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REVIEW ARTICLE

Research progress on immunotherapy targeting the tumor immune microenvironment for cholangiocarcinoma*

Tianyin Ma[1, 2], Zhang Chen (Co-first author)[1, 2], Yibo Chai[1, 2], Xiangdong Gongye[1, 2], Peng Xia[1, 2], Chenming Qu[1, 2], Ming Tian[1, 2] (✉), Yufeng Yuan[1, 2, 3] (✉)

[1] Department of Hepatobiliary and Pancreatic Surgery, Zhongnan Hospital of Wuhan University, Wuhan 430071, China
[2] Clinical Medicine Research Center for Minimally Invasive Procedure of Hepatobiliary & Pancreatic Diseases of Hubei Province, Wuhan 430071, China
[3] TaiKang Center for Life and Medical Sciences, Wuhan University, Wuhan 430071, China

Abstract

Cholangiocarcinoma (CCA) is the second most common hepatobiliary cancer, and its incidence has increased significantly in recent years. CCA has poor prognosis owing to the limited diagnosis and treatment options. The tumor immune microenvironment (TIME), which comprises immune cells, cytokines, and chemokines, plays a significant role in cancer progression, the evasion of immune surveillance, and therapeutic responses. Immunotherapeutic strategies targeting the TIME offer the potential for the recognition and eradication of CCA. This review discusses the cellular and molecular components of the TIME in CCA and immunotherapeutic strategies targeting it.

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Cholangiocarcinoma (CCA), a malignant tumor originating from the biliary duct, has poor prognosis because of limited diagnostic and treatment options [1, 2]. Current treatment options for CCA include surgery, chemotherapy, and radiotherapy. However, delayed diagnosis, high recurrence rates, and a lack of effective systemic therapies compromise the prognosis [3]. The tumor immune microenvironment (TIME) is a multifaceted system comprising immune cells, cytokines, and chemokines that actively interact with neoplastic cells. These intricate interactions critically affect cancer progression, the evasion of immune surveillance, and responses to therapeutic interventions [4]. By modulating host immunity to recognize and eradicate malignant cells, immunotherapy targeting the TIME is a potential therapeutic strategy against several cancers, including CCA [5].

Cellular and molecular components of TIME in CCA

Tumor-associated macrophages (TAMs) and their roles in promoting tumor growth and metastasis

TAMs are abundant in the CCA tumor microenvironment and contribute to tumor progression, angiogenesis, and metastasis by secreting growth factors, cytokines, and chemokines. These key TIME components contribute to disease progression [6]. They also suppress T cells and promote the expansion of immunosuppressive

✉ Correspondence to: Ming Tian. E-mail: Tianm131@163.com; Yufeng Yuan. E-mail: yuanyf1971@whu.edu.cn
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cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) [7]. TAMs are characterized by their remarkable ability to adjust and transform, allowing them to perform pro-tumoral or anti-tumoral actions, as influenced by stimuli within their immediate environment [8]. Their high plasticity renders them important for promoting or suppressing tumor growth. In CCA, TAMs promote tumor growth and metastasis through various mechanisms [6, 9].

First, they promote CCA progression through the expression of immune checkpoint molecules, such as signal regulatory protein alpha (SIRPα) and programmed cell death protein 1 (PD1). Elevated SIRPα and PD1 expression in TAMs is associated with poor prognosis in patients with intrahepatic CCA, suggesting a potential role for these immune checkpoint molecules in immune evasion and tumor progression [9].

Second, TAMs secrete various cytokines and chemokines that promote tumor cell proliferation, angiogenesis, and tissue remodeling [8]. Physiologically, TAMs overexpress matrix metalloproteinase 1 (MMP1), a protein-digesting enzyme that participates in the breakdown of extracellular matrices and facilitates tumor infiltration into surrounding tissues [10]. TAMs can also suppress anti-tumor immune responses by interacting with other immune cells, such as MDSCs and Tregs, within the TIME [8]. This immunosuppressive environment enables tumor cells to escape immune surveillance and promote tumor growth and metastasis. Thus, given the significance of TAMs in the promotion of CCA development, therapies targeting them are being considered as potential therapeutic options for this malignancy [6, 9].

Tumor-infiltrating lymphocytes (TILs) and their roles in tumor immunity

TILs, including $CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells, Tregs, B cells, and natural killer (NK) cells, are essential components of CCA TIME. They infiltrate tumor tissues and play pivotal roles in tumor immunity [2, 11, 12]. Their presence within the TIME influences cancer progression, immune surveillance, and therapeutic responses [4, 13]. $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells are key players in anti-tumor immune responses as they can recognize and bind tumor antigens to induce tumor cell killing. Patients with CCA tend to exhibit better treatment outcomes when their tumors are infiltrated by a high density of TILs, particularly $CD8^+$ T cells [11, 12]. The LEL subtype of Epstein-Barr virus-associated intrahepatic cholangiocarcinoma is linked with good survival outcomes, possibly due to the activation of tumor-infiltrating B and $CD8^+$ T cells [14]. However, immunosuppressive factors in the TIME can suppress the activity of these T cells, resulting in immune evasion and tumor progression [2].

Tregs are a subset of TILs that suppress anti-tumor immune responses by inhibiting effector T cell functions, leading to a pro-tumorigenic environment [12]. Tregs are strongly associated with poor prognosis in all types of CCA [15]. Owing to their potentially important role in tumor progression, especially in lymph node metastasis, the $FoxP3^+/CD8^+$ ratio is considered an important marker of the immune environment in intrahepatic cholangiocarcinoma (ICC) [16, 17].

B cells and NK cells also contribute to tumor immune responses. B cells produce tumor-specific antibodies and serve as antigen-presenting cells, whereas NK cells are innate immune cells that kill tumor cells without prior sensitization. However, their roles in CCA have not been fully elucidated [2].

Overall, TILs play crucial roles in shaping anti-tumor immune responses, and their presence within the TIME is vital. The elucidation of their complex interplay and function will aid the development of novel immunotherapeutic strategies for the treatment of this disease. By investigating the multifaceted roles of TILs in CCA, valuable insights that can ultimately lead to improved treatment outcomes using immunotherapy may be gained [2, 11].

MDSCs and their immunosuppressive effects

MDSCs accumulate in the TIME of CCA and contribute to immunosuppression by inhibiting the activation and functioning of effector T cells, promoting tumor growth and immune evasion [4]. MDSCs are a heterogeneous population of immature myeloid cells that expand in response to tumor-derived factors. They suppress immune responses by inhibiting the functions of T cells, NK cells, and dendritic cells, thereby promoting tumor progression and metastasis. The gut microbiome regulates hepatocytes to form an immunosuppressive environment through the accumulation of $CXCR2^+$ polymorphonuclear MDSCs to promote CCA [18]. Although the significance of MDSCs in CCA are yet to be conclusively determined, they can be regulated by cancer-associated fibroblasts to enhance cancer stemness [19].

Immune checkpoint molecules and their roles in immune evasion

Immune checkpoint molecules play a crucial role in regulating the immune system and maintaining self-tolerance. However, these molecules can be exploited by cancer cells, including CCA cells, to evade immune surveillance and promote tumor progression. In CCA, immune checkpoints such as programmed death-1 (PD-1), programmed death ligand-1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) play significant roles in immune evasion [20].

PD-1 is a receptor on activated T cell surfaces, whereas PD-L1 is a ligand on tumor and antigen-presenting cells. Interactions between PD-1 and PD-L1 suppress T cell activation and proliferation, thereby dampening anti-tumor immune responses [20]. In extrahepatic CCA, PD-L1, TILs, and human leukocyte antigen have shown potential for immunotherapy [21].

CTLA-4, an immune checkpoint molecule, is expressed on T cells and competes with the co-stimulatory molecule CD28 to bind B7 ligands (CD80 and CD86) on antigen-presenting cells. This competition results in the inhibition of T cell activation and the suppression of immune responses against cancer cells [22]. Thus, targeting immune checkpoint molecules is a promising therapeutic strategy for CCA. Immune checkpoint inhibitors (ICIs) such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies have shown great potential in preclinical studies and clinical trials, providing a rationale for further investigation of their use for patients with CCA [22–24].

Cytokines and chemokines in the TIME and their effects on tumor progression and immune responses

Cytokines and chemokines, including transforming growth factor-beta (TGF-β), interleukin-6 (IL-6), and chemokine (C-C motif) ligand 2 (CCL2), play vital roles in shaping the TIME in CCA. They regulate immune cell recruitment, infiltration, and activation, thereby influencing tumor progression and the overall immune response. Chemokine (C-C motif) ligand 20 (CCL20), a crucial player in CCA cell migration, promotes tumor progression [25]. Similarly, CXCL12 expression in intrahepatic CCA is associated with metastasis and a poor prognosis [26]. These chemokines attract immune cells to tumor sites and modulate immune responses.

Cytokines, such as interleukins and interferons, also play essential roles in the TIME. They promote or suppress tumor growth and modulate immune responses against cancer cells. Pro-inflammatory cytokines stimulate immune cell activation and enhance anti-tumor responses, whereas anti-inflammatory cytokines suppress immune cell functions and promote tumor immune evasion. According to Guo *et al*., the absence of AT-rich interactive domain 1A (ARID1A) within the SWItch/sucrose nonfermentable (SWI/SNF) complex impairs TGF-β signaling activation in biliary epithelial cells. These alterations lead to the development of CCA, originating from biliary cells [27]. Elucidating the roles of specific cytokines and chemokines in the TIME in CCA will aid the development of immunotherapeutic strategies to improve patient outcomes. Li *et al*. showed that the cGGNBP2-184aa protein, produced by the IL-6-induced *cGGNBP2* gene, promotes ICC progression through a positive feedback loop. Therefore, cGGNBP2-184aa can be used as an auxiliary treatment for clinical interventions targeting IL-6/STAT3 signaling in ICC [28].

Immunotherapeutic strategies targeting the TIME in CCA

Modulation of TAMs to enhance anti-tumor immune responses

Targeting TAMs is a promising strategy for enhancing anti-tumor immune responses in CCA. Inhibiting TAM recruitment or polarization towards the immunosuppressive phenotype or promoting their reprogramming towards a pro-inflammatory, anti-tumor phenotype might improve the efficacy of immunotherapy in CCA [7]. One approach to modulate TAMs is to block the colony-stimulating factor 1 receptor (CSF1R), which is involved in TAM recruitment and survival. The inhibition of CSF1R reduces TAM infiltration in various tumor models, leading to suppressed tumor growth and enhanced anti-tumor immune responses [29, 30]. However, the efficacy of CSF1R blockade could depend on the tumor model and the timing of treatment [31].

Combination therapies targeting TAMs and other immune cells have shown promising outcomes for CCA. Loeuillard *et al*. reported that targeting TAMs and granulocytic-MDSCs improved the efficacy of PD-1 blockade in CCA, leading to enhanced tumor control and improved survival outcomes [32]. This demonstrates the potential of combining TAM-targeting strategies with other immunotherapies, such as immune checkpoint inhibitors, to achieve better therapeutic outcomes in CCA. In summary, the modulation of TAMs is a potential approach for enhancing anti-tumor immune responses in CCA. Further studies and clinical trials are needed to optimize TAM-targeting strategies and explore their potential when combined with other immunotherapies.

Adoptive T-cell transfer and its potential for targeting TILs

Adoptive T cell transfer (ACT) is a promising immunotherapeutic strategy for CCA that involves the isolation, expansion, and reinfusion of tumor-specific T cells into patients to enhance anti-tumor immune responses. The use of chimeric antigen receptor (CAR) T cells is a novel cellular therapeutic approach in which autologous T cells are harvested from a patient and genetically modified to express CARs. CAR-T cell therapy is thus a type of adoptive T-cell transfer with great potential for CCA treatment [33, 34].

Anti-mucin 1 (MUC1) CAR T cells have been developed for adoptive T cell therapy for CCA. They have been shown to exert cytotoxic effects against MUC1-expressing CCA cells *in vitro* [35]. The anti-MUC1 CAR T cells expressing a PD-1-CD28 switch receptor exhibited

enhanced cytotoxicity against CCA cells, overcoming the immunosuppressive effects of PD-1 signaling [36].

Another promising target for CAR T cell therapy in CCA is CD133, a cancer stem cell marker. Fourth-generation CAR T cells targeting CD133 exhibit anti-tumor effects against CCA cells *in vitro* and *in vivo* [37]. Further, T cells secreting αCD133-αCD3 bispecific T cell engager (BiTE) have anti-tumor activities against CCA, with the potential to redirect T cells to target CD133-positive tumor cells [38].

To overcome the challenge of instability of T lymphocyte cytotoxicity on combining gemcitabine with cytotoxic T lymphocytes, Methi *et al*. developed a recombinant PD-L1xCD3 BiTE, and the results showed that the combination of gemcitabine and PD-L1xCD3 BiTE could promote T lymphocyte cytotoxicity against CCA cells *in vitro*, suggesting potential synergistic effects between chemotherapy and immunotherapy [39]. However, CAR T immunotherapy targeting human epidermal growth factor receptor 2 (HER2) in patients with advanced CCA only led to disease stabilization in four of nine patients [40].

Tumor vaccines induce immune activation through tumor antigens

Tumor vaccines are designed to stimulate immune responses against tumor-specific antigens, thereby promoting tumor-specific T cell activation and expansion. Messenger RNA (mRNA) vaccines have gained significant attention because of their potential for precise and personalized cancer immunotherapy [41]. These vaccines deliver mRNA encoding tumor-associated antigens, which are then translated into proteins by host cells. These antigens are then presented to the immune system, activating immune responses against the tumor cells expressing them [42].

In CCA, studies have aimed to identify tumor antigens and immune subtypes suitable for mRNA vaccine development. Huang *et al*. identified 13 CCA-specific antigens and three immune subtypes that can be targeted by personalized mRNA vaccines [43]. Tang *et al*. proposed a precise pipeline for mRNA vaccine development for CCA, including tumor antigen identification, the design of mRNA constructs, and assessments of vaccine efficacy in preclinical models [41].

Studies on bile duct cancer vaccines have also included DNA and protein vaccines [44, 45], with one study noting that protein vaccines could downregulate PD-L1 gene expression and suppress CCA carcinogenesis, relative to the effects of DNA vaccines [45]. Although CCA vaccines are in the early developmental stages, studies highlight the potential of mRNA vaccines to target tumor antigens and induce immune activation against CCA cells. Studies and clinical trials should be performed to assess the safety, efficacy, and optimal combination of tumor antigens for personalized mRNA vaccines in patients with CCA.

ICIs target immune checkpoints in CCA

ICIs are promising immunotherapeutic agents for CCA that target immune checkpoints to enhance anti-tumor immune responses. Immune checkpoints such as PD-1, PD-L1, and CTLA-4 play critical roles in tumor cell immune evasion [20]. ICIs block these checkpoints, enabling the immune system to effectively recognize and attack tumor cells [23].

Nivolumab, an anti-PD-1 antibody, is a potential treatment option for biliary tract cancers, including CCA [23]. A single-center study found that both pembrolizumab and nivolumab exert effective anticancer effects when combined with lenvatinib [46]. Durvalumab, an anti-PD-L1 antibody, is also a potential therapeutic option for CCA [24]. TOPAZ-1, the first phase 3 trial, which involved 685 patients with inoperable, locally advanced, recurrent, or metastatic biliary tract cancer who were randomized to receive durvalumab or a placebo, showed the benefits of immunotherapy for improved overall survival, in combination with chemotherapy, creating a new standard of care [47]. Clinical and molecular analyses of advanced biliary tract cancers treated with immune checkpoint blockade have shown that elevated PD-L1 expression, TILs, and microsatellite instability are potential markers of the response to ICIs [21, 48]. Ipilimumab, an anti-CTLA-4 antibody, binds to the CTLA-4 antigen, resulting in anti-tumor immune responses. This allows the body to attack cancerous cells, and it is less likely to harm healthy tissues. A phase 2 clinical trial found that a combination of nivolumab and ipilimumab could improve therapeutic outcomes in patients with advanced biliary tract cancers [49]. However, the addition of nivolumab to chemotherapy or ipilimumab did not improve the 6-month progression free survival (PFS) [50].

Various clinical trials have investigated the efficacy and safety of combining ICIs with other targeted therapies or chemotherapies for CCA treatment [22, 51]. The combination of MEK inhibitors and ICIs has shown promising activity against various solid tumors, especially in the presence of KRAS or p53 mutations [52, 53]. These combination therapies enhance the anti-tumor effects of ICIs and improve patient outcomes [51]. In summary, ICIs that target immune checkpoints are potential therapeutic strategies for CCA. Clinical trials should be performed to determine the optimal patient population, combination therapies, and biomarkers for predicting responses to these treatments.

Bifunctional fusion proteins inhibit TGF-β and enhance anti-PD-L1 outcomes

Bifunctional fusion proteins that target TGF-β and PD-L1 have been developed to enhance the efficacy of anti-PD-L1 treatment [54]. High expression is associated with CCA invasion and metastasis [55]. By inhibiting TGF-β1-mediated mesenchymalization, these fusion proteins can modulate the immunosuppressive effects of TGF-β in the TIME while enhancing anti-tumor immune responses through PD-L1 blockade [56]. Inhibiting TGF-β has the ability to rejuvenate exhausted cytotoxic T cells, augmenting the efficacy of anti-PD-L1 treatment [57]. Bintrafusp alfa (M7824) is an innovative and unique protein consisting of a human anti-PD-L1 IgG1 monoclonal antibody and two extracellular domains of TGF-β receptor II. It is considered the first bifunctional fusion protein in its class. Preclinical studies have shown promising results [54, 56] and long-term follow-up safety and efficacy data for bintrafusp alfa in patients with pretreated CCA have been reported [58]. Currently, two newly developed drugs, SHR-1701 and YM101 [59, 60], have been designed to target PD-L1 and TGF-β in cancer treatment. Clinical studies have reported that SHR-1701 has a favorable therapeutic effect on solid tumors, such as CCA [61]. In contrast, YM101 is used in conjunction with other drugs to reduce immune exclusion in tumors and enhance their therapeutic effects against cancer [62]. While similar drugs, such as JS201, GS-19, PM8001, and NRT6003, are still in the preclinical research stage, further studies and clinical trials should be performed to assess the potential of bifunctional fusion proteins for CCA treatment [4].

Conclusions and perspectives

Immunotherapy targeting the TIME is a promising therapeutic approach for CCA treatment. The cellular and molecular components of the TIME, such as TAMs, TILs, MDSCs, Tregs, immune checkpoint molecules, and cytokines/chemokines, play critical roles in tumor progression, immune evasion, and therapeutic responses. Various immunotherapeutic strategies, including the modulation of TAMs, ACT, tumor vaccines, ICIs, and bifunctional fusion proteins, have shown great potential for CCA therapy in preclinical studies and clinical trials.

However, overall response rates to immunotherapies remain modest, and some patients experience immune-related adverse events. Studies should aim to optimize treatment regimens, identify predictive biomarkers of treatment responses, and develop rational combination therapies that enhance the efficacy of immunotherapy while minimizing toxicity. In addition, an elucidation of the complex interactions among tumor cells, immune cells, and the TIME is essential for the development of novel therapeutic strategies and personalized treatment approaches for patients with CCA [4, 5, 63].

In conclusion, studies on immunotherapy targeting the TIME in CCA have provided valuable insights and promising therapeutic strategies that can potentially improve the outcomes of patients with CCA. As the understanding of the TIME and its components continues to grow, we anticipate the development of more effective and personalized immunotherapeutic approaches. Researchers and clinicians should continue to collaborate in designing and executing well-conducted preclinical studies and clinical trials, with the goal of improving the prognosis and quality of life of patients with CCA [4, 5, 63].

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

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

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Not applicable.

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Not applicable.

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