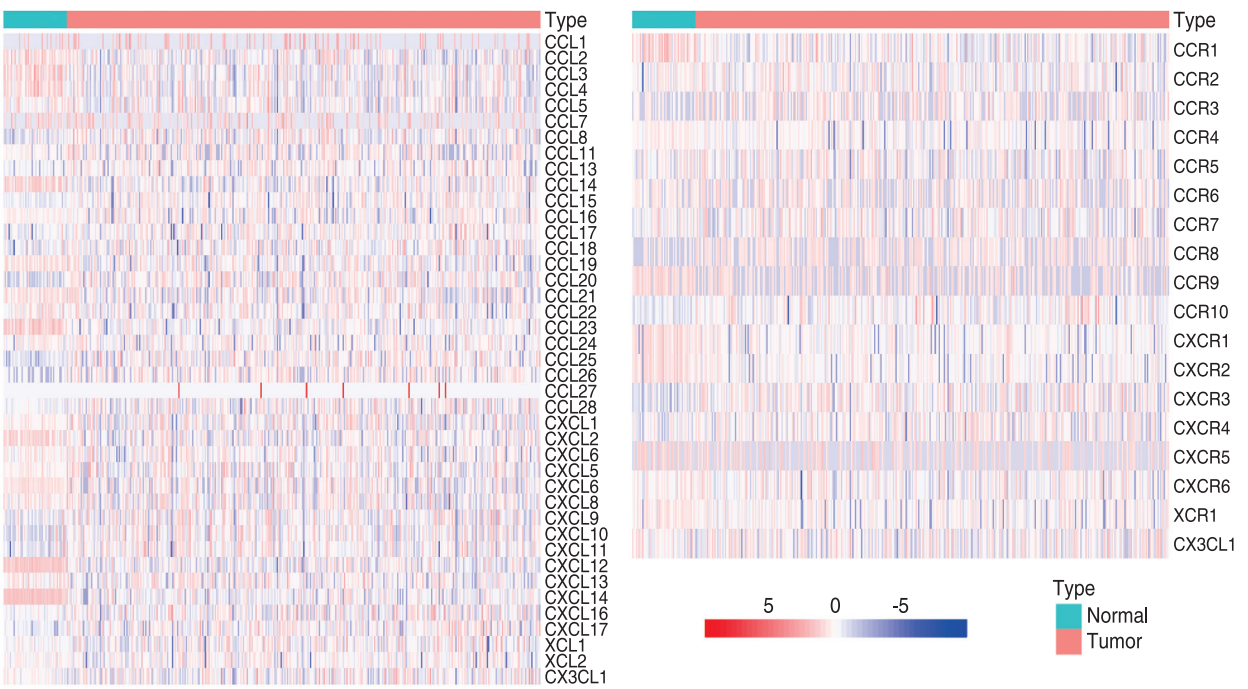

Fig. 1 Cytokine-chemokine profile in LIHC and normal liver tissues. The heat map shows cytokine and chemokine gene expression across TCGA-LIHC (tumor tissue, *n* = 371; normal tissue, *n* = 50). Datasets were analyzed using UCSC Xena (https:--xenabrowser.net-datapages-). LIHC: liver hepatocellular carcinoma; TCGA: The Cancer Genoma Atlas

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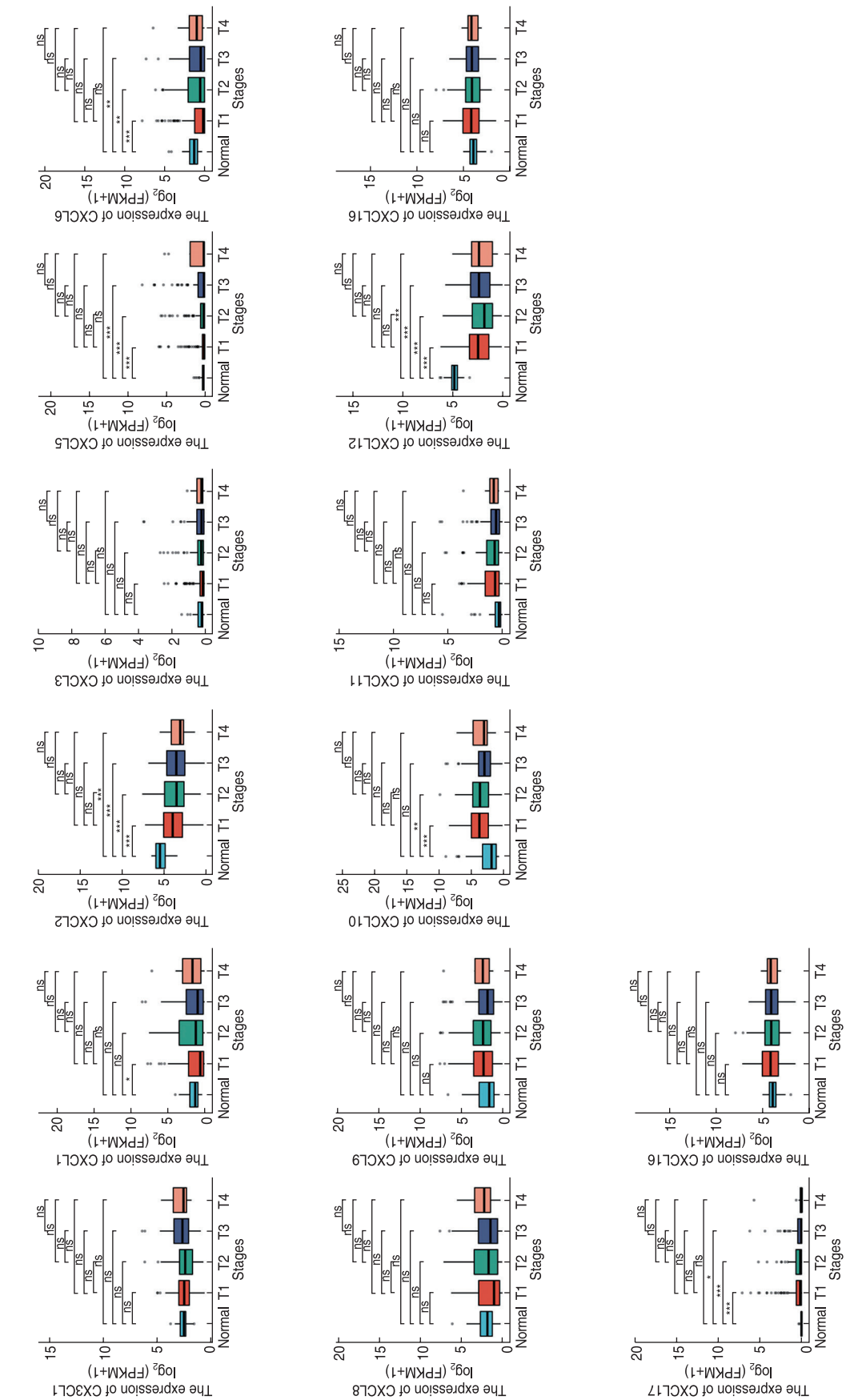
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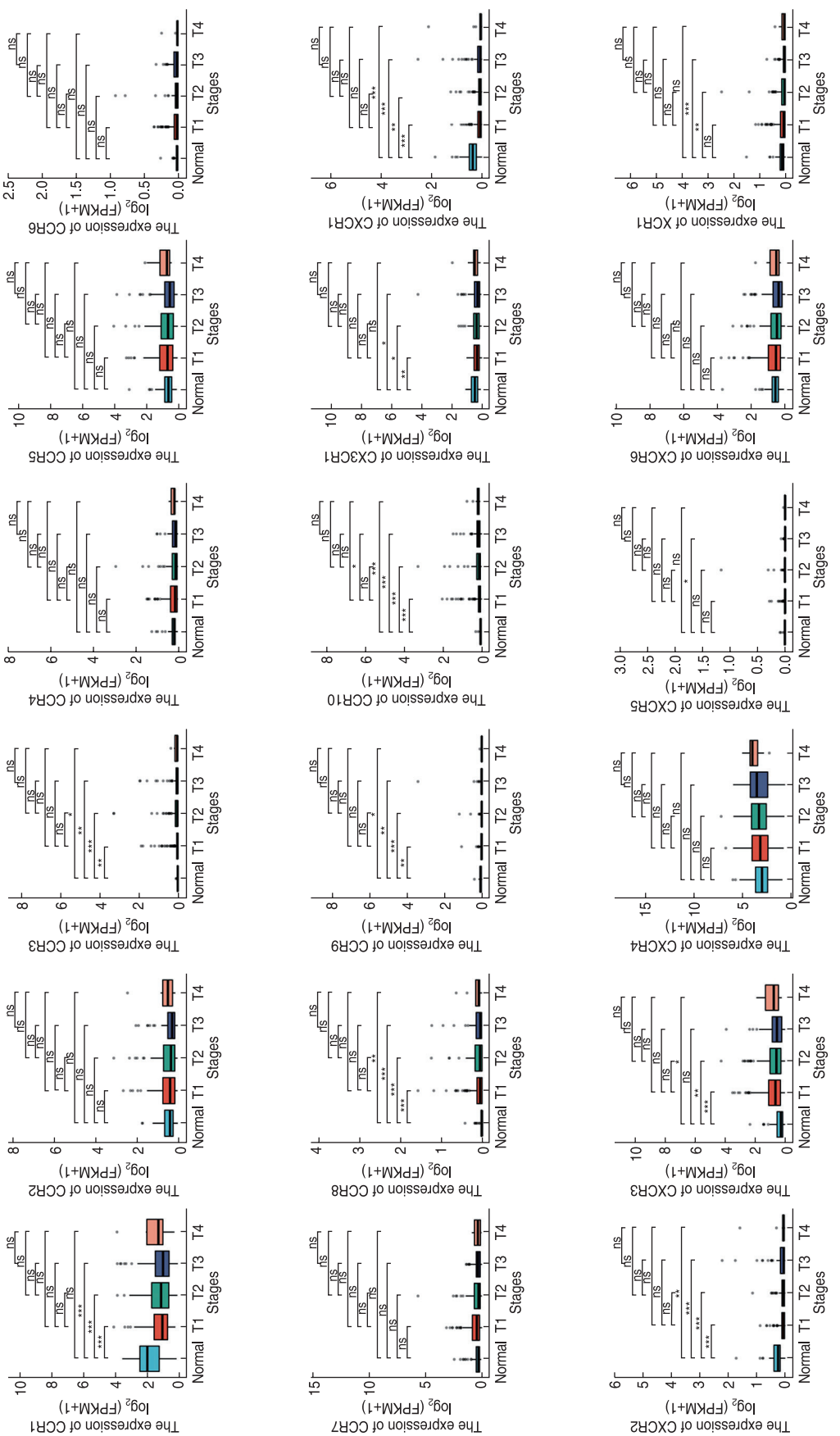

Fig. 2 The clinical correlation of chemokines and chemokine receptors with tumor stage in HCC. (a) Correlation between HCC tumor stage and CCL chemokines and XCL chemokines; (b) Correlation between the HCC tumor stage and CXCL chemokines; (c) Correlation between the HCC tumor stage and chemokine receptors. Datasets were obtained from TCGA-LIHC (tumor tissue, $n = 371$; normal tissue, $n = 50$; stage T1, $n = 183$; stage T2, $n = 95$; stage T3, $n = 80$; stage T4, $n = 13$). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. HCC: hepatocellular carcinoma; LIHC: liver hepatocellular carcinoma; TCGA: The Cancer Genoma Atlas

functions at different stages of HCC progression [8-10]. The composition of the immune TME may determine the anti- and protumorigenic effects of CCL2-CCR2 signaling. During early HCC, there are not enough $CCR2^+$ inhibitory immune cells in the TME (such as $CCR2^+$ Treg cells) to antagonize the interaction of CCL2 with CCR2 on $CD8^+$ T cells. Therefore, CCL2-CCR2 may protect against tumor initiation in the early stages of HCC. However, immunosuppressive cells in the TME of advanced HCC impair the recruitment of $CD8^+$ T cells, thus abolishing their antitumor effects [9]. CCL2 is highly expressed in HCC and is an independent prognostic factor of overall survival [11].

CCL5 (also known as regulated upon activation of normal T cell expressed and secreted factor, RANTES) may also play a dual role in HCC. CCL5 restores immune surveillance in β-catenin-driven HCC cells by recruiting $CD103^+$ dendritic cells and antigen-specific $CD8^+$ T cells [12]. Furthermore, CCL5 and CCL4 can attract γδ T cells to HCCs. γδ T cells have cytotoxic antitumor activity and regulate the infiltration and differentiation of $CD8^+$ T cells [13]. However, CCL5 is overexpressed in HCC compared to adjacent tissues and is associated with proliferation, migration, and epithelial-mesenchymal transition (EMT) in HCC [14]. Furthermore, CCL5 is overexpressed in circulating tumor cells (CTCs) and enhances the migration ability of CTCs by recruiting Treg cells [15].

CCL17 (also known as thymus and activation-regulated chemokine, TARC) and CCL22 (also known as macrophage-derived chemokine, MDC) share 37% homology in their amino acid sequences. CCR4 is a receptor for both CCL17 and CCL22 [16]. Both CCL17 and CCL22 are potent chemoattractants for trafficking Treg cells into the TME in HCC. Treg cells are involved in immune response disruption; therefore, these chemokines create a microenvironment conducive to metastasis [17, 18]. CCL17- and CCL22-recruited Treg cells also participate in the construction of an inhibitory immune environment for HBV-associated HCC [19].

CXCL8 (also known as interleukin-8, IL-8) is a pro-inflammatory chemokine with multiple protumorigenic roles in HCC. CXCL8 specifically binds to CXCR1 (IL-8 receptor [IL-8R] A or CD181) and CXCR2 (IL-8RB). Upstream NF-κB signaling promotes the production of CXCL8, which triggers activation of PI3K-MAPK signaling in HCC cells, thereby mediating proliferation, angiogenesis, and migration [20-22]. CXCL8 is overexpressed in HCCs and in highly metastatic HCC cell lines [20, 23]. Further, higher expression of CXCL8 may predict poor prognosis in HCC patients [23, 24].

CXCL9 (also known as monokine induced by γ interferon, MIG), CXCL10 (also known as interferon γ-induced protein 10, IP-10), and CXCL11 (also known as interferon-inducible T-cell alpha chemoattractant, ITAC) are all Th1-activating chemokines and selective ligands for CXCR3 [25]. These three chemokines are potent chemotaxis regulators of $CD8^+$ T cells and other effector immune cells by binding to CXCR3 in an autocrine or paracrine manner. They are secreted by tumor cells and $CD8^+$ T cells either dependent or independent of interferon-γ (IFN-γ) stimulation [26-28]. The expressions of CXCL9, CXCL10, and CXCL11 are closely associated with overall survival in HCC and sensitivity to immune therapy [29]. A detailed study of these three chemokines in HCC will be presented later.

The chemokine CXCL12 (also known as stromal cell-derived factor-1, SDF-12) binds primarily to CXC receptor 4 (CXCR4 or CD184) [30]. Hepatoma cells and hepatic stellate cells (HSCs) are the primary sources of CXCL12 [31, 32]. Some studies have noted CXCR4 expression in HCC tissue but not in normal liver tissue. In HCC, CXCR4 is expressed in multiple cell types, such as lymphocytes, HSCs, MDSCs, tumor cells, and other stromal cell types [31, 33, 34]. CXCL12-CXCR4 signaling in tumor cells promotes pathological angiogenesis, survival, invasion, and immune evasion surveillance [31]. Higher CXCR4 expression is positively associated with aggressive tumor behavior and poor prognosis [35].

It is worth mentioning that most chemokines showed tumor-promoting effects in most studies. Chemokines such as CCL14 (also known as hemofiltrate C-C chemokine-1) may be associated with tumor suppression. Zhu *et al*. observed that CCL14 is downregulated in HCC tissues, and low expression of CCL14 in HCC is associated with poor prognosis [36].

Role of chemokines and chemokine receptors in the HCC immune microenvironment

Chemokines mediate remodeling of the TME by recruiting immune cells and regulating their motility and function (Fig. 3). Here, we highlight the role of chemokines in immune cells in HCC.

Chemokines, chemokine receptors, and TAMs

Macrophages infiltrating the TME are called TAMs. Liver macrophages consist of liver-resident macrophages termed KCs and monocyte-derived macrophages recruited from the peripheral blood or bone marrow [37]. KCs originate from yolk sac-derived specific progenitor cells and seed in the liver. KCs have no migratory characteristics but do have phagocytic capacity and maintain liver homeostasis as a critical part of the innate immune system in the liver [38]. Multiple chemokines, as well as colony-stimulating factor 1 (SCF1), recruit peripheral monocytes into the TME and expand the macrophage pool during disease progression [39]. Of the

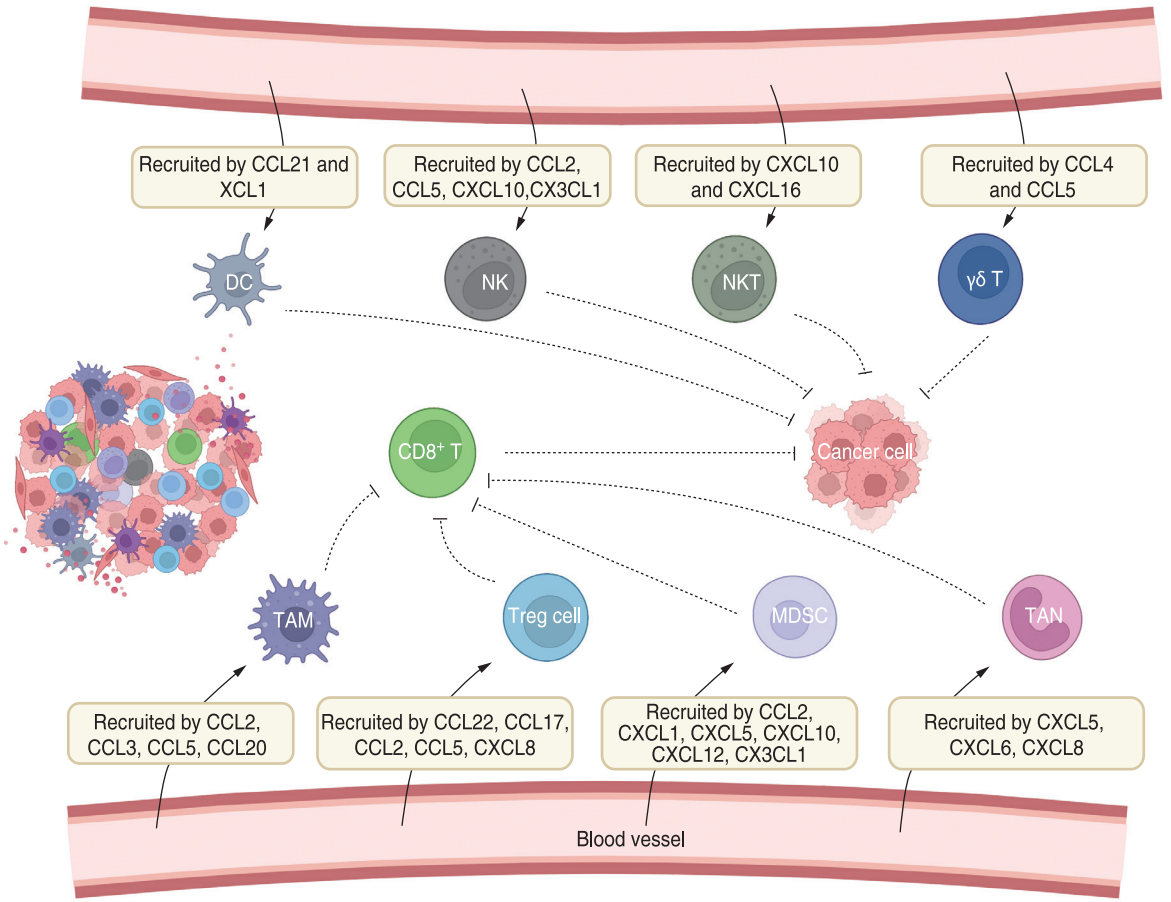

Fig. 3 Recruitment of immune cells or stromal cells by chemokines within the tumor microenvironment. DC: dendritic cell; NK: natural killer; NKT: natural killer T; TAM: tumor-associated macrophage; Treg: regulatory T; MDSC: myeloid-derived suppressor cells; TAN: tumor-associated neutrophil

chemokines involved in hepatocellular carcinogenesis, CCL2 has the greatest ability to recruit immature myeloid cells (iMCs), monocytes, macrophages, and TAMs [10, 40]. In pre-malignant liver tissue, senescent and injured hepatocytes secrete CCL2 and recruit $CCR2^+$ monocytes and iMCs into the liver, which differentiate into pro-inflammatory macrophages and inhibit tumor initiation [8]. However, cytokine deregulation can block massive monocyte and iMC maturation, leading to a disordered TME. These abnormally differentiated cells lose their capacity for immune surveillance and instead exert an immunosuppressive effect, leading to immune escape [8]. CCL20 may be an additional chemoattractant signaling pathway that recruits TAMs via CCR6. CCR6+ monocytes-macrophages accumulate in the TME of HCC, and CCL20 expression is positively associated with intratumoral TAMs [41]. Furthermore, hypoxic cancer cells recruit monocytes by secreting CCL20, further stimulating indoleamine-2,3-dioxygenase 1 (IDO) expression in monocytes [42]. These inhibitory IDO^+ monocyte-derived macrophages inhibit T cell responses and promote tumor antigen tolerance [42]. Interferon regulatory factor 8 (IRF8) can inhibit HCC by mediating IFN-γ and PD-1 signaling. IRF8 impedes TAM infiltration by inhibiting CCL20 secretion [43]. Further, CCL3-CCR1 signaling increases KC accumulation in N-nitrosodiethylamine (DEN)-induced hepatocarcinogenesis, suggesting a role for immune cells in this process.

Macrophages also secrete chemokines. Hou *et al.* noted that macrophages may be the primary source of CCL1. They demonstrated that the CCL1-CCR8 axis alters HCC intracellular signaling through epigenetic regulators and mediates crosstalk between HCC cells and macrophages [44]. KCs secrete CCL2 during miR-206-mediated KC M1 polarization. This KC-derived CCL2 promotes $CD8^+$ T cell migration and expansion and impedes tumor progression in the early stage of HCC development [45]. CCL17 and CCL22 are cytokines that can recruit Treg cells and are secreted by M2 TAMs in sorafenib-treated HCC [46, 47], indicating that CCL2 mediates crosstalk between M2 TAMs and Treg cells, which contributes to an immunosuppressive microenvironment. M2 TAM-derived CCL22 can also directly target cancer cells and promote EMT in HCC [48]. Activated $CD4^+$ T cells stimulate macrophages to produce

Table 1 The secreting and targeting cells of chemokines in HCC and their role within TME

Chemokine (Alternate names)	Receptor	Secretory cell	Target cell	Effect	References
CCL1 (I-309)	CCR8 (CD198)	Macrophage	Hepatoma cells	• Promotes the conversion of monocytes to macrophages and mediates the crosstalk between monocytes/ macrophages and HCC cells.	(1)
CCL2 (MCP1)	CCR2 (CD192)	Hepatoma cells KCs TANs NK cells	iMCs TAMs Treg cells MDSCs $CD8^+$ T cells NK cells	• Recruit CCR2+ iMCs into the vicinity of oncogene-induced senescent hepatocytes. • Acts tumor suppressive in early stages of liver tumorigenesis, while promotive during tumor progression. • Facilitates TAMs M1 polarization and increases CD8+ T cells infiltration during the initiation and early stage of HCC while educating the polarization of M2-type TAMs, MDSCs, and Treg cells in advance HCC.	(2-8)
CCL3 (MIP1α)	CCR1 CCR5 (CD195)	Monocytes Hepatoma cells (those stimulated by proinflammatory cytokines) NK cells	Hepatoma cells KCs	• Inhibits hepatoma cell lines proliferation. • Promotes angiogenesis through MMP9 in DEN-induced HCC. • Increases KCs infiltration in DEN-induced HCC.	(8-10)
CCL4 (MIP1β)	CCR1 CCR5	Hepatoma cells	γδ T cells	• Attracts γδ T cells from peripheral blood or peritumor regions into HCC. • Enhances anti-tumor immunity and improves HCC patients' prognosis.	(11)
CCL5 (RANTES)	CCR1 CCR4 (CD194) CCR5	Hepatoma cells CTCs MSCs CAFs NK cells	Treg cells γδ T cells Hepatoma cells NK cells	• Recruits Treg cells to prevent CTCs from immune clearance. • Attracts γδ T cells and NK cells from peripheral blood or peritumor regions into HCC. • Induces EMT and promotes the migration and invasion of HCC cells.	(7, 8, 11-14)
CCL14 (HCC-1)	CCR1 CCR3 CCR5	Not mentioned	Hepatoma cells	• Suppresses the proliferation of HCC cells and promotes their apoptosis.	(15)
CCL15 (LKN-1, MIP-5, HCC-2)	CCR1	Hepatoma cells (TNF-α and IFN-γ promote CCL15 production)	Monocytes Hepatoma cells MSCs	• Promotes HCC migration. • Recruits suppressive monocytes, impairing anti-tumor immunity and accelerating tumor proliferation and invasion. • Mediates the homing of MSCs into HCC, which are regarded as a promising delivery of therapeutic genes in anti-HCC therapy.	(16-18)
CCL16 (HCC-4, LEC)	CCR1 CCR8	Not mentioned	Hepatoma cells	• Mediate hepatoma cell adhesion and maximal migration at different concentration.	(19)
CCL17 (TARC)	CCR4	TANs Macrophages Hepatoma cells	Treg cells	• Promotes Treg cells intratumoral infiltration and facilitates HCC neovascularization and progression. • Contributes to sorafenib resistance.	(6, 20, 21)
CCL20 (LARC, MIP-3α)	CCR6 (CD196)	Hepatoma cells Myofibroblasts	TAMs Hepatoma cells $CD19^+$ $CD5^+$ B cells	• Enhances the migratory ability of macrophages and $CD19^+$ $CD5^+$ B cells. • Recruits Tregs and contributes to HCV-related HCC progression. • Enhances the capacity of tumor angiogenesis and migration through the responding B cells. • Promotes aerobic glycolysis in cancer cells. • Induces the expression of IDO of macrophage.	(22-26)

Chemokine (Alternate names)	Receptor	Secretory cell	Target cell	Effect	References
CCL21 (SLC, 6Ckine)	CCR7 (CD197)	Not mentioned	DCs	• Induces the maturation of DCs. • The intra-tumoral administration of CCL21 and anti-CD25 constitutes an anti-tumor environment in TME via altering the profiles of cytokines and immune cells.	(27)
CCL22 (MDC)	CCR4	Hepatoma cells M2-type TAM KCs	Treg cells	• Recruits Treg cells to facilitate immune escape. • Promotes HCC growth and enhances tumor invasiveness through EMT activation. • Promotes venous metastases and the development of portal vein tumor thrombus in HBV+ HCC. • Contribute to HBV-associated sorafenib resistance.	(21, 28-31)
CCL26 (eotaxin-3)	CCR3 (CD193)	CAFs	HSCs	• Recruits HSCs and exacerbates HCC initiation.	(32)
CXCL1 (GROα, MGSA)	CXCR1 (IL-8RA, CD181) CXCR2 (IL-8RB)	Hepatoma cells $CD133^+$ TICs	MDSCs	• Mediates the migration of MDSCs into HCC and subsequent immune escape. • Modulates tumorigenicity and self-renewal properties of CD133+ TICs	(33-35)
CXCL2 (GROβ, MIP2α)	CXCR1	TAMs IIC2s	Neutrophils	• Recruits and sustains the survival of neutrophils in HCC tumor milieus.	(36, 37)
CXCL5 (ENA-78, SCYB5)	CXCR2	Hepatoma cells	TANs Hepatoma cells MDSC	• Activates HCC cells EMT phenotype and promote HCC proliferation and lung metastasis. • Recruits immunosuppressive TANs and MDSCs into the tumor site of HCCs.	(38-41)
CXCL6 (GCP2)	CXCR1 CXCR2	Hepatoma cells	CAFs TANs	• Facilitates HCC cells' stem-like properties. • Activates ERK1/2 signaling in CAFs and mediates the crosstalk between CAFs and TAN, accelerating HCC progression.	(42)
CXCL8 (IL-8)	CXCR1 CXCR2	$CD133^+$TICs TAMs IIC2s Hepatoma cells CAF	Neutrophils Liver TICs Hepatoma cells LESCs M2-type macrophage	• Promotes tumorigenicity, angiogenesis, and self-renewal ability of liver TICs • Recruits neutrophils into HCC. • Induces M2-type macrophage polarization. • Enhances the permeability of LESCs via decreasing tight junctions between cells. • Enhances the capacity of LSECs to induce Treg cells. • Promotes HCC growth, migration, and invasion.	(35-37, 43-46)
CXCL9 (MIG)	CXCR3 (GPR9, CD183)	Hepatoma cells $CD8^+$ T cells	$CXCR3^+$ B cells $CD8^+$ T cells	• Promotes the recruitment of $CXCR3^+$ B cells. • Promotes $CD8^+$ T cells migration into HCC.	(47-49)
CXCL10 (IP10)	CXCR3	Hepatoma cells $CD8^+$ T cells TAMs	CXCR3+ B cells CD8+ T cells Treg cells NK cells MDSCs	• Promotes $CD8^+$ T cells migration into HCC. • Promotes the maturation of CXCR3+ B cells. • Recruits NK cells and NKT cells and enhances their anti-tumor efficiency through promoting IFN-γ secretion. • Recruits Treg cells and MDSCs, and mediates HCC growth and recurrence after liver transplantation.	(48-54)
CXCL11 (ITAC)	CXCR3 CXCR7 (GPR159)	Hepatoma cells $CD8^+$ T cells $\alpha2\delta1^+$ TICs HSCs CAFs	$CXCR3^+$ B cells $CD8^+$ T cells $\alpha2\delta1^+$ TICs Hepatoma cells	• Promotes the recruitment of CXCR3+ B cells and CXCR3+ T cells. • Promotes CD8+ T cells migration into HCC. • Promotes the stemness, proliferation and drug resistance of HCC TICs • Promotes HCC cells migration.	(48, 49, 55-57)

Chemokine (Alternate names)	Receptor	Secretory cell	Target cell	Effect	References
CXCL12 (SDF-1)	CXCR4 (CD184) CXCR7	OV6+ HCC cells Hepatoma cells HSCs	OV6+ HCC cells Hepatoma cells HSCs MDSCs	• Promotes OV6+ cell, a potential stem/progenitor-like cell, self-renewal and migration. • Promotes HCC cells migration and invasion. • Mediates HSCs differentiation to myofibroblasts in HCC and further fibrosis. • Increases Gr1+ myeloid cell infiltration in HCC after sorafenib treatment. • Modulates migration ability of MDSCs and endothelial cells.	(58-61)
CXCL16 (SRPSOX)	CXCR6 (CD186)	LSECs Hepatoma cells	NKT cells Hepatoma cells	• Mediates Simvastatin inhibition of HCC progression via recruiting NKT cells. • Contributes to HCC cell migration and invasion via an autocrine loop.	(62, 63)
CXCL17	Not mentioned	Hepatoma cells	TAMs	• Mediates TAMs polarization towards M2-type.	(64)
CX3CL1 (fractalkine)	CX3CR1 (GPR13)	Hepatoma cells	MDSCs NK cells	• Mediates MDSCs accumulation after CIK cell therapy, resulting in impaired anti-tumor activity. • Recruits NK cells that can function as robust effectors against HCC.	(64, 65)
XCL1	XCR1	NK cells CD8+ T cells	cDC1 cells	• Recruits cDC1 cells for tumor antigens presenting, attracting more CD8+ T cells to exert anti-tumor response.	(66)

CAFs: Cancer-associated fibroblasts; CTCs: circulating tumor cells; DEN: N-nitrosodiethylamine; EMT: Epithelial-mesenchymal transition; HSCs: Hepatic stellate cells; IFN-γ: Interferon-γ; IIC2s: G roup-2 innate lymphoid cells; iMCs: Immature myeloid cells; KCs: Kupffer cells; LESCs: Liver sinusoidal endothelial cells; MDSCs: Myeloid-derived suppressor cells; MSCs: mesenchymal stromal cells (MSC); NK cells: Natural killer cells; TAMs: Tumor-associated macrophages; TANs: Tumor-associated neutrophils; TICs: Tumor-initiating cells; TNF-α: Tumor necrosis factor; Treg cells: Regulatory T cells

CXCL10. After binding to CXCR3, CXCL10 stimulates B cells to transform into IgG-producing plasma cells, which produce IL-6, IL-10, and CCL20 [49]. Gut-derived IL-25 can also promote the secretion of CXCL10 from activated M2 TAMs, mediating the tumorigenesis of HCC [50]. Collectively, TAMs are attracted to tumor sites by chemokines and can communicate with surrounding cells by secreting chemokines to reshape the immune TME (Table 1).

Chemokines, chemokine receptors, and MDSCs

The disordered immune TME of HCC provides the necessary signals for the differentiation of immature myeloid cells into MDSCs with immunosuppressive activity. The levels of MDSCs are closely associated with overall survival, treatment efficacy, and tumor recurrence in HCC [51, 52]. Dysregulated chemokine signaling promotes the recruitment and activation of MDSCs during HCC development. MDSCs can be mobilized into the HCC tumor milieu through CXCL12-CXCR4 signaling [31]. In addition, tumor-associated fibroblast-derived CXCL12 can attract monocytes by binding to CXCR4, and their subsequent differentiation into MDSCs is mediated through leukocyte-derived IL-6-induced STAT3 signaling [53]. CCL2 from tumor cells can also direct MDSC homing in HCC, and CCR2 inhibition impedes MDSC accumulation [10]. CX3CL1-recruited MDSCs decrease the efficacy of cytokine-induced killer cell-based immunotherapy in advanced HCC [54]. The CXCL1-CXCR2 signaling pathway also contributes to the recruitment of MDSCs in HCC, and inhibiting CXCR2 reverses MDSC-mediated immunosuppression [55]. Further, psychological stress can affect tumor progression and clinical outcomes. A recent study indicated that chronic stress enhances MDSC mobilization and immunosuppressive proficiency via CXCL5-CXCR2-Erk signaling, revealing multiple roles for MDSCs in HCC [56].

Chemokines, chemokine receptors, and Treg cells

Treg cells are another group of cells that contribute significantly to the immunosuppressive TME. Identifying the complex signaling network among Treg cells and other immune, stromal, and tumor cells in the tumor milieu is of great therapeutic value.

Treg cells in HCC mainly express CCR4 and are recruited into the TME in response to CCL22 and CCL17. CCR4 is the only receptor for CCL17 [57]. Elevated CCL17 and CCL22 concentrations are associated with increased Treg cell infiltration in HCC [18, 58, 59]. Gao *et al.* noted that the chemokines CCL22 and CCL17 are upregulated by sorafenib, and CCR4+ Treg cells are the primary type of

Treg cells in HBV-associated HCC. These Treg cells are associated with sorafenib resistance and HBV load [19, 47]. HBV infection causes multiple pathological changes, including augmenting TGF-β signaling, which leads to the production of CCL22 and further recruitment of Treg cells [17]. CCL5-attracted Treg cells have also been reported to participate in immune evasion of CTCs by protecting them against immune clearance [15]. CXCL8-CXCR1 signaling provokes the polarization and accumulation of Treg cells to suppress antitumor immunity in HCC. Further, CXCL10, a typical chemoattractant for $CD8^+$ T cells and NKT cells, recruits Treg cells and MDSCs and mediates HCC growth and recurrence after liver transplantation [60, 61].

Chemokines, chemokine receptors, and cytotoxic T lymphocytes

$CD8^+$ T cells, or cytotoxic T lymphocytes, are a population of cytotoxic cells that can kill tumor cells by secreting high levels of IFN-γ, perforin, or protease granzyme B. They can also induce apoptosis via overexpression of FasL or tumor necrosis factor α (TNF-α) [62]. CXCL9, CXCL10, and CXCL11 are the main chemokines that attract $CD8^+$ T cells in HCC. CXCL10 is the most studied $CD8^+$ T cell chemoattractant in HCC. CXCL10 can activate tumor cells and promote IFN-γ secretion from NK cells and NKT cells, forming a positive feedback loop in the TME [26]. A recent study found that increased CXCL9/CXCL10 signaling may be responsible for the increased infiltration of $CD8^+$ T cells in HCC [29]. Kohei *et al*. determined that CXCL10 mediates increased $CD8^+$ T cell infiltration and provides a survival benefit in HCC patients treated with regorafenib and anti-PD-1 combination therapy [63]. In contrast, lower CXCL10 is associated with less $CD8^+$ T cell infiltration [64]. In addition, higher levels of CXCL9, CXCL10, and CXCL11 are associated with better response to PD-1 blockade [65]. Collectively, these findings suggest that the absence of CXCL9, CXCL10, and CXCL11 predisposes patients to HCC development.

Chemokines, chemokine receptors, and unconventional T cells

Unconventional T cells, such as γδ T and NKT cells, are also involved in tumor immunity in many cancers, although studies focusing on their role in HCC are relatively limited. γδ T cells and NKT cells are immune cells with cytotoxic activity, and their infiltration in HCC can enhance antitumor immunity and improve patient outcomes [13]. NKT cells are essential for antitumor immune surveillance in multiple tumor types, and their absence promotes tumor cancer development in HCC [66, 67]. CXCL10-CXCR3 and CXCL16-CXCR6 signaling can augment the migration of NKT cells into HCC. These NKT cells then regulate antitumor responses via the production of IFN-γ [26, 68]. CCL4 and CCL5 can promote the migration of γδ T cells into HCC through binding with CCR1 and CCR5, respectively, and both signaling pathways are associated with better overall survival and less aggressive tumors in HCC [13]. γδ T cells also express CCR2 and can be recruited to tumors by CCL2 [69]. However, intratumoral infiltration of γδ T cells is substantially impaired in HCC, which is partly mediated by Treg cells [70].

Chemokines, chemokine receptors, and NK cells

NK cells are innate immune system effector cells and play an indispensable role in tumor immune surveillance. The inflammatory cytokines TNF-α, IFN-γ, and Toll receptor-like ligands stimulate HCC cells and macrophages to secrete NK-trafficking chemokines that bind to receptors on NK cells, such as CXCL10, which binds to CXCR3 on NK cells [26]. Activated NK cells produce more IFN-γ, the best-characterized cytokine produced by NK cells. IFN-γ is a potent immune effector involved in multiple immune responses [71]. IFN-γ released by NK cells enhances the production of CXCL10, forming a positive feedback loop to block tumorigenesis [72]. CCL2, CCL5, and CX3CL1 can also chemoattract NK cells and enhance their cytotoxicity by binding to CCR2, CCR5, and CX3CR1, respectively [41, 72, 73]. Interestingly, human NK cells can secrete CCL2, CCL3, and CCL5

Table 2 Chemokine-targeted therapies in HCC

Target	Drug name	Combination strategy	Reference or Clinical trial number (disease)
CXCL12-CXCR4	AMD3100 (Plerixafo)		NCT01711073 (End-stage liver disease, excluding HCC)
		AMD3100+ Sorafenib	(104)
		AMD3100+ Sorafenib+ anti-PD-1 antibody	(111)
	RU486 (Metabolite)	RU486+ Sorafenib	(112)
CCL2-CCR2	RDC018		(11)
	BMS-813160	BMS-813160+ Nivolumab	NCT04123379 (NSCLC, HCC)
CXCL8-CXCR1-2	BMS-986253		NCT04123379 (NSCLC, HCC)

upon activation during the early stages of tumor growth. Therefore, these chemokines provide a mechanism to communicate between the innate immune response and the $CD8^+$ T cell-mediated immune response [74].

Chemokines, chemokine receptors, and tumor-associated neutrophils

Tumor-associated neutrophils (TANs) have emerged as essential players during tumorigenesis, mediating both pro- and antitumorigenic processes, depending on the composition of the TME. However, an increase in TANs tends to be associated with poor clinical outcomes in most cancers, including HCC [18, 75]. CXCL5 is the primary chemokine that mediates TAN infiltration in HCC [18, 76]. Sorafenib induces the recruitment of TANs via CXCL5 signaling. These TANs further increase the infiltration of macrophages and Treg cells by secreting CCL2 and CCL17, thereby mediating metastasis, neovascularization, and sorafenib resistance in HCC [18]. TANs can also augment the stem cell characteristics of HCC cells, which leads to higher levels of CXCL5, forming a positive feedback loop. Recently, CXCL16 was shown to contribute to the recruitment of TANs, facilitating tumor progression in middle -and late-stage HCC [77].

Treatment

Considering the crucial roles of chemokines and their receptors in HCC initiation and progression, efforts have been made to target chemokines in cancer. Although multiple preclinical studies have focused on chemokine signaling, few agents that directly target a single chemokine or chemokine receptor have been used clinically. This may due to the redundant nature of chemokine networks and cellular heterogeneity, which is not fully understood. Here, we summarize several single and combined strategies that focus on chemokine signaling in HCC.

CXCL12 and CXCR4 inhibition

The CXCL12-CXCR4 axis is one of the most studied chemokine axes and is regarded as a promising target in multiple cancer types [30, 108]. Sorafenib is the standard therapy for advanced HCC and was approved by the FDA in 2007 [109]. Although sorafenib prolongs survival in HCC, its efficacy is severely compromised because of the development of resistance. CXCL12 and CXCR4 may participate in sorafenib resistance [104]. Chen *et al*. observed that sorafenib-induced hypoxia increases the expression of CXCR4 and CXCL12, which increases the infiltration of immunosuppressive $Gr\text{-}1^+$ myeloid cells and HSCs [104]. AMD3100 (plerixafor) is a potent, specific antagonist of CXCR4 [110]. The combined administration of sorafenib and AMD3100 significantly slows murine tumor growth and alleviates hypoxia-induced tumor fibrosis, which decreases sorafenib resistance [104]. Chen *et al*. found that AMD3100 can also decrease the fraction of $F4\text{-}80^+$ TAMs and $CD4^+/CD25^+/FoxP3^+$ Treg cells in sorafenib-treated HCC murine models [111]. Furthermore, combination therapy of anti-PD-1 antibody, sorafenib, and AMD3100 increased $CD8^+$ T cell penetration and activation, ultimately delaying HCC progression better than the combinations of two of these drugs [111]. Zheng *et al*. found that metapristone (RU486 metabolite) reduces CXCR4 expression, which interrupts CXCL12-CXCR4 signaling and related downstream tumor-promoting signaling in HCC. Metapristone also enhances the anti-proliferative efficacy of sorafenib [112]. Moreover, to overcome the adverse side effects and poor pharmacokinetics of chemotherapy drugs, self-assembling nanocarriers to deliver sorafenib and metapristone into tumor tissue have been developed. The combined delivery of sorafenib and metapristone via CXCR4-targeted NPs significantly prolongs circulation time and enhances tumor absorption, leading to a stronger inhibitory effect of sorafenib [112].

CCL2-CCR2 inhibition

As discussed above, tumor cell-derived CCL2 recruits $CCR2^+$ immunosuppressive cells into the TME and induces TAM polarization toward the M2 phenotype. Li *et al*. validated that blocking CCL2-CCR2 signaling using the CCR2 antagonist RDC018 (US patent: US 8431590 B2) decreases the infiltration of TAMs, especially M2 TAMs, while enhancing peripheral $CD8^+$ T cells and cytotoxic $CD8^+$ TILs in a murine model [11]. Further, administration of a CCR2 antagonist significantly suppresses murine HCC growth and metastasis and prevents postsurgical recurrence in a $CD8^+$ T cell-dependent manner [11]. BMS-813160 is a potent and selective CCR2/5 dual antagonist. Several clinical trials are examining the combination of BMS-813160 and an anti-PD-1 antibody (nivolumab) in patients with non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma, and HCC (NCT04123379 and NCT03496662).

CXCL8-CXCR1/2 inhibition

The CXCL8-CXCR1/2 axis is involved in multiple malignant biological processes during HCC development, including tumor growth, angiogenesis, migration, and invasion [22, 91, 98]. CXCL8 and its receptors CXCR1/2 may be therapeutic targets in HCC. An anti-IL-8 mAb significantly increases overall survival and impairs angiogenesis in an HCC murine model [22]. Another study confirmed that IL-8 neutralizing antibodies can eliminate the pro-angiogenic and pro-tumorigenic activity of $CD133^+$ TICs in HCC [91]. A phase 2 clinical trial examining the administration of nivolumab and an anti-IL-8 monoclonal antibody (BMS-986253) in

patients with NSCLC or HCC is ongoing (NCT04123379). Reparixin, an investigational allosteric inhibitor of the IL-8 receptor CXCR1/2, is also being examined in clinical trials [113]. Reparixin inhibits HCC growth and metastasis by attenuating M2 polarization of TAMs and blocking EMT [98]. Reparixin also represses the stem cell features of HCC cells and enhances their sensitivity to sorafenib [114]. However, no clinical trials have examined reparixin in HCC.

Discussion

The HCC TME consists of carcinoma cells and multiple tumor-resident cells. These cells recruit various immune cells that express specific receptors from the peripheral blood or bone marrow by secreting cytokines [115]. Immune cells also secrete a variety of cytokines to reshape the TME. The process of tumorigenesis is modulated by cancer cells, tissue-resident cells, and immune cells. Chemokines, a specific subfamily of cytokines, act as messengers among these components. They shuttle between different or similar cells to regulate tumor initiation and development [5]. Because chemokines have tumor-promoting functions in HCC, including angiogenesis, invasion, migration, proliferation, and EMT, they are attractive therapeutic targets [116]. A series of preclinical experiments have examined the roles of chemokine inhibition in HCC. However, there are few studies examining chemokine inhibition as monotherapy. We posit two reasons for this. The first is the high degree of redundancy in chemokine signaling. When a specific chemokine signal is blocked, other chemokine signals can compensate to some extent and abolish the blocking effect. The second is that cellular functions are spatiotemporally heterogeneous. Cells of different subtypes and cells in various stages of tumor development display diverse responses to chemokine stimulation. This remains a blind spot to identifying the underlying mechanism of chemokine signaling. However, despite these limitations, chemokines are attractive therapeutic targets, and chemokine-targeted therapies will continue to be assessed clinically.

The application of ICIs has profoundly improved the treatment landscape of HCC. However, despite their success, primary or acquired resistance to ICIs has decreased their effectiveness in patients with HCC [117]. Multiple studies have demonstrated the contribution of chemokine signaling to ICI resistance [3]. Therefore, the combination of chemokine-targeted therapy and ICIs may be a therapeutic strategy. This combination has already been shown to achieve a meaningful clinical response. In addition, analysis of multi-dimensional "spatiotemporal" axes of HCC samples using single-cell techniques can further clarify the spatiotemporal heterogeneity during HCC development. Clarifying the various immune cellular subtypes and their responsiveness to chemokines will facilitate precise chemokine-targeted therapy.

In conclusion, chemokine-targeted therapy provides a transformative therapeutic avenue for HCC treatment. Future research should clarify the value of chemokine-targeted therapy in combination with other therapeutic options, including ICIs, and the functional heterogeneity of chemokines across time and different cell subsets.

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Conflicts of interest

The authors indicated no potential conflicts of interest.

Author contributions

Conceptualization, Y.W. and L.X.; writing - original draft preparation, Y.W.; writing - review and editing, Y.W. and L.X.; bioinformatics analysis, Y.W. and M.S.; visualization, TZ and Y.F; supervision, X.J., M.X., and D.L.; funding acquisition, L.X. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The datasets generated and analyzed during the current study are available from the China Drug Trials Repository (http://www.chinadrugtrials.org.cn/index). Chemokine expression information was obtained and analyzed using UCSC Xena (https://xenabrowser.net/datapages/).

Ethical approval

Not applicable.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.
2. Tang W, Chen Z, Zhang W, et al. The mechanisms of sorafenib resistance in hepatocellular carcinoma: theoretical basis and therapeutic aspects. Signal Transduct Target Ther. 2020;10;5(1):87.
3. Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. Annu Rev Pathol. 2021;16:223-249.
4. Jenne CN, Kubes P. Immune surveillance by the liver. Nat Immunol. 2013;14(10):996-1006.
5. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. Nat

Rev Immunol. 2017;17(9):559-572.
6. Miller MC, Mayo KH. Chemokines from a structural perspective. Int J Mol Sci. 2017;18(10):2088.
7. Liu LZ, Zhang Z, Zheng BH, et al. CCL15 Recruits Suppressive Monocytes to Facilitate Immune Escape and Disease Progression in Hepatocellular Carcinoma. Hepatology. 2019;69(1):143-159.
8. Eggert T, Wolter K, Ji J, et al. Distinct functions of senescence-associated immune responses in liver tumor surveillance and tumor progression. Cancer Cell. 2016;30(4):533-547.
9. Liu N, Wang X, Steer CJ, et al. MicroRNA-206 promotes the recruitment of CD8+ T cells by driving M1 polarisation of Kupffer cells. Gut. 2021:gutjnl-2021-324170.
10. Wang D, Li X, Li J, et al. APOBEC3B interaction with PRC2 modulates microenvironment to promote HCC progression. Gut. 2019;68(10):1846-1857.
11. Li X, Yao W, Yuan Y, et al. Targeting of tumour-infiltrating macrophages via CCL2/CCR2 signalling as a therapeutic strategy against hepatocellular carcinoma. Gut. 2017;66(1):157-167.
12. Ruiz de Galarreta M, Bresnahan E, Molina-Sánchez P, et al. β-catenin activation promotes immune escape and resistance to anti-PD-1 therapy in hepatocellular carcinoma. Cancer Discov. 2019;9(8):1124-1141.
13. Zhao N, Dang H, Ma L, et al. Intratumoral γδ T-cell infiltrates, chemokine (C-C Motif) ligand 4/chemokine (C-C Motif) ligand 5 protein expression and survival in patients with hepatocellular carcinoma. Hepatology. 2021;73(3):1045-1060.
14. Singh SK, Mishra MK, Rivers BM, et al. Biological and clinical significance of the CCR5/CCL5 axis in hepatocellular carcinoma. Cancers (Basel). 2020;12(4):883.
15. Sun YF, Wu L, Liu SP, et al. Dissecting spatial heterogeneity and the immune-evasion mechanism of CTCs by single-cell RNA-seq in hepatocellular carcinoma. Nat Commun. 2021;12(1):4091.
16. Yoshie O, Matsushima K. CCR4 and its ligands: from bench to bedside. Int Immunol. 2015;27(1):11-20.
17. Yang P, Li QJ, Feng Y, et al. TGF-β-miR-34a-CCL22 signaling-induced Treg cell recruitment promotes venous metastases of HBV-positive hepatocellular carcinoma. Cancer Cell. 2012;22(3):291-303.
18. Zhou SL, Zhou ZJ, Hu ZQ, et al. Tumor-associated neutrophils recruit macrophages and T-regulatory cells to promote progression of hepatocellular carcinoma and resistance to Sorafenib. Gastroenterology. 2016;150(7):1646-1658.e17.
19. Gao Y, You M, Fu J, et al. Intratumoral stem-like CCR4+ regulatory T cells orchestrate the immunosuppressive microenvironment in HCC associated with hepatitis B. J Hepatol. 2022;76(1):148-159.
20. Sun F, Wang J, Sun Q, Li F, Gao H, Xu L, Zhang J, *et al*. Interleukin-8 promotes integrin β3 upregulation and cell invasion through PI3K/Akt pathway in hepatocellular carcinoma. J Exp Clin Cancer Res 2019;38:449.
21. Fang T, Lv H, Lv G, et al. Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. Nat Commun. 2018;9(1):191.
22. Liu Y, Zhang Y, Wang S, et al. Prospero-related homeobox 1 drives angiogenesis of hepatocellular carcinoma through selectively activating interleukin-8 expression. Hepatology. 2017;66(6):1894-1909.
23. Huang Y, Yang X, Meng Y, et al. The hepatic senescence-associated secretory phenotype promotes hepatocarcinogenesis through Bcl3-dependent activation of macrophages. Cell Biosci. 2021;11(1):173.
24. Chan SL, Chan AW, Chan AK, et al. Systematic evaluation of circulating inflammatory markers for hepatocellular carcinoma. Liver Int. 2017;37(2):280-289.
25. Tokunaga R, Zhang W, Naseem M, et al. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy. Cancer Treat Rev. 2018;63:40-47.
26. Yan Y, Zheng L, Du Q, et al. Interferon regulatory factor 1(IRF-1) activates anti-tumor immunity via CXCL10/CXCR3 axis in hepatocellular carcinoma (HCC). Cancer Lett. 2021;506:95-106.
27. Sasaki K, Nishina S, Yamauchi A, et al. Nanoparticle-mediated delivery of 2-Deoxy-D-Glucose induces antitumor immunity and cytotoxicity in liver tumors in mice. Cell Mol Gastroenterol Hepatol. 2021;11(3):739-762.
28. Chow MT, Ozga AJ, Servis RL, et al. Intratumoral activity of the CXCR3 chemokine system is required for the efficacy of anti-PD-1 therapy. Immunity. 2019;50(6):1498-1512.e5.
29. Fang Y, Liu W, Tang Z, et al. Monocarboxylate transporter 4 inhibition potentiates hepatocellular carcinoma immunotherapy through enhancing T cell infiltration and immune attack. Hepatology. 2022 Jan 19.
30. Teicher BA, Fricker SP. CXCL12 (SDF-1)/CXCR4 pathway in cancer. Clin Cancer Res. 2010;16(11):2927-2931.
31. Xu Y, Fang F, Jiao H, et al. Activated hepatic stellate cells regulate MDSC migration through the SDF-1/CXCR4 axis in an orthotopic mouse model of hepatocellular carcinoma. Cancer Immunol Immunother. 2019;68(12):1959-1969.
32. Tsai CN, Yu SC, Lee CW, et al. SOX4 activates CXCL12 in hepatocellular carcinoma cells to modulate endothelial cell migration and angiogenesis in vivo. Oncogene. 2020;39(24):4695-4710.
33. García-Irigoyen O, Latasa MU, Carotti S, et al. Matrix metalloproteinase 10 contributes to hepatocarcinogenesis in a novel crosstalk with the stromal derived factor 1/C-X-C chemokine receptor 4 axis. Hepatology. 2015;62(1):166-178.
34. Yang J, Zhang L, Jiang Z, et al. TCF12 promotes the tumorigenesis and metastasis of hepatocellular carcinoma via upregulation of CXCR4 expression. Theranostics. 2019;9(20):5810-5827.
35. Ghanem I, Riveiro ME, Paradis V, et al. Insights on the CXCL12-CXCR4 axis in hepatocellular carcinoma carcinogenesis. Am J Transl Res. 2014;6(4):340-352.
36. Zhu M, Xu W, Wei C, et al. CCL14 serves as a novel prognostic factor and tumor suppressor of HCC by modulating cell cycle and promoting apoptosis. Cell Death Dis. 2019;10(11):796.
37. Dou L, Shi X, He X, et al. Macrophage phenotype and function in liver disorder. Front Immunol. 2020;10:3112.
38. Gomez Perdiguero E, Klapproth K, Schulz C, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature. 2015;518(7540):547-551.
39. Zhu Y, Yang J, Xu D, et al. Disruption of tumour-associated macrophage trafficking by the osteopontin-induced colony-stimulating factor-1 signalling sensitises hepatocellular carcinoma to anti-PD-L1 blockade. Gut. 2019;68(9):1653-1666.
40. Tan S, Zhao J, Sun Z, et al. Hepatocyte-specific TAK1 deficiency drives RIPK1 kinase-dependent inflammation to promote liver fibrosis and hepatocellular carcinoma. Proc Natl Acad Sci U S A. 2020;117(25):14231-14242.
41. Chew V, Lai L, Pan L, et al. Delineation of an immunosuppressive gradient in hepatocellular carcinoma using high-dimensional proteomic and transcriptomic analyses. Proc Natl Acad Sci U S A. 2017;114(29):E5900-E5909.
42. Ye LY, Chen W, Bai XL, et al. Hypoxia-induced epithelial-to-mesenchymal transition in hepatocellular carcinoma induces an immunosuppressive tumor microenvironment to promote metastasis.

Cancer Res. 2016;76(4):818-830.
43. Wu H, Li Y, Shi G, et al. Hepatic interferon regulatory factor 8 expression suppresses hepatocellular carcinoma progression and enhances the response to anti-programmed cell death protein-1 therapy. Hepatology. 2022 Jan 6.
44. Hou PP, Luo LJ, Chen HZ, et al. Ectosomal PKM2 promotes HCC by inducing macrophage differentiation and remodeling the tumor microenvironment. Mol Cell. 2020;78(6):1192-1206.e10.
45. Liu N, Wang X, Steer CJ, et al. MicroRNA-206 promotes the recruitment of CD8+ T cells by driving M1 polarisation of Kupffer cells. Gut. 2021:gutjnl-2021-324170.
46. Hefetz-Sela S, Stein I, Klieger Y, et al. Acquisition of an immunosuppressive protumorigenic macrophage phenotype depending on c-Jun phosphorylation. Proc Natl Acad Sci U S A. 2014;111(49):17582-17587.
47. Gao Y, Fan X, Li N, Du C, Yang B, Qin W, Fu J, *et al*. CCL22 signaling contributes to sorafenib resistance in hepatitis B virus-associated hepatocellular carcinoma. Pharmacol Res. 2020;157:104800.
48. Yeung OW, Lo CM, Ling CC, *et al*. Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma. J Hepatol. 2015;62:607-616.
49. Wei Y, Lao XM, Xiao X, *et al*. Plasma Cell Polarization to the Immunoglobulin G Phenotype in Hepatocellular Carcinomas Involves Epigenetic Alterations and Promotes Hepatoma Progression in Mice. Gastroenterology 2019;156:1890-1904.e1816.
50. Gao Y, Fan X, Li N, et al. CCL22 signaling contributes to sorafenib resistance in hepatitis B virus-associated hepatocellular carcinoma. Pharmacol Res. 2020;157:104800.
51. Zhang X, Fu X, Li T, et al. The prognostic value of myeloid derived suppressor cell level in hepatocellular carcinoma: A systematic review and meta-analysis. PLoS One. 2019;14(12):e0225327.
52. Chang CJ, Yang YH, Chiu CJ, et al. Targeting tumor-infiltrating Ly6G+ myeloid cells improves sorafenib efficacy in mouse orthotopic hepatocellular carcinoma. Int J Cancer. 2018;142(9):1878-1889.
53. Deng Y, Cheng J, Fu B, et al. Hepatic carcinoma-associated fibroblasts enhance immune suppression by facilitating the generation of myeloid-derived suppressor cells. Oncogene. 2017;36(8):1090-1101.
54. Yu SJ, Ma C, Heinrich B, et al. Targeting the crosstalk between cytokine-induced killer cells and myeloid-derived suppressor cells in hepatocellular carcinoma. J Hepatol. 2019;70(3):449-457.
55. Li YM, Liu ZY, Wang JC, et al. Receptor-interacting protein kinase 3 deficiency recruits myeloid-derived suppressor cells to hepatocellular carcinoma through the chemokine (C-X-C Motif) ligand 1-chemokine (C-X-C Motif) receptor 2 axis. Hepatology. 2019;70(5):1564-1581.
56. Cao M, Huang W, Chen Y, et al. Chronic restraint stress promotes the mobilization and recruitment of myeloid-derived suppressor cells through β-adrenergic-activated CXCL5-CXCR2-Erk signaling cascades. Int J Cancer. 2021;149(2):460-472.
57. Heiseke AF, Faul AC, Lehr HA, et al. CCL17 promotes intestinal inflammation in mice and counteracts regulatory T cell-mediated protection from colitis. Gastroenterology. 2012;142(2):335-345.
58. Liu N, Chang CW, Steer CJ, et al. MicroRNA-15a/16-1 prevents hepatocellular carcinoma by disrupting the communication between Kupffer cells and regulatory T cells. Gastroenterology. 2022;162(2):575-589.
59. Wiedemann GM, Knott MM, Vetter VK, et al. Cancer cell-derived IL-1α induces CCL22 and the recruitment of regulatory T cells. Oncoimmunology. 2016;5(9):e1175794.
60. Liu H, Ling CC, Yeung WHO, et al. Monocytic MDSC mobilization promotes tumor recurrence after liver transplantation via CXCL10/TLR4/MMP14 signaling. Cell Death Dis. 2021;12(5):489.
61. Li CX, Ling CC, Shao Y, et al. CXCL10/CXCR3 signaling mobilized-regulatory T cells promote liver tumor recurrence after transplantation. J Hepatol. 2016;65(5):944-952.
62. St Paul M, Ohashi PS. The roles of CD8+ T cell subsets in antitumor immunity. Trends Cell Biol. 2020;30(9):695-704.
63. Shigeta K, Matsui A, Kikuchi H, et al. Regorafenib combined with PD1 blockade increases CD8 T-cell infiltration by inducing CXCL10 expression in hepatocellular carcinoma. J Immunother Cancer. 2020;8(2):e001435.
64. Huang XY, Zhang PF, Wei CY, et al. Circular RNA circMET drives immunosuppression and anti-PD1 therapy resistance in hepatocellular carcinoma via the miR-30-5p/snail/DPP4 axis. Mol Cancer. 2020;19(1):92.
65. Wu X, Gu Z, Chen Y, et al. Application of PD-1 blockade in cancer immunotherapy. Comput Struct Biotechnol J. 2019;17:661-674.
66. Vivier E, Ugolini S, Blaise D, et al. Targeting natural killer cells and natural killer T cells in cancer. Nat Rev Immunol. 2012;12(4):239-252.
67. Mossanen JC, Kohlhepp M, Wehr A, et al. CXCR6 inhibits hepatocarcinogenesis by promoting natural killer T- and CD4+ T-cell-dependent control of senescence. Gastroenterology. 2019;156(6):1877-1889.e4. .
68. Ji G, Ma L, Yao H, et al. Precise delivery of obeticholic acid via nanoapproach for triggering natural killer T cell-mediated liver cancer immunotherapy. Acta Pharm Sin B. 2020;10(11):2171-2182.
69. Lança T, Costa MF, Gonçalves-Sousa N, et al. Protective role of the inflammatory CCR2/CCL2 chemokine pathway through recruitment of type 1 cytotoxic γδ T lymphocytes to tumor beds. J Immunol. 2013;190(12):6673-6680.
70. Yi Y, He HW, Wang JX, et al. The functional impairment of HCC-infiltrating γδ T cells, partially mediated by regulatory T cells in a TGFβ- and IL-10-dependent manner. J Hepatol. 2013;58(5):977-983.
71. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. Nat Rev Cancer. 2016;16(1):7-19.
72. Chew V, Chen J, Lee D, et al. Chemokine-driven lymphocyte infiltration: an early intratumoural event determining long-term survival in resectable hepatocellular carcinoma. Gut. 2012;61(3):427-438.
73. Chen EB, Zhou ZJ, Xiao K, et al. The miR-561-5p/CX3CL1 signaling axis regulates pulmonary metastasis in hepatocellular carcinoma involving CX3CR1+ natural killer cells infiltration. Theranostics. 2019;9(16):4779-4794.
74. Dorner BG, Scheffold A, Rolph MS, et al. MIP-1alpha, MIP-1beta, RANTES, and ATAC/lymphotactin function together with IFN-gamma as type 1 cytokines. Proc Natl Acad Sci U S A. 2002;99(9):6181-6186.
75. Jaillon S, Ponzetta A, Di Mitri D, et al. Neutrophil diversity and plasticity in tumour progression and therapy. Nat Rev Cancer. 2020;20(9):485-503.
76. Zhou SL, Yin D, Hu ZQ, et al. A positive feedback loop between cancer stem-like cells and tumor-associated neutrophils controls hepatocellular carcinoma progression. Hepatology. 2019;70(4):1214-1230.
77. Song M, He J, Pan QZ, et al. Cancer-associated fibroblast-mediated cellular crosstalk supports hepatocellular carcinoma progression. Hepatology. 2021;73(5):1717-1735.
78. Tacke F. Targeting hepatic macrophages to treat liver diseases. J Hepatol. 2017;66(6):1300-1312.
79. Yang X, Lu P, Fujii C, et al. Essential contribution of a chemokine,

CCL3, and its receptor, CCR1, to hepatocellular carcinoma progression. Int J Cancer. 2006;118(8):1869-1876.
80. Lu P, Nakamoto Y, Nemoto-Sasaki Y, et al. Potential interaction between CCR1 and its ligand, CCL3, induced by endogenously produced interleukin-1 in human hepatomas. Am J Pathol. 2003;162(4):1249-1258.
81. Liu J, Chen S, Wang W, et al. Cancer-associated fibroblasts promote hepatocellular carcinoma metastasis through chemokine-activated hedgehog and TGF-β pathways. Cancer Lett. 2016;379(1):49-59.
82. Gao Y, Zhou Z, Lu S, et al. Chemokine CCL15 mediates migration of human bone marrow-derived mesenchymal stem cells toward hepatocellular carcinoma. Stem Cells. 2016;34(4):1112-1122.
83. Li Y, Wu J, Zhang W, et al. Identification of serum CCL15 in hepatocellular carcinoma. Br J Cancer. 2013;108(1):99-106.
84. Howard OM, Dong HF, Shirakawa AK, et al. LEC induces chemotaxis and adhesion by interacting with CCR1 and CCR8. Blood. 2000;96(3):840-845.
85. He H, Wu J, Zang M, et al. CCR6+ B lymphocytes responding to tumor cell-derived CCL20 support hepatocellular carcinoma progression via enhancing angiogenesis. Am J Cancer Res. 2017;7(5):1151-1163.
86. Yuan Q, Zhang J, Liu Y, et al. MyD88 in myofibroblasts regulates aerobic glycolysis-driven hepatocarcinogenesis via ERK-dependent PKM2 nuclear relocalization and activation. J Pathol. 2021 Dec 19.
87. Ouaguia L, Moralès O, Aoudjehane L, et al. Hepatitis C virus improves human tregs suppressive function and promotes their recruitment to the liver. Cells. 2019;8(10):1296.
88. Chen L, Zhou S, Qin J, et al. Combination of SLC administration and Tregs depletion is an attractive strategy for targeting hepatocellular carcinoma. Mol Cancer. 2013;12(1):153.
89. Luo Q, Wang CQ, Yang LY, et al. FOXQ1/NDRG1 axis exacerbates hepatocellular carcinoma initiation via enhancing crosstalk between fibroblasts and tumor cells. Cancer Lett. 2018;417:21-34.
90. Xia S, Wu J, Zhou W, et al. SLC7A2 deficiency promotes hepatocellular carcinoma progression by enhancing recruitment of myeloid-derived suppressors cells. Cell Death Dis. 2021;12(6):570.
91. Tang KH, Ma S, Lee TK, et al. CD133(+) liver tumor-initiating cells promote tumor angiogenesis, growth, and self-renewal through neurotensin/interleukin-8/CXCL1 signaling. Hepatology. 2012;55(3):807-820.
92. Peng ZP, Jiang ZZ, Guo HF, et al. Glycolytic activation of monocytes regulates the accumulation and function of neutrophils in human hepatocellular carcinoma. J Hepatol. 2020;73(4):906-917.
93. Xu X, Ye L, Zhang Q, et al. Group-2 innate lymphoid cells promote HCC progression through CXCL2-neutrophil-induced immunosuppression. Hepatology. 2021;74(5):2526-2543.
94. Haider C, Hnat J, Wagner R, et al. Transforming growth factor-β and Axl induce CXCL5 and neutrophil recruitment in hepatocellular carcinoma. Hepatology. 2019;69(1):222-236.
95. Zhou SL, Zhou ZJ, Hu ZQ, et al. CXCR2/CXCL5 axis contributes to epithelial-mesenchymal transition of HCC cells through activating PI3K/Akt/GSK-3β/Snail signaling. Cancer Lett. 2015;358(2):124-135.
96. Zhou SL, Dai Z, Zhou ZJ, et al. Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma. Hepatology. 2012;56(6):2242-2254.
97. Zhang C, Gao Y, Du C, et al. Hepatitis B-induced IL8 promotes hepatocellular carcinoma venous metastasis and intrahepatic Treg accumulation. Cancer Res. 2021;81(9):2386-2398.
98. Xiao P, Long X, Zhang L, et al. Neurotensin/IL-8 pathway orchestrates local inflammatory response and tumor invasion by inducing M2 polarization of Tumor-Associated macrophages and epithelial-mesenchymal transition of hepatocellular carcinoma cells. Oncoimmunology. 2018;7(7):e1440166.
99. Liu RX, Wei Y, Zeng QH, et al. Chemokine (C-X-C motif) receptor 3-positive B cells link interleukin-17 inflammation to protumorigenic macrophage polarization in human hepatocellular carcinoma. Hepatology. 2015;62(6):1779-1790.
100. Monnier J, Boissan M, L'Helgoualc'h A, et al. CXCR7 is up-regulated in human and murine hepatocellular carcinoma and is specifically expressed by endothelial cells. Eur J Cancer. 2012;48(1):138-148.
101. Zhang Y, Zhao W, Li S, et al. CXCL11 promotes self-renewal and tumorigenicity of α2δ1+ liver tumor-initiating cells through CXCR3/ERK1/2 signaling. Cancer Lett. 2019;449:163-171.
102. Liu G, Sun J, Yang ZF, et al. Cancer-associated fibroblast-derived CXCL11 modulates hepatocellular carcinoma cell migration and tumor metastasis through the circUBAP2/miR-4756/IFIT1/3 axis. Cell Death Dis. 2021;12(3):260.
103. Yang W, Wang C, Lin Y, et al. OV6+ tumor-initiating cells contribute to tumor progression and invasion in human hepatocellular carcinoma. J Hepatol. 2012;57(3):613-620.
104. Chen Y, Huang Y, Reiberger T, et al. Differential effects of sorafenib on liver versus tumor fibrosis mediated by stromal-derived factor 1 alpha/C-X-C receptor type 4 axis and myeloid differentiation antigen-positive myeloid cell infiltration in mice. Hepatology. 2014;59(4):1435-1447.
105. Yu Z, Guo J, Liu Y, et al. Nano delivery of simvastatin targets liver sinusoidal endothelial cells to remodel tumor microenvironment for hepatocellular carcinoma. J Nanobiotechnology. 2022;20(1):9.
106. Gao Q, Zhao YJ, Wang XY, et al. CXCR6 upregulation contributes to a proinflammatory tumor microenvironment that drives metastasis and poor patient outcomes in hepatocellular carcinoma. Cancer Res. 2012;72(14):3546-3556.
107. Song G, Shi Y, Zhang M, et al. Global immune characterization of HBV/HCV-related hepatocellular carcinoma identifies macrophage and T-cell subsets associated with disease progression. Cell Discov. 2020;6(1):90.
108. Walenkamp AME, Lapa C, Herrmann K, et al. CXCR4 ligands: the next big hit? J Nucl Med. 2017;58(Suppl 2):77S-82S.
109. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378-390.
110. DiPersio JF, Uy GL, Yasothan U, et al. Plerixafor. Nat Rev Drug Discov. 2009;8(2):105-106.
111. Chen Y, Ramjiawan RR, Reiberger T, et al. CXCR4 inhibition in tumor microenvironment facilitates anti-programmed death receptor-1 immunotherapy in sorafenib-treated hepatocellular carcinoma in mice. Hepatology. 2015;61(5):1591-1602.
112. Zheng N, Liu W, Li B, et al. Co-delivery of sorafenib and metapristone encapsulated by CXCR4-targeted PLGA-PEG nanoparticles overcomes hepatocellular carcinoma resistance to sorafenib. J Exp Clin Cancer Res. 2019;38(1):232.
113. Schott AF, Goldstein LJ, Cristofanilli M, et al. Phase Ib pilot study to evaluate reparixin in combination with weekly paclitaxel in patients with HER-2-negative metastatic breast cancer. Clin Cancer Res. 2017;23(18):5358-5365.
114. Kahraman DC, Kahraman T, Cetin-Atalay R. Targeting PI3K/Akt/mTOR pathway identifies differential expression and functional role of IL8 in liver cancer stem cell enrichment. Mol Cancer Ther. 2019;18(11):2146-2157.
115. Li Q, Wang C, Wang Y, et al. HSCs-derived COMP drives hepatocellular carcinoma progression by activating MEK/ERK and PI3K/AKT signaling pathways. J Exp Clin Cancer Res.

2018;37(1):231.
116. Ehling J, Tacke F. Role of chemokine pathways in hepatobiliary cancer. Cancer Lett. 2016;379(2):173-183.
117. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. Nat Rev Dis Primers. 2021;7(1):6.

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REVIEW ARTICLE

Metabolic reprogramming drives homeostasis and specialization of regulatory T cells in cancer

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Abstract

Transcription factor forkhead box P3 (Foxp3)+ regulatory T (Treg) cells are receiving increasing attention because this unique subset of T cells is characterized by exerting negative regulatory function of cellular immune responses. The resultant suppression of anti-tumor immunity in the tumor microenvironment (TME) is regarded as a major obstacle to immunotherapies in a plethora of cancers. Thus, an integrated understanding of the intrinsic correlation between tumors and Treg cell biology is urgently required. This review focuses on the peculiar biochemical effects of tumor metabolic environments on Tregs and how Tregs orchestrate internal metabolic switches and altered metabolic pathways and molecules to survive and function after the remodeling of homeostasis and specialization, providing new directions for immunotherapies.

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Regulatory T (Treg) cells, an inhibitory subpopulation of $CD4^+$ T lymphocytes, are indispensable for the homeostasis of the immune system and maintenance of immune tolerance in the body. They serve as a crucial barrier to anti-tumor immunity and cancer immunotherapy by directly and indirectly suppressing the proliferation, activation, and differentiation of $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells specifically against neoantigens or shared antigens in the tumor microenvironment (TME). Treg cells lack the expression of CD127 and express high levels of the α subunit of the IL-2 receptor (CD25), the key molecule for homeostasis of Tregs, and the lineage-specifying transcription factor forkhead box P3 (Foxp3), which is essential for Treg cell development and immunosuppressive function [1–3]. Notably, the molecular and functional phenotypes of Treg cells do not always remain stable but undergo a beneficial shift for Tregs to better adapt to the specific resident tissues, neighboring cells, and micro-environment [4–6]. When exposed to IL-6 with or without IL-1β and IL-23 *in vitro* or during autoimmune arthritis *in vivo*, these $Foxp3^+$ Tregs downregulate Foxp3 expression and overexpress Th17 genes, including IL-17, IL-22, IL-23R, and RORγt, reacquiring characteristics of Th17 cells, a subset of T helper cells implicated in the pathogenesis of autoimmune diseases, such as multiple sclerosis, psoriasis, and systemic lupus erythematosus (SLE) [7]. In the TME, effector Treg (eTreg) cells distinguished by $CD45RA^-FOXP3^{hi}CD127^{low}CD25^{hi}CD4^+$ constitute the major subsets that restrain antitumor adaptive immune responses through cellular and humoral mechanisms [8] and are correlated with poorer prognosis in various types of cancers [9–12]. These tumor-infiltrating eTreg cells express multiple co-inhibitory receptors on their surface, including cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), lymphocyte activation gene 3 protein (LAG3), T cell immunoglobulin mucin receptor 3 (TIM3), and neuropilin 1 (NRP1), and secrete abundant immunosuppressive cytokines, such as IL-10, IL-35, and transforming growth factor-β (TGF-β), which downregulate the activity of both antigen-presenting cells (APCs) and effector T (Teff) cells [13].

Recent studies have highlighted the central role of metabolism in driving phenotypic and functional homeostasis and specialization of Tregs in cancer. Distinct from other T cell subtypes, Treg cells exhibit a prominent capacity to promptly adapt to the TME characterized by

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fierce nutrient competition, low pH, limited oxygen, and accumulation of metabolites, and even proliferate and exert immunosuppression in a less unaffetcted manner [14–15]. In this review, we will discuss what and how specific metabolites and metabolic pathways in the TME remodel, stabilize, specialize, and provide Treg cells surviving advantages at transcriptional, epigenetic, and cellular levels and identify the key candidate metabolic determinants that can be potentially targeted alone or in combination with current immunotherapies for better survival of patients diagnosed with carcinoma.

Tumor resident Tregs

Treg cells can be divided into two subgroups based on their originating source sites: thymus-derived Treg (tTreg) cells naturally originating from the thymus [also called natural Treg (nTreg) cells] and maturing through high-avidity interactions with MHC class II/self-peptide complexes and stimulation by IL-2 signaling; induced Treg (iTreg) cells differentiated from conventional T (Tconv) cells in the periphery [also called peripherally-derived Treg (pTreg) cells]. The iTregs can be induced *in vivo* or generated after T cell receptor (TCR) stimulation in the presence of TGF-β and IL-2 or retinoic acid *in vitro*, which frequently lack or have restricted expression of Helios and NRP1 involved in the maintenance of Treg stability and suppressive function [16–18].

The specific origins of tumor-resident Treg cells and mechanisms of Treg repertoire formation in cancer remain unclear and debated. A systematic analysis of nTreg and iTreg accumulation revealed that nTreg cells that migrated into the TME favorably recognized self-antigens, while iTreg cells preferentially recognized the same tumor-specific antigens (TSA) [19]. Recent results of T-cell receptor and single-cell transcriptome sequencing suggested that Tregs in human breast cancer may stem considerably from antigen-experienced Tconv conversion into secondary-induced Tregs through intratumoral activation [20]. This is supported by another study that explored the association between peripheral blood Treg II cells and intratumoral Treg cells [11]. However, the transcriptional analysis of Treg cells in untreated human breast carcinomas, normal mammary glands, and peripheral blood indicated that the gene expression pattern of tumor-resident Tregs resembled that of normal breast tissues than that of circulating Tregs [21]. Further comparison of the TCR repertoire of Treg cells from breast cancer and normal breast tissues in matched patients exhibited little overlap, excluding the possibility that tumor-infiltrating Tregs originate from local expansion of the tissue-resident Treg population [21], except for tumor-draining lymph nodes [22]. Moreover, tumor-associated Tregs in human bladder cancer possess a private TCR repertoire distinct from other $CD4^+$ T cells, suggesting that these immune-suppressing T cells are unlikely to be converted from other effector $CD4^+$ T cells in the TME [23]. In summary, these results revealed that tumor-resident Tregs with suppressive capacity might mainly originate from the periphery or tumor-draining lymph nodes and are subsequently recruited to the TME.

Tumor-infiltrating Treg cells directly promote tumor immune evasion, exhibiting distinct phenotypic and functional profiles, such as IL-2 consumption, secretion of granzymes and perforins, immunosuppressive cytokines (e.g., IL-10, IL-35, and TGF-β), and through the expression of multiple checkpoint inhibitory molecules, such as CTLA-4, PD-1, TIGIT, T cell immunoglobulin, mucin domain-containing protein 3 (TIM-3), and LAG-3 [24]. Moreover, they depend on the ectoenzymes CD39 and CD73 to degrade extracellular adenosine triphosphate (ATP) or adenosine diphosphate (ADP) to immune-suppressive adenosine [25].

Intriguingly, Tregs show stunning adaptive capabilities to metabolic changes in the TME by regulating several signaling pathways, among which the PI3K/Akt signaling pathway plays a central role. Once activated by upstream TCR and IL-2 signaling in Treg cells, PI3K/Akt signaling directly controls the phosphorylation of Foxo (Foxo1 or Foxo3a) transcription factors and blocks their nuclear translocation [26–27]. When Akt is hypoactivated, nuclear Foxo abundance is correspondingly elevated on the promoter regions of Foxp3, giving rise to Foxp3 expression and stability of tTregs [28–30], This process can be mediated by Sema4a in a PTEN-dependent manner [31]. The mTOR signaling is the upstream of metabolic reprogramming. Furthermore, Akt can affect the mTOR signaling including mTORC1and mTORC2, is affected by Akt [32]. Akt phosphorylates TSC2 to relieve TSC complex inhibition of mTORC1, which has been reported to be essential for Foxp3 expression at low doses [33]. This is in agreement with previous observations that co-stimulation with TCR and IL-2 *in vitro* could reverse the anergic state of freshly isolated Treg cells by re-activating the Akt-mTOR signaling inhibited by PTEN [34].

Metabolic profiles in the TME

Tumor cells are characterized by their unparalleled competence in the uptake of oxygen and various other nutrients and outstanding resilience to local environmental changes through metabolic reprogramming to sustain proliferative signaling, evade growth suppressors, resist cell death, enable replicative immortality, induce angiogenesis, and activate invasion and metastasis [35]. In 2016, Pavlova and colleagues concluded that six tumor-associated metabolic modifications occur in the TME: (1) deregulation of glucose and amino acid metabolism, (2) altered nutrient uptake, (3) utilization of intermediates from the citric acid cycle (TCA cycle)/glycolysis for

the biosynthesis of nicotinamide adenine dinucleotide phosphate (NADPH), (4) increased nitrogen requirement, (5) variations in the regulation of metabolite-dependent gene expression, and (6) interactions between metabolic pathways within the TME [36]. Due to the unlimited consumption of these nutrients, the tumor microenvironment always presents with a hypoxic, acidic, and glucose-low state, which is unsuitable for the survival of large numbers of normal cells. In particular, tumor cells prefer aerobic glycolysis for glucose metabolism when compared with normal cells, even under oxygen-rich conditions, which is called the Warburg effect [37]. Glycolysis is also promoted by high expression levels of hypoxia-inducible factor (HIF) induced in hypoxia, and the final metabolic product lactic acid, generated in this process, is exported through monocarboxylate transporter (MCT) on the cell membrane to the TME together with the carbonic acid formed during oxidative phosphorylation, contributing to the formation of low pH environments [38–40]. In addition, de novo synthesis of fatty acids in cancer cells is elevated, and the limited availability of free fatty acids can control the proliferation of these cells [41–42]. Glutamine metabolism is also enhanced [43], but the role of the metabolism of other amino acid vary among tumors owing to their vast heterogeneity [44–46]. Together, these metabolic changes occurring in the TME are mainly mediated by genetic variations, epigenetic modifications, and metabolic reprogramming in tumor cells, facilitating their proliferation, metastasis, and invasion. Notably, these metabolic changes also force chain changes in other cells infiltrating the TME, subsequently causing the re-construction of immune responses favoring cancers, strengthening the activities of cells inhibiting immunity, such as Treg and Macrophage 2.

Less glucose, more stability

Unlike Teff cells, which show significant dependence on glucose for survival, proliferation, differentiation, and function, Treg cells exhibit striking plasticity and acclimation capacity when confronted with a glucose switch [47], that is, uptake glucose more efficiently than Teff cells when needed [48] and remain functionally and phenotypically stable in glucose-restricted environments. Glucose is used for energy production in Tregs via oxidative phosphorylation (OXPHOS) and glycolysis, and its availability in the specific resident environment directly determines the dominant metabolic pathway [49]. Treg cells exhibit significantly higher levels of glycolysis than Teff cells in the glucose-poor TME [50], thus inducing cellular senescence and suppression of responder T cells through crosstalk, which can be reversed by activating TLR8 signaling-mediated reprogramming of glucose metabolism [51]. Compelling evidence has shown that glycolysis and OXPHOS directly and indirectly control Treg activity through transcriptional and epigenetic modulations and involvement in various critical intracellular signaling pathways that determine the fate of tumor-infiltrating immune cells [52–57]. Glycolytic activity in Tregs varies considerably among resident tissues, Treg populations, and disease models depending on the expression of upstream enzymes (e.g., phosphofructokinase-1, glycogen phosphorylase inhibitor) and glucose transporters [e.g., GLUT1(glucose transporter 1) and GLUT3 (glucose transporter 3)][51, 55, 58–60]. The migratory capacity to secondary lymphoid organs and proliferating activities both rely on mTORC2-mediated upregulation of glycolysis [61–62], favoring Treg survival in a hostile environment. However, when a certain concentration is exceeded, glucose impairs the suppressive function and stability of Tregs [63]. A recent study illustrated a direct relationship between tumor glycolysis and intratumoral Treg cell stability by comparing their phenotypes and functions in high and low glycolytic TME and concluded that Tregs with greater availability of glucose were less stable [64]. The deprivation of glucose through the deficiency of GLUT1 mostly mediates glucose uptake in Tregs. While, it favors iTreg cell differentiation from Tconv cells by driving Foxp3 expression [65]. In addition, it retains stronger suppression function ex vivo in glycolysis-defective tumors compared to control Treg cells [64]. Despite conflicting results on Glut1 expression probably resulting from heterogeneous tissues and Treg populations, this glucose transporter is deleterious to the stability and suppressive capacity of Tregs, particularly in the TME [55, 60, 65–66]. Notably, glucose uptake through Glut1 indispensably guarantees the expansion and proliferation of Tregs [64], suggesting that this metabolite exerts different effects and is important at different stages of Treg growth and development.

How does glucose drive the regulation of Treg function and homeostasis?

Characterized as a suppressive marker of Tregs, Foxp3 acts as a direct functioning target and a critical switch point mediating the interaction between epigenetic modifications and metabolic modulations. Foxp3 suppresses c-Myc expression and glycolysis, enhances oxidative phosphorylation, and increases nicotinamide adenine dinucleotide oxidation [67], whereas the deficiency of this transcription factor results in augmented aerobic glycolysis and oxidative phosphorylation [52]. However, these Treg cells could restore their suppressive function caused by Foxp3 deficiency through the inhibition of rapamycin-insensitive companion of mTOR (RICTOR) or mTORC2 depletion [52]. Mammalian target of rapamycin (mTOR), consisting of complexes I (mTORC1) and II (mTORC2), coordinates the transcriptional programs and cellular metabolism of Tregs, thereby integrating

metabolite availability and growth factor signaling. The discrepancy between the *in vitro* anergic state of Treg cells and their proliferative capability *in vivo* partly lies in activating mTOR signaling *in vivo* [68]. Specifically, PTEN deficiency upregulates mTORC2 signaling, thus increasing glycolytic activities and reducing the stability and suppressive functions of Tregs [69]. Similarly, PI3K-Akt-mTORC1 signaling induced by Toll-like receptor (TLR) signals also increased glycolysis and impaired the suppressive capacity of Tregs and is opposed by Foxp3/Foxo1 to diminish glycolysis and anabolic metabolism [28, 50, 55]. Interestingly, the elevation of PI3K mediated mTOR signaling has minimal effects on tTregs but dramatically increases glycolysis and reduces the expression of Foxp3 in normally oxidative iTregs [58], accompanied by di- and trimethylation of lysine 4 of histone H3 (H3K4me2 and -3) near the Foxp3 transcription start site and within the 5` untranslated region [33]. In agreement with this, mTOR signaling was also reported to be a crucial downstream signal upon TCR activation for regulating T cell de novo differentiation into Tregs, associated with decreased signal transducer and activator of transcription factor activation and failure to upregulate lineage-specific transcription factors [70].

Instead of glycolysis, OXPHOS appears to be the key fuel source for driving the functions of Tregs and is directly regulated by Foxp3 [71]. In addition, Treg suppressive function was reported to be impaired through Treg-specific deletion of mitochondrial complex III in adult mice, with no impact on the expression levels of Foxp3 or the number of FoxP3+ Tregs [72]. In contrast, blocking OXPHOS with oligomycin reduces FOXP3 expression and IL-10 production in Treg cells [64], in agreement with previous findings [66, 72], whereas forcing glycolysis in Treg cells makes them more susceptible to loss of stability. Dysfunctional Tregs in mice lacking the metabolic sensor Lkb1 led to disrupted mitochondrial metabolism and subsequent lethal autoimmunity, similarly underlying the essential role of OXPHOS in their suppressive function [73–74]. The genetic ablation of Tfam (mitochondrial transcription factor A, essential for mitochondrial respiration and mitochondrial DNA replication, transcription, and packaging) in Tregs impairs Treg maintenance in non-lymphoid tissues and in tumors by enhancing methylation in the Treg cell specific demethylation region of the Foxp3 locus [75], revealing potential interactions between Foxp3 and OXPHOS. Another linking molecule, the nuclear factor of activated T cells (NFAT), binds to the non-coding sequence 2 (CNS2) of the enhancer upstream of the Foxp3 gene and promotes its expression [76]; it is activated by metabolic reactive oxygen species (mROS) produced during OXPHOS [77].

The role of fatty acid oxidation (FAO)

FAO (producing acetyl-CoA, which enters the mitochondrial tricarboxylic acid (TCA) cycle) and glycolysis both provide substrates for the OXPHOS process to maintain suppressive functions of Tregs [60]. Under inflammatory conditions, regulatory T cells preferably rely on exogenous fatty acids (primarily oleic acid) rather than fatty acids endogenously generated through acetyl-CoA carboxylase 1 (ACC1)-mediated de novo fatty acid synthesis for FAO [78–79]. In the TME, although the relative abundance of free fatty acids across various cancers is still debated, men could confirm that the high levels of free fatty acid in some cancers (e.g., in RHOA mutated gastric cancer) confer Tregs surviving and proliferative advantages towards Teffs [80], inferring the vital roles of exogenous fatty acids for Tregs and the existing lipid metabolic reprogramming. However, endogenous fatty acids are also indispensable for the proliferation, stability, and specialization of intratumoral Tregs [60, 81–82]. Intratumoral Tregs upregulate the expression of fatty acid transporters CD36 and SLC27A1 and activate PPAR-β pathways to increase lipid uptake and decrease glucose oxidation, orchestrating the survival and suppressive functions of intratumoral T cells [81, 83]. In the colorectal cancer (CRC) TME, accumulation of OX40+ Tregs in the visceral adipose tissue (VAT) of obese CRC patients suggested that these Tregs might migrate from their 'transit stations' full of polyunsaturated fatty acids into the TME via chemotaxis [84]. Mechanistically, lipid uptake and oxidation affect the homeostasis and function of Tregs through direct and indirect pathways [85-88]. The pharmacological inhibition of FAO with etomoxir targeting carnitine palmitoyltransferase 1a (CPT1a) and using an shRNA approach to reduce CPT1a levels in Tregs both resulted in the abrogation of FoxP3 expression (the determining factor of Treg homeostasis and function) and promoted differentiation and proliferation of Tregs rather than Teffs [85]. The elevated levels of Foxp3 in turn upregulated components of all the electron transport complexes and enhanced ATP generation through FAO-fueled OXPHOS metabolism, forming a positive feedback loop between FAO and Treg-suppressive function [71, 79]. Additional evidence supporting the indispensable roles of FAO in Tregs indicates that dysregulated mitochondrial metabolism is responsible for the impaired suppressive function of Tregs after inhibition of lipid uptake by targeting FABP5, the key regulator of lipid uptake and intracellular trafficking [88]. It is interesting to note that FABP-mediated lipid metabolism in plasmacytoid DCs (pDCs) also supports the appropriate generation of regulatory T cells (Tregs) in the TME [89]. Moreover, AMPK signaling inhibition was reported to facilitate fatty acid entry into the mitochondria, thus promoting OXPHOS and enforcing Treg function and proliferation

[56], in agreement with previous observations that iTreg cells have high levels of activated AMPK and FAO [66]. In contrast, Tregs also rely on the activation of transcription factor SREBP-dependent de novo lipid biosynthesis to facilitate subsequent TCR-induced maturation and maintain the expression of PD-1 [82,89]. Notably, short-chain rather than long-chain fatty acids could help stabilize the expression of Foxp3 [87], strengthen mitochondrial oxidative capabilities [90], promote differentiation of Tregs [91], and limit anti-CTLA4 therapies [92], probably by downregulating the expression of histone deacetylases, such as HDAC6 and HDAC9 [87], which were reported to destabilize Foxp3 protein [93]. However, one must concede that the precise mechanisms by which FAO reprograms the functions and homeostasis of Tregs still require further investigation.

Amino acids

Amino acids are used as substrates in multiple metabolic pathways, and some of them or their derivatives, including glutamic acid, tryptophan, kynurenine, arginine, and isoleucine, have been found to play decisive roles in the differentiation, proliferation, and stabilization of Tregs [94–98]. Glutamine not only fuels the TCA cycle to provide essential energy for cancer growth [43] but also facilitates maintenance of the intracellular glutathione (a tripeptide of cysteine, glutamate, and glycine) pool through cysteine-glutamate transport, thus preventing reactive oxygen species (ROS) accumulation and counteracting oxidative stress [99]. Thus, intratumoral Tregs have given up competence for this metabolite and adapted to survive and function in glutamine-low microenvironment, deprivation of glutamine in the media, or addition of the glutaminase inhibitor 6-diazo-5-oxo-l-norleucine (DON) oppositely increased Foxp3 expression and contributed to the suppression of these T cells [100–101]. Notably, as the tumor cells consumed glutamine and exported glutamate in exchange for cystine, the resulting high-glutamate microenvironment also favors the accumulation and function of Tregs, and VEGF blockade could further increase glutamate levels, revealing potential mechanisms contributing to failures of VEGF blockade therapies in glioblastoma [102].

Indoleamine 2,3-dioxygenase (IDO), an enzyme that catabolizes the amino acid tryptophan and mediates its conversion to kynurenine, has also been shown to be positively correlated with the differentiation and proliferation of Tregs [103–104]. Resting $CD4^+CD25+Foxp3^+$ Tregs can be directly activated to exert potent suppressor activity by a small population of pDCs expressing IDO [105]. Various types of tumor cells also highly express IDO, thus elevating the concentration of kynurenine in the TME [106–107], which has been reported to drive the generation of Tregs and tolerogenic myeloid cells by interacting with the ligand-activated transcription factor aryl hydrocarbon receptor (AHR) [94]. Blockade of AHR signaling could reverse Treg–macrophage interplay-dependent immunosuppression and delay the progression of IDO-overexpressing tumors with (better efficacy) or without the combination of PD-1 therapies [94]. Moreover, IDO also functions in Tregs by inhibiting the mTOR/Akt axis and upregulating FoxO3 upon activation, partly accompanied by upregulation of PD-1 and subsequent activation of PTEN, which finally blocks phosphorylation of Akt on its other activating site in a so-called positive-feedback loop to favor Treg differentiation/function/stability [108]. Other amino acids, such as isoleucine [98], leucine [109], and arginine [109], were all shown to be essential for sustaining Treg proliferation; the latter two amino acids probably act as key inducers activating mTORC1 signaling in Tregs to license Treg function [109].

Significantly, multiple solute carrier (SLC) molecules mediating amino acid transport across the plasma membrane have been identified to directly control Treg activity and are involved in the regulation of immune responses in the tumor microenvironment [110]. SlC3A2-deficient Treg cells show impaired branched-chain amino acid (BCAA)-induced activation of the mTORC1 pathway and an altered metabolic state, followed by decreased replication *in vivo* and low cell density [98]. Knockdown or pharmacological inhibition of the cystine/glutamate antiporter SLC7A11 proved to be deleterious to proliferation and function rather than the viability of TCR-stimulated human Tregs through the leptin-mTOR axis [111]. However, there is also evidence that the deletion of amino acid transporters ASCT2 and SLC7A5, transporters of glutamine and leucine, has no impact on Treg differentiation [112–113].

Impacts of other metabolic factors on Tregs in the TME

Hypoxia

It has been earlier observed that the suppressing capability and homeostasis of nTregs were enhanced under simulated hypoxic conditions [114]. The elevated expression of the intratumoral chemokine CCL-28 in the hypoxic TME facilitated the recruitment of Tregs [115]. In response to hypoxic microenvironments, hypoxia-inducible factor 1α (HIF-1α) and HIF-2α binding to hypoxia response elements (HREs) is increased [116], acting as the central node to coordinate the hypoxic regulation of Treg activity. HIF-1α was found to promote glycolytic-driven migration of Tregs by directing glucose away from mitochondria, leaving Tregs dependent on fatty acids for mitochondrial metabolism [83]. In contrast, crosstalk can be found between HIF-2α and HIF-1α. HIF-2α represses HIF-1α expression. HIF-1α is upregulated in HIF-2α-KO (Knockout) Treg cells [116]. Foxp3-conditional KO of

HIF-2α in mice led to restricted growth of MC38 colon adenocarcinoma and metastases of B16F10 melanoma, revealing its distinct effects on strengthening Treg-mediated suppression of antitumor immune responses [116]. Interestingly, the roles of HIF-1α in regulating the suppression and homeostasis of Tregs are diametrically opposite in the TME compared to those in inflammatory environments [117–118]. Depletion of HIF-1α in Treg cells inhibits downstream glucose transport and glycolysis and restores Foxp3 stability *in vivo* or *ex vivo*, thus increasing mitochondrial metabolism, such as oxidative phosphorylation, and enhancing their suppressive capacity [38, 83, 117, 119]. However, it remains doubtful whether Tregs that restore high consumption of oxygen in hypoxic environments could adapt to such drastic energetic switches and sustain prolonged survival. HIF-1α KO mice bearing GL-261 brain tumors showed increased survival compared to WT mice [61, 83], suggesting a delicate balance between survival and function in plastic Tregs.

Acidity

The major source of intra-tumoral acidification arises from the large amounts of lactate produced by tumor cells through anaerobic, oxygen-limited metabolism pathways. Typically, lactate has long been described as a waste metabolite that directly enhances tumor cell motility and assists tumor immune escape by inducing apoptosis of naïve T cells and ultimately inhibiting antitumor immunity [120]. However, recent studies have revealed the significant role of lactic acid as a primary circulating TCA substrate in tumors and other cells [121–122]. Conventional Teff cells sustain glycolytic flux by converting pyruvate into lactate and utilizing the consequently generated NAD nicotinamide adenine dinucleotide. Tregs, as previously mentioned, nevertheless, prefer to maintain the NADH/NAD+ ratio by oxidizing exogenous lactate and endogenous pyruvate in the mitochondria instead of converting pyruvate to lactic acid because they do not rely on high-rate glycolysis to fuel cellular activities [67]. Therefore, Tconv cells, rather than Tregs, struggle to proliferate and function in the presence of extracellular lactic acid or sodium lactate because they are unable to excrete the lactate produced due to loss of gradient [67]. Although Tregs display the ability to uptake exogenous lactic acid, the results *in vitro* by depleting MCT1-a, Although Tregs display the ability to uptake exogenous lactic acid, the results from an in vitro study indicated that the MCT1-a (Monocarboxylate transporter1-a) may not be indispensable for Treg function [63]. Combined with previous observations that lactate drove naive T cell polarization and increased the frequencies of iTregs in the TME in a TGF-β-dependent manner [123] but did not affect Treg function and stability *in vitro* after manual addition of lactate [64], and that lactate enhanced tryptophan metabolism and kynurenine production by pDCs [96], we hypothesize that lactate indirectly helps maintain the relative advantages of Tregs by suppressing Tconv cells and stimulating Treg-associated cells and pathways. Moreover, lactic acid also contributes to tumor growth by inducing the expression of vascular endothelial growth factor and M2-like polarization of tumor-associated macrophages in a HIF-1α dependent way [124]. It would be interesting to investigate the deeper links between lactate, Tregs, and other immune cells.

Concluding remarks

Despite the plethora of metabolic threats, such as nutrient restriction, acidity, and oxidative stress, imposed by the TME on infiltrating immune cells, Tregs display strikingly adaptive competencies to survive, proliferate, and function. In this review, we provide a comprehensive understanding of how Tregs deal with these metabolic stresses based on recent publications. As described above, Treg cells show broad heterogeneity in glucose and lipid metabolism within various contextual features, which may not be limited to a certain pathway. For example, conflicting results have been observed regarding the impact of glycolysis on the proliferation and suppressive function of Treg cells [52–57]. Further research utilizing frontier technologies is needed to investigate the epigenetic and molecular signaling mechanisms driven by metabolic reprogramming, which have been demonstrated to affect the differentiation of Tregs and their suppressive and migratory properties. Considering the wide and profound involvement of metabolic reprogramming in TME, targeting metabolism is viewed as a promising therapeutic approach for cancer therapy. One can envision that these approaches may synergize with current effective anti-tumor strategies.

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References

1. Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory t cells. Immunity. 2013;38(3):414-423.
2. Sakaguchi S, Sakaguchi N, Asano M, et al. Immunologic self-tolerance maintained by activated t cells expressing il-2 receptor alpha-chains (cd25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995; 155(3):1151-1164.
3. Hori S, Nomura T, Sakaguchi S. Control of regulatory t cell development by the transcription factor foxp3. Science. 2003; 299(5609):1057-1061.
4. Zhou X, Bailey-Bucktrout SL, Jeker LT, et al. Instability of the transcription factor foxp3 leads to the generation of pathogenic memory t cells in vivo. Nat Immunol. 2009;10(9):1000-1007.
5. Komatsu N, Okamoto K, Sawa S, et al. Pathogenic conversion of foxp3+ t cells into th17 cells in autoimmune arthritis. Nat Med. 2014; 20(1):62-68.
6. Lucca LE, Dominguez-Villar M. Modulation of regulatory t cell function and stability by co-inhibitory receptors. Nat Rev Immunol. 2020;20(11):680-693.
7. Yang XO, Nurieva R, Martinez GJ, et al. Molecular antagonism and plasticity of regulatory and inflammatory t cell programs. Immunity. 2008;29(1):44-56.
8. Togashi Y, Shitara K, Nishikawa H. Regulatory t cells in cancer immunosuppression–implications for anticancer therapy. Nat Rev Clin Oncol. 2019;16(6):356-371.
9. Saito T, Nishikawa H, Wada H, et al. Two foxp3(+)cd4(+) t cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med. 2016;22(6):679-684.
10. Echarti A, Hecht M, Büttner-Herold M, et al. Cd8+ and regulatory t cells differentiate tumor immune phenotypes and predict survival in locally advanced head and neck cancer. Cancers (Basel). 2019; 11(9):1398.
11. Wang L, Simons DL, Lu X, et al. Connecting blood and intratumoral t cell activity in predicting future relapse in breast cancer. Nat Immunol. 2019;20(9):1220-1230.
12. Bonnal RJP, Rossetti G, Lugli E, et al. Clonally expanded eomes tr1-like cells in primary and metastatic tumors are associated with disease progression. Nat Immunol. 2021;22(6):735-745.
13. Kumar P, Bhattacharya P, Prabhakar BS. A comprehensive review on the role of co-signaling receptors and treg homeostasis in autoimmunity and tumor immunity. J Autoimmun. 2018; 95: 77-99.
14. Pedroza-Gonzalez A, Verhoef C, Ijzermans JNM, et al. Activated tumor-infiltrating cd4+ regulatory t cells restrain antitumor immunity in patients with primary or metastatic liver cancer. Hepatology. 2013; 57(1):183-194.
15. Bader JE, Voss K, Rathmell JC. Targeting metabolism to improve the tumor microenvironment for cancer immunotherapy. Mol Cell. 2020; 78(6):1019-1033.
16. Shevach EM, Thornton AM. Ttregs, ptregs, and itregs: Similarities and differences. Immunol Rev. 2014;259(1):88-102.
17. Coombes JL, Siddiqui KRR, Arancibia-Cárcamo CV, et al. A functionally specialized population of mucosal cd103+ dcs induces foxp3+ regulatory t cells via a tgf-beta and retinoic acid-dependent mechanism. J Exp Med. 2007;204(8):1757-1764.
18. Nakagawa H, Sido JM, Reyes EE, et al. Instability of helios-deficient tregs is associated with conversion to a t-effector phenotype and enhanced antitumor immunity. Proc Natl Acad Sci USA. 2016; 113(22):6248-6253.
19. Schreiber TH, Wolf D, Bodero M, et al. Tumor antigen specific itreg accumulate in the tumor microenvironment and suppress therapeutic vaccination. Oncoimmunology. 2012;1(5):642-648.
20. Xydia M, Rahbari R, Ruggiero E, et al. Common clonal origin of conventional t cells and induced regulatory t cells in breast cancer patients. Nat Commun. 2021;12(1):1119.
21. Plitas G, Konopacki C, Wu K, et al. Regulatory t cells exhibit distinct features in human breast cancer. Immunity. 2016;45(5):1122-1134.
22. Núñez NG, Tosello Boari J, Ramos RN, et al. Tumor invasion in draining lymph nodes is associated with treg accumulation in breast cancer patients. Nat Commun. 2020;11(1):3272.
23. Oh DY, Kwek SS, Raju SS, et al. Intratumoral cd4 t cells mediate anti-tumor cytotoxicity in human bladder cancer. Cell. 2020;181(7):1612-1625.
24. Tanaka A, Sakaguchi S. Regulatory t cells in cancer immunotherapy. Cell Res. 2017;27(1):109-118.
25. Maj T, Wang W, Crespo J, et al. Oxidative stress controls regulatory t cell apoptosis and suppressor activity and pd-l1-blockade resistance in tumor. Nat Immunol. 2017;18(12):1332–1341.
26. Fan MY, Turka LA. Immunometabolism and pi(3)k signaling as a link between il-2, foxp3 expression, and suppressor function in regulatory t cells. Front Immunol. 2018; 29(9):69.
27. Hedrick SM, Hess Michelini R, Doedens AL, et al. Foxo transcription factors throughout t cell biology. Nat Rev Immunol. 2012;12(9):649-661.
28. Ouyang W, Liao W, Luo CT, et al. Novel foxo1-dependent transcriptional programs control t(reg) cell function. Nature. 2012; 491(7425):554-559.
29. Ouyang W, Beckett O, Ma Q, et al. Foxo proteins cooperatively control the differentiation of foxp3+ regulatory t cells. Nat Immunol. 2010;11(7):618-627.
30. Kerdiles YM, Stone EL, Beisner DR, et al. Foxo transcription factors control regulatory t cell development and function. Immunity. 2010; 33(6):890-904.
31. Delgoffe GM, Woo S-R, Turnis ME, et al. Stability and function of regulatory t cells is maintained by a neuropilin-1-semaphorin-4a axis. Nature. 2013;501(7466):252-256.
32. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. Front Oncol. 2014;4:64.
33. Sauer S, Bruno L, Hertweck A, et al. T cell receptor signaling controls foxp3 expression via pi3k, akt, and mtor. Proc Natl Acad Sci USA. 2008;105(22):7797-7802.
34. Walsh PT, Buckler JL, Zhang J, et al. Pten inhibits il-2 receptor-mediated expansion of cd4+ cd25+ tregs. J Clin Invest. 2006;116(9): 2521-2531.
35. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;144(5):646-674.
36. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metab. 2016;23(1):27-47.
37. Liberti MV, Locasale JW. The warburg effect: How does it benefit cancer cells? Trends Biochem Sci. 2016;41(3):211-218.
38. Nagao A, Kobayashi M, Koyasu S, et al. Hif-1-dependent reprogramming of glucose metabolic pathway of cancer cells and its therapeutic significance. Int J Mol Sci. 2019;20(2):238.
39. Baltazar F, Pinheiro C, Morais-Santos F, et al. Monocarboxylate transporters as targets and mediators in cancer therapy response. Histol Histopathol. 2014;29(12):1511-1524.
40. Huber V, Camisaschi C, Berzi A, et al. Cancer acidity: An ultimate frontier of tumor immune escape and a novel target of immunomodulation. Semin Cancer Biol. 2017;43:74-89.

41. Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. Nature. 2012;491(7424):364-373.
42. Currie E, Schulze A, Zechner R, et al. Cellular fatty acid metabolism and cancer. Cell Metab. 2013;18(2):153-161.
43. Son J, Lyssiotis CA, Ying H, et al. Glutamine supports pancreatic cancer growth through a kras-regulated metabolic pathway. Nature. 2013;496(7443):101-105.
44. Sivanand S, Vander Heiden MG. Emerging roles for branched-chain amino acid metabolism in cancer. Cancer Cell. 2020;37(2):147-156.
45. Li Z, Zhang H. Reprogramming of glucose, fatty acid and amino acid metabolism for cancer progression. Cell Mol Life Sci. 2016;73(2): 377-392.
46. Cha YJ, Kim ES, Koo JS. Amino acid transporters and glutamine metabolism in breast cancer. Int J Mol Sci. 2018;19(3):907.
47. Johnson MO, Siska PJ, Contreras DC, *et al*. Nutrients and the microenvironment to feed a t cell army. Semin Immunol. 2016;28(5): 505-513.
48. Ho PC, Bihuniak JD, Macintyre AN, et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor t cell responses. Cell. 2015; 162(6):1217-1228.
49. Sasidharan Nair V, Saleh R, Toor SM, et al. Metabolic reprogramming of t regulatory cells in the hypoxic tumor microenvironment. Cancer Immunol Immunother. 2021;70(8):2103-2121.
50. Xu R, Wu M, Liu S, et al. Glucose metabolism characteristics and tlr8-mediated metabolic control of cd4 treg cells in ovarian cancer cells microenvironment. Cell Death Dis. 2021;12(1):22.
51. Li L, Liu X, Sanders KL, et al. Tlr8-mediated metabolic control of human treg function: A mechanistic target for cancer immunotherapy. Cell Metab. 2019; 29(1):103-123.
52. Charbonnier LM, Cui Y, Stephen-Victor E, et al. Functional reprogramming of regulatory t cells in the absence of foxp3. Nat Immunol. 2019; 20(9):1208-1219.
53. Zeng H, Chi H. Mtor signaling in the differentiation and function of regulatory and effector t cells. Curr Opin Immunol. 2017;46:103-111.
54. de Kivit S, Mensink M, Hoekstra AT, et al. Stable human regulatory t cells switch to glycolysis following tnf receptor 2 costimulation. Nat Metab. 2020;2(10):1046-1061.
55. Gerriets VA, Kishton RJ, Johnson MO, et al. Foxp3 and toll-like receptor signaling balance t cell anabolic metabolism for suppression. Nat Immunol. 2016;17(12):1459-1466.
56. Herzig S, Shaw RJ. Ampk: Guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol, 2018;19(2):121-135.
57. Chou WC, Guo Z, Guo H, et al. Aim2 in regulatory t cells restrains autoimmune diseases. Nature. 2021;591(7849):300-305.
58. Priyadharshini B, Loschi M, Newton RH, et al. Cutting edge: Tgf-β and phosphatidylinositol 3-kinase signals modulate distinct metabolism of regulatory t cell subsets. J Immunol. 2018;201(8):2215-2219.
59. Bruzzaniti S, Bocchino M, Santopaolo M, et al. An immunometabolic pathomechanism for chronic obstructive pulmonary disease. Proc Natl Acad Sci USA. 2019;116(31):15625-15634.
60. Pacella I, Procaccini C, Focaccetti C, et al. Fatty acid metabolism complements glycolysis in the selective regulatory t cell expansion during tumor growth. Proc Natl Acad Sci USA. 2018;15(28): E6546-E6555.
61. Kishore M, Cheung KCP, Fu H, et al. Regulatory t cell migration is dependent on glucokinase-mediated glycolysis. Immunity. 2018 17;48(4):831-832
62. Zeng H, Yang K, Cloer C, et al. Mtorc1 couples immune signals and metabolic programming to establish t(reg)-cell function. Nature. 2013;499(7459):485-490.
63. Watson MJ, Vignali PDA, Mullett SJ, et al. Metabolic support of tumour-infiltrating regulatory t cells by lactic acid. Nature. 2021; 591(7851):645-651.
64. Zappasodi R, Serganova I, Cohen IJ, et al. Ctla-4 blockade drives loss of t stability in glycolysis-low tumours. Nature. 2021;591(7851): 652-658.
65. Macintyre AN, Gerriets VA, Nichols AG, et al. The glucose transporter glut1 is selectively essential for cd4 t cell activation and effector function. Cell Metab. 2014;20(1):61-72.
66. Michalek RD, Gerriets VA, Jacobs SR, 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(6): 3299-3303.
67. Angelin A, Gil-de-Gómez L, Dahiya S, et al. Foxp3 reprograms t cell metabolism to function in low-glucose, high-lactate environments. Cell Metab. 2017;25(6):1282-1293.
68. Procaccini C, De Rosa V, Galgani M, et al. An oscillatory switch in mtor kinase activity sets regulatory t cell responsiveness. Immunity. 2010;33(6):929-941.
69. Shrestha S, Yang K, Guy C, et al. Treg cells require the phosphatase pten to restrain th1 and tfh cell responses. Nat Immunol. 2015;16(2): 178-187.
70. Delgoffe GM, Kole TP, Zheng Y, et al. The mtor kinase differentially regulates effector and regulatory t cell lineage commitment. Immunity. 2009;30(6):832-844.
71. Howie D, Cobbold SP, Adams E, et al. Foxp3 drives oxidative phosphorylation and protection from lipotoxicity. JCI Insight. 2017; 2(3):e89160.
72. Weinberg SE, Singer BD, Steinert EM, et al. Mitochondrial complex iii is essential for suppressive function of regulatory t cells. Nature. 2019;565(7740):495-499.
73. Yang K, Blanco DB, Neale G, et al. Homeostatic control of metabolic and functional fitness of t cells by lkb1 signalling. Nature. 2017; 548(7669):602–606.
74. He N, Fan W, Henriquez B, et al. Metabolic control of regulatory t cell (treg) survival and function by lkb1. Proc Natl Acad Sci USA. 2017; 114(47):12542-12547.
75. Fu Z, Ye J, Dean JW, et al. Requirement of mitochondrial transcription factor a in tissue-resident regulatory t cell maintenance and function. Cell Rep. 2019;28(1):159-171.
76. Li X, Liang Y, LeBlanc M, et al. Function of a foxp3 cis-element in protecting regulatory t cell identity. Cell. 2014;158(4):734-748.
77. Sena LA, Li S, Jairaman A, et al. Mitochondria are required for antigen-specific t cell activation through reactive oxygen species signaling. Immunity. 2013; 38(2):225-236.
78. Berod L, Friedrich C, Nandan A, et al. De novo fatty acid synthesis controls the fate between regulatory t and t helper 17 cells. Nat Med. 2014;20(11):1327-1333.
79. Pompura SL, Wagner A, Kitz A, et al. Oleic acid restores suppressive defects in tissue-resident FOXP3 Tregs from patients with multiple sclerosis. J Clin Invest. 2021;131(2):e138519.
80. Kumagai S, Togashi Y, Sakai C, et al. An oncogenic alteration creates a microenvironment that promotes tumor progression by conferring a metabolic advantage to regulatory t cells. Immunity. 2020;53(1):187-203.
81. Wang H, Franco F, Tsui YC, et al. Cd36-mediated metabolic adaptation supports regulatory t cell survival and function in tumors. Nat Immunol. 2020;21(3):298-308.
82. Lim SA, Wei J, Nguyen TM, et al. Lipid signalling enforces functional specialization of t cells in tumours. Nature. 2021;591(7849):306-311.

83. Miska J, Lee Chang C, Rashidi A, et al. Hif-1α is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of tregs in glioblastoma. Cell Rep. 2019; 27(1):226-237
84. Donninelli G, Del Cornò M, Pierdominici M, et al. Distinct blood and visceral adipose tissue regulatory t cell and innate lymphocyte profiles characterize obesity and colorectal cancer. Front Immunol. 2017;9(8);643.
85. O'Connor RS, Guo L, Ghassemi S, et al. The cpt1a inhibitor, etomoxir induces severe oxidative stress at commonly used concentrations. Sci Rep. 2018;8(1):6289.
86. Gualdoni GA, Mayer KA, Göschl L, et al. The amp analog aicar modulates the treg/th17 axis through enhancement of fatty acid oxidation. FASEB J. 2016;30(11):3800-3809.
87. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic treg cell homeostasis. Science. 2013;341(6145):569-573.
88. Field CS, Baixauli F, Kyle RL, et al. Mitochondrial integrity regulated by lipid metabolism is a cell-intrinsic checkpoint for Treg suppressive function. Cell Metab. 2020;31(2):422-437.
89. Liu C, Chikina M, Deshpande R, et al. Treg cells promote the srebp1-dependent metabolic fitness of tumor-promoting macrophages via repression of cd8 t cell-derived interferon-γ. Immunity. 2019;51(2):381-397.
90. Raud B, Roy DG, Divakaruni AS, et al. Etomoxir actions on regulatory and memory t cells are independent of cpt1a-mediated fatty acid oxidation. Cell Metab. 2018;28(3):504-515.
91. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory t cells. Nature. 2013;504(7480):446-450.
92. Coutzac C, Jouniaux JM, Paci A, et al. Systemic short chain fatty acids limit antitumor effect of ctla-4 blockade in hosts with cancer. Nat Commun. 2020;11(1):2168.
93. Beier UH, Akimova T, Liu Y, et al. Histone/protein deacetylases control foxp3 expression and the heat shock response of t-regulatory cells. Curr Opin Immunol. 2011;23(5):670-678.
94. Campesato LF, Budhu S, Tchaicha J, et al. Blockade of the ahr restricts a treg-macrophage suppressive axis induced by l-kynurenine. Nat Commun. 2020;11(1):4011.
95. Kurniawan H, Franchina DG, Guerra L, et al. Glutathione restricts serine metabolism to preserve regulatory t cell function. Cell Metab. 2020;31(5):920-936
96. Raychaudhuri D, Bhattacharya R, Sinha BP, et al. Lactate induces pro-tumor reprogramming in intratumoral plasmacytoid dendritic cells. Front Immunol. 2019;10:1878.
97. Lowe MM, Boothby I, Clancy S, et al. Regulatory t cells use arginase 2 to enhance their metabolic fitness in tissues. JCI Insight. 2019; 4(24):e129756.
98. Ikeda K, Kinoshita M, Kayama H, et al. Slc3a2 mediates branched-chain amino-acid-dependent maintenance of regulatory t cells. Cell Rep. 2017;21(7):1824-1838.
99. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative stress in cancer. Cancer Cell. 2020;38(2):167-197.
100. Klysz D, Tai X, Robert PA, et al. Glutamine-dependent α-ketoglutarate production regulates the balance between t helper 1 cell and regulatory t cell generation. Sci Signal. 2015;8(396):ra97.
101. Metzler B, Gfeller P, Guinet E. Restricting glutamine or glutamine-dependent purine and pyrimidine syntheses promotes human t cells with high foxp3 expression and regulatory properties. J Immunol. 2016;196(9):3618-3630.
102. Long Y, Tao H, Karachi A, et al. Dysregulation of glutamate transport enhances treg function that promotes vegf blockade resistance in glioblastoma. Cancer Res. 2020;80(3):499-509.
103. Sharma MD, Hou DY, Liu Y, et al. Indoleamine 2,3-dioxygenase controls conversion of foxp3+ tregs to th17-like cells in tumor-draining lymph nodes. Blood. 2009;113(24):6102-6111.
104. Baban B, Chandler PR, Sharma MD, et al. Ido activates regulatory t cells and blocks their conversion into th17-like t cells. J Immunol. 2009;183(4):2475-2483.
105. Sharma MD, Baban B, Chandler P, et al. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature tregs via indoleamine 2,3-dioxygenase. J Clin Invest. 2007;117(9):2570-2582.
106. Zhai L, Bell A, Ladomersky E, et al. Immunosuppressive ido in cancer: Mechanisms of action, animal models, and targeting strategies. Front Immunol. 2020;16(11):1185.
107. Munn DH, Mellor AL. Ido in the tumor microenvironment: Inflammation, counter-regulation, and tolerance. Trends Immunol. 2016;37(3):193-207.
108. Munn DH, Sharma MD, Johnson TS, et al. IDO, PTEN-expressing Tregs and control of antigen-presentation in the murine tumor microenvironment. Cancer Immunol Immunother. 2017;66(8):1049-1058.
109. Shi H, Chapman NM, Wen J, et al. Amino acids license kinase mtorc1 activity and treg cell function via small g proteins rag and rheb. Immunity. 2019;51(6):1012-1027.
110. Wang W, Zou W. Amino acids and their transporters in t cell immunity and cancer therapy. Mol Cell. 2020;80(3):384-395.
111. Procaccini C, Garavelli S, Carbone F, et al. Signals of pseudo-starvation unveil the amino acid transporter slc7a11 as key determinant in the control of treg cell proliferative potential. Immunity. 2021;54(7).
112. Nakaya M, Xiao Y, Zhou X, et al. Inflammatory t cell responses rely on amino acid transporter asct2 facilitation of glutamine uptake and mtorc1 kinase activation. Immunity. 2014;40(5):692-705.
113. Sinclair LV, Rolf J, Emslie E, et al. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for t cell differentiation. Nat Immunol. 2013;14(5):500-508.
114. Ben-Shoshan J, Maysel-Auslender S, Mor A, et al. Hypoxia controls cd4+cd25+ regulatory t-cell homeostasis via hypoxia-inducible factor-1alpha. Eur J Immunol. 2008;38(9):2412-2418.
115. Facciabene A, Peng X, Hagemann IS, et al. Tumour hypoxia promotes tolerance and angiogenesis via ccl28 and t(reg) cells. Nature. 2011;475(7355):226-230.
116. Hsu TS, Lin YL, Wang YA, et al. Hif-2α is indispensable for regulatory t cell function. Nat Commun. 2020;11(1):5005.
117. Hsiao HW, Hsu TS, Liu WH, et al. Deltex1 antagonizes hif-1α and sustains the stability of regulatory t cells in vivo. Nat Commun. 2015; 19(6):6353.
118. Clambey ET, McNamee EN, Westrich JA, et al. Hypoxia-inducible factor-1 alpha-dependent induction of foxp3 drives regulatory t-cell abundance and function during inflammatory hypoxia of the mucosa. Proc Natl Acad Sci USA. 2012;109(41):E2784-E2793.
119. Lee JH, Elly C, Park Y, et al. E3 ubiquitin ligase vhl regulates hypoxia-inducible factor-1α to maintain regulatory t cell stability and suppressive capacity. Immunity. 2015;42(6):1062-1074.
120. Xia H, Wang W, Crespo J, et al. Suppression of fip200 and autophagy by tumor-derived lactate promotes naïve t cell apoptosis and affects tumor immunity. Sci Immunol. 2017;2(17):eaan4631.
121. Hui S, Ghergurovich JM, Morscher RJ, et al. Glucose feeds the tca

cycle via circulating lactate. Nature. 2017;551(7678):115-118.
122. Li N, Kang Y, Wang L, Huff S, et al. Alkbh5 regulates anti-pd-1 therapy response by modulating lactate and suppressive immune cell accumulation in tumor microenvironment. Proc Natl Acad Sci U SA. 2020;117(33):20159-20170.
123. Comito G, Iscaro A, Bacci M, et al. Lactate modulates cd4 t-cell polarization and induces an immunosuppressive environment, which sustains prostate carcinoma progression via tlr8/mir21 axis. Oncogene. 2019;38(19):3681-3695.
124. Colegio OR, Chu NQ, Szabo AL, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature. 2014;513(7519):559-563.

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REVIEW ARTICLE

Recent advances in targeted therapy for ovarian cancer

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Abstract

The global burden of ovarian cancer is gradually increasing while patients still suffer from relatively limited treatment options. With recent advances in the decoding of the molecular landscape of ovarian cancer, more options in targeted strategy were offered and can therefore be tailored in different clinical settings for individual patient. Targeting of the abnormal angiogenesis process is the first significant clinical breakthrough which revolutionized the treatment of advanced ovarian cancer, followed by the advent of poly-(ADP)-ribose polymerase (PARP) inhibitors. These two strategies represented by bevacizumab and olaparib respectively underwent tests of numerous clinical trials. In recent years, immune checkpoint inhibitors (ICIs) have been incorporated into the blueprint of ovarian cancer treatment though the effectiveness still left much to be desired. Herein, we systematically outlined recent advances in targeted therapy for ovarian cancer and summarized the landmark clinical trials for each targeted therapy including angiogenesis inhibitors, PARP inhibitors and ICIs.

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Ovarian cancer is one of the female reproductive tract neoplasms with the highest case fatality rate [1]. Global cancer statistics estimated that in 2020 ovarian cancer accounted for 313 959 new cases and 207 252 deaths worldwide [2]. The malignancy onset is generally insidious, with lack of typical symptoms and effective screening methods [3–4]. Therefore, by the time of clinical diagnosis most patients already present with advanced disease, often characterized by extensive dissemination in the pelvis and abdominal cavity, which may develop into malignant ascites, posing significant challenges to surgeons and oncologists. Cytoreductive surgery followed by platinum-based chemotherapy for 6–8 cycles is currently the primary therapeutic strategy for ovarian cancer [5]. Neoadjuvant chemotherapy is an alternative option for patients with bulky stage III or IV disease; however, for poor surgical candidate patients, no gross residual disease (R0) is unlikely to be achieved solely through primary cytoreduction [6]. Ovarian cancer patients have a 70% chance of relapse within 2 years after reaching a clinical complete response (CR) [7], or even multiple regressions accompanied by a gradually shortened platinum-free intervals (PFI), thereby inevitably developing platinum resistance. Due to recent progress in better understanding the biological and molecular features underpinning ovarian cancer, a generation of novel targeted drugs has been developed, gradually shaping a new treatment landscape for ovarian cancer. Among these, anti-angiogenic agents and poly-(ADP)-ribose polymerase (PARP) inhibitors have demonstrated significant potential, both in randomized controlled trials (RCTs) and clinical practice [8]. In addition immunotherapy, despite having modest effects when used as single agent [9], possibly due to the immunosuppressive tumor microenvironment (TME) of ovarian cancers [10], still holds great potential in ovarian cancer research.

Targeting angiogenesis in ovarian cancer

Abnormal angiogenesis is considered to be a hallmark of multiple malignancies [11]. Accumulated evidence has demonstrated that angiogenesis is associated with an

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unfavorable prognosis in patients with ovarian cancer [12]. The formation of new blood vessels facilitates tumor progression, and is stimulated and regulated by a series of growth factors, the most clinically relevant of which is the vascular endothelial growth factor (VEGF) [13]. The angiopoietin axis is another signaling pathway contributing to angiogenesis [14]. Angiopoietin 1 and 2 (Ang 1&2) regulate vascularization and tissue remodeling by interacting with the tyrosine kinase receptor Tie2. Hence, the vascular endothelial growth factor receptor (VEGFR) and angiopoietin pathways are promising anti-angiogenic targets.

Bevacizumab

Bevacizumab was the first humanized recombinant monoclonal IgG antibody developed against angiogenesis, and the first targeted drug approved by the Food and Drug Administration (FDA) for the treatment of ovarian cancer. Bevacizumab targets all known VEGF subtypes, thereby inhibiting VEGFR pathway activation. Two landmark randomized controlled trials (RCTs) have confirmed bevacizumab's efficacy in first-line treatment of ovarian cancer, both when combined with standard chemotherapy and when used as a single-agent for maintenance.

In the Gynecologic Oncology Group (GOG)-218 study, 1873 patients with newly diagnosed stage IV, or stage III cancer who failed to achieve R0 resection, were randomized in three groups: (1) a control group, receiving standard chemotherapy plus placebo (2–22 cycles); (2) a bevacizumab-initiation group, receiving standard chemotherapy plus bevacizumab (2–6 cycles), followed by placebo (7–22 cycles); and (3) a bevacizumab-throughout group, receiving standard chemotherapy plus bevacizumab (2–22 cycles). The median progression-free survival (PFS) for the above groups was 10.3, 11.2, and 14.1 months, respectively. Compared to chemotherapy alone the bevacizumab-throughout group achieved better PFS, although no apparent difference in overall survival (OS) between the two groups was observed [hazard ratio (HR) = 0.717; $P < 0.001$].

A second large, randomized, phase III trial, the International Collaboration on Ovarian Neoplasms (ICON7), enrolled 1528 newly diagnosed patients with either high-risk early (IA–IIA) or advanced (IIB–IV) stage disease. These patients were treated with standard chemotherapy, or chemotherapy in combination with bevacizumab plus bevacizumab maintenance for 12 additional cycles. The addition and maintenance therapy with bevacizumab significantly improved the PFS (HR = 0.81; $P < 0.004$); however, this benefit did not translate into an improvement in OS. Further exploratory analysis revealed that the high-risk subgroup with stage IV disease or inoperable/sub-optimally debulked (> 1 cm) stage III disease benefited the most from concomitant bevacizumab treatment, with a significant improvement in median OS (39.7 *vs.* 30.2 months; $P = 0.03$). It is worth mentioning that when the ICON7 high-risk definition was applied to the GOG-0218 cohort, no benefit in OS was observed in the respective GOG-0218 subgroup. However, it already has been established that the GOG-0218 stage IV subgroup alone did receive a meaningful benefit in OS compare to the control arm [HR = 0.72; 95% confidence interval (CI): 0.53–0.97] [15]. Bevacizumab was generally well-tolerated by patients in both trials, despite specific toxicities (hypertension, gastrointestinal perforation, thrombo embolism, etc.) and a slight reduction in health-related quality of life (HRQoL) [16].

Based on these seminal trials, bevacizumab was approved as first-line treatment in combination with standard chemotherapy, and as maintenance therapy for patients with advanced-stage ovarian cancer (IIIB, IIIC, and IV), by both the European Medicines Agency (EMA) and FDA in 2011 and 2018 respectively. Further, real-world observational studies, including the ROBOT and JGOG3022 trials, have validated its efficacy and safety in a clinical setting [17–18].

In addition, there is substantial clinical evidence that bevacizumab demonstrates efficacy in relapsed ovarian cancer. The OCEANS and GOG-0213 trials recruited platinum-sensitive patients with recurrent disease [19–20]. Both studies demonstrated a significantly prolonged PFS when patients were treated with a combination of chemotherapy and bevacizumab, compared to standard chemotherapy alone. The AURELIA trial was the first to explore the efficacy of bevacizumab in combination with chemotherapy in patients with platinum-resistant recurrent ovarian cancer. In this trial, 361 patients were randomly assigned to two arms: (1) a group receiving single-agent chemotherapy; and (2) a group receiving single-agent chemotherapy plus bevacizumab. The addition of bevacizumab significantly improved PFS (6.7 *vs.* 3.4 months; HR = 0.48; $P < 0.001$) and ORR (11.8% *vs.* 27.3%; $P = 0.001$). The trend in OS, however, was not significant (13.3 *vs.* 16.6 months, HR = 0.85; $P < 0.174$) [21].

Bevacizumab's optimal treatment dosage, timing, and duration, remain to be determined by additional pre-clinical and clinical studies [22]. Moreover, biomarkers associated with bevacizumab response and patient prognosis are currently being investigated, and warrant further validation [23].

Tyrosine kinase inhibitors (TKIs)

Tyrosine kinases play a pivotal role in many biological processes, including angiogenesis, cell proliferation, and cell cycle [24–25]. TKIs prevent kinases from catalyzing the phosphorylation of tyrosine residues on their substrates, thereby blocking the activation of downstream signaling

pathways [25].

Sorafenib

Sorafenib is an oral TKI originally developed as a Raf inhibitor that has since shown affinity for various kinases, including VEGFR2 and VEGFR3 [26]. A multicenter phase II trial investigated the efficacy and tolerability of sorafenib in patients with recurrent or persistent ovarian cancer. The 71 patients received sorafenib 400 mg orally twice per day, revealing that sorafenib yielded modest benefits at the cost of substantial toxicity [27]. The TRIAS study enrolled 174 platinum-resistant ovarian cancer patients, previously treated with two or fewer chemotherapy lines. Patients were randomized on a 1:1 basis to receive: (1) topotecan in combination with sorafenib; or (2) topotecan plus placebo; a significant improvement in both PFS (6.7 *vs.* 4.4 months; HR = 0.60; P = 0.0018) and OS (17.4 *vs.* 10 months; HR = 0.65; P = 0.017) were observed in the sorafenib combination arm [28].

Pazopanib

Pazopanib is a TKI targeting VEGFR, platelet-derived growth factor receptor (PDGFR), c-kit, and c-fms [29]. Pazopanib is poorly tolerated when combined with cytotoxic therapy. Yet, in the AGO-OVAR 16 study, a phase III clinical trial of 940 stage II–IV patients, pazopanib significantly improved PFS when used as first-line maintenance therapy following chemotherapy (17.9 *vs.* 12.3 months; HR = 0.77; P = 0.021); no significant difference in OS was observed, however [30]. MITO 11, another randomized, non-blinded, phase II trial, demonstrated that weekly therapy with pazopanib in combination with paclitaxel significantly prolonged PFS in patients with platinum-resistant or refractory advanced ovarian cancer (6.35 *vs.* 33.49 months; HR = 0.42; P = 0.0002) [31]. The trade-off between pazopanib's modest efficacy and adverse effects warrant further investigation. Of note, the 2019 NCCN guidelines no longer recommended pazopanib as first-line maintenance therapy; however, its use is still recommended in the recurrent ovarian cancer setting.

Nintedanib

Nintedanib is an oral TKI targeting VEGFR, PDGFR, and fibroblast growth factor receptor (FGFR) [32], and its efficacy in the first-line ovarian cancer setting was investigated by the AGO-OVAR12 study. The 1366 postoperative chemotherapy-naive patients with stage IIB–IV ovarian cancer were randomized to receive standard chemotherapy in combination with either nintedanib or placebo, followed by maintenance treatment with each agent. The results indicated that the nintedanib combination approach significantly prolonged PFS from 16.6 to 17.3 months (HR = 0.84; P = 0.0239) [33]. Surprisingly, the low-risk subgroup benefited the most from nintedanib combination, which contradicted findings of trials evaluating other TKIs; therefore, these results warrant further investigation. In the latest results reported by the AGO-OVAR12 trial, PFS improvement appeared consistent, although it did not translate into OS benefit [34].

Cediranib

Cediranib is a highly potent VEGFR inhibitor, exerting similar inhibitory activity to PDGFR and c-kit [35]. The ICON-6 trial investigated the efficacy of cediranib in patients with platinum-sensitive recurrent ovarian cancer. Four hundred fifty-six patients were randomized to receive either of the following: (1) chemotherapy in combination with placebo, followed by placebo maintenance; (2) chemotherapy in combination with cediranib, followed by placebo maintenance; and (3) chemotherapy in combination with cediranib, followed by cediranib maintenance. PFS in the above groups was 8.7, 9.9, and 11 months, respectively. However, increased adverse reactions during cediranib maintenance therapy may reduce patient compliance [36]. Of note, the ICON-6 data released at the 2013 European Society of Medical Oncology (ESMO) annual meeting demonstrating a significant improvement in PFS and OS (20.3% *vs.* 17.6%; HR = 0.70; P = 0.0419), were the first data reporting an OS benefit as a result of combining chemotherapy with anti-angiogenic agents.

Angiopoietin axis inhibitor

Trebananib is a newly developed peptibody that neutralizes both Ang1 and Ang2 through interaction with the Tie2 receptor, thereby inhibiting endothelial sprouting, and decreasing blood vessel density and vascular permeability [37–38].

TRINOVA-3 is a randomized placebo-controlled phase III clinical trial investigating trebananib in combination with single-agent weekly paclitaxel in patients with recurrent ovarian cancer. One thousand fifteen patients were selected and randomized to receive either of the following: (1) 6 cycles of paclitaxel and carboplatin plus weekly trebananib, followed by trebananib maintenance for up to 18 additional months; and (2) 6 cycles of paclitaxel and carboplatin plus weekly trebananib, and placebo maintenance. Unfortunately, no significant benefit in PFS was observed in TRINOVA-3, thereby diminishing the utility of trebananib in first line management of ovarian cancer [39].

The TRINOVA-1 trial assessed the addition of trebananib to single-agent weekly paclitaxel in patients with recurrent ovarian cancer. In this setting, median PFS was significantly prolonged in the trebananib group compared toplacebo (7.2 *vs.* 5.4 months; HR = 0.66; P < 0.0001) [40]. A later study evaluating HRQoL in TRINOVA-1, reported that the improvement in PFS in the trebananib arm did not significantly compromise patients' HRQoL [41]. However, the clinical applications of

trebananib require further investigation.

Poly-(ADP)-ribose polymerase (PARP) inhibitors

The advent of PARP inhibitors has fundamentally transformed the clinical management of patients with ovarian cancer carrying mutationsin the *BRCA1/2* genes. DNA single-strand breaks (SSBs) are common DNA damage events [42]; PARP recognizes and orchestrates the repair of SSBs, thereby maintaining DNA stability [43]. PARP inhibition leads to persistent unresolved SSBs, which during DNA replication give rise to stalled replication forks and subsequent accumulation of double-strand breaks (DSBs). Cells with homologous recombination deficiency (HRD), such as *BRCA1/2*-mutant cells, cannot efficiently repair these DSBs, thus giving rise to the "synthetic lethality" phenotype [44].

Olaparib

Olaparib was the first PARP inhibitor introduced in the clinical setting by the FDA in 2014 and has since transformed the landscape of ovarian cancer treatment [45].

The SOLO-1 trial compared olaparib maintenance treatment to placebo in a front-line setting, among newly diagnosed patients with *BRCA1/2* mutations (388 patients with germline mutations, and 2 with somaticmutations). A total of 391 International Federation of Gynecology and Obstetrics (FIGO) stage III or IV patients who previously achieved complete or partial response to platinum-based chemotherapy were randomly assigned to receive olaparib 300 mg twice a day or placebo tablets as maintenance therapy until disease progression. When the median follow-up duration reached 40.7 months, 60% of the patients in the olaparib arm achieved the primary endpoint of PFS, compared to 27% in the placebo arm (HR = 0.3; $P < 0.001$) [46]. In the latest 5-year follow-up of the SOLO-1 study, the PFS benefit was sustained (56 *vs.* 13.8 months; HR = 0.33) beyond the end of treatment, with an extension of median progression-free survival past 4.5 years [47]. Based on the robust SOLO-1 data, the FDA and EMA have approved olaparib as a new standard of care.

The SOLO-1 trial excluded patients receiving bevacizumab-containing therapy. These patients were explored by the PAOLA-1 study, which included bevacizumab in both treatment arms. Eight hundred and six newly diagnosed ovarian cancer patients who responded to first-line platinum-taxane chemotherapy plus bevacizumab were eligible for inclusion, regardless of surgical outcome and *BRCA* status. At a median follow-up of 22.9 months, the addition of olaparib yielded a 5.5 months PFS benefit (22.1 *vs.* 16.6 months; HR = 0.59; $P < 0.0001$). Subgroup analysis revealed that patients with HRD tumors demonstrated a robust prolonged PFS (37.2 *vs.* 17.7 months; HR = 0.33). The disease progression or death hazard ratio was 0.43 in patients with *BRCA* wild-type and HRD-positive tumors (PFS 28.1 *vs.* 16.6 months) [48]. The results of the PAOLA-1 trial prompted the FDA to approve olaparib in combination with bevacizumab as first-line maintenance for HRD-positive ovarian cancer patients.

Pivotal clinical trials of olaparib in the setting of relapsed ovarian cancer include Study 19, SOLO-2, and SOLO-3. Study 19 is an international randomized phase II trial that enrolled 265 patients with recurrent ovarian cancer and unselected *BRCA* status. Participants were treated with either olaparib or placebo, and the trial met the primary endpoint of PFS (8.4 *vs.* 4.8 months; HR = 0.35; $P < 0.001$). In apre-planned retrospective analysis, the *BRCA* mutation sub-group in the olaparib arm showed substantially improved PFS (11.2 *vs.* 4.3 months; HR = 0.18; $P < 0.0001$). Interestingly, the non-*BRCA* mutation subgroup also obtained a significant benefit in PFS (7.4 *vs.* 5.5 months; HR = 0.54; $P = 0.0075$), albeit less pronounced [49]. As a landmark trial, the encouraging data from Study 19 led to EMA approval of olaparib for maintenance therapy in a recurrent setting, regardless of *BRCA* status. The SOLO-2 trial prospectively evaluated the efficacy of olaparib in patients with *BRCA* mutations. The 295 patients with recurrent disease were randomized to receive olaparib or placebo, and olaparib maintenance was associated with significantly prolonged PFS (19.1 *vs.* 5.5%; HR = 0.30; $P < 0.0001$) [50]. The SOLO-3 study was the first phase III trial evaluating the efficacy and safety of olaparib monotherapy compared to chemotherapy, in patients with germline *BRCA* mutations. The SOLO-3 olaparib arm achieved a significantly higher BICR-assessed ORR compared to the standard chemotherapy arm (72.2% *vs.* 51.4%, OR = 2.53; $P = 0.002$); BICR-assessed PFS also favored the olaparib arm (13.4 *vs.* 9.2 months, HR = 0.62; $P = 0.013$). In a sub-group analysis, patients who had received two prior lines of treatment seemed to benefit the most, with an OR of 3.44 [51].

Niraparib

Niraparib is a potent selective inhibitor of the PARP1/2 nuclear proteins [52]. The efficacy and safety of niraparib were examined in the QUADRA study, a single-arm phase II trial. The 463 patients were enrolled and stratified according to their HRD and germline *BRCA* mutation status tests. The trial met its primary endpoint, resulting in an ORR of 29% among HRD-positive subpopulations, 39% among platinum-sensitive *BRCA*-positive patients, and 27% among platinum-resistant *BRCA*-positive patients. Hematological toxicity was the most common drug-related adverse event, and was effectively managed

by dose modification [53].

The NOVA study explored the role of niraparib maintenance therapy in relapsed, platinum-sensitive ovarian cancer. In this randomized phase III trial, 553 patients with high-grade serous, platinum-sensitive ovarian cancer were categorized based on their *BRCA* mutation status in two cohorts, gBRCA and non-gBRCA. Homologous recombination capacity was tested in 345 non-gBRCA-mutated patients to identify the HRD subpopulation of ovarian cancers. There was a significant improvement in PFS regardless of gBRCA and HRD status, including 21.0 vs. 5.5 months in the gBRCA cohort (HR = 0.27); 12.9 *vs.* 3.8 months in the non-gBRCA HRD cohort (HR = 0.38); and 9.3 vs. 3.9 months in the HR proficient cohort (HR = 0.45) [54].

The satisfactory outcomes of NOVA prompted the investigation of niraparib in a front-line setting. The PRIMA study is a double-blinded, randomized phase III trial investigating the efficacy of niraparib monotherapy as maintenance therapy in newly diagnosed ovarian cancer. Seven hundred thirty-three patients were classified based on their HRD status, and randomized to receive maintenance therapy with either niraparib or placebo. The overall population achieved a modest benefit in median PFS (13.8 *vs.* 8.2 months, HR = 0.62; $P < 0.0001$), which was more pronounced among patients with HRD tumors (21.9 *vs.* 10.4 months; HR = 0.43; $P < 0.001$) [55]. The PRIMA results were concordant with the results obtained from the NOVA trial.

Rucaparib

Rucaparib is another oral, small-molecule inhibitor of PARP1/2/3, approved by the FDA for clinical applications [56]. Its approval was mainly based on two open-label, multicenter, single-arm clinical trials. The ARIEL 2 study, which is comprised of two parts, is a multicenter phase II trial investigating the effectiveness of rucaparib in pretreated ovarian cancer patients. Part 1 of ARIEL 2 included 204 patients previously treated with ≥ 1 line of chemotherapy, while Part 2 included patients previously treated with 3 or 4 lines. The findings of Part 1 indicated that HR-related gene status could determine responders to PARP inhibitors regardless of *BRCA* status. The *BRCA*-mutated subgroup had the longest PFS (12.8 months; HR = 0.27, $P < 0.0001$), followed by the *BRCA* wild-type and loss of heterozygosity (LOH) high subgroup (5.7 months; HR = 0.62, $P = 0.011$); the *BRCA* wild-type and LOH low subgroup had a PFS of 5.2 months [57]. Results of the ARIEL 2 Part 2 are still pending. In the latest analysis of data derived by both parts of ARIEL 2, it was reported that RAD51C and RAD51D status correlated with meaningful clinical activity of rucaparib, similar to that of *BRCA* status in high-grade ovarian cancer [58].

Rucaparib was also evaluated in the Study 10 trial, which consisted of three parts: part 1 established the recommended dose of rucaparib in a dose dependent manner; part 2A enrolled 42 pretreated platinum-sensitive patients with a germline *BRCA* mutation, investigated ORR based on RECIST, and reached an ORR at 60%; part 2B enrolled 40 patients previously treated with 3 or 4 lines of chemotherapy. Part 2B results of Study 10, however, are still pending [59].

The ARIEL 3 study provided further evidence that rucaparib could be used as standard of care for patients with ovarian cancer, in a second- or later-line maintenance setting. This randomized multicenter phase 3 trial recruited 564 patients with platinum-sensitive disease who had previously received ≥ 2 platinum-based chemotherapy regimens. The overall population was divided into the following three nested cohorts for subgroup analysis: (1) patients with *BRCA* mutations; (2) patients with HRD disease; and (3) the intention-to-treat population. The biomarkers established by the ARIEL 2 study were employed in the interpretation of data obtained from ARIEL 3. The median PFS for *BRCA*-mutated patients was 16.6 months in the rucaparib arm, compared to 5.4 months in the placebo arm (HR = 0.23; $P < 0.0001$). For the HRD cohort, median PFS was 13.6 *vs.* 5.4 months (HR = 0.32; $P < 0.0001$). The intention-to-treat cohort reached a PFS of 10.8 months versus 5.4 months (HR = 0.36; $P < 0.0001$). Thus, rucaparib monotherapy in the second- or later-line maintenance achieved significant improvement in PFS across all three sub-groups [60]. The currently ongoing ARIEL 3 trial compares rucaparib with standard chemotherapy for relapsed ovarian cancer patients with *BRCA* mutations who were previously treated with ≥ 2 lines of chemotherapy regimens [61].

Immune checkpoint inhibitors (ICIs)

ICIs have profoundly enriched and revolutionized the treatment landscape of various cancers. By releasing inhibitory brakes present on T cells, ICIs induce a robust antitumor effect by harnessing both the innate and adaptive arms of the human immunesystem [62]. Unfortunately, not all tumor types and patients respond to ICIs, and even patients that initially respond can develop acquired resistance. FDA-approved ICIs can be classified into three categories based on their target: monoclonal antibodies targeting the cytotoxic T lymphocyte-associated protein 4 (CTLA-4), monoclonal antibodies targeting the programmed cell death protein 1 (PD-1), and monoclonal antibodies targeting the programmed cell death one ligand 1 (PD-L1). To date, the efficacy of ICIs as single agents in preclinical studies and clinical trials of ovarian cancer remains poor. This is mainly due to the immunosuppressive microenvironment of ovarian cancer. Nonetheless, conventional chemotherapies can

stimulate anticancer immunity, leading to the possibility that synergistic combinatorial regimens may potentially enhance the effectiveness of ICIs monotherapy [63]. ICIs entering Phase III clinical trials include nivolumab, avelumab, and atezolizumab.

The NINJA study is a multicenter randomized phase III study, investigating the efficacy and safety of nivolumab against chemotherapy in platinum-resistant ovarian cancer patients in Japan. Three hundred sixteen patients were randomly assigned to receive either nivolumab or gemcitabine/pegylated liposomal doxorubicin (PLD). Unfortunately, the study failed its primary endpoint, observing no significant difference between the two arms (HR = 1.0, $P = 0.808$); in fact, median PFS was longer in the gemcitabine/PLDNINJA arm (2.0 *versus* 3.8 months; HR = 1.5; $P = 0.002$) [64].

JAVELIN 200 is a multicenter three-arm randomized phase III trial. A total of 566 platinum-resistant or -refractory patients were randomized to receive (1) avelumab monotherapy; (2) avelumab in combination with PLD; and (3) PLD monotherapy. The median OS for the above three groups was 11.8, 15.7, and 13.1 months, respectively, and the median PFS 1.9, 3.7, and 3.5 months, respectively. Neither avelumab monotherapy nor combination therapy significantly prolonged PFS or OS compared to PLD. This outcome suggests that proper patient selection is necessary in future studies [65].

IMagyn050 is a multicenter placebo-controlled phase III trial, investigating the addition of atezolizumab to standard chemotherapy and bevacizumab as first-line treatment for advanced ovarian cancer. The 1301 patients were randomized to receive atezolizumab combined with standard chemotherapy plus bevacizumab, or placebo with standard chemotherapy plus bevacizumab. The median PFS in the intention-to-treat subpopulation was 19.5 versus 18.4 months respectively (HR = 0.92; stratified log-rank $P = 0.28$). In the PD-L1 positive subpopulation, PFS was 20.8 versus 18.5 months (HR = 0.80; $P = 0.038$). This limited benefit in PFS did not translate into a statistically significant extension in OS [65].

Summary

The molecular targeted therapies introduced in this article are gradually creating a paradigm shift in the clinical management of ovarian cancer. While some have demonstrated great success in both preclinical and clinical settings, others warrant further validation and investigation. Exploring biomarkers that can predict prognosis and response, selecting patient populations more likely to benefit from particular treatments, and designing rational drug combinations and optimal dosages, are of paramount importance and should be a priority. In this review, we underline critical studies on targeted therapies for the treatment of ovarian cancer. We anticipate that ample evidence addressing the aforementioned issues will be reported by currently ongoing and future studies and trials.

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Author contributions

All authors contributed to data acquisition, data interpretation, and reviewed and approved the final version of this manuscript.

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References

1. Cancer stat facts: ovarian cancer. https://seer.cancer.gov/statfacts/html/ovary.html. Accessed 2 Feb 2022.
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249.
3. Lheureux S, Braunstein M, Oza AM. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. CA Cancer J Clin. 2019;69(4):280-304.
4. Menon U, Karpinskyj C, Gentry-Maharaj A. Ovarian cancer prevention and screening. Obstet Gynecol. 2018;131(5):909-927.
5. Bristow RE, Tomacruz RS, Armstrong DK, et al. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. J Clin Oncol. 2002;20(5):1248-1259.
6. Kuroki L, Guntupalli SR. Treatment of epithelial ovarian cancer. BMJ. 2020;371:m3773.
7. Gadducci A, Cosio S, Conte PF, et al. Consolidation and maintenance treatments for patients with advanced epithelial ovarian cancer in complete response after first-line chemotherapy: a review of the literature. Crit Rev Oncol Hematol. 2005;55(2):153-166.
8. Gadducci A, Cosio S. Randomized clinical trials and real world prospective observational studies on bevacizumab, PARP inhibitors, and immune checkpoint inhibitors in the first-line treatment of advanced ovarian carcinoma: A critical review. Anticancer Res. 2021;41(10):4673-4685.
9. Matulonis UA, Shapira-Frommer R, Santin AD, et al. Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study. Ann Oncol. 2019;30(7):1080-1087.
10. Yigit R, Massuger LF, Figdor CG, et al. Ovarian cancer creates a suppressive microenvironment to escape immune elimination.

Gynecol Oncol. 2010;117(2):366-372.
11. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-674.
12. Alvarez AA, Krigman HR, Whitaker RS, et al. The prognostic significance of angiogenesis in epithelial ovarian carcinoma. Clin Cancer Res. 1999;5(3):587-591.
13. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;473(7347):298-307.
14. Gavalas NG, Liontos M, Trachana SP, et al. Angiogenesis-related pathways in the pathogenesis of ovarian cancer. Int J Mol Sci. 2013;14(8):15885-15909.
15. Karam A, Ledermann JA, Kim JW, et al. Fifth Ovarian Cancer Consensus Conference of the Gynecologic Cancer InterGroup: first-line interventions. Ann Oncol. 2017;28(4):711-717.
16. Stark D, Nankivell M, Pujade-Lauraine E, et al. Standard chemotherapy with or without bevacizumab in advanced ovarian cancer: quality-of-life outcomes from the International Collaboration on Ovarian Neoplasms (ICON7) phase 3 randomised trial. Lancet Oncol. 2013;14(3):236-243.
17. Wu PY, Cheng YM, Shen MR, et al. Real-world study of adding bevacizumab to chemotherapy for ovarian, tubal, and peritoneal cancer as front-line or relapse therapy (ROBOT): 8-year experience. Front Oncol. 2020;10:1095.
18. Komiyama S, Kato K, Inokuchi Y, et al. Bevacizumab combined with platinum-taxane chemotherapy as first-line treatment for advanced ovarian cancer: a prospective observational study of safety and efficacy in Japanese patients (JGOG3022 trial). Int J Clin Oncol. 2019;24(1):103-114.
19. Aghajanian C, Blank SV, Goff BA, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol. 2012;30(17):2039-2045.
20. Coleman RL, Brady MF, Herzog TJ, et al. Bevacizumab and paclitaxel-carboplatin chemotherapy and secondary cytoreduction in recurrent, platinum-sensitive ovarian cancer (NRG Oncology/Gynecologic Oncology Group study GOG-0213): a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2017;18(6):779-791.
21. Pujade-Lauraine E, Hilpert F, Weber B, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol. 2014;32(13):1302-1308.
22. Colombo N, Conte PF, Pignata S, et al. Bevacizumab in ovarian cancer: Focus on clinical data and future perspectives. Crit Rev Oncol Hematol. 2016;97:335-348.
23. Haunschild CE, Tewari KS. Bevacizumab use in the frontline, maintenance and recurrent settings for ovarian cancer. Future Oncol. 2020;16(7):225-246.
24. Jiao Q, Bi L, Ren Y, et al. Advances in studies of tyrosine kinase inhibitors and their acquired resistance. Mol Cancer. 2018;17(1):36.
25. Huang L, Jiang S, Shi Y. Tyrosine kinase inhibitors for solid tumors in the past 20 years (2001-2020). J Hematol Oncol. 2020;13(1):143.
26. Escudier B, Worden F, Kudo M. Sorafenib: key lessons from over 10 years of experience. Expert Rev Anticancer Ther. 2019;19(2):177-189.
27. Matei D, Sill MW, Lankes HA, et al. Activity of sorafenib in recurrent ovarian cancer and primary peritoneal carcinomatosis: a gynecologic oncology group trial. J Clin Oncol. 2011;29(1):69-75.
28. Chekerov R, Hilpert F, Mahner S, et al. Sorafenib plus topotecan versus placebo plus topotecan for platinum-resistant ovarian cancer (TRIAS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol. 2018;19(9):1247-1258.
29. McLachlan J, Banerjee S. Pazopanib in ovarian cancer. Expert Rev Anticancer Ther. 2015;15(9):995-1005.
30. du Bois A, Floquet A, Kim JW, et al. Incorporation of pazopanib in maintenance therapy of ovarian cancer. J Clin Oncol. 2014;32(30):3374-3382.
31. Pignata S, Lorusso D, Scambia G, et al. Pazopanib plus weekly paclitaxel versus weekly paclitaxel alone for platinum-resistant or platinum-refractory advanced ovarian cancer (MITO 11): a randomised, open-label, phase 2 trial. Lancet Oncol. 2015;16(5):561-568.
32. Khalique S, Banerjee S. Nintedanib in ovarian cancer. Expert Opin Investig Drugs. 2017;26(9):1073-1081.
33. du Bois A, Kristensen G, Ray-Coquard I, et al. Standard first-line chemotherapy with or without nintedanib for advanced ovarian cancer (AGO-OVAR 12): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. 2016;17(1):78-89.
34. Ray-Coquard I, Cibula D, Mirza MR, et al. Final results from GCIG/ENGOT/AGO-OVAR 12, a randomised placebo-controlled phase III trial of nintedanib combined with chemotherapy for newly diagnosed advanced ovarian cancer. Int J Cancer. 2020;146(2):439-448.
35. Tang W, McCormick A, Li J, et al. Clinical pharmacokinetics and pharmacodynamics of cediranib. Clin Pharmacokinet. 2017;56(7):689-702.
36. Ledermann JA, Embleton AC, Raja F, et al. Cediranib in patients with relapsed platinum-sensitive ovarian cancer (ICON6): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2016;387(10023):1066-1074.
37. Liontos M, Lykka M, Dimopoulos MA, et al. Profile of trebananib (AMG386) and its potential in the treatment of ovarian cancer. Onco Targets Ther. 2014;7:1837-1845.
38. Eroglu Z, Stein CA, Pal SK. Targeting angiopoietin-2 signaling in cancer therapy. Expert Opin Investig Drugs. 2013;22(7):813-825.
39. Vergote I, Scambia G, O'Malley DM, et al. Trebananib or placebo plus carboplatin and paclitaxel as first-line treatment for advanced ovarian cancer (TRINOVA-3/ENGOT-ov2/GOG-3001): a randomised, double-blind, phase 3 trial. Lancet Oncol. 2019;20(6):862-876.
40. Monk BJ, Poveda A, Vergote I, et al. Anti-angiopoietin therapy with trebananib for recurrent ovarian cancer (TRINOVA-1): a randomised, multicentre, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. 2014;15(8):799-808.
41. Fujiwara K, Monk BJ, Lhommé C, et al. Health-related quality of life in women with recurrent ovarian cancer receiving paclitaxel plus trebananib or placebo (TRINOVA-1). Ann Oncol. 2016;27(6):1006-1013.
42. Caldecott KW. DNA single-strand break repair and spinocerebellar ataxia. Cell. 2003;112(1):7-10.
43. Ray Chaudhuri A, Nussenzweig A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. Nat Rev Mol Cell Biol. 2017;18(10):610-621.
44. Lord CJ, Tutt AN, Ashworth A. Synthetic lethality and cancer therapy: lessons learned from the development of PARP inhibitors. Annu Rev Med. 2015;66:455-470.
45. Kim G, Ison G, McKee AE, et al. FDA approval summary: olaparib monotherapy in patients with deleterious germline BRCA-mutated advanced ovarian cancer treated with three or more lines of chemotherapy. Clin Cancer Res. 2015;21(19):4257-4261.
46. Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J

Med. 2018;379(26):2495-2505.
47. Banerjee S, Moore KN, Colombo N, et al. Maintenance olaparib for patients with newly diagnosed advanced ovarian cancer and a BRCA mutation (SOLO1/GOG 3004): 5-year follow-up of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2021;22(12):1721-1731.
48. Ray-Coquard I, Pautier P, Pignata S, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. N Engl J Med. 2019;381(25):2416-2428.
49. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366(15):1382-1392.
50. Pujade-Lauraine E, Ledermann JA, Selle F, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18(9):1274-1284.
51. Penson RT, Valencia RV, Cibula D, et al. Olaparib versus nonplatinum chemotherapy in patients with platinum-sensitive relapsed ovarian cancer and a germline BRCA1/2 mutation (SOLO3): A randomized phase III trial. J Clin Oncol. 2020;38(11):1164-1174.
52. Caruso D, Papa A, Tomao S, et al. Niraparib in ovarian cancer: results to date and clinical potential. Ther Adv Med Oncol. 2017;9(9):579-588.
53. Moore KN, Secord AA, Geller MA, et al. Niraparib monotherapy for late-line treatment of ovarian cancer (QUADRA): a multicentre, open-label, single-arm, phase 2 trial. Lancet Oncol. 2019;20(5):636-648.
54. Mirza MR, Monk BJ, Herrstedt J, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med. 2016;375(22):2154-2164.
55. González-Martín A, Pothuri B, Vergote I, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2019;381(25):2391-2402.
56. Colomba E, Pautier P, Pommeret F, et al. Rucaparib in the landscape of PARP inhibition in ovarian cancer. Expert Rev Anticancer Ther. 2019;19(6):437-446.
57. Swisher EM, Lin KK, Oza AM, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncol. 2017;18(1):75-87.
58. Swisher EM, Kwan TT, Oza AM, et al. Molecular and clinical determinants of response and resistance to rucaparib for recurrent ovarian cancer treatment in ARIEL2 (Parts 1 and 2). Nat Commun. 2021;12(1):2487.
59. Kristeleit R, Shapiro GI, Burris HA, et al. A phase I-II study of the oral PARP inhibitor rucaparib in patients with germline BRCA1/2-mutated ovarian carcinoma or other solid tumors. Clin Cancer Res. 2017;23(15):4095-4106.
60. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10106):1949-1961.
61. ARIEL4: A study of rucaparib versus chemotherapy BRCA mutant ovarian, fallopian tube, or primary peritoneal cancer patients. https://clinicaltrials.gov/ct2/show/NCT02855944. Accessed 2 Feb 2022.
62. Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. Annu Rev Pathol. 2021;16:223-249.
63. Galluzzi L, Humeau J, Buqué A, et al. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. Nat Rev Clin Oncol. 2020;17(12):725-741.
64. Hamanishi J, Takeshima N, Katsumata N, et al. Nivolumab versus gemcitabine or pegylated liposomal doxorubicin for patients with platinum-resistant ovarian cancer: open-label, randomized trial in Japan (NINJA). J Clin Oncol. 2021;39(33):3671-3681.
65. Pujade-Lauraine E, Fujiwara K, Ledermann JA, et al. Avelumab alone or in combination with chemotherapy versus chemotherapy alone in platinum-resistant or platinum-refractory ovarian cancer (JAVELIN Ovarian 200): an open-label, three-arm, randomised, phase 3 study. Lancet Oncol. 2021;22(7):1034-1046.

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REVIEW ARTICLE

Gene mutation analysis and immune checkpoint therapy in head and neck squamous cell carcinoma*

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Abstract

Immune checkpoint inhibitors (ICI), represented by blocked programmed cell death-1 (PD-1), is a group of novel medicines for anti-tumor immunotherapy. It has been approved by the U.S. Food and Drug Administration (FDA) in recent years for relapsed or metastatic head and neck squamous cell carcinoma (HNSCC), and brings promising treatment prospects. However, the instability caused by tumor gene mutations significantly compromises the therapeutic effect of ICI. Therefore, the identification and analysis of HNSCC gene mutations can further guide and optimize the application of ICIs in HNSCC. In this study, we preliminarily described the clinical research progress of ICI therapy and the potential immune escape mechanism in HNSCC. An overview of complete HNSCC gene mutation results was generated from the bioinformatics study of TCGA database to further explain and analyze the relevant molecular mechanisms, which may aid in designing future personalized therapeutic strategies for HNSCC patients.

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Key words: head and neck squamous cell carcinoma (HNSCC); immune checkpoint inhibitor; gene mutation

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of malignant tumor worldwide. Although there has been significant progress recently in chemotherapy and adjuvant radiotherapy, there is no noticeable increase in the five-year survival rate among HNSCC patients, with metastasis and recurrence being one of the main causes for the poor prognosis and low survival rate of HNSCC patients [1]. In recent years, immunotherapy has gradually become a popular research topic because of its high effectiveness. Immune checkpoint inhibitors (ICIs), represented by blocking programmed cell death-1 (PD-1), have made breakthrough progress in the treatment of solid tumors such as lung cancer and melanoma, which not only reduces the efficiency of tumor metastasis and recurrence, but also effectively extends the survival time of patients [2–3]. Since 2016, two ICI therapies targeting relapsed and metastatic HNSCC, namely nivolumab and pembrolizumab, have been approved for marketing by the U.S. Food and Drug Administration (FDA). However, while the ICI therapeutic approach achieves better curative effects, drug resistance and serious adverse reactions were observed after long-term treatment. Consequently, the need to develop personalized treatment by adapting individual factors in HNSCC cases is enhanced. Recent studies have shown that the mutational gene phenotypes of cancer patients not only compromise the therapeutic efficiency of standardized drugs, but are also closely related to post-treatment adverse effects. Therefore, analysis of gene mutations in HNSCC patients can optimize individual therapy strategies and provide more precise and personalized ICI treatment plans [1, 4–6].

HNSCC and ICI treatment

Mechanism of HNSCC immune evasion

Although the immune evasion mechanism for HNSCC in the PD-1/PD-L1 pathway has not yet been determined, a literature review indicates that the mechanisms could be as follows [7–8]: 1. Induction of T cell apoptosis: The interaction between PD-L1 on the tumor surface and PD-1 from effector T cells causes either loss of T cell

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response or apoptosis, or both occur simultaneously; 2. By promoting immune tolerance, PD-L1 binds to CD80 on cytotoxic T cells to inhibit the immune response; 3. Via tumor cell activity regulation: Reverse transmission of the PD-L1 biological signaling pathway can prevent tumor cells from entering the apoptotic state; 4. Via inhibition of T cell proliferation, the PD-1/PD-L1 pathway inhibits PI3K/Akt/mTOR and Ras/MEK/Erk pathways, which further leads to the downregulation of amino acid and sugar metabolism, increase in fatty acid oxidation, enhancement of T cell differentiation, and induction of T cell depletion.

Clinical research of ICI treatment

The results of the Phase III trial (CheckMate 141) suggest that nivolumab treatment has better efficacy than standard treatment [9]. The results showed that the median overall survival (mOS) of the nivolumab group [7.5 months, 95% confidence interval (CI): 5.5-9.1] was significantly longer than the group receiving standard treatment (5.1 months, 95% CI: 4.0–6.0) (HR = 0.70; 97.73% CI: 0.51–0.96; P = 0.01). Another anti-PD-1 therapy, pembrolizumab, has also shown good results in a phase Ib clinical trial (KEYNOTE-012) in HNSCC patients. With an objective response rate (ORR) of 18% and median progression-free survival (mPFS) of 2 months, the mOS period was extended to 13 months [10]. A phase II clinical study (KEYNOTE-055) further reported the following results [11]: 16% of ORR, same mPFS (2.1 months), and a 2-month median response time. Nonetheless, the mOS outcome was shorter than that in the phase I record (8 months). Although further data from the phase III clinical trial (KEYNOTE-040) showed no significant prolongation of patients' OS with pembrolizumab treatment [12], the figures still indicate that patients with positive PD-L1 expression had better survival upon receiving pembrolizumab (mOS 11.6 months) than the low expression group (mOS 8.7 months), proving that PD-L1 can be used as an important prognostic factor for HNSCC patients upon ICI treatment. Furthermore, the identification and analysis of biomarker expression would benefit doctors in developing personalized ICI treatment strategies for HNSCC patients. However, it is worth noting that HNSCC patients treated with nivolumab and pembrolizumab both experienced adverse reactions such as fatigue, nausea, and loss of appetite. The records of the Checkmate 141 trial showed that 58.9% of patients treated with nivolumab experienced adverse reactions, among which 13.1% had grade 3 to 4 adverse reactions. Several HNSCC patients (62%–64%) receiving pembrolizumab experienced adverse reactions, with 9%–17% of them falling in grade 3–4. In order to reduce the resistance to standard treatments, optimize treatment efficacy, and monitor adverse reactions, several ICI clinical combination treatments are being developed (Table 1).

HNSCC mutation

Summary of gene mutation results

The HNSCC patient gene mutation data were downloaded from the TCGA database based on four processing software. The "maftools" package in the R software was used to draw waterfall diagrams of the mutation results processed by the mutect. The top 30 genes with higher mutation probability were enriched in the waterfall chart, with the mutation types and probabilities of related genes in each sample. Different mutation types are represented by different colors, including frame-del mutations, nonsense mutations, missense mutations, frame shift ins, splice sites, frame shift del, start site mutations (translation start site), and multiple mutations coexist (multiple hits). The top three genes with the highest mutation probabilities were *TP53*, *TTN*, and *FAT1*, with mutation probabilities of 66%, 35%, and 21%, respectively (Fig. 1).

Analysis of gene mutation

Fig. 2a showed a total of nine common types of mutations in HNSCC samples, which is different from

Table 1 Ongoing clinical trials of Immune checkpoint inhibitors on HNSCC

Agent	Immune checkpoint	Combination	Phase	Clinical trials / NCT number	No.of patients	Predict time of completion
Relatlimab	LAG-3	Nivolumab	Phase I /II	NCT01968109	1500	December 31, 2023
Nivolumab	PD-1	Relatlimab	Phase II/III	NCT03470922	700	March 16, 2022
INCAGN02390	TIM-3	/	Phase I	NCT03652077	41	January 31, 2021
Pembrolizumab	PD-1	AST-008	Phase II	NCT03684785	130	September 30, 2021
AMP-110	B7-H4	/	Phase I	NCT01878123	26	July, 2014
Nivolumab	PD-1	Surgery, Radiotherapy/Chemoradiotherapy	Phase II	NCT03721757	120	November 2023
Nivolumab	PD-1	Ipilimumab	Phase II	NCT03406247	140	February 2024
Nivolumab	PD-1	Ipilimumab	Phase III	NCT02741570	947	February 4, 2026
Nivolumab	PD-1	Ipilimumab	Phase II	NCT02823574	675	January, 2024
Ipilimumab/ Nivolumab	PD-1/CTL-4	INCAGN01876	Phase I /II	NCT03126110	45	October, 2021

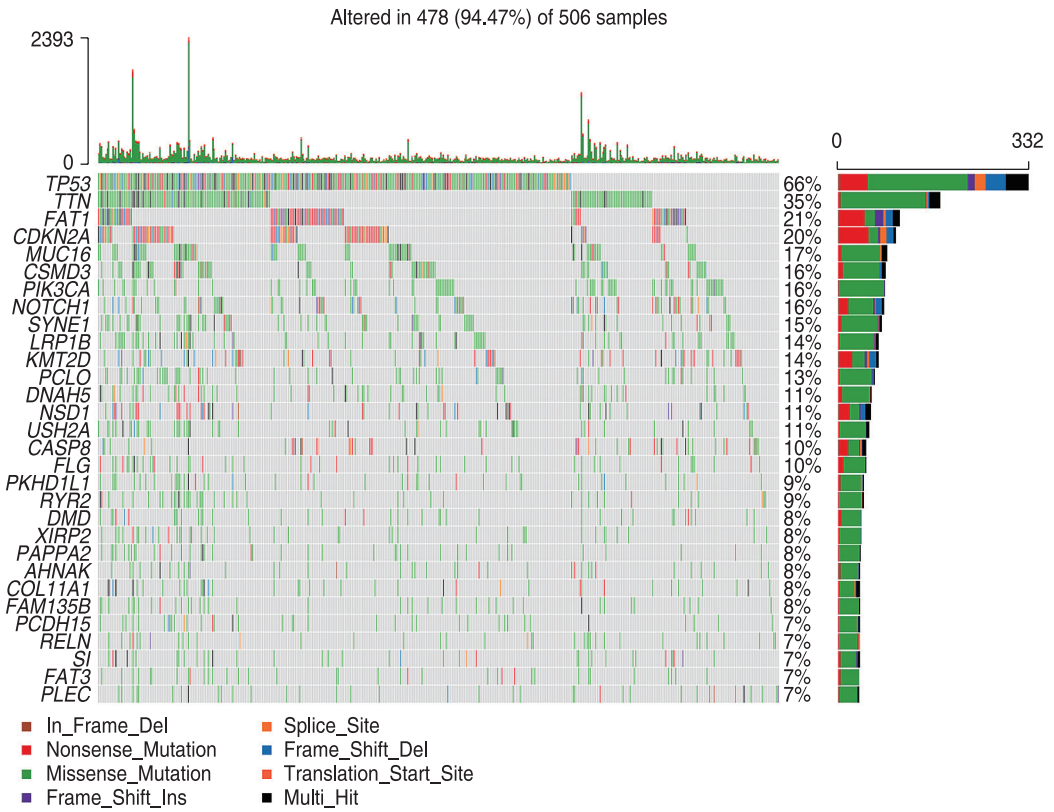

Fig. 1 Waterfall plot of tumor mutation

the analytical results of specific gene mutations in Fig. 1. It provides an overview of all high-probability mutation types in HNSCC patients, including missense mutations, nonsense mutations, frame shift del, frame shift ins, frame del, frame ins, splice sites, nonstop mutations, and translation start sites, of which missense mutations have the highest proportion. In addition, mutations at single nucleotide sites occurred more frequently than insertions or deletions (Fig. 2b). Among them, C > T mutations are the most commonly found single-nucleotide variant (SNV) types in HNSCC (Fig. 2c). Fig. 2d and 2e further summarize the total number of mutations with categories for each sample. The top 10 genes with the highest mutation probabilities in HNSCC samples were identified as *TP53* (66%), *TTN* (35%), *FAT1* (21%), *MUC16* (17%), *CDKN2A* (20%), *CSMD3* (16 %), *SYNE1* (15%), *LRP1B* (14%), *NOTCH1* (16%), and *PIK3CA* (16%) (Fig. 2f). According to previous research, HNSCC is a heterogeneous tumor and is related to classic pathogenic factors such as smoking and drinking [13]. Therefore, the appearance of tobacco-related genes such as TP53 and *CDKN2A* with higher mutation frequency in HNSCC patients in this habit and behavior independent prediction validated the design of the study.

Correlation analysis of mutant genes

The correlation analysis between genes with higher mutation probability revealed mutually exclusive relationships as the most common predictive correlation, while the co-expression relationship was more significant (Fig. 3). Among them, the green color represents the co-expression relationship of the two genes, and the red color represents the mutual exclusion relationship; the significance of the correlation was non-significant, significant ($P < 0.05$), and highly significant ($P < 0.001$). Among them, there is a highly significant positive correlation between *TP53* and *CDKN2A* genes ($P < 0.001$), which highlights the potential for further exploration as a possible key theoretical research direction.

HNSCC mutant genes and ICI therapy

Following the discoveries above, the mechanism between higher mutation frequency genes and ICI therapy in HNSCC was further explored and analyzed.

Higher mutation frequency genes

TP53 gene

The latest research results in 2021 show that the immune-related gene prognostic index (IRGPI) can be

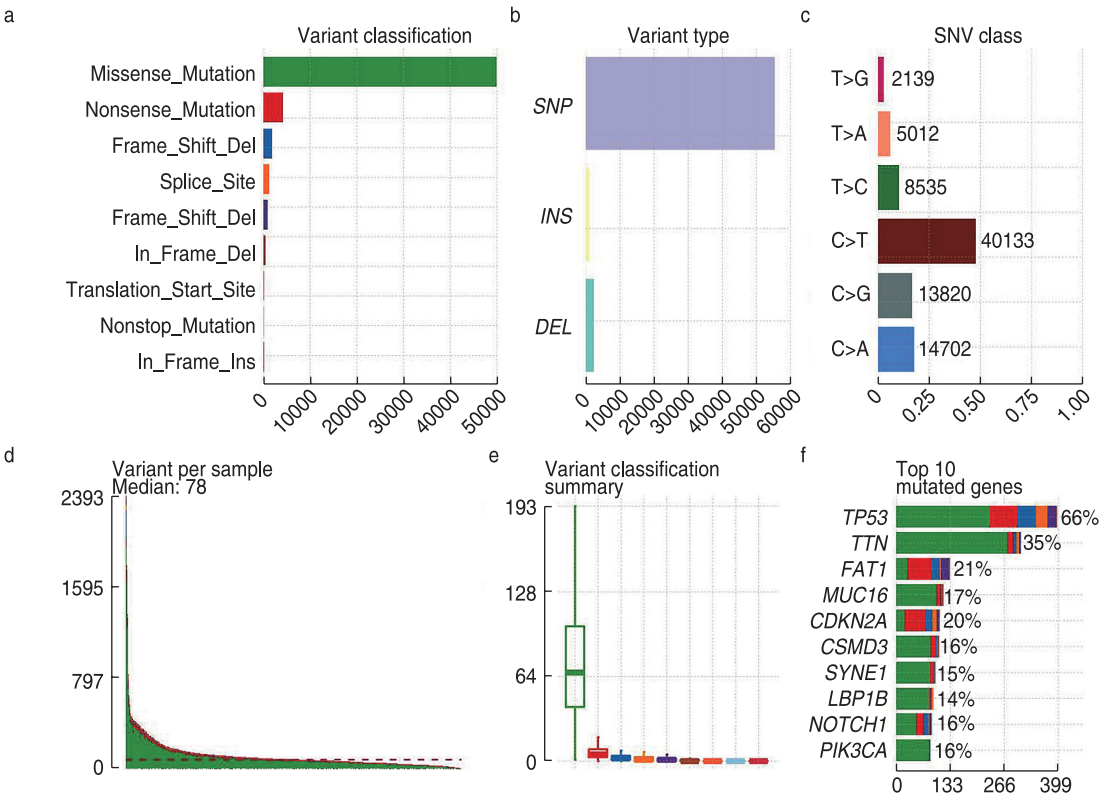

Fig. 2 Summary of mutation type. (a) Among the variant classification category, missense mutation accounted for the majority; (b) SNP is the most frenquecy variant type in HNSCC; (c) In SNV class, C > T account for 40133 cases and is the most commom SNV type in HNSCC; (d) The total mutation number in each sample; (e) Box plots of each variant classification in each sample; (f) Top 10 mutated genes in HNSCC with the variant frenquency. SNP, single nucleotide polymorphism; SNV, single nucleotide variants

used as a predictive marker of post-ICI treatment efficacy in HNSCC patients. A higher IRGPI indicates better treatment outcomes of the patients; in contrast, low IRGPI indicates poor ICI treatment effect. In this study, it was seen that there was a significant negative correlation between the mutation frequency of the *TP53* gene in HNSCC patients with IRGPI expression, further indicating that the former can predict the ICI treatment effect of HNSCC patients in the opposite way [14]. According to the literature, the underlying mechanism of this correlation may be as follows: HPV is one of the triggers of HNSCC. The viral genome integration into the host cell genome causes E6 and E7 to express viral oncoproteins, which leads to the degradation of TP53, inactivation of tumor suppressor retinoblastoma protein, and activation of the immunosuppressive pathway to allow tumor escape [13, 15–16]. Therefore, the occurrence of *TP53* gene mutations can affect their interaction with viral oncoproteins. The efficacy of ICI therapy changes accordingly by regulating the degradation efficiency of TP53 [17].

PI3KCA gene

The IRGPI article reported that there is a significant correlation between the low mutation frequency of *PI3KCA* and *IRGPI* in HNSCC patients, while the high-frequency mutation group had no such correlation. It also predicted that the prognostic results are similar to the *TP53* mutation frequency, which suggests that HNSCC patients in the low mutation frequency group of *PI3KCA* tend to obtain better ICI treatment effects. In addition, compared with the wild-type with HNSCC in a mouse model, the *PI3K* knockout can regulate T cells and immune checkpoint markers (PD-L1, PD-1) by affecting the functions of myeloid cells and T cell expression, thereby increasing the expression of anti-tumor cytotoxic molecules (IFN-γ, IL-17). These results indicate that the inhibition of *PI3K* can regulate the expression of tumor-related immune cells, indicating that the use of *PI3K* inhibitors in combination with ICI can further enhance the therapeutic effect of HNSCC [18]. In 2020, Novartis invented the world's first *PIK3CA* mutation medicine, Piqray, which was approved for the Canadian market, targeting advanced breast cancer. The phase III clinical trial results reveal significantly prolonged mPFS of patients (11.0 months vs 5.7 months) who underwent combined treatment of Piqray and fulvestrant, while the ORR increased nearly 2 times (36% vs. 16%) [19], proving that targeting *PI3KCA* mutations is significantly promising for tumor treatment. Although there have

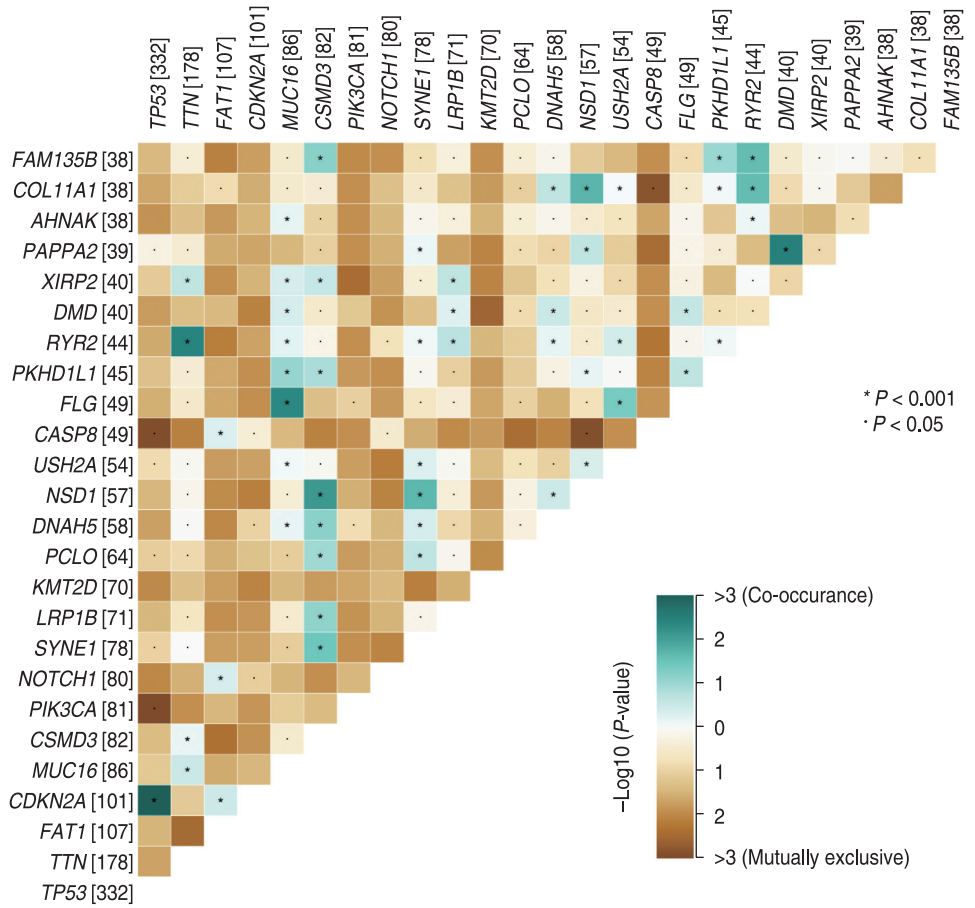

Fig. 3 Correlation analysis of mutated genes

been few studies on the correlation between post-ICI HNSCC and *PI3KCA* mutation types, we assume that by analyzing the type and frequency of *PI3K* mutations in HNSCC samples, the therapeutic effect of *PI3K* inhibitors can be further determined to identify novel strategies for ICI treatment combination.

NOTCH1 gene

The results of 126 HNSCC patients who underwent ICI treatment suggested that the frequency of *NOTCH1* mutation is related to the immune response to PD-1/L1 inhibitors, and the high frequency of *NOTCH1* mutation was more likely to occur in HPV-negative anti-PD-1/PD-L1 responders ($P < 0.05$) [20]. A recent study showed that *NOTCH1* mutation can be used as a new biomarker for lung cancer patients receiving ICI treatment, which consistently refers to the *NOTCH1* mutation as an important predictor of ICI treatment effect; however, the relevant mechanism remains unclear [21].

Signal pathways related to mutant genes

Wnt signaling pathway

Considering the highly frequent mutant genes (e.g., *FAT1* and *NOTCH1*), the results in Fig. 3 show a significant positive correlation between these two genes. The reason for this co-expression correlation could be that both *FAT1* and *NOTCH1* genes exist in the abnormally activated Wnt signaling pathway, which is involved in the development of tumors. Studies have shown that the Wnt signaling pathway can cooperate or antagonize other signaling pathways to further regulate tumor proliferation, migration, and invasion. Additionally, its constitutive expression can eliminate T cells in tumor tissues and contribute to resistance to ICI treatment [22]. An HNSCC study demonstrated that *FAT1* and *NOTCH1* are upstream conditional factors of the Wnt signaling pathway. Mutations in these two genes can lead to the loss of the core component of pathway-β-catenin, inhibiting the cancer process [17]. Therefore, an in-depth study of *FAT1* and *NOTCH1* mutations in the Wnt pathway can help further the understanding of the mechanism of drug resistance in HNSCC patients.

Hippo-YAP signaling pathway

The *PI3KCA* gene is located in the PI3K/Akt/mTOR signaling pathway, a classic pathway of immune

resistance after ICI treatment. There have been studies that have found that the *PI3KCA* gene is highly likely to be closely related to another tumor immune pathway, namely the Hippo-YAP signaling pathway. The high expression of *PI3KCA* in HNSCC patients is related to nuclear YAP localization, which can activate downstream target genes to promote the growth of HNSCC tumor cells, causing HNSCC patients to have a higher tumor recurrence rate [23]. In contrast, the latest research in 2021 shows that YAP expression is negatively correlated with the prognosis of patients with solid tumors, which can mediate the resistance to anti-PD-1 treatments and become a biological predictor of the efficacy of anti-PD-1 treatments [24]. Consequently, we speculate that the high frequency of *PI3KCA* mutations in HNSCC patients will affect the expression of YAP, thereby further affecting the tumor recurrence rate and patients' ICI treatment effect.

Discussion

This is the first comprehensive discussion on the analysis of HNSCC mutation results based on the TCGA database that analyzes the effects of ICI treatment and related immune mechanisms in patients. To date, ICIs still have limitations in the application of HNSCC. Although ICI has made breakthroughs in other solid tumor treatment options, the clinical data for its application are still insufficient. Studies have shown that the mechanism of PD-L1 expression in HNSCC and other solid tumors may be partially different, and not all HNSCC patients are PD-L1 positive; hence, more data are required to further guide clinical applications. On the other hand, drug tolerance leads to a decline in long-term therapeutic effects, which triggers the alteration towards a combination of multiple immune checkpoint inhibitors currently under development. Theoretically, a customized treatment plan according to the patient's marker expression or gene mutation case can improve the curative effect of HNSCC more precisely while reducing side effects and other adverse reactions. Nevertheless, the present supportive technology system for precision medical treatment setup in our country, such as the establishment of biobanks, bioinformatics collection and analysis, and big data analysis technology, are not yet mature. Therefore, this article collected the gene mutation information of HNSCC patients in public databases, conducted preliminary analysis through bioinformatics, and elaborated the relevant mechanisms, aiming to provide better treatment plans for HNSCC patients under the guidance of precise treatment plans in the future.

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Conflicts of interest
The authors indicated no potential conflicts of interest.

Author contributions
Hua Yang and Yuxue Wei contributed to data acquisition and data analysis, and Gangli Liu took responsibility for the data interpretation and reviewed and approved the final version of this manuscript.

Data availability statement
Not applicable.

Ethical approval
Not applicable.

References

1. Chen SH, Hsiao SY, Chang KY, et al. New insights into oral squamous cell carcinoma: from clinical aspects to molecular tumorigenesis. Int J Mol Sci. 2021;22(5):2252.
2. Singh S, Hassan D, Aldawsari HM, et al. Immune checkpoint inhibitors: a promising anticancer therapy. Drug Discov Today. 2020;25(1):223-229.
3. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Nat Commun. 2020;11(1):3801.
4. Lee TW, Lai A, Harms JK, et al. Patient-derived xenograft and organoid models for precision medicine targeting of the tumour microenvironment in head and neck cancer. Cancers. 2020; 12(12):3743.
5. Nishikawa D, Sasaki E, Suzuki H, et al. Treatment outcome and pattern of recurrence of sinonasal squamous cell carcinoma with EGFR-mutation and human papillomavirus. J Craniomaxillofac Surg. 2021;49(6):494-500.
6. Chahoud J, Gleber-Netto FO, McCormick BZ, et al. Whole-exome sequencing in penile squamous cell carcinoma uncovers novel prognostic categorization and drug targets similar to head and neck squamous cell carcinoma. Clin Cancer Res. 2021;27(9):2560-2570.
7. Zandberg DP, Strome SE. The role of the PD-L1:PD-1 pathway in squamous cell carcinoma of the head and neck. Oral Oncol. 2014; 50(7):627-32.
8. Majc B, Novak M, Jerala NK, et al. Immunotherapy of glioblastoma: current strategies and challenges in tumor model development. Cells. 2021;10(2):265.
9. Ferris RL, Blumenschein Jr G, Fayette J, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med. 2018;81:45-51.
10. Seiwert TY, Burtness B, Mehra R, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. Lancet Oncol. 2016;17(7):956-965.

11. Bauml J, Seiwert TY, Pfister DG, et al. Pembrolizumab for platinum- and cetuximab-refractory head and neck cancer: results from a single-arm, phase II study. J Clin Oncol. 2017;35(14):1542-1549.
12. Cohen E, Harrington K, Le Tourneau C, et al. Pembrolizumab (pembro) vs standard of care (SOC) for recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC): phase 3 KEYNOTE-040 trial. Ann Oncol. 2017;28:628.
13. Solomon B, Young RJ, Rischin D. Head and neck squamous cell carcinoma: Genomics and emerging biomarkers for immunomodulatory cancer treatments. Semin Cancer Biol. 2018;52(Pt 2):228-240.
14. Chen Y, Li ZY, Zhou GQ, et al. An immune-related gene prognostic index for head and neck squamous cell carcinoma. Clin Cancer Res. 2021;27(1):330-341.
15. Biton J, Mansuet-Lupo A, Pécuchet N, et al. TP53, STK11, and EGFR mutations predict tumor immune profile and the response to anti–PD-1 in lung adenocarcinoma. Clin Cancer Res. 2018;24(22):5710-5723.
16. Offin M, Chan JM, Tenet M, et al. Concurrent RB1 and TP53 alterations define a subset of EGFR-mutant lung cancers at risk for histologic transformation and inferior clinical outcomes. J Thorac Oncol. 2019;14(10):1784-1793.
17. Leemans CR, Snijders PJ, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer. 2018;18(5):269-282.
18. Anderson K, Ryan N, Alkhimovitch A, et al. Inhibition of PI3K isoform p110γ increases both anti-tumor and immunosuppressive responses to aggressive murine head and neck squamous cell carcinoma with low immunogenicity. Cancers. 2021;13(5):953.
19. Rugo H, André F, Yamashita T, et al. Time course and management of key adverse events during the randomized phase III SOLAR-1 study of PI3K inhibitor alpelisib plus fulvestrant in patients with HR-positive advanced breast cancer. Ann Oncol. 2020;31(8):1001-1010.
20. Hanna GJ, Lizotte P, Cavanaugh M, et al. Frameshift events predict anti-PD-1/L1 response in head and neck cancer. JCI Insight. 2018;3(4):e98811.
21. Zhang K, Hong X, Song Z, et al. Identification of deleterious NOTCH mutation as novel predictor to efficacious immunotherapy in NSCLC. Clin Cancer Res. 2020;26(14):3649-3661.
22. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature. 2015;523(7559):231-235.
23. García-Escudero R, Segrelles C, Dueñas M, et al. Overexpression of PIK3CA in head and neck squamous cell carcinoma is associated with poor outcome and activation of the YAP pathway. Oral Oncol. 2018;79:55-63.
24. Yu M, Peng Z, Qin M, et al. Interferon-γ induces tumor resistance to anti-PD-1 immunotherapy by promoting YAP phase separation. Mol Cell. 2021;81(6):1216-1230.

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ORIGINAL ARTICLE

A metabolism-associated gene signature with prognostic value in colorectal cancer*

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Abstract

Objective In this study, our goal was to explore the role of metabolism-associated genes in colorectal cancer (CRC) and construct a prognostic model for patients with CRC.

Methods Differential expression analysis was conducted using RNA-sequencing data from The Cancer Genome Atlas (TCGA) dataset. Enrichment analyses were performed to determine the function of dysregulated metabolism-associated genes. The protein-protein interaction (PPI) network, Kaplan-Meier curves, and stepwise Cox regression analyses identified key metabolism-associated genes. A prognostic model was constructed using LASSO Cox regression analysis and visualized as a nomogram. Survival analyses were conducted in the TCGA and Expression Omnibus (GEO) cohorts to demonstrate the predictive ability of the model.

Results A total of 332 differentially expressed metabolism-associated genes in CRC were screened from the TCGA cohort. Differentially expressed metabolism-associated genes mainly participate in the metabolism of nucleoside phosphate, ribose phosphate, lipids, and fatty acids. A PPI network was constructed out of 328 key genes. A prognostic model was established based on five prognostic genes (*ALAD*, *CHDH*, *ISYNA1*, *NAT1*, and *P4HA1*) and was demonstrated to predict survival in the TCGA and GEO cohorts accurately.

Conclusion The metabolism-associated prognostic model can predict the survival of patients with CRC. Our work supplements previous work focusing on determining prognostic factors of CRC and lays a foundation for further mechanistic exploration.

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Key words: colorectal cancer (CRC); prognostic; metabolism; RNA-seq; The Cancer Genome Atlas (TCGA)

Affecting over one million people globally, colorectal cancer (CRC) is among the top three cancers diagnosed most frequently in men and women [1]. Although numerous novel technologies and strategies for CRC diagnosis and treatment have been developed, approximately 10% of cancer-related deaths are still caused by CRC, and the overall survival of patients with CRC remains poor [2]. Many prognostic factors, such as various gene mutations, non-coding RNAs, expression of PD-L1, the neutrophil-to-lymphocyte ratio, and anatomic stage have been demonstrated to predict the survival of patients with CRC over the past decade [3–4]. However, only a few prognostic factors are effective because of the large extent of heterogeneity in CRC, which calls for identifying other prognostic factors.

Alterations in metabolic activities can help cells obtain and maintain malignant properties, facilitating tumor initiation, growth, or progression. Extensive studies on metabolic alterations in cancer cells began with the observation of the Warburg effect. These studies have highlighted that reprogrammed metabolism is a hallmark of cancer [5–6]. The exploration of cancer metabolism offers a new perspective on tumorigenesis. Furthermore, metabolism-associated genes have been shown to have prognostic value in various tumors. For example, a mutation in the gene coding the metabolic enzyme

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isocitrate dehydrogenase (*IDH*) may indicate a favorable prognosis for gliomas [7]. The prognostic value of a signature reflecting glucose metabolism has been validated in patients with breast cancer through integrative analysis [8]. High expression levels of genes involved in glycolysis may indicate shorter median survival in patients with pancreatic cancer, but the high expression levels of genes involved in cholesterol synthesis may have the opposite effect [9].

Several studies have shown that metabolism is closely related to colorectal oncogenesis [10–12]. Furthermore, other studies have also identified a few prognostic metabolism-associated genes in colorectal cancer [13]. Nevertheless, since various metabolic alterations, such as the biosynthesis and metabolism of glucose, lipids, amino acids, and triphosadenine, play a role in tumor initiation and progression, the metabolism-associated genes involving in prognosis of CRC patients are far from fully explored. In this study, we analyzed CRC RNA-sequencing data from The Cancer Genome Atlas (TCGA) database from different perspectives and discovered five metabolism-associated genes that are independently related to survival in CRC patients. Additionally, a prognostic model was generated, and its prognostic value was confirmed in GSE39582 and TCGA.

Materials and methods

Data collection and processing

RNA-sequencing data files and corresponding clinical and pathological characteristics of patients with CRC were collected from the TCGA database, including a total of 44 normal samples and 568 tumor samples. Microarray data (GSE39582) with 585 samples from the Gene Expression Omnibus (GEO) database were downloaded as the validation cohort. Patients who were followed up for less than a month were excluded. We obtained metabolism-associated genes from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using the Gene Set Enrichment Analysis (GSEA) website (https://www.gsea-msigdb.org). The R package "Limma" was used to conduct differential gene expression analysis (version 3.6.2). Metabolism-associated genes that met the "adjusted *P*-value < 0.05 and |log fold change| > 0.5" thresholds were selected for further analysis. Volcano plots and heatmaps were generated to visualize the differentially expressed genes (DEGs).

Gene Ontology (GO) and KEGG enrichment analyses of DEGs

To gain insight into the possible biological functions of the differentially expressed metabolism-associated genes, the R package "clusterProfiler" was used to perform GO enrichment and KEGG pathway analyses with a threshold of both a *P*- and *Q*-value < 0.05.

Protein-protein interaction (PPI) network construction

Because proteins mediate most of the biological functions, a PPI network was constructed using STRING (http://string-db.org) to elucidate protein interactions. Cytoscape, a visualization tool, was used to construct the PPI network. Proteins that did not interact with any other proteins were considered relatively useless and were removed from the network. The metabolism-associated genes participating in the PPI network were identified as key genes.

Identification and validation of prognostic genes

The log-rank test and univariate Cox regression analysis were conducted to identify candidate prognostic genes from the key genes screened from the PPI network. Multivariate Cox regression analysis was also performed to determine whether the candidate prognostic genes could be independent prognostic indicators. Genes with a *P*-value < 0.05 in all of the above analyses were ultimately considered prognostic genes. Differential expression of these five genes was confirmed from different perspectives. Unpaired samples were discarded, and differential expression analysis was performed between 44 paired tumor and peritumoral tissues for these five genes in the TCGA cohort to avoid the effect caused by the large difference between the number of tumor and normal samples. The prognostic genes were also verified in GSE39582 using GraphPad Prism 7.0 software.

Construction and analysis of the prognostic model

The prognostic metabolism-associated genes identified from the above analyses were analyzed using LASSO Cox regression analysis with the R package "glmnet" to generate the prognostic model. The established model was presented as a formula, and the risk score of each sample was calculated using regression coefficients and mRNA expression levels of the prognostic genes. Patients were assigned to the high- and low-risk groups, with the median risk score used as the classification criterion. Kaplan-Meier survival curves were drawn to compare the outcomes of the high- and low-risk groups. The heat map, survival state diagram, and risk curve were generated according to the risk score. Then, univariate and multivariate Cox proportional regression analyses were conducted to determine the role of the risk score in outcome prediction. The "survminer" and "survival" R packages were utilized to perform the above survival analyses. To evaluate the ability of the model to predict survival, receiver operating characteristic (ROC) curves

were created using the R package "survival ROC". A nomogram was then generated based on the prognostic genes to predict patient survival using the "rms package" in the R software, and calibration curves were used to assess the deviation of predicted from actual survival.

Results

Identification of differentially expressed metabolism-associated genes

The workflow of this study is shown in Fig. 1. The RNA-sequencing data collected from TCGA included 568 tumor samples and 44 adjacent normal samples. After extracting the expression values of 961 metabolism-associated genes, we identified 332 DEGs that contained 160 downregulated genes and 172 upregulated genes (Fig. 2).

Functions of differentially expressed metabolism-associated genes and the PPI network

GO function and KEGG pathway enrichment analyses were performed on dysregulated genes to investigate their biological functions and lay the foundation for further mechanistic exploration. Bar and circle plots were also generated. The top 30 enriched GO terms and pathways are presented. The upregulated genes were mainly related to the biosynthesis and metabolism of nucleoside phosphate, ribose phosphate, and purine (Fig. 3). The downregulated genes were mostly involved in the metabolism of various lipids and acids (Fig. 4). Because the interactions between proteins are essential in most biological functions, a PPI network was constructed to determine the significant metaboslism-associated genes in biological processes. The PPI network comprised 328 nodes and 3574 edges after removing disconnected nodes (Fig. 5). The mean node degree of the network was 22, and the maximum node degree of protein nodes in the network was 85.

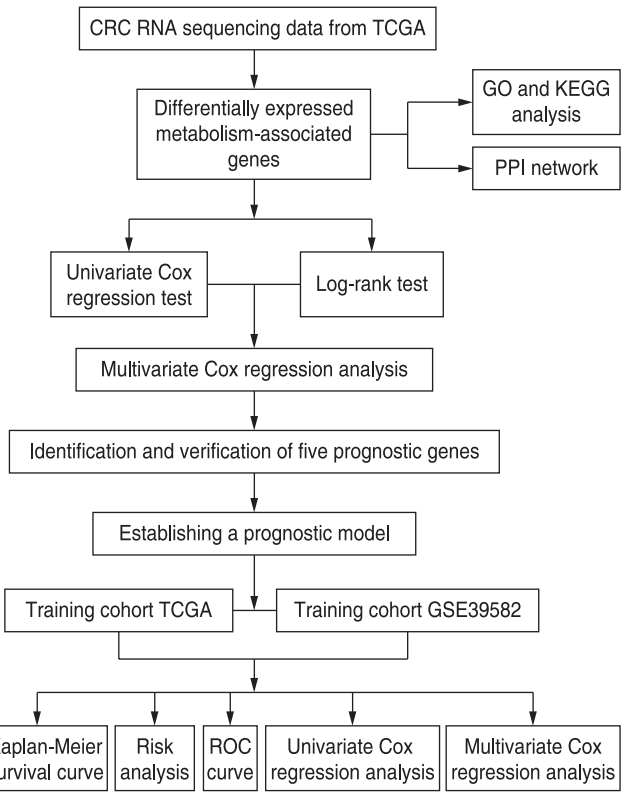


Fig. 1 Workflow for this study

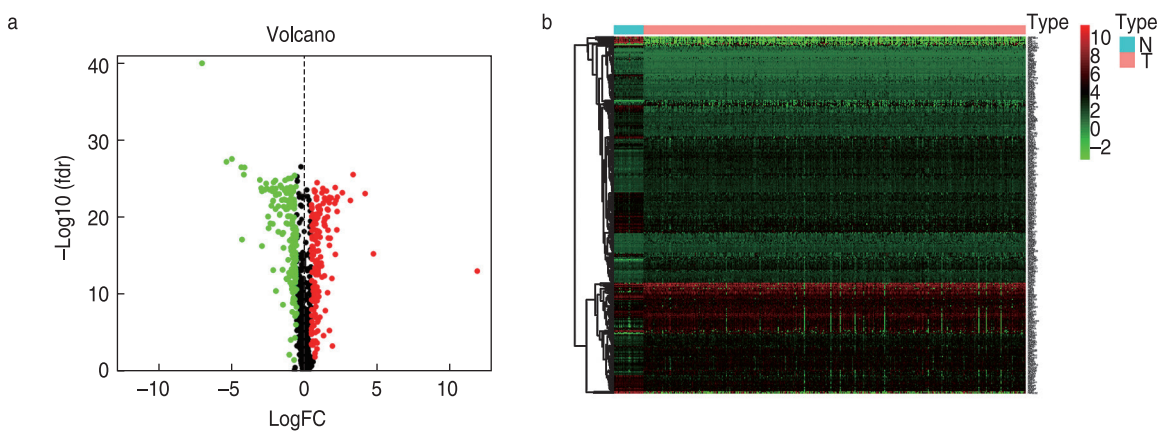


Fig. 2 Analyses of differently expressed genes. (a) The volcano plot of differentially expressed metabolism-associated genes between colorectal cancer and normal tissues in the TCGA database. A total of 160 downregulated genes are displayed in green, and 172 upregulated genes are displayed in red. (b) Heat map of differentially expressed metabolism-associated genes between colorectal cancer and normal tissues in the TCGA database. TCGA = The Cancer Genome Atlas

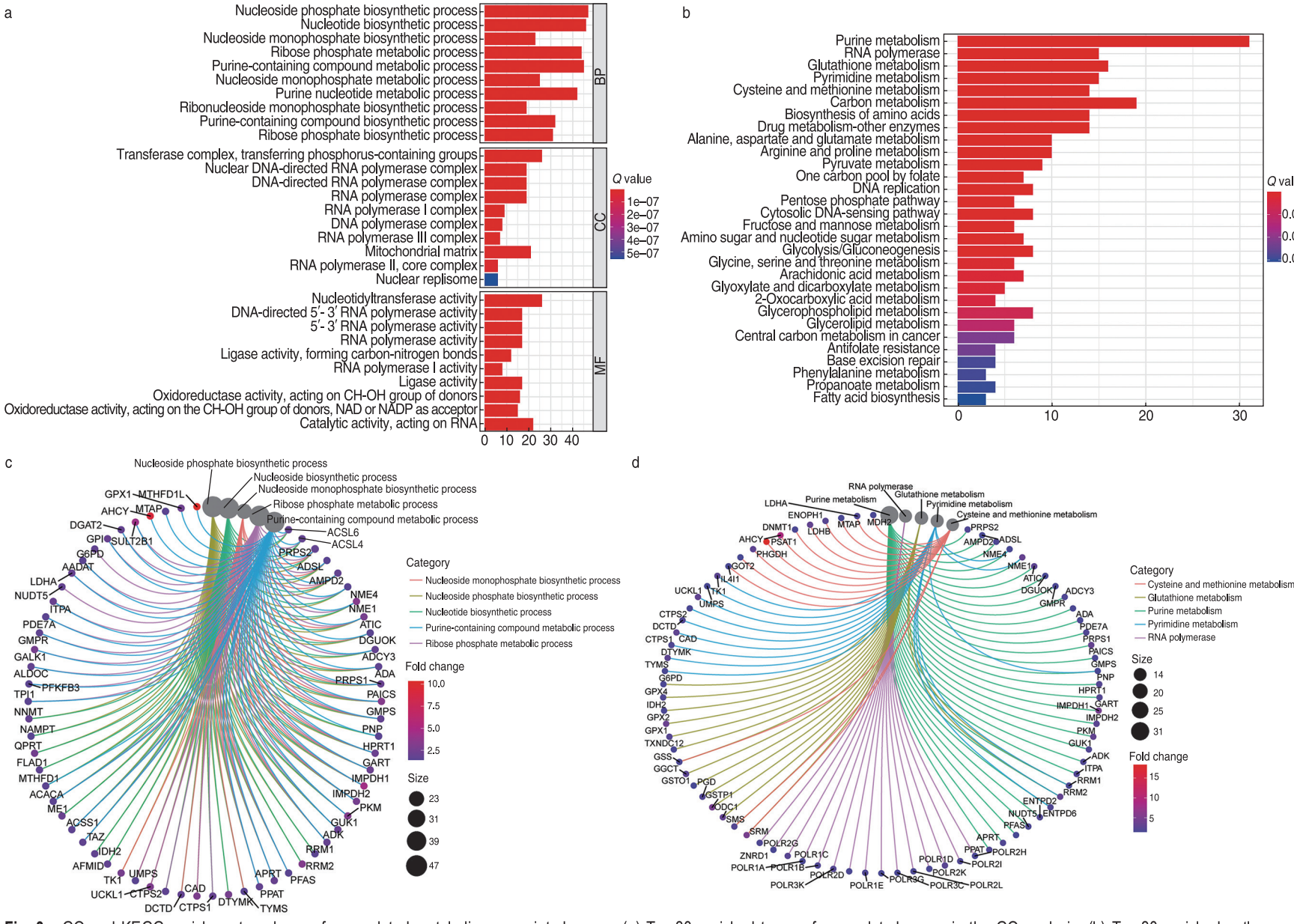


Fig. 3 GO and KEGG enrichment analyses of upregulated metabolism-associated genes. (a) Top 30 enriched terms of upregulated genes in the GO analysis. (b) Top 30 enriched pathways of upregulated genes in the KEGG analysis. (c) Upregulated genes involved in the top five enriched terms in the GO analysis. (d) Upregulated genes involved in the top five enriched pathways in the KEGG analysis. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes

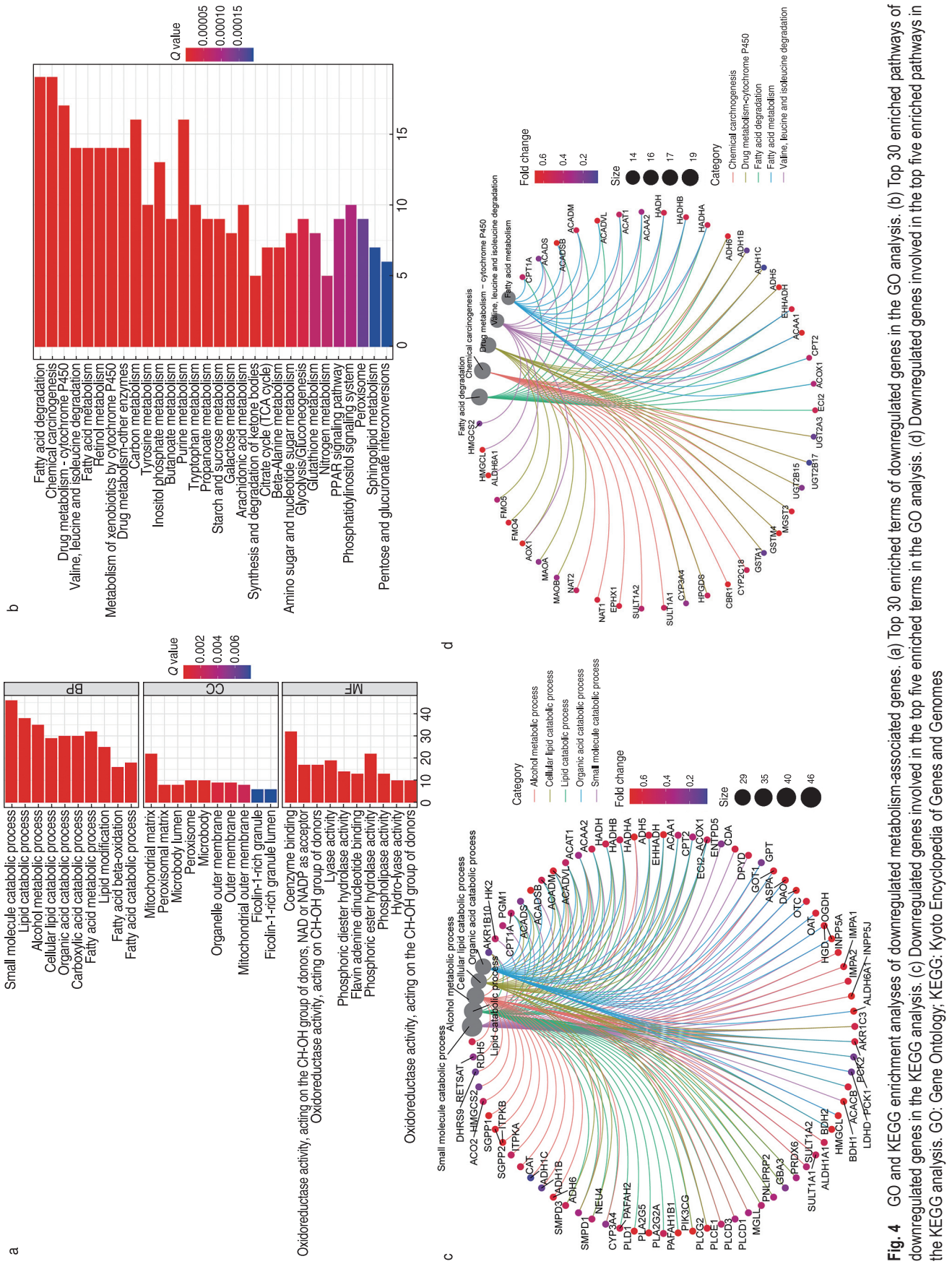


Fig. 4 GO and KEGG enrichment analyses of downregulated metabolism-associated genes. (a) Top 30 enriched terms of downregulated genes in the GO analysis. (b) Top 30 enriched pathways of downregulated genes in the KEGG analysis. (c) Downregulated genes involved in the top five enriched terms in the GO analysis. (d) Downregulated genes involved in the top five enriched pathways in the KEGG analysis. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes

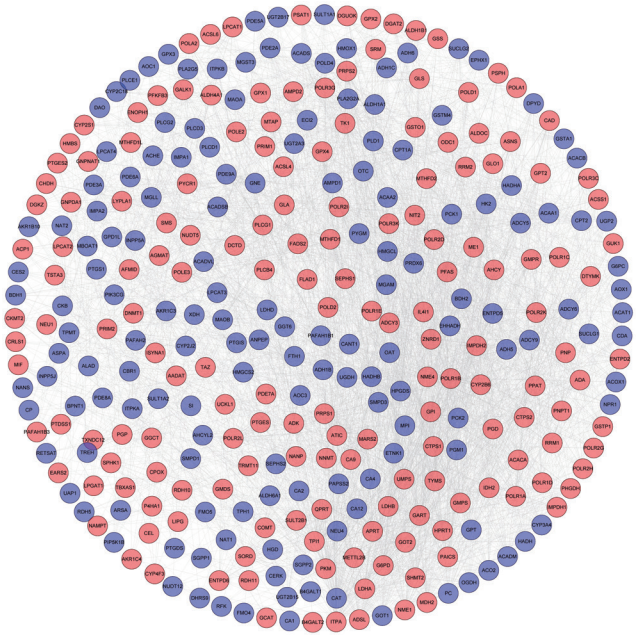


Fig. 5 PPI network analysis. Protein-protein interaction network of differentially expressed metabolism-associated genes. Green dots represent downregulated genes with a fold change of less than 0.5. Red dots represent upregulated genes with fold changes greater than 0.5. PPI: protein-protein interaction

Identification and validation of prognostic metabolism-associated genes

Seventeen genes were acquired using the log-rank test and univariate Cox regression analysis (Fig. 6a). These 17 genes could be significant prognostic factors. However, it is unknown whether their influence on survival is unaffected by other vital characteristics, such as age and stage. Therefore, multivariate Cox regression analysis was conducted, and we obtained five genes (*ALAD*, *CHDH*, *ISYNA1*, *NAT1*, and *P4HA1*) that independently affected overall survival (Fig. 6b). High expression of *CHDH* and *NAT1* was observed to be associated with a lower risk of death with hazard ratios < 1 in both univariate and multivariate Cox analyses, whereas high expression of *ALAD*, *ISYNA1*, and *P4HA1* had negative effect on survival. Thus, we hypothesized that *CHDH* and *NAT1* are tumor suppressor genes, whereas *ALAD*, *P4HA1*, and *ISYNA1* are oncogenes.

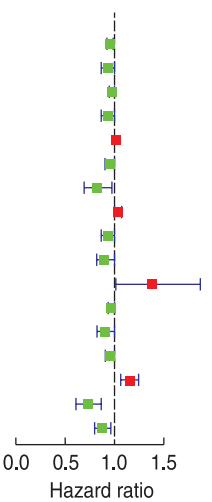
The results of differential expression analysis performed between paired tumor and peritumoral tissues in the TCGA cohort for these five genes confirmed our initial findings (Fig. 7a–7e), demonstrating that the initial differential expression analysis was unaffected by differences in the total sample number between tumor and normal tissues. The expression patterns of these genes were verified using another database. In accordance with the TCGA results, the expression levels of *CHDH*, *P4HA1*, and *ISYNA1* in the validation cohort GSE39582 were significantly elevated in colorectal carcinomas compared to peritumoral tissues, whereas the expression levels of *ALAD* and *NAT1* were lower in tumor tissues (Fig. 7f–7j).

Construction and analysis of prognostic models

A LASSO Cox regression model consisting of regression coefficients and mRNA expression levels of prognostic genes was constructed. The following formula was used to calculate the risk scores: (–0.1025 × Exp *CHDH*) + (0.0242 × Exp *P4HA1*) + (0.1748 × Exp *ALAD*) + (–0.3568 × Exp *NAT1*) + (0.0226 × Exp *ISYNA1*). Patients were assigned

a

	P value	Hazard ratio
ADH5	0.020	0.964 (0.935–0.994)
ACOX1	0.036	0.934 (0.875–0.996)
GOT2	0.024	0.978 (0.959–0.997)
CHDH	0.026	0.933 (0.877–0.992)
P4HA1	0.033	1.018 (1.001–1.035)
GNPNAT1	0.035	0.954 (0.913–0.997)
NANAP	0.021	0.822 (0.695–0.971)
ISYNA1	< 0.001	1.038 (1.018–1.058)
GPD1L	0.046	0.936 (0.877–0.999)
PAFAH2	0.024	0.900 (0.821–0.986)
ACACB	0.038	1.381 (1.017–1.876)
SUCLG2	0.006	0.975 (0.958–0.993)
HMGCL	0.032	0.908 (0.831–0.992)
NAMPT	0.030	0.958 (0.921–0.996)
ALAD	< 0.001	1.156 (1.073–1.245)
NAT1	< 0.001	0.728 (0.613–0.864)
NAT2	0.005	0.877 (0.801–0.961)



b

	P value	Hazard ratio
Age	< 0.001	1.055 (1.031–1.079)
Gender	0.653	0.903 (0.578–1.411)
Stage	0.264	1.507 (0.734–3.096)
T	0.103	1.538 (0.917–2.578)
M	0.062	2.641 (0.952–7.325)
N	0.849	1.045 (0.661–1.654)
ADH5	0.469	1.013 (0.978–1.050)
ACOX1	0.140	0.933 (0.852–1.023)
GOT2	0.563	1.008 (0.982–1.034)
CHDH	< 0.001	0.841 (0.765–0.924)
P4HA1	0.021	1.029 (1.004–1.053)
GNPNAT1	0.512	0.978 (0.914–1.046)
NANP	0.229	0.860 (0.673–1.099)
ISYNA1	0.005	1.033 (1.010–1.056)
GPD1L	0.421	1.046 (0.937–1.169)
PAFAH2	0.285	1.090 (0.931–1.277)
ACACB	0.114	1.405 (0.922–2.140)
SUCLG2	0.246	1.019 (0.987–1.052)
HMGCL	0.060	0.877 (0.766–1.005)
NAMPT	0.213	0.970 (0.924–1.018)
ALAD	0.013	1.129 (1.026–1.241)
NAT1	0.019	0.685 (0.499–0.940)
NAT2	0.867	0.986 (0.835–1.164)

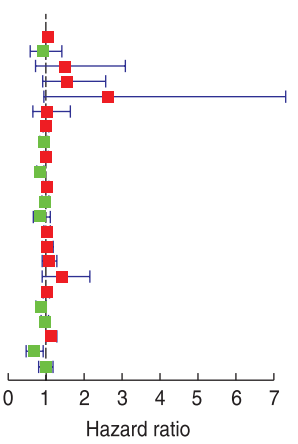


Fig. 6 Univariate Cox regression analysis and multivariate Cox regression analysis of key genes. (a) Seventeen candidate prognostic genes with a *P*-value < 0.05 in both the log-rank test and univariate Cox regression analysis. (b) Results of multivariate Cox regression analysis of 17 candidate prognostic genes. High-risk genes are shown in red, and low-risk genes are in green

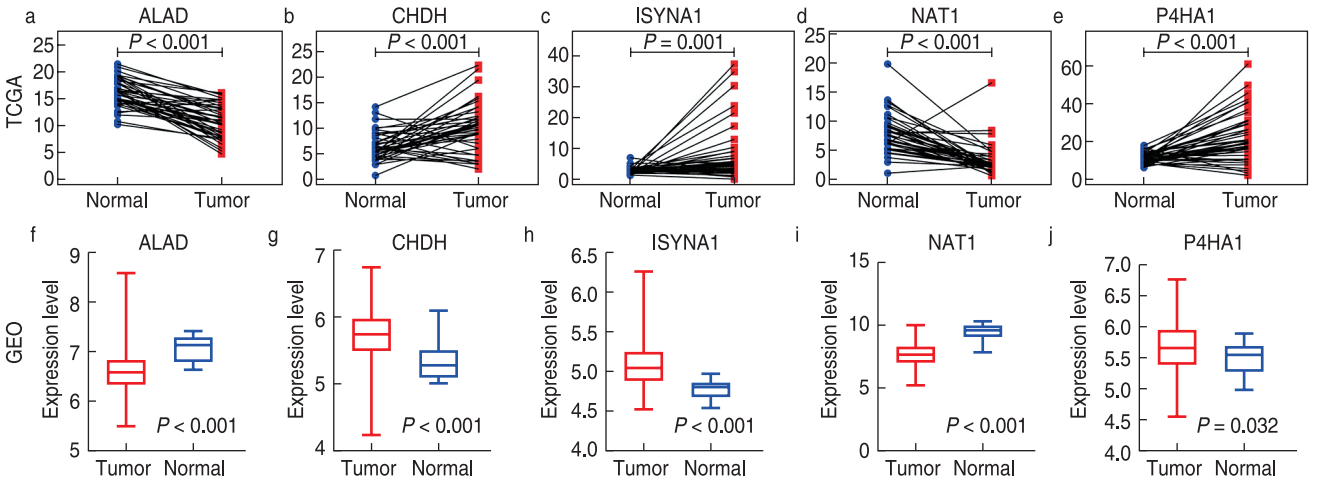


Fig. 7 Validation of five prognostic genes in the TCGA and GEO databases. (a–e) Differential expression of five prognostic genes between paired tumor and normal tissues in the TCGA cohort. (f–j) Differential expression of five prognostic genes in the CRC samples and control samples in GSE39582, and gene expression underwent log2 transformation. GEO: Gene Expression Omnibus database; CRC: colorectal cancer

to the high- or low-risk groups, with the median risk score being the classification criterion. The difference in survival probability between these two groups was statistically significant in both the TCGA ($P < 0.01$; Fig. 8a) and GEO cohorts ($P < 0.01$; Fig. 8b). Patients in the low-risk group were more likely to live longer. In the TCGA cohort, univariate (HR = 3.029, $P < 0.01$; Fig. 8c) and multivariate Cox regression analyses (HR = 2.485, $P < 0.01$; Fig. 8e) showed that the risk score was negatively associated with the overall survival of CRC patients, regardless of confounding factors, such as age, sex, and stage. For the GEO cohort, univariate Cox regression analysis suggested that the overall survival of patients with CRC was significantly related to the risk score (HR = 1.231, $P = 0.026$; Fig. 8d). However, multivariate Cox regression analysis did not yield the same results (HR = 1.076, $P = 0.439$; Fig. 8f). The areas under the ROC curve were 0.744 and 0.569 for the TCGA cohort (Fig. 8g) and GEO cohorts, respectively (Fig. 8h), indicating that the prognostic model was powerful. The difference in the expression levels of the five prognostic genes between the high- and low-risk groups was not statistically significant in the TCGA (Fig. 9a) and GEO cohorts (Fig. 9b). Patients ranked by risk score in the TCGA (Fig. 9c) and GEO (Fig.

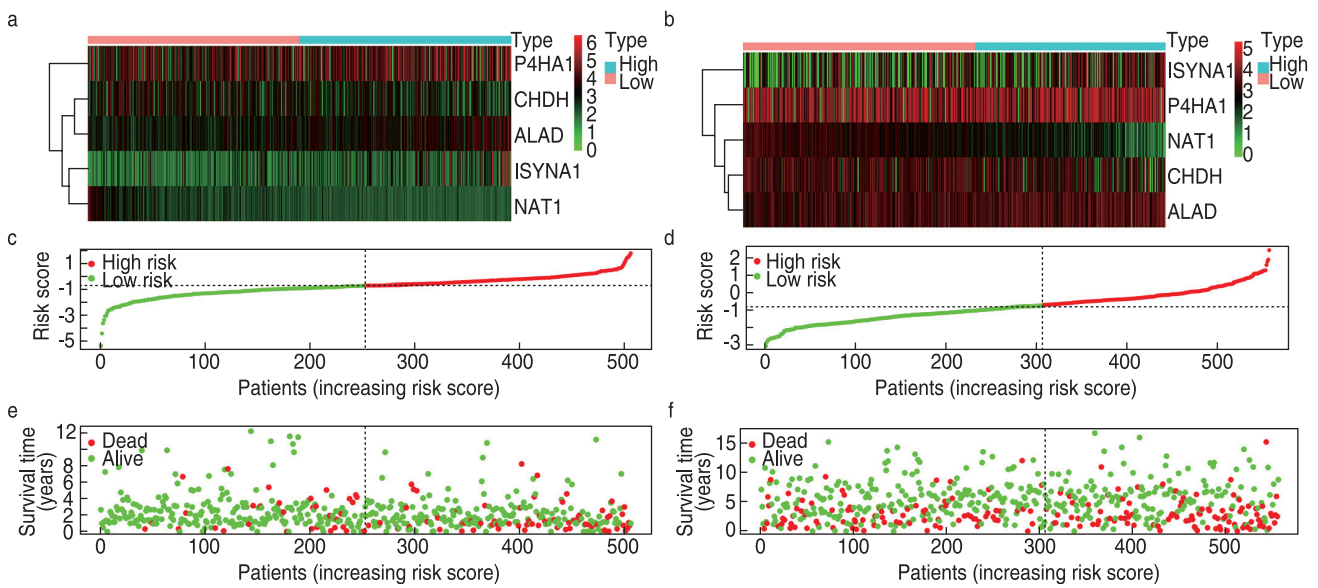


Fig. 9 Risk analyses of the prognostic model. Expression of five prognostic genes in the high- and low-risk groups in the TCGA cohort (a) and GEO cohort (b). Patients ranked by risk scores in the TCGA cohort (c) and GEO cohort (d). Survival status of patients ranked by risk score in the TCGA cohort (e) and GEO cohort (f). TCGA: The Cancer Genome Atlas; GEO: Gene Expression Omnibus database

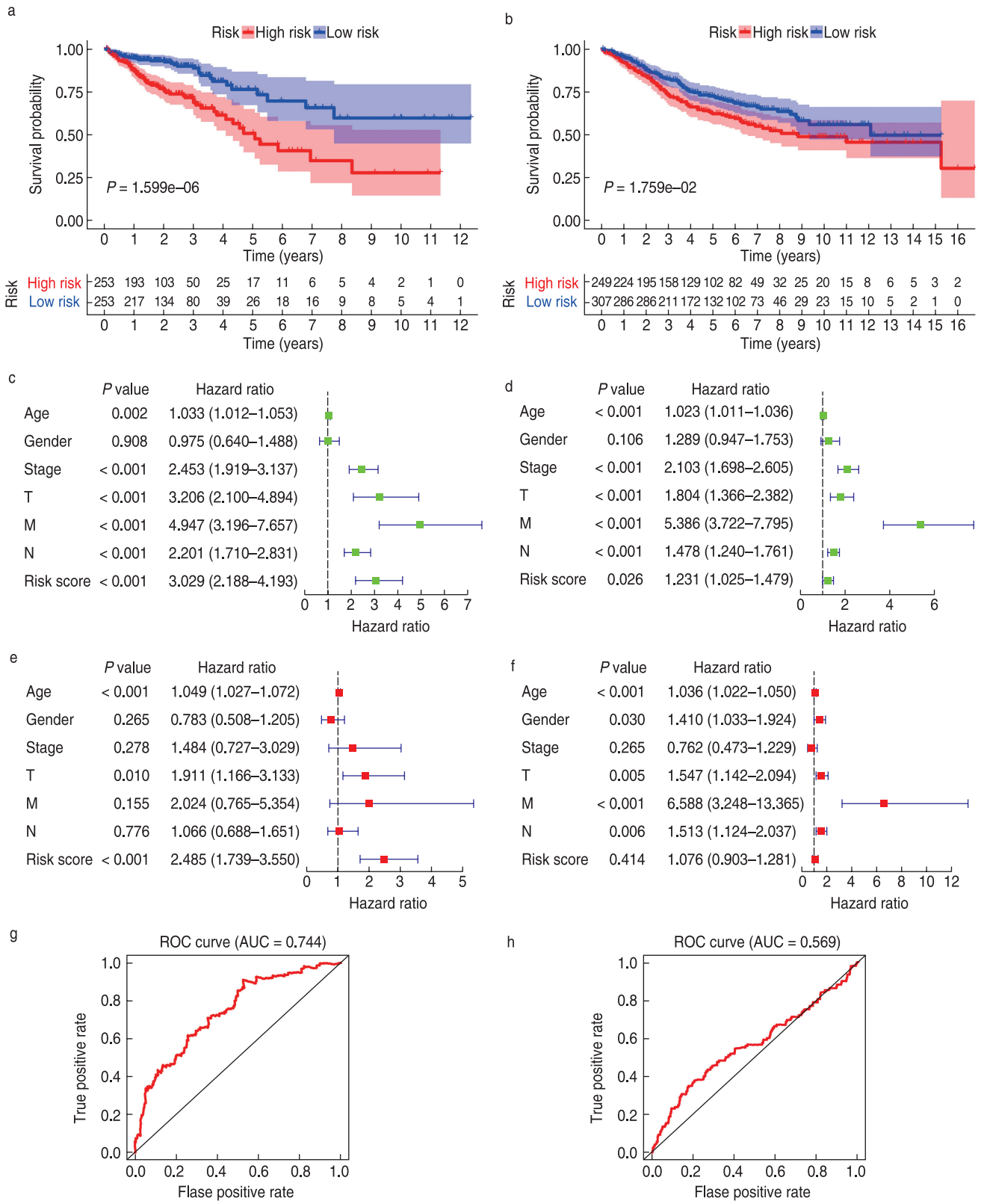


Fig. 8 Construction and verification of the prognostic model. Kaplan-Meier survival curves of the high- and low-risk groups in the TCGA cohort (a) and GEO cohort (b). Univariate Cox regression analysis of risk score and clinicopathological variables in TCGA cohort (c) and GEO cohort (d). Multivariate Cox regression analysis of risk score and clinicopathological variables in the TCGA cohort (e) and GEO cohort (f). ROC curves of the risk score in the TCGA cohort (g) and GEO cohort (h). TCGA: The Cancer Genome Atlas; GEO: Gene Expression Omnibus database

9d) cohorts are displayed. Surviving patients decreased with an increase in the risk score (Fig. 9e and 9f), consistent with the results of the Kaplan-Meier curve and stepwise Cox regression analyses. A nomogram based on the prognostic model was plotted to predict the survival of patients with CRC (Fig. 10a), and calibration curves showed that the predicted survival of the nomogram was consistent with actual survival (Fig. 10b and 10c).

Discussion

CRC accounts for a large porportion of gastrointestinal tumors and poses a huge threat to global health. The overall survival of patients with CRC depends on many risk factors. Recently, numerous prognostic biomarkers have been developed for CRC, but only a few of them have been applied clinically. Therefore, it is necessary to identify more potential prognostic factors. Cancer metabolism is an important segment of the malignant transition. The link between the gut microbiome and colon carcinogenesis may also be mediated by altered metabolism [14]. Numerous studies have confirmed the prognostic value of metabolism-associated genes in various tumors. Therefore, there is a need to explore the metabolism-associated genes that play a role in the outcome of patients with CRC.

In this study, using TCGA data, we conducted an integrative analysis to offer a well-rounded landscape of 961 metabolism-associated genes in CRC. The possible mechanisms underlying oncogenesis were explored using functional enrichment and PPI network analyses. Additionally, we identified five prognostic metabolism-associated genes (*ALAD*, *CHDH*, *ISYNA1*, *NAT1*, and *P4HA1*) through stepwise statistical analyses and

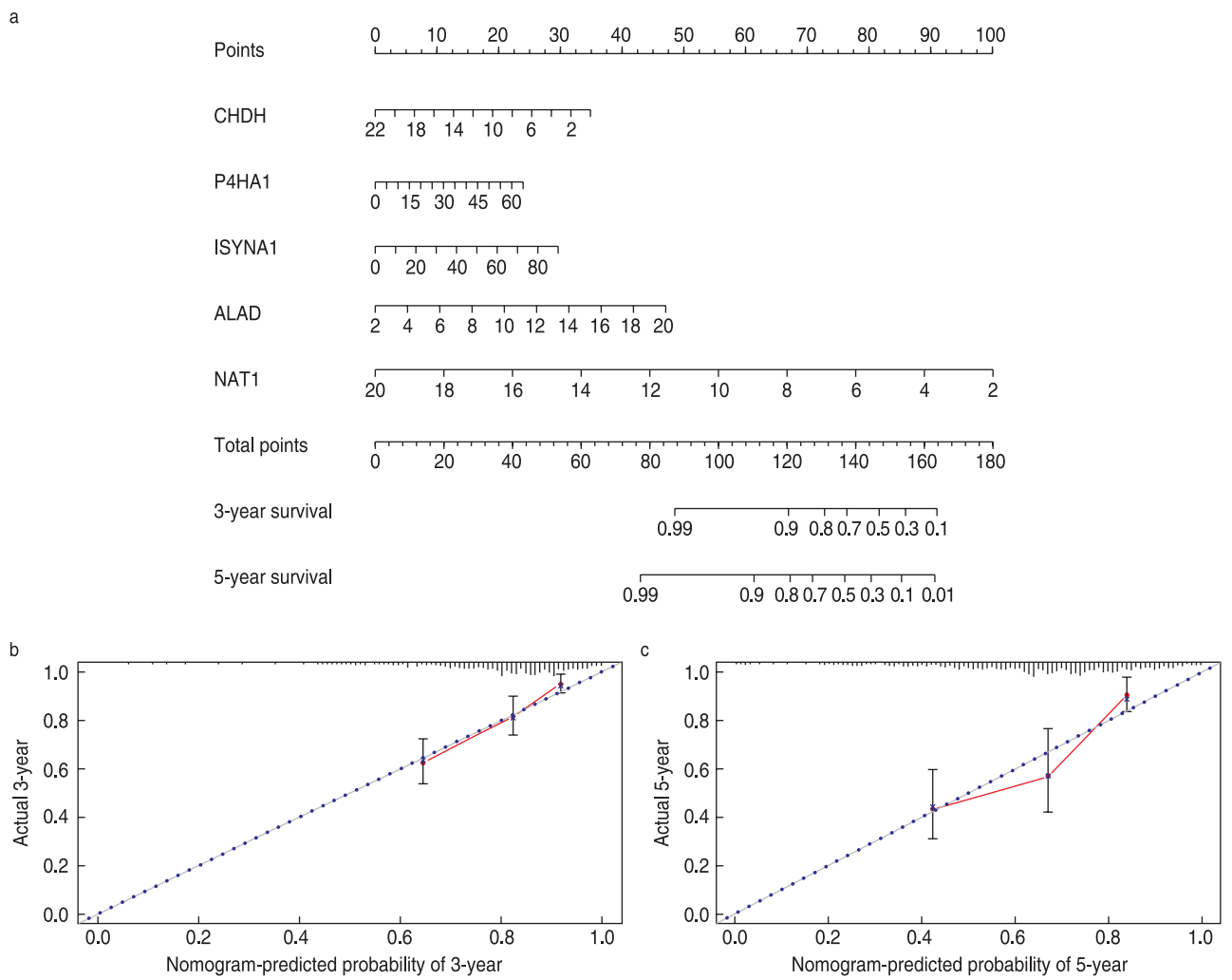


Fig. 10 Nomogram and calibration curves of the prognostic model. (a) Nomogram based on five prognostic genes for predicting the 3-year and 5-year overall survival probability of patients with colorectal cancer. (b) A 3-year calibration plot of the nomogram. (c) A 5-year calibration plot of the nomogram

constructed a prognostic model that performed well in the GEO dataset.

Functional enrichment analysis of differentially expressed metabolism-associated genes revealed that these genes are closely related to the biosynthesis and metabolism of nucleoside phosphate, ribose phosphate, DNA, and RNA polymerase, and the metabolism of lipids and acids. The involvement of nucleotide metabolism has been illustrated in senescence [15], which could determine cancer cell fate. There is increasing evidence that lipid metabolism often affects cancer cells in different ways [16–19]. Cancer cells have an added demand for amino acids and fatty acids to aid their rapid proliferation and increased communication. An earlier study also demonstrated that higher expression levels of genes involved in DNA replication were related to poorer survival in patients with CRC [20]. These findings imply that the functions and pathways discovered in our study are worth exploring.

Given that proteins are the direct mediators of vital biological processes, genes screened from the PPI network are more likely to provide crucial functions and are considered key genes. The node degree of a protein represents the number of proteins with which they interact. None of the five prognostic genes in this study ranked among the genes with the highest node degrees in the PPI network; this could be explained by the paucity of studies on these genes. Thus, future studies must focus on how these genes are involved.

The prognostic genes identified in our study have been shown to impact tumor development in different ways. ALAD is also known as aminolevulinate dehydratase, and its major function is to synthesize heme and inhibit the 26S proteasome. A recent study suggested that ALAD expression level was lower in breast cancer tissues than in normal breast tissues. Increased *ALAD* expression level was correlated with longer disease-free survival of patients with breast cancer, which could be caused by inhibiting the epithelial-mesenchymal transition [21]. Other studies have indicated that genetic variation in *ALAD* is related to the risk of urologic neoplasms and brain tumors [22–23]. In our study, *ALAD* expression negatively affected the overall survival of colorectal cancer patients. Choline dehydrogenase (CHDH), found in the mitochondria, participates in the mitophagy and transformation of betaine aldehyde [24]. A study showed that *ALAD* had positive effects on the overall survival of patients with head and neck squamous cell carcinoma [25]. It could be hypothesized that *ALAD* functions as a tumor suppressor gene. Additionally, *CHDH* variants were correlated with the risk of pancreatic cancer [26].

In our study, inositol-3-phosphate synthase 1 (ISYNA1) was upregulated in colorectal carcinomas compared to that in para-tumor tissues, and its expression was correlated with poorer overall survival. Research evidence has also demonstrated that the mRNA level of ISYNA1 is higher in bladder carcinomas than in para-tumor tissues and that patients with higher expression level of ISYNA1 tended to have higher pathological grades [27]. *ISYNA1* functions as a regulator of proliferation and apoptosis [27]. Additionally, low *ISYNA1* expression level indicated poorer prognoses for patients with pancreatic cancer, which was correlated with p21 inhibition [28]. It is also worth mentioning that *ISYNA1* is associated with the p53 mutation in several tumors, which indicates its significant role in tumorigenesis [29].

NAT1 (N-acetyltransferase 1) can metabolize carcinogens, and its impact on tumor development has been elucidated in numerous studies. Zhao *et al* found that patients with luminal breast cancer had higher expression level of *NAT1* and that *NAT1* could facilitate bone metastasis via a downstream pathway [30]. *NAT1* was also an indicator of response to chemotherapy in patients with breast cancer [31]. Shi *et al* discovered that high expression level of *NAT1* could predict longer overall survival of patients with colon adenocarcinoma through the analyses of the RNA-seq dataset of colon adenocarcinoma (COAD) in TCGA [32]. The positive effect of *NAT1* on the prognosis of patients with CRC was shown by univariate and multivariate Cox regression analyses in our study. Thus, we hypothesized that *NAT1* could function as a tumor suppressor gene in CRC. The functions of *NAT1* in carcinogenesis have also been indicated in bladder cancer and pediatric acute lymphoblastic leukemia [33–34]. However, the underlying mechanisms have not been explored in depth.

As a key gradient of prolyl 4-hydroxylase, P4HA1 (prolyl 4-hydroxylase subunit alpha 1) is essential for collagen synthesis. *P4HA1* is necessary for tumor development. *P4HA1* was demonstrated to regulate the stemness of breast cancer cells and accelerate distant metastasis [35]. Another study on pancreatic cancer showed that the P4HA1 knockdown could reduce stemness in cancer cells and enhance the response to chemotherapy [36]. *P4HA1* has also been shown to be correlated with unfavorable outcomes in patients with high-grade gliomas and head and neck squamous cell carcinoma [37–38]. A recent study demonstrated that the proliferation and invasion of cancer cells could be remarkably promoted by *P4HA1*, and the malignancy of CRC cells could be reduced by *P4HA1* inhibition [39]. However, the prognostic value of *P4HA1* in CRC has not yet been verified. Our study revealed that P4HA1 was upregulated in CRC tissues and that patients with higher P4HA1 expression level had poorer outcomes.

Although all five genes affected overall survival, the effect of a single gene on patient survival was limited. Because it is far from sufficient for one gene to predict patient survival, we constructed a prediction model based on the prognostic genes. Based on the prognostic genes, a

nomogram was used to predict the survival of patients with CRC. The prognostic model we established performed well in both TCGA and GEO cohorts. Overall, we explored the underlying mechanisms of the differentially expressed metabolism-associated genes in CRC, identified five prognostic genes (*ALAD*, *CHDH*, *ISYNA1*, *NAT1*, and *P4HA1*), and constructed a prognostic model via a series of bioinformatics analyses. Although some studies have demonstrated the roles of *CHDH*, *P4HA1*, *ISYNA1*, *ALAD*, and *NAT1* in tumor initiation and progression, few of them have studied the prognostic value of these genes in CRC. The limitation of our study was that all conclusions are drawn from data in public databases, and as such, *in vivo* and *in vitro* experiments were required for further verification and mechanistic exploration. However, our work provides insight into metabolism-associated genes in CRC from multiple perspectives and will lay the foundation for further studies.

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Conflict of interest

The authors indicated no potential conflicts of interest.

Author contributions

Conceptualization: LY Xiao and B Liu; Design and methodology: LY Xiao, YB Huang, W Qin, CF Liu, H Qiu, and B Liu; Data analysis and figure plotting: LY Xiao, YB Huang, and W Qin; Writing-original draft: LY Xiao and B Liu; Writing-review & editing: H Qiu, B Liu, and XL Yuan; Supervision: B Liu and XL Yuan.

Data availability statement

The datasets analyzed in this study are available from TCGA (https://portal.gdc.cancer.gov/) and GEO (http://www.ncbi.nlm.nih.gov/geo).

Ethical approval

Not applicable.

References

1. Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. Lancet. 2019;394(10207):1467-1480.
2. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
3. Liu SW, Ma R, Cao HX, et al. Prognostic significance of PD-L1 expression in patients with colorectal cancer: a meta-analysis. Oncol Transl Med. 2019;5(2):68-74.
4. Yu QQ, Qiu H, Zhang MS, et al. Prognostic value of neutrophil-to-lymphocyte ratio in metastatic colorectal cancer. Oncol Transl Med. 2016;2(2):75-79.
5. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-674.
6. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metab. 2016;23(1):27-47.
7. Cohen AL, Holmen SL, Colman H. IDH1 and IDH2 mutations in gliomas. Curr Neurol Neurosci Rep. 2013;13(5):345.
8. Kang S, Kim EH, Hwang JE, et al. Prognostic significance of high metabolic activity in breast cancer: PET signature in breast cancer. Biochem Biophys Res Commun. 2019;511(1):185-191.
9. Karasinska JM, Topham JT, Kalloger SE, et al. Altered gene expression along the glycolysis-cholesterol synthesis axis is associated with outcome in pancreatic cancer. Clin Cancer Res. 2020;26(1):135-146.
10. Montrose DC, Zhou XK, Kopelovich L, et al. Metabolic profiling, a noninvasive approach for the detection of experimental colorectal neoplasia. Cancer Prev Res (Phila). 2012;5(12):1358-1367.
11. Brown DG, Rao S, Weir TL, et al. Metabolomics and metabolic pathway networks from human colorectal cancers, adjacent mucosa, and stool. Cancer Metab. 2016;4:11.
12. Denkert C, Budczies J, Weichert W, et al. Metabolite profiling of human colon carcinoma – deregulation of TCA cycle and amino acid turnover. Mol Cancer. 2008;7:72.
13. Yuan Y, Chen J, Wang J, et al. Development and clinical validation of a novel 4-gene prognostic signature predicting survival in colorectal cancer. Front Oncol. 2020;10:595.
14. Datta S, Chowdhury S, Roy HK. Metabolism, microbiome and colorectal cancer. Aging (Albany NY). 2017;9(4):1086-1087.
15. Aird KM, Zhang G, Li H, et al. Suppression of nucleotide metabolism underlies the establishment and maintenance of oncogene-induced senescence. Cell Rep. 2013;3(4):1252-1265.
16. Abramson HN. The lipogenesis pathway as a cancer target. J Med Chem. 2011;54(16):5615-5638.
17. Santos CR, Schulze A. Lipid metabolism in cancer. FEBS J. 2012;279(15):2610-2623.
18. Röhrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. Nat Rev Cancer. 2016;16(11):732-749.
19. Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. Nature. 2012;491(7424):364-373.
20. Pillaire MJ, Selves J, Gordien K, et al. A 'DNA replication' signature of progression and negative outcome in colorectal cancer. Oncogene. 2010;29(6):876-887.
21. Ge J, Yu Y, Xin F, et al. Downregulation of delta-aminolevulinate dehydratase is associated with poor prognosis in patients with breast cancer. Cancer Sci. 2017;108(4):604-611.
22. Neslund-Dudas C, Levin AM, Rundle A, et al. Case-only gene-environment interaction between ALAD tagSNPs and occupational lead exposure in prostate cancer. Prostate. 2014;74(6):637-646.
23. Rajaraman P, Stewart PA, Samet JM, et al. Lead, genetic susceptibility, and risk of adult brain tumors. Cancer Epidemiol Biomarkers Prev. 2006;15(12):2514-2520.
24. Park S, Choi SG, Yoo SM, et al. Choline dehydrogenase interacts with SQSTM1/p62 to recruit LC3 and stimulate mitophagy. Autophagy. 2014;10(11):1906-1920.
25. Wu ZH, Tang Y, Zhou Y. A metabolic gene signature to predict overall survival in head and neck squamous cell carcinoma. Mediators Inflamm. 2020;2020:6716908.

26. Chittiboyina S, Chen Z, Chiorean EG, et al. The role of the folate pathway in pancreatic cancer risk. PLoS One. 2018;13(2):e0193298.
27. Guo X, Li HH, Hu J, et al. ISYNA1 is overexpressed in bladder carcinoma and regulates cell proliferation and apoptosis. Biochem Biophys Res Commun. 2019;519(2):246-252.
28. Zhou L, Sheng W, Jia C, et al. Musashi2 promotes the progression of pancreatic cancer through a novel ISYNA1-p21/ZEB-1 pathway. J Cell Mol Med. 2020;24(18):10560-10572.
29. Koguchi T, Tanikawa C, Mori J, et al. Regulation of myo-inositol biosynthesis by p53-ISYNA1 pathway. Int J Oncol. 2016;48(6):2415-2424.
30. Zhao C, Cai X, Wang Y, et al. NAT1 promotes osteolytic metastasis in luminal breast cancer by regulating the bone metastatic niche via NF-κB/IL-1B signaling pathway. Am J Cancer Res. 2020;10(8):2464-2479.
31. Minchin RF, Butcher NJ. Trimodal distribution of arylamine N-acetyltransferase 1 mRNA in breast cancer tumors: association with overall survival and drug resistance. BMC Genomics. 2018;19(1):513.
32. Shi C, Xie LY, Tang YP, et al. Hypermethylation of N-Acetyltransferase 1 is a prognostic biomarker in colon adenocarcinoma. Front Genet. 2019;10:1097.
33. Hernández-González O, Ortiz-Zamudio JJ, Rodríguez-Pinal CJ, et al. Genetic polymorphisms of arylamine N-acetyltransferases 1 and 2 and the likelihood of developing pediatric acute lymphoblastic leukemia. Leuk Lymphoma. 2018;59(8):1968-1975.
34. El Kawak M, Dhaini HR, Jabbour ME, et al. Slow N-acetylation as a possible contributor to bladder carcinogenesis. Mol Carcinog. 2020;59(9):1017-1027.
35. Xiong G, Stewart RL, Chen J, et al. Collagen prolyl 4-hydroxylase 1 is essential for HIF-1α stabilization and TNBC chemoresistance. Nat Commun. 2018;9(1):4456.
36. Cao XP, Cao Y, Li WJ, et al. P4HA1/HIF1α feedback loop drives the glycolytic and malignant phenotypes of pancreatic cancer. Biochem Biophys Res Commun. 2019;516(3):606-612.
37. Hu WM, Zhang J, Sun SX, et al. Identification of P4HA1 as a prognostic biomarker for high-grade gliomas. Pathol Res Pract. 2017;213(11):1365-1369.
38. Li Q, Shen Z, Wu Z, et al. High P4HA1 expression is an independent prognostic factor for poor overall survival and recurrent-free survival in head and neck squamous cell carcinoma. J Clin Lab Anal. 2020;34(3):e23107.
39. Agarwal S, Behring M, Kim HG, et al. Targeting P4HA1 with a small molecule inhibitor in a colorectal cancer PDX model. Transl Oncol. 2020;13(4):100754.

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