

Igniting cold tumors of intrahepatic cholangiocarcinoma: An insight into immune evasion and tumor immune microenvironment

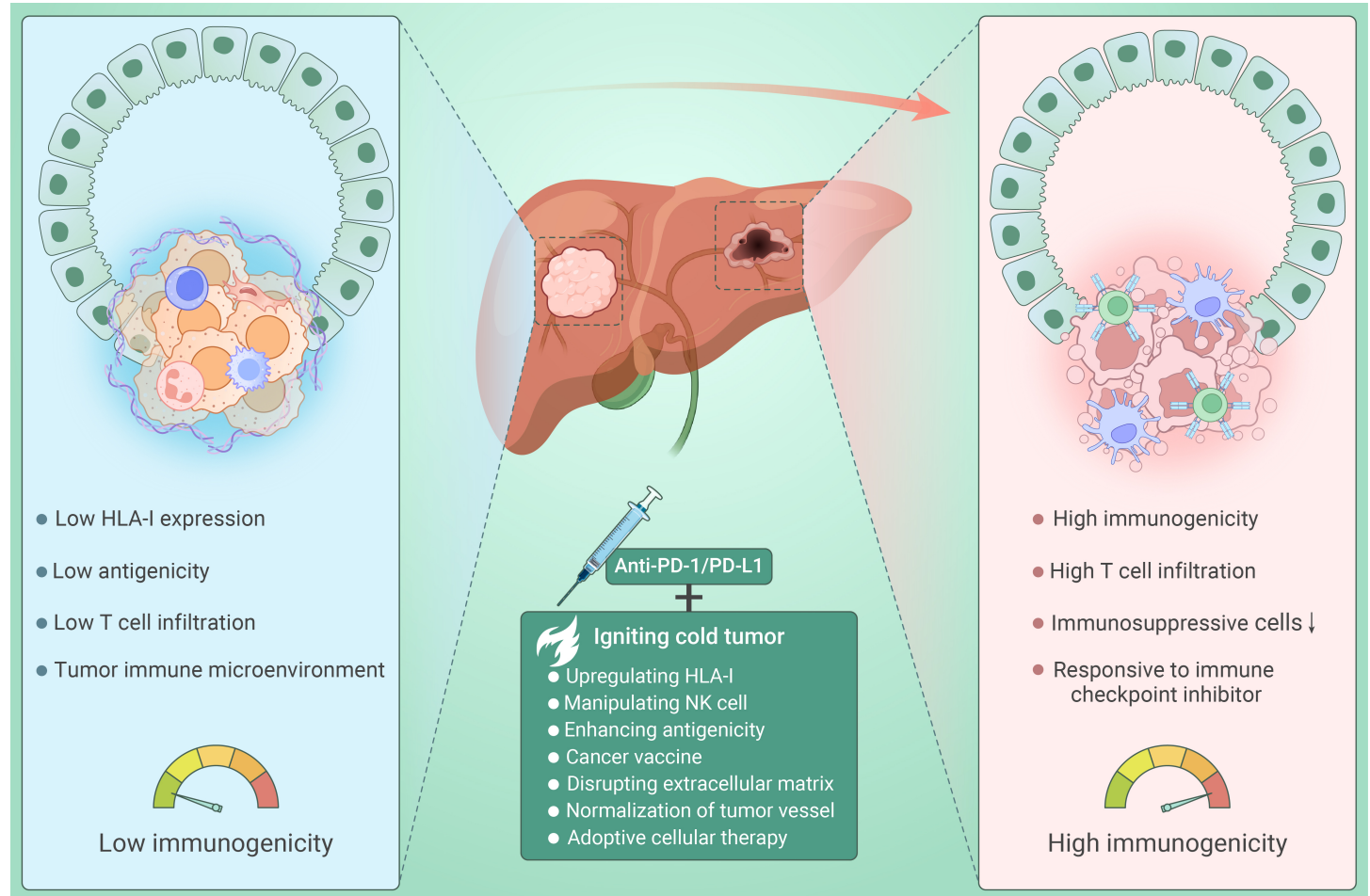
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Most intrahepatic cholangiocarcinomas (ICCs) present as cold tumors.
- Immune evasion contributes to the cold phenotype of ICCs.
- Cold ICCs are associated with an immunosuppressive tumor immune microenvironment.
- Igniting cold ICCs can potentiate the efficacy of immunotherapy.

Igniting cold tumors of intrahepatic cholangiocarcinoma: An insight into immune evasion and tumor immune microenvironment

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Intrahepatic cholangiocarcinoma (ICC) is a rare hepatobiliary cancer that originates from the epithelium of the intrahepatic bile duct. The various treatments for ICC, such as chemotherapy, radiotherapy, and locoregional therapy, confer only modest improvements in survival rates. Immunotherapy, although revolutionary in cancer treatment, has found limited application in the treatment of ICCs due to the “cold” nature of these tumors, which is marked by scant T-cell infiltration. This characteristic makes immune checkpoint inhibitors (ICIs) unsuitable for the majority of ICC patients. Therefore, comprehensively understanding the mechanisms underlying these “cold” tumors is crucial for harnessing the potential of immunotherapy for treating ICC patients. This paper explores immune evasion mechanisms and the complex tumor immune microenvironment of ICC. This study provides a comprehensive overview of therapeutic strategies aimed at activating cold tumors and enhancing their immunogenicity. Furthermore, potential and promising targets for cancer vaccines and adoptive cellular therapy in the context of ICC are discussed. This endeavor strives to reveal new pathways for innovative immunotherapy strategies, with a focus on overcoming the key challenge of triggering an effective immune response in ICC patients.

INTRODUCTION

Biliary tract cancer (BTC) remains a significant health challenge, particularly in regions with a high prevalence. BTC can be anatomically classified into subtypes, including intrahepatic, perihilar, and distal carcinoma as well as gallbladder cancer, each of which presents unique clinical complexities.¹ The incidence of intrahepatic cholangiocarcinoma (ICC) is on the rise, presenting distinct challenges. Patients with early-stage ICC often remain asymptomatic, leading to difficulties in early detection.^{1–3} While surgery is the cornerstone of treatment, its applicability is limited.⁴ Despite the availability of diverse therapeutic approaches, such as chemotherapy, radiotherapy, and locoregional therapy, the prognosis for a considerable proportion of ICC patients with locally advanced and/or metastatic disease remains poor.^{1,5,6} This situation underscores an urgent, unmet need for the development of effective systemic therapies that can meaningfully bridge this clinical gap.

The advent of immune checkpoint inhibitors (ICIs) has ushered in a new era of cancer therapy, transforming the treatment landscape. However, the application of ICIs for treating ICC has yielded only incremental benefits.⁷ In contrast to cancers such as non-small-cell lung cancer, melanoma, and colorectal cancer, which respond favorably to immunotherapy, understanding the poor response of ICC to ICIs could unveil novel therapeutic targets for advancing immunotherapy.^{8–10} ICC is characterized by deficient T-cell infiltration and is an immunologically “cold” cancer type, which severely impedes the effectiveness of ICIs since restoring T-cell function is central to the

success of ICI treatment. In addition to the limited T-cell presence, cold ICCs exhibit reduced antigenicity and immunogenicity.^{2,11} These attributes collectively contribute to the shortcomings of ICI monotherapy for treating ICC.¹² Acknowledging that tumor tissue is a complex, organized ecosystem comprising malignant cells, the tumor immune microenvironment (TIME) encapsulates immunological components within the tumor milieu.^{13–15} In cold ICCs, the TIME is characterized by the prevalence of immunosuppressive cell populations, and sophisticated cell–cell interactions orchestrated by molecular pathways intricately shape the response to immunotherapy.^{16,17} Together, the mechanisms of immune evasion and the intricate TIME cooperate to define the cold microenvironment intrinsic to ICC. Gaining a more nuanced understanding of this phenotype could help to illuminate the resistance mechanisms underpinning existing immunotherapy strategies, ultimately steering the design of innovative methods to invigorate this immunologically cold tumor.

In this review, we explore the mechanisms contributing to the cold phenotype of ICC, shedding light on the specific immune escape and TIME characteristics of these tumors. Furthermore, we consider emerging immunotherapy strategies capable of redefining ICC as an entity with enhanced immunogenicity. In addition, we discuss for the first time potential therapeutic targets yet to be investigated within the context of ICC. This endeavor broadens the horizons of immunotherapy, introducing additional therapeutic options to augment the efficacy of ICIs.

MOST ICCs PRESENT AS COLD TUMORS

The prevailing theory describing the immunophenotypes of the tumor microenvironment involves three distinct profiles: the immune-desert, immune-excluded, and immune-inflamed phenotypes.¹¹ In the immune-desert phenotype, a conspicuous absence of CD8⁺ T cells characterizes the TIME. Conversely, in the immune-excluded phenotype, immune cells are present but predominantly confined to the tumor stroma, with limited infiltration into the tumor core.¹⁸ Tumors with the immune-desert or immune-excluded phenotype are collectively known as cold tumors and exhibit resistance to ICIs. In stark contrast, tumors with the immune-inflamed phenotype, often termed “hot tumors”, exhibit promising clinical outcomes in response to ICI therapy (Figure 1).¹⁸

The efficacy of ICI therapy hinges on the reactivation of CD8⁺ T cells within the tumor. Consequently, the limited infiltration of CD8⁺ T cells in cold tumors restricts the effectiveness of ICI therapy. Intriguingly, studies have revealed that the immune-desert phenotype is prevalent in approximately 45% of ICCs.² The immune-excluded type accounts for approximately 42.7%.¹⁹ These findings underscore that a substantial proportion of ICCs are characterized as cold tumors. In these cold ICCs, gene signatures related to acquired immu-

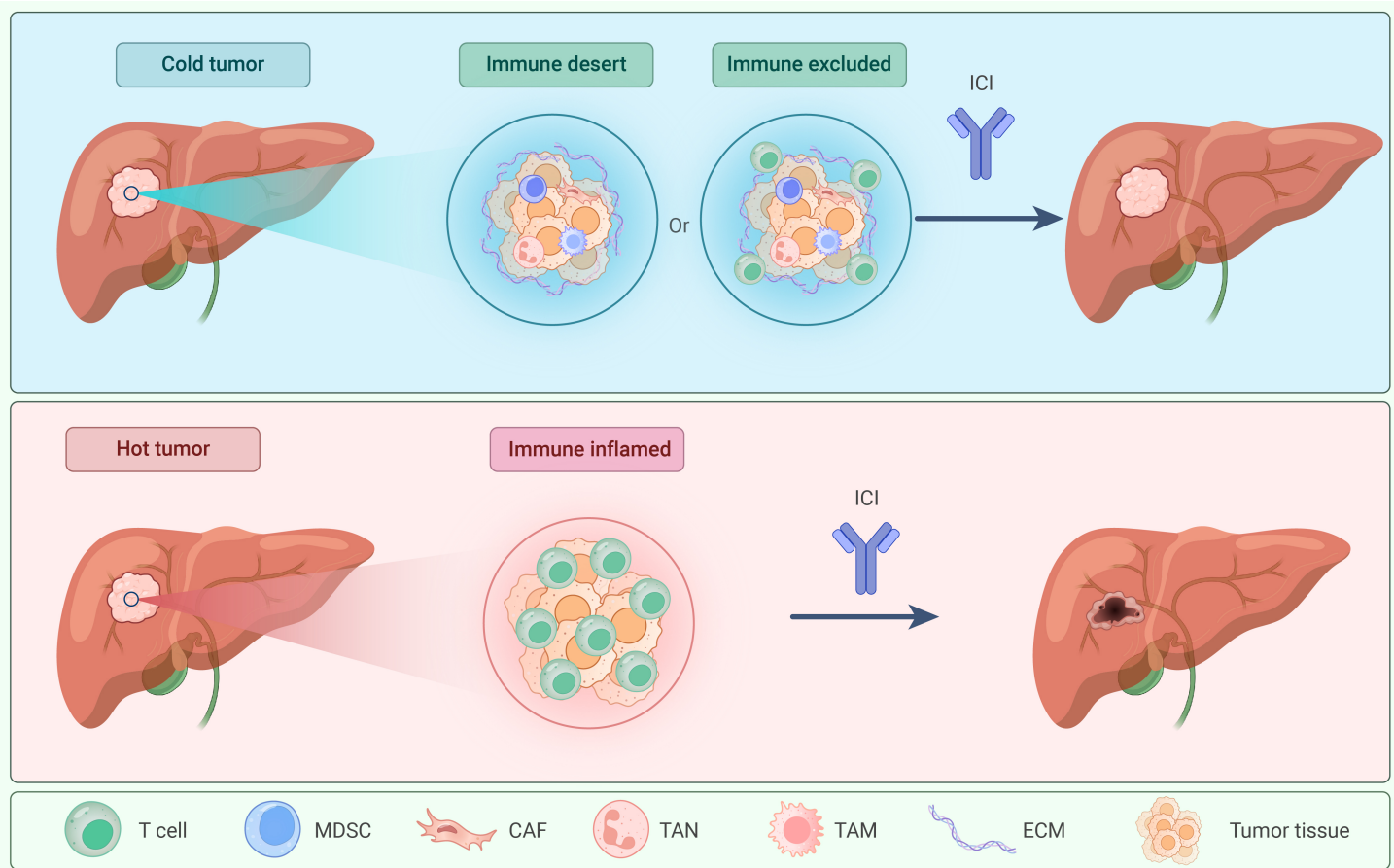


Figure 1. Characteristics of the three immunophenotypes of ICC As shown in the left panel, cold tumors, which exhibit an immune-desert phenotype or immune-excluded phenotype, exhibit an unfavorable response to ICI therapy. The main trait of the immune-desert phenotype is the paucity of CD8⁺ T cells in the tumor microenvironment. The immune-excluded phenotype exhibits abundant immune cells, which are restricted to the tumor stroma. Cold tumors account for a large proportion of ICCs. As shown in the right panel, a “hot tumor”, also referred to as the immune-inflamed phenotype, is associated with favorable outcomes after ICI therapy. The immune-inflamed phenotype shows abundant immune cell infiltration inside the tumor core. ICC, intrahepatic cholangiocarcinoma; ICI, immune checkpoint inhibitor.

nity and monocytic lineages are notably deficient. Additionally, cell populations indicative of immunologically cold microenvironments predominate in the ICC landscape.²⁰ Parallel investigations have further illuminated the immunological backdrop of cold ICCs, revealing scant T-cell infiltration at the tumor margin and an absence of immune cells within the tumor mass itself.² In line with these observations, the overarching pattern suggests that a majority of ICCs exhibit characteristics typical of cold tumors. Functionally, ICCs demonstrate considerable attenuation of both tumor and stromal immune signaling, reinforcing the concept of an immunological desert.² This body of evidence collectively strengthens the assertion that most ICCs exhibit traits emblematic of cold tumors and characterized by compromised antitumor immune responses.

IMMUNE EVASION MEDIATED BY COLD TUMORS

In addition to sparse T-cell infiltration, cold tumors exhibit characteristics such as low levels of HLA-I and reduced antigenicity. These features contribute to the immune evasion of cold tumors. Consequently, both the number and function of T cells in cold tumors decrease, leading to a poor response to ICIs.¹⁷ Understanding the acquisition of immune evasion mechanisms is conducive to the development of novel immunotherapy strategies aimed at activating cold ICCs. In this chapter, we explore how ICCs employ these strategies to establish a cold tumor phenotype.

Defects in HLA-I and antigenicity

Cancer cells become recognizable to tumor-specific CD8⁺ T cells through the recognition of peptide-HLA-I complexes. Thus, a deficiency in HLA-I expression could be a critical factor hindering the identification of tumor antigens and ultimately leading to immune evasion. The downregulation or absence of HLA-I is a recurrent observation in ICCs. In an *in vitro* study, two

types of cholangiocarcinoma (CCA) cells, CC-SW-1 and CC-LP-1, exhibited HLA-I expression in 60% and 45% of cells, respectively, while no HLA-I expression was detected in the remaining cells. Similarly, HLA-I deficiency has been detected in patient samples.²¹ A further investigation of 27 ICC patients revealed that 16 had reduced HLA-I expression, whereas 6 had no detectable HLA-I expression. In contrast to tumors with higher HLA-I levels, tumors with reduced HLA-I expression were associated with more advanced disease stages. Additionally, a correlation between CD8⁺ T-cell presence and HLA-I expression has been observed in fibrous septa and tumor lobules.²² Another study reinforced these findings by establishing a similar connection between the outer border area of ICC tissue, HLA-I expression, and the number of CD8⁺ T cells.²³

The composition of HLA-I includes the invariant β 2-microglobulin protein (B2M) gene and three HLA genes (*HLA-A*, *HLA-B* and *HLA-C*). Tumor cells employ various genetic, transcriptional, and posttranslational mechanisms to manipulate components of the antigen-processing machinery (APM), ultimately leading to reduced HLA-I expression.²⁴ Notably, the loss of heterozygosity (LOH) in HLA-I is a frequently observed feature of tumors that is associated with immunosurveillance evasion. This process leads to the loss of certain HLA-I allotypes, potentially leading to diminished diversity in the peptide repertoire presented.²⁴ Clinicogenomic data for various cancers have revealed that HLA-I LOH varies among cancer types, with 17% of patients exhibiting HLA-I LOH. Remarkably, in CCA, the prevalence of HLA-I LOH ranges from 10%-20%.²⁵ In a comprehensive study, Lin *et al.*²⁶ classified the tumor microenvironment of ICC into three subtypes based on immune infiltration: sparsely infiltrated, heterogeneously infiltrated, and highly infiltrated. The authors found the frequency of HLA-I LOH to be the highest in highly infiltrated tumors (71.4%), followed by heterogeneously infiltrated tumors (34.6%) and sparsely infiltrated tumors (33.3%), indicating that HLA-I LOH is a

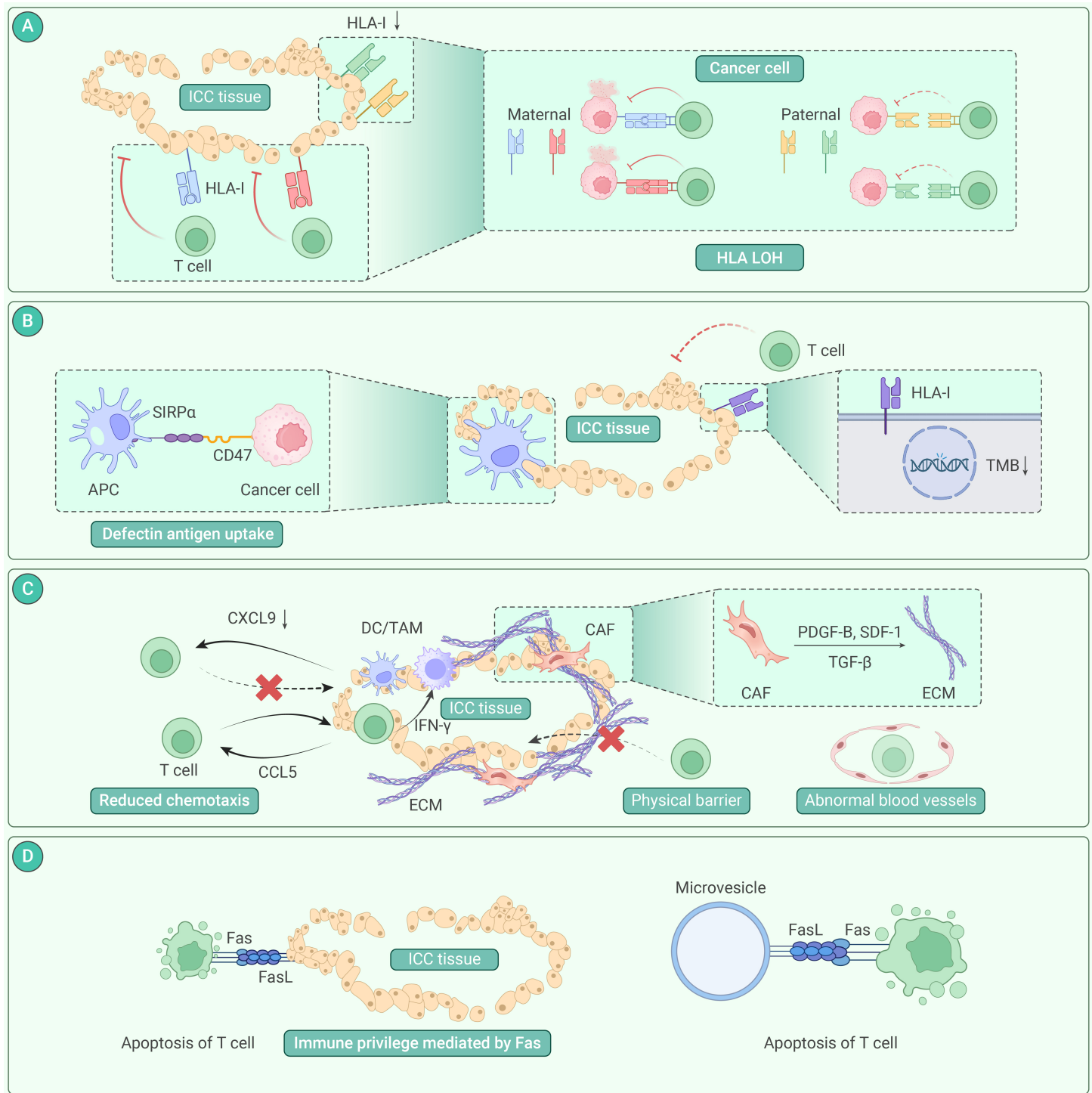


Figure 2. Mechanisms of immune evasion in cold ICCs (A) HLA LOH is expected to result in a reduction in the diversity of the repertoire of presented peptides, thereby impeding the onset of the immune response. (B) Defects in antigen uptake mediated by the CD47/SIRP α axis decrease antigen presentation. Additionally, ICCs with a low TMB are associated with a low neoantigen load, which prevents T cells from recognizing tumor cells. (C) Tumor cell-derived CCL5 enables T-cell trafficking in tumors. Despite the necessity of this approach, the expression of CCL5 by tumor cells is insufficient. IFN- γ released by T cells further activates TAMs and DCs to produce CXCL9. However, the role of CXCL9 as an amplifier of T-cell engraftment in tumors is dampened. Low perfusion and slack endothelial cells lead to poor T-cell infiltration into the tumor. A dense ECM in tumor tissues hinders the infiltration of T cells into the tumor core. (D) Cancer cells express FasL to induce apoptosis in Fas-bearing T cells. Similarly, microvesicles containing functional FasL have been shown to induce T-cell apoptosis. ICC, intrahepatic cholangiocarcinoma; HLA, human leukocyte antigen; LOH, loss of heterozygosity; APC, antigen-presenting cell; SIRP α , signal regulatory protein- α ; TMB, tumor mutation burden; DC, dendritic cell; TAM, tumor-associated macrophage; ECM, extracellular cellular matrix; CAF, cancer-associated fibroblast; PDGF-B, platelet-derived growth factor-B; SDF-1, stromal-derived factor-1; TGF- β , transforming growth factor- β .

recurrent phenomenon in ICC. Loss of B2M copy number was shown to play a role in the loss of surface HLA-I expression, particularly in highly infiltrated tumors (Figure 2A).

During the antigen processing pathway, proteasomes break down endogenous proteins into peptides that are subsequently transported into the endoplasmic reticulum (ER) via transporters associated with antigen processing

(TAPs). In the ER, these peptides are loaded onto HLA-I molecules. Consequently, disruptions in TAP can impede antigen processing.²⁴ In ICC, a decrease in the copy number of *TAP1/TAP2* has been identified as a factor that hampers the antigen presentation process.²⁶

Defects in antigen uptake. CD47, a transmembrane protein, has garnered increased amounts of attention due to its widespread overexpression in vari-

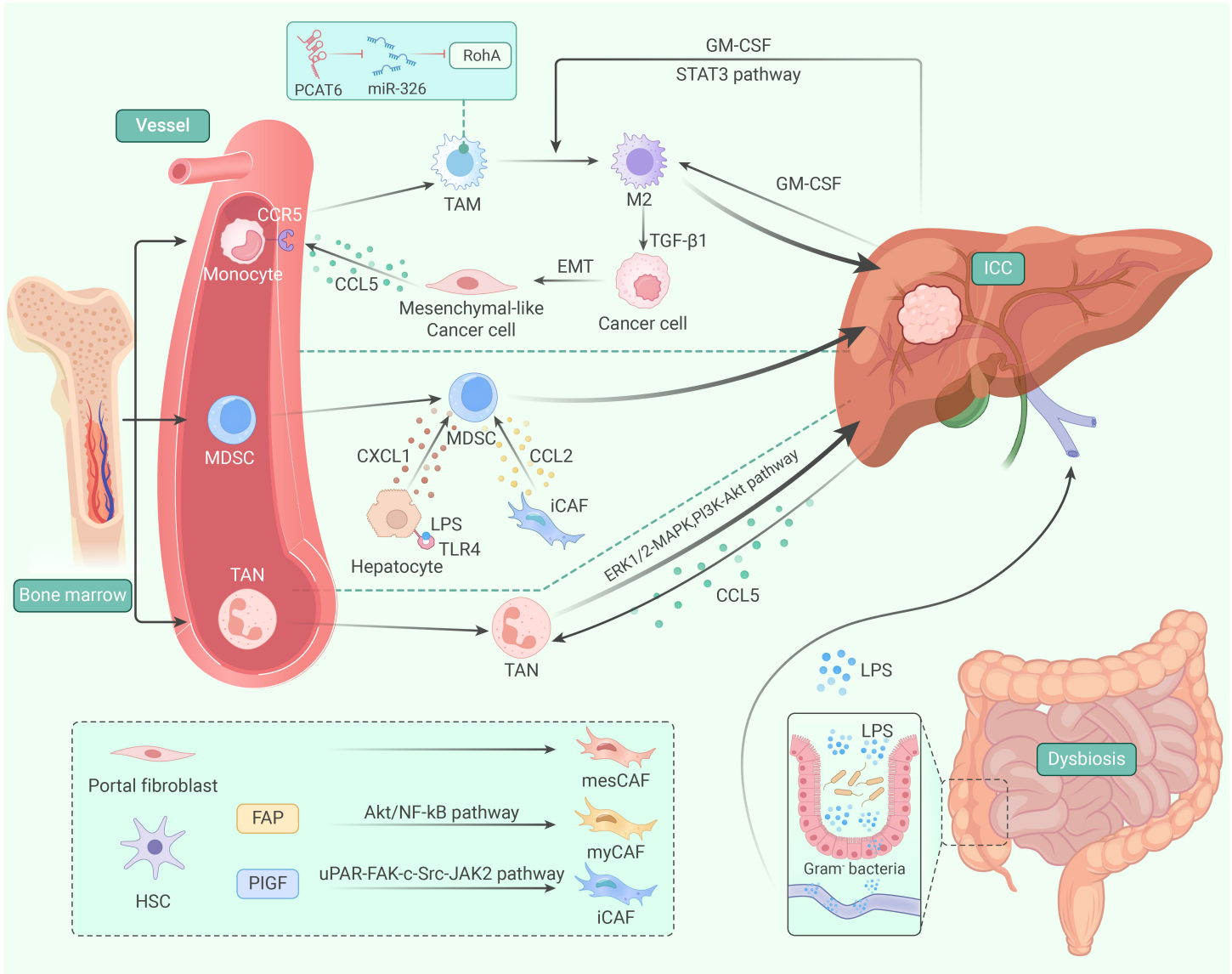


Figure 3. Origin, recruitment and phenotypic alteration of cellular components in ICC Ascertaining the source of cellular components is conducive to developing novel immunotherapy strategies for treating ICC. ICC, intrahepatic cholangiocarcinoma.

ous cancer types. Its ligand, signal regulatory protein- α (SIRP α), is abundantly expressed on antigen-presenting cells (APCs), including dendritic cells (DCs) and macrophages.²⁷ A pivotal interplay takes place between APCs and CD47/SIRP α : the interaction between SIRP α and CD47 transmits a “don’t eat me” signal to the APC, effectively shielding tumor cells from being phagocytosed by disrupting antigen uptake. Consequently, the upregulation of CD47 endows neoplasm cells with a means to evade immune surveillance.²⁸ Bioinformatic analyses have shown that an intensified CD47/SIRP α signaling profile correlates with unfavorable prognoses of ICCs.²⁹ In parallel, an *in vitro* study underscored the disruptive potential of the CD47/SIRP α interaction, which impedes macrophage-mediated phagocytic activity in CCA (Figure 2B).³⁰

Lack of neoantigens. Insufficient T-cell recognition due to the dearth of tumor antigens contributes in part to T-cell priming deficiencies. Tumor antigens can be broadly categorized into two groups: tumor-associated antigens (TAAs) and neoantigens, also referred to as tumor-specific antigens (TSAs). TAAs encompass “self-antigens” that are inappropriately overexpressed on tumor cells, such as cancer-testicular antigens, differentiated antigens, and “nonself” antigens of viral origin.³¹ Despite their potential to induce limited antitumor immune responses, T cells targeting TAAs might be eliminated during their maturation owing to central immune tolerance mechanisms operating within the thymus.³² Therefore, neoantigens, which exhibit

increased HLA affinity and potent immunogenicity, have emerged as ideal targets for the identification of tumor cells harboring these unique antigens by T cells.

In cancer cells, somatic mutations can give rise to neoantigens, making them recognizable to the immune system.³³ Significantly, tumors that accrue a greater number of neoantigens tend to have a greater somatic mutation burden.³⁴ The altered amino acids stemming from these mutations may enhance the ability of T cells to bind with HLA molecules, thereby prompting T-cell responses.³⁴ The metric for quantifying the number of somatic mutations per megabase of the genome is known as the tumor mutation burden (TMB).³⁵ Essentially, the TMB serves as a gauge of the neoantigen load in the tumor. A higher TMB level in tumors is correlated with an elevated neoantigen burden, enhancing the potential for immune system priming.^{36,37} Notably, a threshold of 10 mutations per megabase of DNA is commonly used to define a high TMB.³⁸ Compared to tumor types with a high TMB, ICC has a low TMB, at 1.29 mutations per megabase.³⁵ Moreover, only an average of 52.0% of clonal neoantigens are actually expressed in ICCs.²⁶ Comprehensive whole-exome RNA-seq has unveiled the mechanisms behind the low antigenicity of ICC. The prevailing trend in sparsely infiltrated ICC involves the dominant copy number loss of clonal neoantigens, whereas in heterogeneously infiltrated ICC, a more frequent occurrence lies in the silencing of mutated genes bearing neoantigens (Figure 2C).²⁶

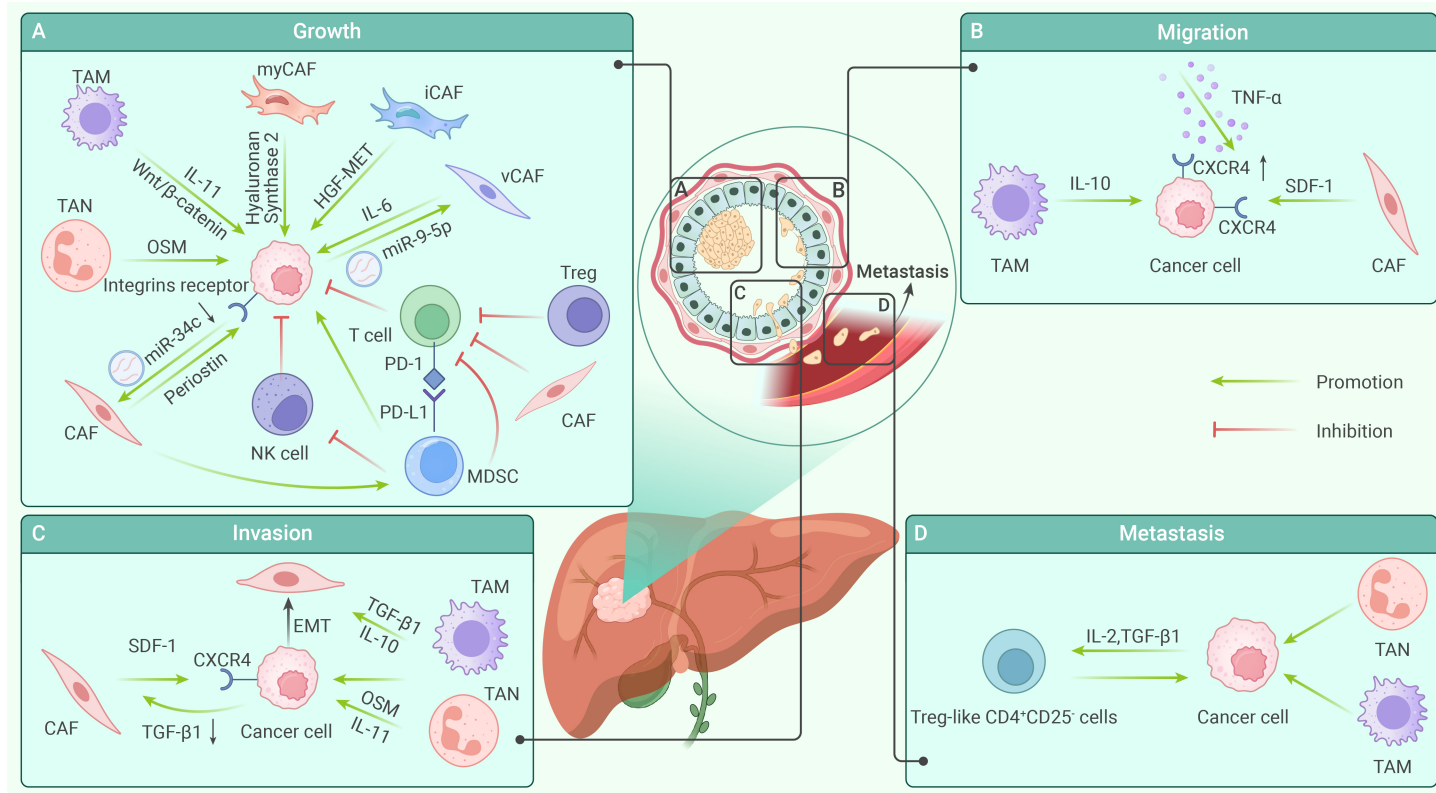


Figure 4. Tumor immune microenvironment of ICC Cellular components, including tumor-associated macrophages, cancer-associated fibroblasts, tumor-associated neutrophils, marrow-derived suppressor cells, and regulatory T cells, collaboratively orchestrate the tumor immune microenvironment, which favors tumor growth, invasion, migration, metastasis and inhibition of antitumor immunity. ICC, intrahepatic cholangiocarcinoma.

Poor T-cell infiltration

Prior to eliminating cancer cells, activated immune cells must first infiltrate tumor sites and navigate into the parenchyma. Any disruption in this intricate, multifaceted process can culminate in insufficient T-cell infiltration, thereby compromising the efficacy of immunotherapy.³⁹

Reduced chemotaxis. Upon activation by DCs within the lymph nodes, effector T cells embark on their journey to infiltrate tumor sites, guided by multiple chemokines.⁴⁰ The process of chemotaxis, which supports T-cell recruitment into tumor tissue, has been elucidated. Tumor cell-derived CCL5 enables T-cell trafficking in tumors. However, the expression of CCL5 by tumor cells alone is often insufficient. Tumor antigen recognition by tumor-specific T cells triggers the release of IFN- γ , which in turn activates tumor-associated macrophages (TAMs) and DCs to produce CXCL9. CXCL9 acts as an amplifier of T-cell engraftment in tumors. Defects at any stage of this loop can result in disorders in T-cell infiltration.^{41,42} A deficiency in CXCL9 within ICC tissues has been identified as a contributing factor to T-cell and NK-cell exclusion (Figure 2C).⁴³

Abnormal blood vessels. Blood vessels serve as conduits for effector T cells to infiltrate the tumor microenvironment.⁴⁰ Anomalies in these vessels can hinder the extravasation of T cells.¹⁷ Within tumors, blood vessels often exhibit distorted, dilated, and scattered morphologies. Histologically, the connections between adjacent endothelial cells are loose. In addition, leaky tumor blood vessels arise from the abnormal detachment of pericytes, cells that envelop vessels and regulate vascular permeability.³⁹ These intravascular and extravascular factors collectively contribute to the dysfunctional circulation of tumor vessels.³⁹ In the case of ICC, which is a hypovascular cancer, tumor vessels appear to collapse, leading to inadequate perfusion.⁴⁴ Increased expression of thrombospondin (THBS)1, THBS2, and pigment epithelium-derived factor (PEDF) in the extracellular fluid of ICC plays a role in curbing vessel morphogenesis and viability (Figure 2C).⁴⁵ Notably, cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM1) and vascular cell adhesion molecule-1 (VCAM1) are important for recruiting T cells.⁴⁶ A decreased expression of these molecules can lead to endothelial anergy and impede T-cell trafficking.⁴⁷ However, such a mechanism has not

been documented in ICC. In summary, restoring aberrant vascular morphology and hemodynamics has emerged as a promising strategy for enhancing T-cell infiltration.

The extracellular matrix (ECM): physical barrier. The tumor stroma functions as a formidable physical barrier that impedes the infiltration of T cells.¹⁷ An intricate network of dense tumor stroma can effectively suppress the migration of T cells toward the tumor core.⁴⁸ A well-established hallmark of ICC is desmoplasia, which is significantly influenced by transforming growth factor- β (TGF- β).^{49,50} The rigidity of the ECM within the tumor microenvironment substantially impedes T-cell infiltration, leading to an inadequate anti-cancer immune response. This, in part, elucidates the limited effectiveness of ICIs for treating ICC.⁵¹ Collagen fiber levels are elevated in ICC tissues, while elastic and reticular fiber levels are decreased.⁵² Throughout cancer development, several ECM proteins undergo considerable dysregulation, resulting in biochemical shifts within the tumor stromal milieu.⁵³ Moreover, periostin plays a major role in determining the importance of the ECM in desmoplastic malignant tumors, including ICC. Functionally, periostin plays a role in various aspects of ECM formation, including collagen cross-linking, fibrogenesis, and fibrillogenesis.⁵⁴ α -Smooth muscle actin (SMA)⁺ myofibroblastic cancer-associated fibroblasts (CAFs) are the principal cellular source of periostin, and TGF- β 1 is one of its inducers.⁵⁴

Notably, cancer-associated myofibroblasts constitute a predominant cellular component within the desmoplastic stroma.⁵⁵ These myofibroblasts can generate factors such as platelet-derived growth factor-B (PDGF-B), stromal-derived factor-1 (SDF-1), and TGF- β , all of which influence the profibrogenic response and ECM synthesis (Figure 2C).⁵⁵

Immune privilege mediated by Fas. The concept of “immune privilege” is well recognized in specific tissues, such as the testis, anterior chamber of the eye, and placenta, where the local microenvironment effectively restricts lymphocyte infiltration to prevent the onset of autoimmunity.⁵⁶ The phenomenon of lymphocyte apoptosis in tumor tissues is driven by the interaction between Fas, which is expressed on lymphocytes, and FasL, which is constitutively expressed on normal tissues. This interaction ultimately results in the apoptosis or programmed cell death of Fas-bearing lymphocytes

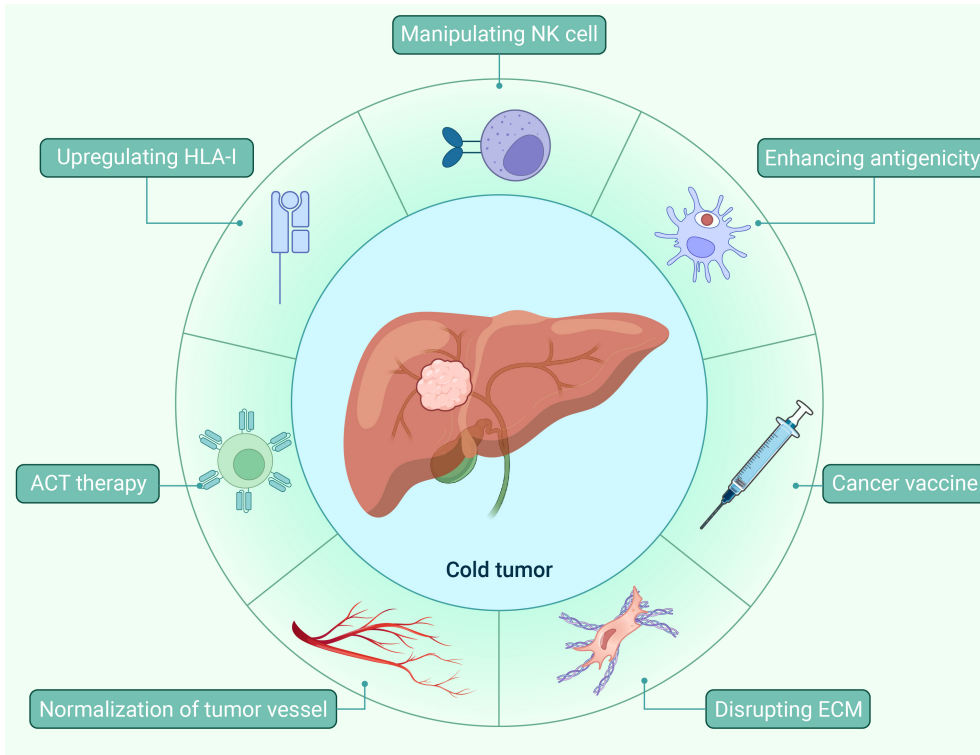


Figure 5. Summary of the therapeutic strategies used to treat cold ICCs.

study highlighted how prostate cancer-associated transcript 6 (PCAT6) polarizes M2 macrophages by inhibiting miR-326 and activating the RohA signaling pathway.⁶⁸

Myeloid-derived suppressor cells (MDSCs), a group of immature myeloid cells with potent immunosuppressive activity and diminished antigen presentation capacity, play a crucial role in the TIME.⁶⁹ In mouse models, impaired gut barrier integrity in conditions such as colitis and primary sclerosing cholangitis leads to an influx of gram-negative bacteria and lipopolysaccharide (LPS) into the liver, subsequently inducing the expression of CXCL1 in hepatocytes via a TLR4-dependent mechanism. The CXCL1/CXCR2 axis facilitates the accumulation of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) in the liver.⁷⁰ MDSCs can also be recruited by CAFs. The role of CCL2 as a mediator of MDSC recruitment has been confirmed in several types of tumors.⁷¹⁻⁷³ In ICC, fibroblast activation protein (FAP)⁺ CAFs are a major source of CCL2 and are suggested to

within the tumor microenvironment.⁵⁶ Likewise, a parallel strategy is employed by cold tumors, utilizing Fas-mediated apoptosis of T cells to evade direct contact with immune cells and thus contribute to the exclusion of T cells.⁵⁷ Extensive investigations of FasL expression have revealed broader and more intense FasL expression in well-differentiated ICCs, while FasL levels tend to decrease in poorly differentiated ICCs.⁵⁸ These findings suggest that the apoptosis of Fas-bearing T cells is an early event in the progression of ICC.⁵⁹ Notably, two distinct CCA cell lines have been classified as Fas^L (low Fas expression) and Fas^H (high Fas expression). In these CCA cells, the relationship between Fas and FasL expression is reciprocal: Fas^H CCA cells exhibit low FasL levels, whereas Fas^L CCA cells express high FasL levels. An increase in FasL expression in Fas^L CCA cells is responsible for the elimination of Fas-bearing T cells. Additionally, microvesicles containing functional FasL have been identified as agents that induce T-cell apoptosis (Figure 2D).⁶⁰

ROLES OF TUMOR IMMUNE MICROENVIRONMENT IN ICC

Recruitment and phenotypic alteration of cellular components

The constituents of the TIME originate from extratumoral tissues and are subsequently recruited to the tumor site, where they undergo phenotypic transformation.⁶¹ Understanding these dynamic and intricate processes holds significant promise for devising effective therapeutic strategies (Figure 3).⁶²

Bone marrow-derived monocytes are macrophages that initially reside within healthy tissues. In response to certain stimuli, these cells differentiate into distinct M1 and M2 phenotypes.⁶¹ The M2 phenotype, known for promoting carcinogenesis, progression, and metastasis, is implicated in various mechanisms.⁶¹ Compelling evidence points to the recruitment of macrophages to ICC tumor sites, where they transition to the M2-TAM phenotype.⁶³⁻⁶⁵ Notably, granulocyte-macrophage colony-stimulating factor (GM-CSF), sourced from tumors, has emerged as a primary mediator of myelopoiesis, recruitment, and polarization of TAMs.²⁰ M2-secreted TGF- β 1 can induce epithelial-mesenchymal transition (EMT) in CCA cells through the atypical protein kinase C γ (aPKC γ)-NF- κ B signaling pathway. Notably, CCA cells undergoing aPKC γ -induced EMT exhibit elevated CCL5 levels, regulating the activation and recruitment of macrophages. This macrophage-aPKC γ -CCL5 feedback loop suggests interplay between M2 macrophages and CCA cells in shaping the protumor microenvironment.⁶⁶ HuCCT1 tumor cell supernatant (TCS) polarizes macrophages toward the M2 phenotype through the signal transducer and activator of transcription 3 (STAT3) pathway.⁶⁷ Another

recruit MDSCs.^{49,74}

Like TAMs, tumor-associated neutrophils (TANs) assume varying roles in the TIME, contingent on their phenotype. TANs can be classified as protumoral (N2) or antitumoral (N1) phenotypes. Originating in the bone marrow, TANs are recruited to cancerous tissues.⁷⁵ CXCL5 derived from ICCs has been shown to attract neutrophils through the PI3K-Akt and ERK1/2-MAPK signaling pathways.⁷⁶

The diverse cellular types within the liver's CAF population reflect their multisource origins. Derived from hepatic HSCs, CAFs can be categorized as myofibroblastic (myCAFs) or inflammatory and growth factor-enriched (iCAFs).⁷⁷ Placental growth factor (PlGF) can activate the Akt/NF- κ B pathway, facilitating a myofibroblast-like phenotype.⁴⁴ FAP activates STAT3 through the uPAR-FAK-c-Src-JAK2 pathway to induce iCAF formation.⁴⁹ Mesothelial CAFs (mesCAFs), which express portal fibroblasts/mesothelial markers, are derived from portal fibroblasts.⁷⁷ Another possible source of CAFs in the ICC, albeit unproven, is circulating bone marrow-derived precursor cells.⁵⁵ CCA cells produce PDGF-D, which recruits CAFs through binding to PDGFR β and subsequent activation of Rho GTPase and JNK.⁷⁸ MiR-206 suppression is implicated in CCA, and its reduction is linked to the transformation of the CAF phenotype.⁷⁹

Roles of cellular components during tumor growth, invasion and metastasis

The intricate molecular communication among cellular components within the TIME creates a complex web of cellular crosstalk, ultimately fostering the development, progression, and metastasis of ICC.⁸⁰ This section provides a comprehensive overview of the roles played by these immunosuppressive cells in orchestrating the tumor niche (Figure 4).

The Wnt/ β -catenin signaling pathway is important for the carcinogenesis of multiple cancers. In a macrophage line, exposure to LPS led to elevated Wnt3 expression at both the mRNA and protein levels. Conditioned media from LPS-activated macrophages triggered the accumulation of β -catenin in CCA cells.⁸¹ TAMs have also been closely implicated in invasion and metastasis in cancer. TGF- β 1 secreted by M2 macrophages induces EMT in CCA cells via the NF- κ B signaling pathway.⁶⁶ *In vitro* studies have shown that coculture of M2 macrophages and ICC cells results in increased invasion, EMT, and migration of cancer cells, facilitated by IL-10/STAT3 signaling.^{63,82} Moreover, the interplay between TANs and TAMs in facilitating the progression of ICC has been revealed.⁸³ Coculture of TANs and TAMs *in vitro*

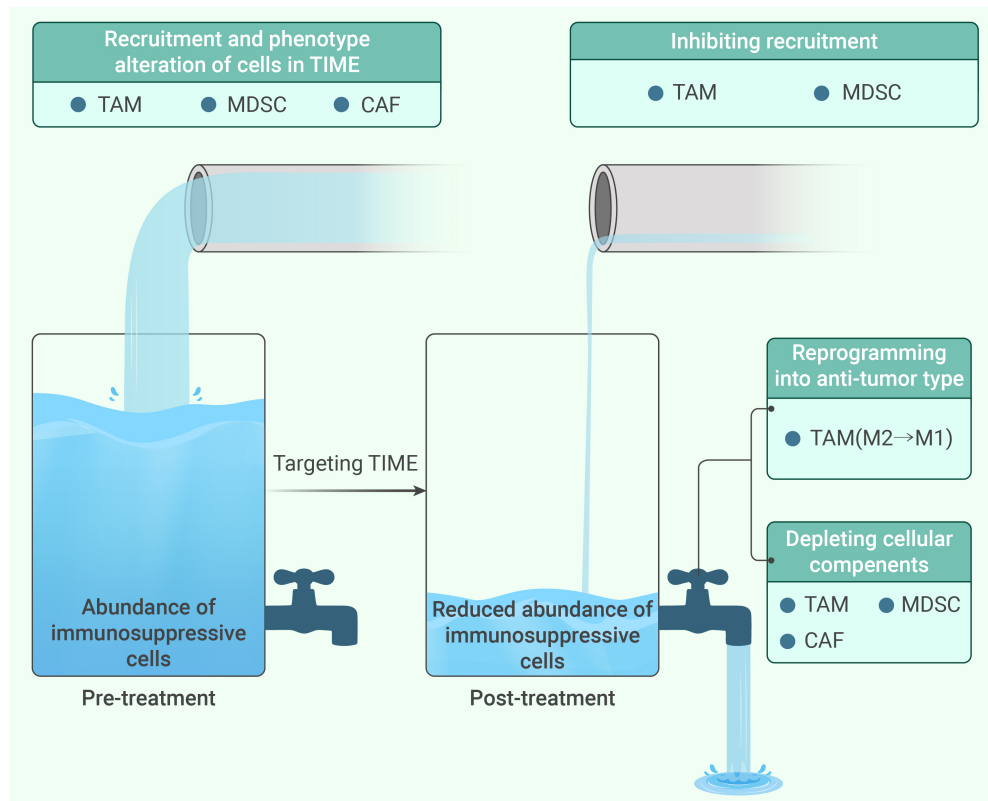


Figure 6. Therapeutic strategies targeting cellular components Left panel: When the water flows into the tank, the water level in the tank will rise. The abundance of immunosuppressive cells in the tumor immune microenvironment increases via tumor-mediated recruitment of these cells. Right panel: Inhibiting the recruitment of cellular components (preventing water from flowing into the tank) and reprogramming into an antitumor phenotype or depleting cellular components (turning on the faucet to drain water) are the goals of reducing the abundance of immunosuppressive cells.

promoted the proliferation, invasion, and colony formation of ICC cells. TANs and TAMs were found to produce elevated levels of oncostatin M (OSM) and IL-11, which activated STAT3, consistent with the *in vitro* results. *In vivo* studies confirmed that TANs and TAMs promoted ICC growth and metastasis in a mouse xenograft model.⁸³ aPKC- ι ⁺ CCA cells were shown to produce IL-2 and TGF- β 1, inducing Treg-like CD4⁺CD25⁺ cells through the aPKC- ι /P-Sp1/Snail signaling pathway, thereby promoting CCA metastasis.⁸⁴

An increasing body of evidence suggests the role of CAFs in cancer cell invasion and metastasis. The local balance between TGF- β 1 and SDF-1 influences CCA invasion. TGF- β 1 secreted from CAA cells downregulates SDF-1 secretion by CAFs, whose interaction with CXCR4 expressed on CCA cells leads to invasion.⁸⁵ In fact, TGF- β 1 expression is either focal or negative in CCA cells, while fibroblasts along the invasive front exhibit SDF-1 expression.⁸⁵ Coculture of CCA cells with CAFs significantly boosts the migratory activity of CCA cells compared to monoculture.⁸⁶ Mechanistically, fibroblasts release SDF-1, which acts on CXCR4 expressed on ICC cells and promotes ICC migration. Remarkably, TNF- α appears to enhance ICC migration by augmenting CXCR4 expression.⁸⁷

Periostin has been observed to be overexpressed in CAFs, inducing tumorigenesis in epithelial cells through interactions with integrin receptors.⁸⁸ CAFs also interact with endothelial cells, playing a critical role in promoting their differentiation and thus facilitating the development of ICCs. CAFs contribute to proinflammatory processes during the early and middle stages of ICC development, whereas tumor growth promoted by CAFs occurs in the late stage of ICC.⁸⁹ myCAFs were found to express hyaluronan synthase 2, facilitating ICC, while iCAFs promoted ICC growth via the HGF-MET pathway.⁷⁷ MicroRNAs enclosed in exosomes have been identified as critical for mediating CAF-cancer cell communication. Tumor-derived exosomal miR-34c dampen CAF activation by targeting the Wnt signaling pathway. HuCCT-1 cells exhibit decreased miR-34c expression in exosomes, thereby fostering CCA progression.⁹⁰ ICC-derived exosomal miR-9-5p induces the expression of IL-6 in vascular cancer-associated fibroblasts (vCAFs), leading to the upregulation of enhancer of zeste homolog 2 (EZH2) and facilitating tumor progression.⁹¹

Immune escape mediated by cellular components

The role of MDSCs in promoting ICC depends on their inhibitory effects on NK-cell function.⁷⁰ Regarding T-cell inhibition, a study of resected human

FOXP3 expression, indicating that high CTLA-4 levels are correlated with an abundance of Tregs.⁹⁵ In tumor-infiltrating Tregs, enrichment of mesenchyme homeobox 1 (MEOX1) has been found to be responsible for reprogramming circulating Tregs to acquire the epigenetic and transcriptional landscape of tumor-infiltrating Tregs, revealing an activated phenotype within the TIME. MEOX1 increased the surface level of CTLA-4. Additionally, the same research team suggested that CTLA-4 exhibited enhanced interaction with CD80/86, indicating that Treg-mediated immunosuppression in ICC may depend on CTLA-4.⁹⁶

Supernatants from FAP⁺ CAFs were shown to enhance the ability of MDSCs to suppress T-cell proliferation. Additionally, these supernatants could inhibit antitumor immunity mediated by CD8⁺ T cells (Figure 4).⁴⁹

STRATEGIES PROVOKING ANTI-TUMOR IMMUNITY

The acquisition of immune evasion in cold ICC contributes to the prediction of the response to ICIs. Given the low immunogenicity and response rate to ICIs, transforming these tumors into hot ICCs is a promising strategy (Figure 5; Table S1).

Restoring antigen presentation machinery

Treatment with ICIs can induce T-cell dysfunction when antigen stimulation is absent. Therefore, a promising strategy to potentiate ICI efficacy, particularly in cancers with low baseline levels of HLA-I, is to increase the level of HLA-I on cancer cells.⁹⁷

In vitro treatment with trametinib has been shown to upregulate PD-L1 and HLA-I in ICC cells. This upregulation enhances the immunogenicity of ICC. Compared with monotherapy, the combination of trametinib and anti-PD-1 therapy reduces the tumor burden in ICC models and improves survival in SB1 tumor-bearing mice. The combination treatment also elicits a durable immune response, as trametinib plus anti-PD-1 therapy establishes strong immune memory against ICC *in vivo*.⁹⁸ Pretreatment with gemcitabine increases the expression of HLA-I on KKKU-213 cells, potentially reinforcing antigen presentation and enhancing the function of CTLs.⁹⁹ Similarly, gemcitabine combined with IFN- γ induces the upregulation of HLA-I in human ICC cells in a dose-dependent manner.¹⁰⁰

NK cells are suppressed when their inhibitory killer cell immunoglobulin-like receptor (KIR) interacts with HLA-I expressed by cancer cells.¹⁰¹ However, in epithelial cancers, the expression of histocompatibility complex (MHC) class I

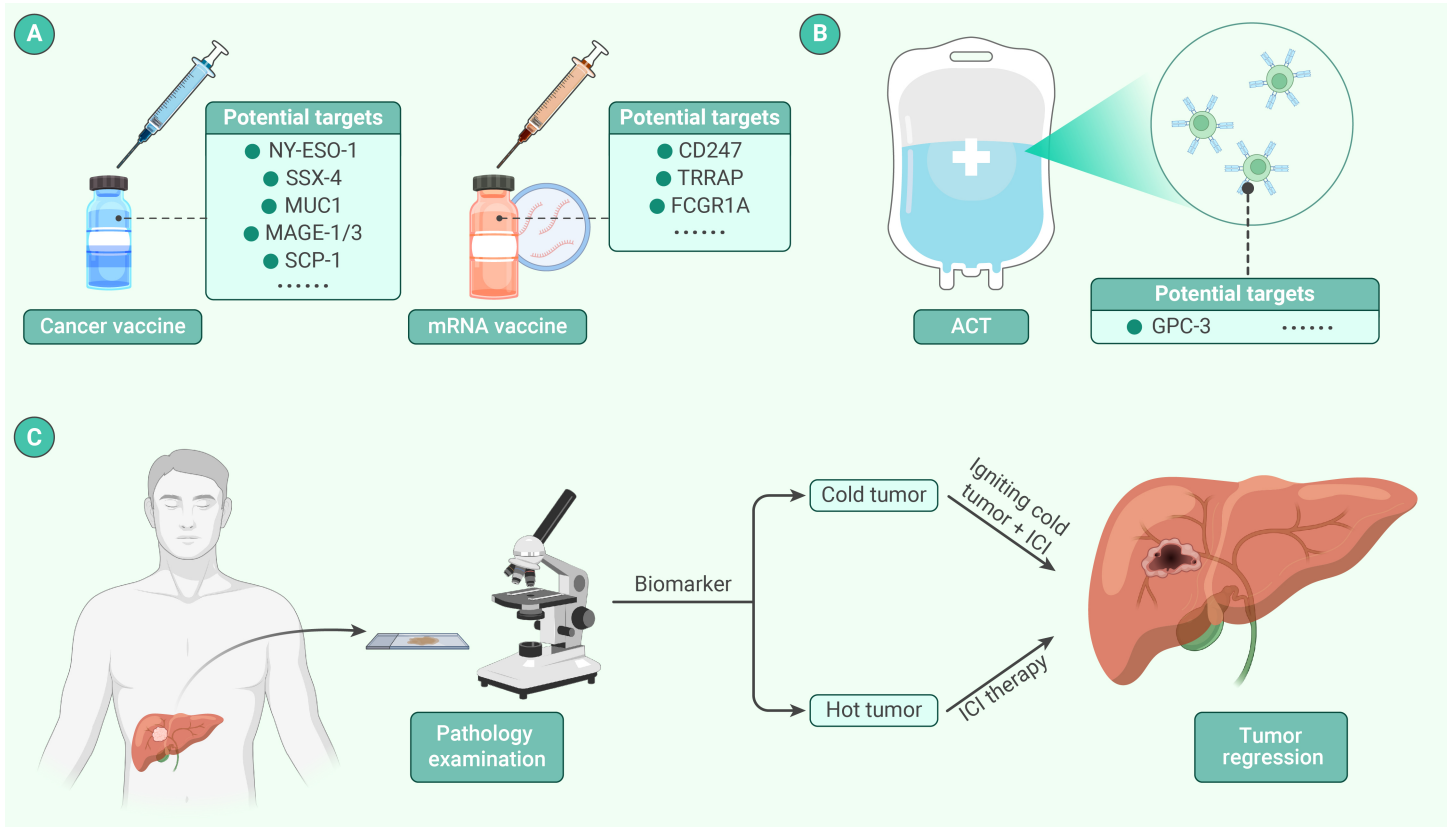


Figure 7. Potential targets and biomarkers of novel immunotherapies for ICC (A) NY-ESO-1, MAGE-1, MAGE-3, SSX-4, SCP-1 and MUC-1 may serve as promising targets for cancer vaccines. CD247, FCGR1A and TRRAP are potential candidates for mRNA vaccines. (B) Based on their ability to elicit an immune response, GPC-3 might be utilized to design a novel target for ACT for treating ICC. (C) Identifying reliable biomarkers to distinguish cold ICCs from hot ICCs is consistent with the concept of normalizing anticancer immunity. However, the predictive value of *IDH1/2* mutations in discriminating cold from hot ICCs deserves further investigation. NY-ESO-1, New York esophageal squamous cell carcinoma 1; MAGE-1, melanoma antigen-encoding gene 1; SSX-4, synovial sarcoma X 4; SCP-1, synaptosomal complex protein 1; FCGR1A, fragment crystallizable (Fc) fragment of IgG receptor 1A; TRRAP, transformation/transcription domain associated protein; ACT, adoptive cellular therapy; GPC-3, glypican-3; *IDH1/2*, isocitrate dehydrogenase 1/2; ICC, intrahepatic cholangiocarcinoma; ICI, immune checkpoint inhibitor.

polypeptide-related sequence (MIC) A and MICB is increased. These molecules can activate NK cells via interaction with NKG2D, even under low-HLA-I conditions. Therefore, manipulating the cytotoxicity of NK cells under low-HLA-I conditions is a complementary therapeutic approach.^{24,102} 7C6, a kind of mAb, can maintain MICA and MICB expression on cancer cells, enabling NKG2D-dependent NK-cell activation and mediating antibody-dependent cytotoxicity (ADCC).¹⁰³ Interestingly, ICC tissues exhibit higher MICA/B expression than nontumoral tissues, justifying the rationale for utilizing the NKG2D-MICA/B axis. Administration of 7C6 dramatically increases IFN- γ production and degranulation of tumor-infiltrating NK cells in both HuCCT-1 cells and autologous ICC patient target cells.¹⁰³

Enhancement of antigenicity

The dysfunction of APCs partly explains the low antigenicity of ICCs, as tumor antigens must be captured, processed, and presented by APCs. Functional APCs are crucial for the recognition of tumor antigens by the immune system and for eliciting a tumor-specific immune response. Stimulating APCs has been demonstrated to improve the response to ICI therapy. In the context of ICC, the effectiveness of anti-PD-1 therapy can be markedly enhanced by engaging DCs and macrophages through CD40 agonists. Preclinical studies using mouse models have shown that monotherapy with a PD-1 antagonist alone has limited efficacy. However, when combined with a CD40 agonist, there is a more profound reduction in tumor burden and a significantly improved long-term survival rate. This combinatorial strategy leverages the synergistic benefits of immune checkpoint blockade and CD40-mediated immune activation, offering a promising therapeutic approach for ICC patients.¹⁰⁴ Additionally, blockade of the CD47-SIRP α axis can enhance macrophage-mediated phagocytosis of cancer cells. Treatment with anti-SIRP α SE5A5 fortified macrophage phagocytosis of KKKU-213 cells in a manner comparable to that achieved with anti-CD47 B6H12.2.³⁰

Cancer vaccines targeting TAAs or TSAs are designed to activate the immune system to identify and kill cancer cells.¹⁰⁵ These vaccines have been demonstrated to expand T cells and facilitate their trafficking to tumor sites.^{12,106} While cancer vaccines have rarely been studied in ICC, limited preclinical small sample clinical studies suggest that they can effectively elicit specific immune responses. For instance, aspartate- β -hydroxylase (ASPH) is widely expressed in cancer cells but is expressed at low levels or is absent in normal tissues. *In vivo* studies have demonstrated that immunization with ASPH-loaded DCs can inhibit tumor growth, reduce tumor volume, limit intrahepatic spread, and restrict T-cell infiltration.¹⁰⁷

Several case reports have provided preliminary evidence of the safety and antitumor effects of cancer vaccines. Phase I and phase I/II trials have shown that four-peptide vaccination and mucin (MUC)1 peptide-loaded DC vaccines are safe and well tolerated.^{108,109} Treatment with tumor peptide- or lysate-pulsed DCs and CD3-activated T cells prolongs survival and controls tumor recurrence.¹¹⁰ Combining autologous tumor lysate-pulsed DCs and *ex vivo*-activated T-cell transfer and surgery has resulted in significantly greater postoperative progression-free survival (PFS) and overall survival (OS) than surgery alone.¹¹¹

Genetic heterogeneity among ICC patients has been confirmed.²⁶ Extensive clinical applications suggest that vaccinations targeting a single cancer antigen may be insufficient to address tumor heterogeneity. Moreover, the spectrum of presented antigens varies among different patients.¹¹² These circumstances emphasize the need for personalized vaccines. A personalized multi-peptide vaccination approach has been employed in the treatment of patients with metastatic ICC. This strategy involves the use of seven epitopes identified through mass spectrometry of HLA ligands and next-generation sequencing techniques. Notably, the results demonstrated robust perforin expression within pulmonary metastatic lesions, indicating the presence of cytotoxic T cells targeting the tumor. Furthermore, there was no

evidence of additional metastasis, and the patient remained free from tumor progression for a minimum duration of 41 months following the initiation of immunotherapy. These findings highlight the potential efficacy of personalized multipptide vaccination in the treatment of metastatic ICC, emphasizing the importance of immune-based therapies in achieving long-term tumor control.¹¹³

Normalization of the stromal component

The presence of desmoplasia, fibroblasts, and aberrant tumor vessels plays a significant role in preventing cancer-specific T cells from infiltrating tumor tissues.^{114,115} Therefore, strategies aimed at normalizing the stroma of ICCs have the potential to enhance the extent of T-cell infiltration.

Boluda *et al.*¹¹⁶ developed a photothermal therapy (PTT) based on remote light-activated nanohyperthermia. This innovative approach involves converting optical energy into heat energy using nanoparticles upon exposure to a near-infrared laser. These authors applied multifunctional iron oxide nanoflowers decorated with gold nanoparticles (GIONFs) to modulate the tumor microenvironment. Both *in vitro* and *in vivo* studies have demonstrated that fibroblasts preferentially internalize GIONFs, resulting in decreased tumor stiffness. Furthermore, three PTT sessions led to a dramatic reduction in the number of α -SMA⁺ cells, indicating depletion of CAFs and tissue softening. Additionally, treatment with miR-195-loaded extracellular vehicles reduced tumor size by suppressing CCA cell growth and desmoplasia, as evidenced by reduced α -SMA staining.¹¹⁷ The efficacy of the antibody (5D11D4) in blocking PlGF was also evaluated, and the results indicated that PlGF blockade led to a reduction in the expression of collagen I and a decrease in tissue stiffness.⁴⁴ Moreover, linsitinib was reported to suppress the expression of collagen I and IV in hTERT- hepatic stellate cells (HSCs).¹¹⁸

In addition to modulating the ECM, PlGF blockade has been found to reopen collapsed vessels and enhance blood perfusion.⁴⁴ Antibodies targeting PEDF, THBS1, and THBS2 restore endothelial cell viability and the ability to form tubes. These findings suggest that normalization of tumor vessels is a viable approach for facilitating the infiltration of T cells into the tumor microenvironment.⁴⁵

Adoptive cellular therapy (ACT)

ACT is a promising approach to address a deficient preexisting immune response.¹¹⁹ T cells can be genetically engineered to express either a T-cell receptor (TCR) or a chimeric antigen receptor (CAR), enabling them to recognize and kill neoplastic cells.¹²⁰ One innovative development involves fourth-generation chimeric antigen receptor (CAR4) T cells designed to target CD133 (anti-CD133 CAR4 T cells). These CAR4 T cells have demonstrated high antitumor efficacy against CCA cells expressing CD133, effectively targeting and eliminating tumor spheroids.¹²¹ Similarly, another CAR4 T-cell therapy targeting MUC1 (anti-MUC1 CAR4 T cells) has exhibited cytotoxicity toward KKKU-213A cells and KKKU-100 cells and effectively lysed KKKU-213A spheroids.¹²² Phanthaphol *et al.*¹²³ engineered CAR T cells targeting integrin $\alpha\beta 6$. They constructed CARs with an integrin $\alpha\beta 6$ -binding peptide (A20) fused to either a second-generation signaling domain (A20-2G CAR) or a fourth-generation signaling domain (A20-4G CAR). Both A20-4G and A20-2G CAR T cells exhibited high efficacy against integrin $\alpha\beta 6$ -positive CCA cells and tumor spheroids. DCs transduced with lentivirus carrying tri-cistronic cDNA sequences (SD-DC-PR) were capable of presenting the cAMP-dependent protein kinase type I- α regulatory subunit (PRKAR1A), which is overexpressed in CCA cells. Autologous effector T cells stimulated by SD-DC-PR exhibited greater cytotoxicity against CCA cells than T cells activated by conventional DCs.¹²⁴

Although clinical data on BTC are scarce, the existing evidence shows promising outcomes. Allogeneic $\gamma\delta$ T-cell immunotherapy has been shown to be safe without adverse effects, resulting in decreased tumor activity.¹²⁵ Another case report indicated that treatment with allogeneic $\gamma\delta$ T cells in combination with locoregional therapy led to longer PFS than locoregional treatment alone.¹²⁶ In one patient with metastatic CCA, TILs containing Th1 cells specific for the mutated *erbB2*-interacting protein (ERBB2IP) were isolated and then adoptively transferred. This approach resulted in a reduction in lesions and prolonged disease stability.¹²⁷

Targeting cellular components in the TIME

The presence of immunosuppressive cells contributes to the “cold” phenotype of ICCs. However, there is hope in reengineering the ICC TIME by targeting these cellular components. Various strategies can be employed to eliminate immune suppressive elements, including inhibiting recruitment, promoting an antitumor phenotype, and depleting these cellular components (Figure 6; Table S2). These approaches hold promise for improving the anticancer immune response in ICC.

Targeting TAMs and turning foes to friends. Depletion of aPKC ζ , a core component of the aPKC ζ -CCL5 feedback loop, effectively prevents the recruitment of M2 macrophages. Codelivery of gemcitabine and aPKC ζ -siRNA via liposomes significantly inhibits the growth of CCA, resulting in a decrease in F4/80⁺ macrophages. Silencing aPKC ζ also reduces the recruitment of macrophages and overcomes CCA-mediated chemoresistance.⁶⁶ Lupeol and stigmasterol, major phytosterols extracted from various herbal plants with anti-inflammatory properties, have been suggested as potential anticancer agents. Compared with monotherapy, dual treatment with lupeol and stigmasterol significantly delays CCA growth in mice. The decreased chemokine production and the subsequent reduced recruitment of macrophages may partly explain the mechanisms underlying this effect.¹²⁸

Phenotypic alteration to the M2 state is essential for TAMs to exert their protumor effects, providing new avenues for treatment. Knockdown of SPARC via si-SPARC suppresses M2 polarization by inhibiting the PI3K/AKT signaling pathway.¹²⁹ Treatment with α -GM-CSF inhibits tumor growth and increases survival in KPPC mice. An *in vitro* study demonstrated that neutralization of GM-CSF significantly dampened macrophage viability, and qRT-PCR analysis revealed decreased expression of genes associated with the M2 phenotype. These results suggested that blocking GM-CSF could reverse M2 polarization of TAMs.²⁰

The application of cold atmospheric plasma (CAP) significantly has been shown to reduce the tumor growth rate and size compared to gemcitabine alone. The role of CAP in modulating the immune compartment has been elucidated. CAP treatment leads to enhanced expression of CCL2 and CCR2, which regulate the chemotaxis of monocytes. CAP also induces the expression of cytokines correlated with the antitumor phenotype of macrophages.¹³⁰

Targeting potent immunosuppressor: MDSC. Targeting MDSCs is an area that merits in-depth investigation due to their potent immunotherapy properties. Strategies for targeting MDSCs include depleting them through antibodies and reducing their chemotaxis. Preventing the recruitment of TAMs and inhibiting TAMs has been found to foster compensatory infiltration of granulocytic MDSCs (G-MDSCs) via enhanced CXCL2 produced by CAFs. These studies suggest that dual blockade of TAMs and MDSCs might effectively release the “brake” that inhibits antitumor immunity. Combining anti-Ly6G (1A8) and anti-PD-1 (G4) with anti-CSF1R (AFS98) has been shown to potentiate anti-PD-1 therapy and prolong survival in a mice model. The LXR/ApoE axis has been shown to mitigate the abundance of MDSCs by inducing apoptosis. The LXR agonist GW3965, in combination with anti-CSF1R and anti-PD-1, significantly reduced the tumor burden compared to that in a control group. These therapeutic modalities also increased the infiltration, activation, and effector function of CD8⁺ T cells.⁹² The depletion of PMN-MDSCs by the 1A8 antibody has been shown to dampen CCA growth in colitis-induced mice.⁷⁰ As mentioned above, the CXCL1/CXCR2 axis is responsible for facilitating PMN-MDSC accumulation. Neutralization of CXCL1 via a-CXCL1 and blockade of CXCR2 via SB225002 reduced the CCA burden in colitis-induced mice and hepatic PMN-MDSCs.⁷⁰ Targeting MDSCs through these various approaches holds promise for enhancing antitumor immunity and improving cancer treatment outcomes.

Destroying the contributor of tumor barrier. Considering the abundance of CAFs in tumors, CAFs are major players in orchestrating the immunosuppressive milieu of ICCs through cellular communication, in addition to forming dense tumor stroma. Thus, targeting CAFs is a promising modality for disrupting the TIME of ICCs.

Gemcitabine-induced changes in the Bcl-2/Bax ratio, which can affect cell apoptosis, have been shown to be reversed by coculture with CAFs.⁷⁹ However, this recovery was not observed in cells treated with exosomal miR-206. *In vivo* studies demonstrated that compared with treatment with gemcitabine alone, treatment with exosomal miR-206 reduced the α -SMA area.

The combination of exosomal miR-206 and gemcitabine led to markedly smaller tumor volumes than monotherapy and prolonged the survival of tumor-bearing mice. Gemcitabine-induced cell apoptosis, which was prevented by CAFs, was reversed by exosomal miR-206, enhancing the chemotherapeutic sensitivity of CCA cells.⁷⁹

Various chemotherapies have the ability to suppress CAFs in the TIME. Everolimus can inhibit the viability of CAFs in a dose-dependent manner. Moreover, everolimus treatment leads to the suppression of multiple cytokines produced by CAFs, including IL-13, IL-8, MIF, MCP-1, and Serpin E1.⁸⁶ Nab-paclitaxel can disrupt surrounding stromal CAFs.¹³¹ Linsitinib decreased the viability of hTERT-HSCs and LX2 cells in a dose-dependent manner by inhibiting IR/IGF1R. Notably, linsitinib abolished the expression of α -SMA in LX2 cells and the expression of the α -SMA mRNA in hTERT-HSCs.¹¹⁸

POTENTIAL TARGETS FOR FUTURE IMMUNOTHERAPY OF ICC

ICC-specific cancer vaccine

In addition to neoantigens, the deregulated expression of cancer testis antigens (CTAs) can induce antitumor immunity. A study involving 20 surgical samples of ICC revealed the expression of CTAs, including New York esophageal squamous cell carcinoma 1 (NY-ESO-1), melanoma antigen-encoding gene 1 (MAGE-1), MAGE-3, synovial sarcoma X 4 (SSX-4), and synaptonemal complex protein 1 (SCP-1), in ICC tissues, while these antigens were undetectable in paired normal tissues.¹³² MUC1, which is typically expressed on epithelial cells and plays a role in protecting epithelial tissues and in signal transduction,¹³³ can become a tumor-specific antigen when aberrantly glycosylated.¹³⁴ Aberrant expression of MUC1 has been observed in multiple cancers, including CCA, compared to normal tissues.^{135,136} The MUC1 level has been correlated with unfavorable prognosis.¹³⁶⁻¹³⁸ Intriguingly, the expression of MUC1 is seldom observed in CCA patients with metastasis.^{139,140} These findings suggest that some ICC patients could benefit from personalized immunotherapy based on their unique expression profiles.

In recent years, mRNA vaccines have emerged as a promising approach in cancer immunotherapy. These vaccines utilize the delivery of mRNAs encoding tumor antigens into APCs, which then express these antigens to stimulate antitumor immunity.¹⁴¹ The noninfectious, nonintegrating and modifiable nature of mRNA makes these vaccines safe and efficient.¹⁴² mRNA vaccines offer the potential for personalized treatment strategies based on individual sequencing data derived from tumor samples, as exemplified by recent advancements in pancreatic cancer research.¹⁴³ For ICCs, the mRNA levels of 3 genes, *CD247*, the fragment crystallizable (*Fc*) fragment of IgG receptor 1A (*FCGR1A*) and transformation/transcription domain associated protein (*TRRAP*), have been identified as promising candidates for the development of an ICC mRNA vaccine based on online databases and bioinformatic approaches (Figure 7).¹⁴⁴

New targets for adoptive cell therapy

Several TAAs have been identified based on the criterion that they can induce a T-cell response in more than three CCA patients but less so in healthy participants.¹⁴⁵ Among these TAAs, glypican-3 (GPC-3) is particularly noteworthy.¹⁴⁵ It is a cell-membrane oncofetal protein anchored by glycosylphosphatidylinositol (GPI) and plays a crucial role in fetal development. While healthy adult tissues rarely express GPC-3, it is highly expressed in various solid malignancies, including hepatocellular carcinoma (HCC).^{146,147} In ICC tissues, high GPC-3 expression is correlated with shorter DFS and OS.¹⁴⁸ To date, most clinical trials of ACT targeting GPC-3 have focused on HCC. However, the therapeutic effects of GPC-3-targeted ACT in ICC need further confirmation and exploration (Figure 7).

CONCLUSION

The majority of ICC patients exhibit unfavorable responses to ICI therapy due to the cold phenotype of these tumors. Our work comprehensively summarizes the factors contributing to the poor immune responses to these tumors, including immune evasion mechanisms and features of the TIME. Additionally, we investigated the corresponding therapeutic modalities that might achieve biological modifications in cold ICCs.

The biological rationale of combining chemotherapy with immunotherapy has been confirmed. The administration of chemotherapy has the potential to enhance the immunogenicity of cold tumors, thereby improving the efficacy of ICIs and generating synergistic effects. The current combination of chemotherapy and immunotherapy for treating ICCs has demonstrated promising outcomes in terms of patient survival prognosis.¹⁴⁹⁻¹⁵²

While cold tumors constitute a significant proportion of ICCs, only a small subset of patients with a hot phenotype benefit from ICI therapy. Indiscriminate transformation of cold tumors is costly, time-consuming and wasteful in these patients. Therefore, developing reliable biomarkers to identify patients with cold ICCs is essential.¹⁵³ *Isocitrate dehydrogenase 1/2 (IDH1/2)* mutations are associated with cold ICCs.¹⁵⁴ Paradoxically, another study demonstrated that patients with *IDH1/2* mutations exhibited increased T-cell infiltration.²⁶ The coexistence of hepatitis B virus infection and neoantigenicity in this study may explain this paradox.²⁶ Despite the promising role of *IDH1/2* in predicting the phenotype of ICC, additional in-depth studies should be performed to verify additional biomarkers (Figure 7).

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AUTHOR CONTRIBUTIONS

X.Z., B.Z. and J.H. was responsible for conceptualization and writing the original draft. J.S., Z.C., J.Z., B.W., E.Z. and S.P. wrote and edited the manuscript. T.W., G.Y., J.C. and M.C. supervised this work. All authors contributed to the article and approved the submitted version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Not applicable.

SUPPLEMENTAL INFORMATION

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