

Review Article

Modulating the Tumor Microenvironment to Enhance Immunotherapy Efficacy in Hepatic Metastases from Colorectal Cancer: The Role of TGF- β Inhibition

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Abstract

Colorectal Cancer (CRC) frequently metastasizes to the liver, where the unique immunosuppressive Tumor Microenvironment (TME) challenges immunotherapy efficacy. Transforming Growth Factor-beta (TGF- β), a cytokine integral to immune regulation, fibrosis and Epithelial-Mesenchymal Transition (EMT), is crucial in creating this hostile TME, facilitating immune evasion and metastasis. TGF- β 's influence on immune cells, including regulatory T-cells (Tregs), contributes to reduced Cytotoxic T Lymphocyte (CTL) infiltration and impaired immune responses within hepatic metastases. Inhibiting TGF- β has emerged as a promising approach to reprogramming the TME, enhancing immune cell infiltration, reducing fibrosis and reversing EMT. Recent studies demonstrate that TGF- β blockade can improve Immune Checkpoint Inhibitor (ICI) responses, particularly in Microsatellite-Stable (MSS) CRC tumors typically resistant to ICIs. However, systemic inhibition presents risks, including potential autoimmunity and fibrosis in non-tumor tissues, highlighting the need for targeted delivery systems, such as nanoparticles, to localize TGF- β inhibition within the TME. Research suggests modulating the gut-liver axis may enhance TGF- β inhibition effects by optimizing immune responses. Combination therapies integrating TGF- β inhibitors with ICIs, cytokine therapies or anti-angiogenic agents show potential to overcome CRC immune resistance. Despite the therapeutic promise, patient selection and biomarker identification remain critical challenges, necessitating further studies to refine and personalize treatment approaches. This review explores the multifaceted role of TGF- β in CRC liver metastasis and discusses strategies for enhancing immunotherapy efficacy through targeted TGF- β modulation.

Keywords: Tumor Microenvironment; Transforming Growth Factor Beta; Colorectal Neoplasms; Liver Neoplasms; Neoplasm Metastasis; Immunotherapy

Introduction

Colorectal Cancer (CRC) remains one of the most prevalent malignancies globally and continues to pose significant clinical challenges, particularly when it progresses to metastatic disease. Despite advancements in diagnostic techniques, surgical interventions and systemic therapies, including chemotherapy and targeted therapy, the prognosis for patients with metastatic Colorectal Cancer (mCRC) remains poor [1-3].

The liver is the most frequent site of metastasis in CRC and the presence of hepatic metastases dramatically reduces survival rates, making liver-directed treatments a central focus in improving outcomes for these patients. Current treatment paradigms, however, are often insufficient, as many patients with liver metastases eventually develop resistance to conventional therapies, necessitating new strategies to combat the disease [4-6].

Immunotherapy has revolutionized the treatment of several cancers, particularly melanoma and lung cancer, by harnessing the body's immune system to attack tumor cells. Immune checkpoint inhibitors, such as those targeting programmed death-1 (PD-1) and its ligand PD-L1, have demonstrated significant efficacy in a subset of cancers by reactivating exhausted T-cells to mount an anti-tumor response [5-7].

However, in mCRC, the success of these therapies has been limited mainly to a small subset of patients with Microsatellite Instability-High (MSI-H) tumors. Most CRC cases, which are Microsatellite Stable (MSS), do not respond well to immune checkpoint inhibitors, primarily due to the immune-excluded phenotype and immunosuppressive nature of these patients' Tumor Microenvironments (TME) [2]. This immune evasion is particularly pronounced in liver metastases, where the TME actively suppresses immune cell infiltration and function [6-8].

The liver is a unique organ in terms of immune regulation. As a primary site for detoxification and filtration of blood, it is constantly exposed to various antigens from the gut, necessitating a finely tuned balance between immune tolerance and activation [9]. While essential for normal liver function, this immunologically tolerant environment also creates a sanctuary for metastatic cancer cells, allowing them to evade immune detection and destruction [10].

The liver's immunosuppressive environment is characterized by the presence of regulatory immune cells, including regulatory T-cells (Tregs), Myeloid-Derived Suppressor Cells (MDSCs) and Tumor-Associated Macrophages (TAMs), all of which are key players in promoting tumor progression and metastasis [11]. This highly suppressive TME is further reinforced by soluble factors such as Transforming Growth Factor-beta (TGF- β), critical in maintaining immune tolerance and promoting tumor immune evasion [12].

Tumor microenvironment plays a pivotal role in CRC's progression, metastasis and immune evasion. It involves a complex interplay between cancer cells, immune cells, stromal cells and extracellular matrix components. Several factors contribute to immune suppression within this microenvironment, including the overexpression of Transforming Growth Factor-beta (TGF- β), a key regulator of immune tolerance and tumor progression [13-15].

TGF- β exerts profound immunosuppressive effects by inhibiting the function of cytotoxic T-cells, promoting the differentiation of regulatory T-cells and inducing Epithelial-Mesenchymal Transition (EMT), all of which facilitate tumor immune evasion and metastasis, particularly to the liver [14-16].

In recent years, TGF- β has emerged as a critical target for therapeutic intervention in CRC, especially in the context of liver metastases. As a site of metastasis, the liver poses a unique immunological challenge due to its role in systemic tolerance and the presence of immune-suppressive cell populations such as Myeloid-Derived Suppressor Cells (MDSCs) and regulatory T-cells (Tregs) [15-17].

These cells are recruited by tumor and modulated by TGF- β signaling to suppress anti-tumor immune responses further. As a result, targeting TGF- β in combination with immunotherapies, such as PD-1/PD-L1 inhibitors, has been proposed as a strategy to overcome resistance to immune checkpoint blockade in metastatic CRC [16-18].

TGF- β is a multifunctional cytokine that regulates various cellular processes, including cell proliferation, differentiation and survival. In the context of cancer, TGF- β signaling is a double-edged sword [19]. It acts as a tumor suppressor in early-stage tumors by inhibiting cell proliferation and inducing apoptosis. However, as cancer progresses, many tumors, including those of CRC, develop mechanisms to bypass the growth-suppressive effects of TGF- β , leading to its transformation into a potent promoter of tumor progression and metastasis [20,21].

In metastatic CRC, particularly in liver metastases, TGF- β is pivotal in creating an immunosuppressive TME that facilitates immune escape and supports metastatic colonization [22]. TGF- β promotes the recruitment and activation of immunosuppressive cell populations, such as Tregs and MDSCs, while simultaneously inhibiting the activity of cytotoxic T-cells and Natural Killer (NK) cells, which are essential for anti-tumor immunity [23].

The role of TGF- β in liver metastasis is not limited to its effects on immune cells. TGF- β also promotes metastatic colonization by inducing EMT, in which epithelial cells acquire invasive mesenchymal properties. EMT is a crucial driver of metastasis in CRC and is associated with resistance to both chemotherapy and immunotherapy [24,25]. By targeting TGF- β , it may be possible to enhance the efficacy of immunotherapies and prevent the metastatic spread of CRC to the liver and other organs. Additionally, TGF- β inhibition may sensitize tumor cells to chemotherapy, further improving outcomes in patients with mCRC [8,26].

TGF- β has been shown to induce Epithelial-Mesenchymal Transition (EMT), a process by which epithelial cancer cells acquire mesenchymal traits, increasing their invasive potential and enabling them to disseminate from the primary tumor to distant organs, such as the liver [12,27].

EMT is not only critical for the initiation of metastasis but also contributes to therapy resistance, particularly to immune checkpoint inhibitors. TGF- β facilitates the generation of cancer stem-like cells, which are highly resistant to conventional therapies and contribute to tumor recurrence and progression by inducing EMT [28]. This dual role of TGF- β in promoting both immune suppression and metastatic progression makes it a desirable target for therapeutic intervention in metastatic CRC [29]. The development of therapeutic strategies targeting TGF- β has garnered significant attention in recent years, particularly in combination with immunotherapy. Preclinical studies have demonstrated that blocking TGF- β can reprogram the TME, making it more conducive to anti-tumor immune responses [14]. TGF- β inhibition has been shown to enhance the infiltration and activity of cytotoxic T-cells within the tumor, reversing the immune-excluded phenotype characteristic of many MSS CRC tumors [30]. By breaking down the fibrotic stroma and disrupting the immunosuppressive signals within the TME, TGF- β inhibitors can potentiate the efficacy of immune checkpoint inhibitors, which have otherwise shown limited success in MSS CRC and liver metastases [25].

Clinical trials are currently investigating the efficacy of TGF- β inhibitors, both as monotherapy and in combination with immune checkpoint inhibitors, in various cancers, including metastatic CRC [29,31]. These studies aim to evaluate whether TGF- β blockade can overcome the inherent resistance of MSS CRC to immunotherapy by enhancing immune infiltration and function within the tumor [32].

Early results from these trials are promising, showing enhanced anti-tumor activity and improved patient outcomes when TGF- β inhibitors are combined with PD-1/PD-L1 blockade. However, the clinical translation of these findings is still in its early stages and challenges remain in optimizing the safety and efficacy of these combination therapies [33-35].

One of the critical challenges in targeting TGF- β is its pleiotropic nature, as it is involved in a wide array of physiological processes beyond cancer. TGF- β plays a crucial role in maintaining tissue homeostasis and immune regulation and its inhibition can lead to adverse effects, including autoimmunity and organ fibrosis [27,36]. Therefore, it is critical to develop more selective inhibitors that can specifically target the tumor-promoting effects of TGF- β without disrupting its physiological functions in normal tissues [32].

Advances in nanomedicine and targeted drug delivery systems offer promising solutions to this challenge by enabling the precise delivery of TGF- β inhibitors to the tumor site, thereby minimizing systemic toxicity and enhancing therapeutic efficacy [34]. In addition to its direct effects on immune cells, TGF- β also influences other components of the TME, including Cancer-Associated Fibroblasts (CAFs) and the Extracellular Matrix (ECM). TGF- β is a crucial tumor fibrosis drive, forming a dense ECM that acts as a physical barrier to immune cell infiltration [23,37].

By targeting TGF- β , it is possible to reduce ECM deposition and stromal stiffening, thereby enhancing the penetration of immune cells into the tumor and facilitating a more effective anti-tumor response [10]. This stromal remodeling is significant in liver

metastases, where the fibrotic nature of the liver's TME further exacerbates immune exclusion and contributes to therapeutic resistance [31].

Evaluating TGF- β inhibitors in combination with immunotherapy is currently underway, with early results showing promising efficacy in several cancer types, including CRC. These trials aim to assess the safety and effectiveness of TGF- β blockade, both as a monotherapy and in combination with PD-1/PD-L1 inhibitors [15]. The success of these trials could pave the way for new treatment paradigms in mCRC, particularly for patients with liver metastases who currently have limited therapeutic options [4].

Despite these advances, several challenges remain in the clinical implementation of TGF- β inhibition. One of the primary concerns is the potential for toxicity, as TGF- β is involved in numerous physiological processes beyond cancer, including tissue homeostasis and immune regulation [38]. Therefore, careful dosing and patient selection will be critical to minimizing adverse effects while maximizing therapeutic benefit. Additionally, identifying predictive biomarkers for response to TGF- β inhibition will be essential to guide patient selection and optimize treatment outcomes [21-23].

Another area of ongoing research is the development of more selective TGF- β inhibitors that can target specific components of the TGF- β signaling pathway without disrupting its beneficial effects in normal tissues [36,39]. Recent advances in nanotechnology and drug delivery systems have shown the potential to enhance the specificity and efficacy of TGF- β inhibitors, reduce off-target effects and improve patient tolerability [34].

The combination of TGF- β inhibition with other therapeutic modalities, such as radiotherapy and chemotherapy, is also being explored. Radiotherapy has been shown to modulate the TME and enhance immune responses and preclinical studies suggest that combining radiotherapy with TGF- β inhibition may further potentiate these effects [12,40]. Similarly, chemotherapy-induced immunogenic cell death could be improved by TGF- β blockade, providing a rationale for combining these therapies in patients with mCRC [8].

Given the role of TGF- β in shaping the immunosuppressive landscape of liver metastases from CRC, a comprehensive understanding of how TGF- β promotes immune evasion and metastasis is essential for developing effective therapeutic strategies [11,38]. This review aims to provide an in-depth analysis of the current state of research on TGF- β inhibition in the context of metastatic CRC, with a particular focus on its potential to enhance the efficacy of immunotherapy. By exploring the latest advancements in preclinical and clinical studies, this review highlights the challenges and opportunities in targeting TGF- β . It offers insights into the future directions of combination therapies for metastatic CRC [4].

This review aims to synthesize the available evidence on the role of TGF- β in the tumor microenvironment of hepatic metastases from CRC and to evaluate the therapeutic potential of TGF- β inhibition in combination with immunotherapy. This review will examine how TGF- β contributes to immune suppression and metastasis, discuss the latest developments in TGF- β -targeted therapies and explore the clinical implications of combining TGF- β inhibitors with immune checkpoint blockade. By providing a comprehensive overview of the field's current state, this review aims to inform future research and guide the development of novel therapeutic approaches for improving outcomes in patients with metastatic CRC.

Methods

This comprehensive review was conducted to investigate the role of the Tumor Microenvironment (TME) and Transforming Growth Factor-beta (TGF- β) in Colorectal Cancer (CRC) liver metastasis and the implications for enhancing immunotherapy efficacy. To achieve this, a systematic literature search was performed across multiple scientific databases, including PubMed, Scopus, Embase, Web of Science, SciELO and Google Scholar, covering the entire database range up to the present. The search strategy employed specific MeSH terms and keywords, including "Tumor Microenvironment," "Transforming Growth Factor-beta," "Colorectal Neoplasms," "Liver Neoplasms," "Neoplasm Metastasis," and "Immunotherapy." Boolean operators (And, Or) were applied to refine search results, optimizing identifying studies relevant to TGF- β 's role in the TME, its effects on metastasis and immunotherapy outcomes in CRC. The review included various study designs, encompassing randomized controlled trials, cohort studies, case-control studies, cross-sectional analyses, case series, systematic reviews, meta-analyses and preclinical studies. These studies were selected based on their focus on TGF- β signaling, immunomodulatory mechanisms, TME dynamics

and therapeutic interventions targeting TGF- β within CRC liver metastasis. The inclusion criteria emphasized studies that explored the molecular interactions within the TME, particularly the roles of TGF- β and immunotherapy and the specific challenges posed by liver metastasis in CRC. Study selection was conducted by two independent reviewers who screened titles and abstracts to ensure comprehensiveness and objectivity of the review. Discrepancies between reviewers were resolved through discussion or, if necessary, by consulting a third reviewer. To minimize bias, the reviewers were blinded to the authors and institutions of the studies during the selection process. A standardized data extraction protocol was used to gather essential information, including study design, population characteristics, key findings and relevant outcomes associated with TGF- β , TME modulation and immunotherapy efficacy in CRC liver metastasis. The extracted data underwent a thematic analysis to organize findings into crucial themes. Central themes included the molecular pathways of TGF- β within the TME, the cytokine's immunosuppressive effects, TME modulation and the potential of TGF- β inhibition in enhancing Immune Checkpoint Inhibitor (ICI) efficacy. The analysis also focused on the structural and immunosuppressive aspects of the TME in liver metastases, as well as therapeutic strategies designed to disrupt these mechanisms and potentiate immune responses. Furthermore, potential pharmacological and gene-editing strategies for TGF- β inhibition were reviewed, along with nanoparticle-based drug delivery systems to improve specificity and reduce systemic side effects. This review aims to provide a detailed synthesis of the current literature, emphasizing the impact of TGF- β on immunotherapy outcomes in CRC liver metastasis. By identifying gaps in knowledge and proposing future research directions, this review seeks to advance understanding of how TGF- β modulation within the TME can improve immunotherapy efficacy for CRC patients with liver metastases.

Results and Discussion

The Tumor Microenvironment (TME) plays a pivotal role in the progression, metastasis and immune evasion of Colorectal Cancer (CRC), particularly in liver metastases, which are the most frequent sites of distant spread. The liver's unique immunological environment, characterized by a delicate balance between immune tolerance and surveillance, presents significant challenges for immunotherapy (Table 1) [41-43].

One of the key drivers of this immunosuppressive TME is Transforming Growth Factor-beta (TGF- β), a multifunctional cytokine involved in immunomodulation, fibrosis and Epithelial-Mesenchymal Transition (EMT) [5]. TGF- β is central to creating an environment that supports tumor progression and metastasis while hindering effective immune responses [24]. As such, inhibiting TGF- β has emerged as a promising therapeutic strategy to enhance the efficacy of Immune Checkpoint Inhibitors (ICIs), which have shown limited success in treating metastatic CRC (mCRC), particularly in the liver [16].

Author	Study	Results
Tauriello, et al., 2018 [1]	Experimental study	The study elucidates the role of TGF- β in promoting immune evasion in colon cancer metastases and diminishing the effectiveness of PD-L1 blockade, suggesting that inhibiting TGF- β could potentiate the response to immunotherapies.
Mariathasan, et al., 2018 [2]	Experimental study	This research demonstrates that TGF- β reduces the efficacy of PD-L1 blockade by facilitating the exclusion of T-cells from the tumor microenvironment, indicating the potential benefits of combining therapies to circumvent TGF- β -mediated resistance.
Huang, et al., 2023 [4]	Drug development study	Describes the development of a multifunctional nanodrug that targets TGF- β to enhance the immune response against colorectal cancer with liver metastases, showing potential to improve outcomes in immunotherapy.
Dodagatta-Marri, et al., 2019 [7]	Clinical and experimental study	Shows that combining α -PD-1 therapy with TGF- β blockade can lead to durable rejection of carcinomas, highlighting the synergistic potential of these therapies in oncological treatment strategies.
O'Malley, et al., 2018 [16]	Observational study	Explores the role of PD-L1 expression on stromal cells in inhibiting CD8+ T-cell antitumor activity in colon cancer, emphasizing its significance in the modulation of the tumor immune microenvironment.
Zboralski, et al., 2017 [17]	Experimental study	Investigates the effects of CXCL12 inhibition on enhancing T-cell infiltration in tumors, which when combined with PD-1 blockade, significantly boosts anti-tumor immunity, suggesting effectiveness in combined immunotherapy settings.
Sun, et al.,	Radiomic	Utilizes radiomic techniques to evaluate CD8+ T-cell infiltration and their response to anti-PD-

2018 [14]	study	1/PD-L1 therapy in metastatic cancers, proposing a novel imaging biomarker approach to predict and monitor treatment responses.
Bald and Smyth, 2018 [22]	Review	Discusses the mechanisms by which TGF- β regulates T-cell infiltration and supports immune evasion in metastatic cancers, stressing the importance of targeting TGF- β in combined immunotherapy approaches.
Wang, et al., 2019 [36]	Genetic study	Focuses on the transcriptomic characteristics of the microsatellite instability subtype of colon cancer, detailing its inherent immunogenic properties and interactions with immune checkpoints, which are crucial for optimizing immunotherapeutic strategies.
Conche, et al., 2023 [45]	Experimental study	Investigates the synergistic effect of inducing ferroptosis in conjunction with MDSC blockades to enhance the sensitivity of hepatic tumors to immunotherapy, representing an innovative treatment avenue for liver metastases.

Table 1: Immunotherapy/tumor microenvironment in colorectal cancer and hepatic metastases.

The inhibition of TGF- β in CRC liver metastasis has garnered increasing attention due to its potential to reverse the immunosuppressive nature of the TME and improve patient outcomes. TGF- β plays a critical role within the TME and at a systemic level, influencing the overall immune response [42-44].

While inhibiting TGF- β may enhance immune-mediated tumor destruction, it also risks disrupting immune homeostasis in healthy tissues, leading to potential side effects such as systemic inflammation, autoimmunity and fibrosis [26,42]. These systemic effects underscore the importance of developing targeted delivery systems or localized inhibition strategies to minimize off-target toxicities while preserving the therapeutic benefits of TGF- β inhibition [45].

Recent studies have demonstrated that systemic inhibition of TGF- β can reverse the immunosuppressive state of the TME, promoting immune cell infiltration and enhancing the efficacy of ICIs. However, the inhibition of TGF- β may also result in heightened immune responses in non-cancerous tissues [20,46].

TGF- β normally functions as a regulator of immune tolerance, preventing overactivation of the immune system and protecting tissues from damage during inflammation. Its inhibition could, therefore, exacerbate inflammatory conditions in healthy tissues, further complicating the therapeutic landscape [14,47]. To address these concerns, targeted delivery systems, such as nanotechnology-based platforms, are being explored to deliver TGF- β inhibitors specifically to the tumor site, reducing systemic exposure and potential adverse effects [38].

The gut-liver axis has also emerged as an area of interest in the context of TGF- β inhibition, particularly in CRC patients. The gut microbiome, a critical regulator of immune responses, has been shown to influence the efficacy of immunotherapy [21]. Dysbiosis or an imbalance in the gut microbiota, can affect immune cell activation and tumor immunogenicity, ultimately impacting therapeutic outcomes. Since TGF- β plays a role in regulating intestinal inflammation and maintaining immune tolerance in the gut, its inhibition could alter the microbiome's composition and function, potentially affecting the efficacy of ICIs in liver metastases [43,48].

Investigating the interplay between TGF- β inhibition and the microbiome represents a novel frontier in cancer immunotherapy research and future studies should focus on optimizing treatment responses by modulating both the microbiome and the TME [17,49]. In the liver, TGF- β inhibition may have unintended consequences beyond the tumor. The liver's immunological role extends to maintaining tolerance through specialized immune cells such as Kupffer and liver-resident dendritic cells. Inhibiting TGF- β may disrupt this immunological balance, leading to inflammation, fibrosis or autoimmunity in healthy liver tissues [33,50]. This highlights the need for selective TGF- β inhibitors or advanced delivery mechanisms to confine the inhibition effects to the tumor site. Nanoparticle-based drug delivery systems offer promising solutions, allowing for the precise targeting of TGF- β inhibitors to the tumor while sparing surrounding healthy tissue [51-53]. Preclinical studies have shown that nanoparticle-based delivery can improve the bioavailability and specificity of TGF- β inhibitors, thereby enhancing therapeutic efficacy while minimizing side effects [10,24].

TGF- β acts with other cytokines and signals pathways in CRC liver metastasis to create a robust immunosuppressive environment. Cytokines such as interleukin-6 (IL-6) and interleukin-10 (IL-10) are also involved in immune regulation and tumor progression, often working in synergy with TGF- β to suppress immune responses. IL-10 has been recognized for its immunosuppressive properties, further contributing to immune cell exclusion from the tumor [52-54]. Understanding how these cytokines interact within the TME could provide new avenues for combination therapies that target multiple immunosuppressive pathways, potentially enhancing the overall efficacy of ICIs [22,35].

TGF- β 's influence extends beyond immune cells to other critical components of the TME, including cancer-associated fibroblasts (CAFs) and the extracellular matrix (ECM). CAFs, activated by TGF- β , contribute to forming a fibrotic stroma that is a physical barrier to immune cell infiltration and limits the penetration of therapeutic agents [48,55].

By inhibiting TGF- β , researchers aim to disrupt the interaction between CRC cells and CAFs, reducing fibrosis and enabling greater immune cell access to the tumor. Stromal remodeling, facilitated by TGF- β inhibition, is a crucial mechanism through which immune exclusion can be reversed and the efficacy of ICIs can be potentiated [45,50].

Gene-editing technologies like CRISPR represent another exciting frontier in CRC liver metastasis treatment. CRISPR-based therapies could specifically target TGF- β expression in tumor cells while sparing healthy tissues, offering a more precise and personalized approach to treatment [17,49].

Although still in its early stages, combining CRISPR-based gene editing with TGF- β inhibitors holds significant promise for overcoming current treatment limitations. These gene-editing approaches could knock down TGF- β signaling in tumor cells, reducing their ability to evade the immune system and making them more susceptible to immune-mediated destruction [40,56]. CAR-T-cell therapies and cancer vaccines also present new opportunities for combination treatments with TGF- β inhibitors. CAR-T-cells have shown great success in hematologic cancers but have faced challenges in solid tumors, partly due to the immunosuppressive TME [25,57].

TGF- β represents a significant barrier to CAR-T-cell efficacy in solid tumors like CRC liver metastasis. Preclinical studies suggest combining TGF- β inhibitors with CAR-T-cell therapy could enhance CAR-T-cell persistence and function, leading to more robust anti-tumor responses. Integrating these therapies could improve the therapeutic landscape for mCRC, making CAR-T-cells more effective in solid tumor settings [49,58].

Angiogenesis, forming new blood vessels, is another critical process influenced by TGF- β in TME. TGF- β promotes abnormal tumor vasculature, which can limit the delivery of immune cells and therapeutic agents to the tumor [16]. By targeting TGF- β , it may be possible to normalize the tumor vasculature, improving immune cell infiltration and enhancing the efficacy of ICIs, chemotherapy and radiotherapy [29]. Preclinical models have shown that combining TGF- β inhibition with anti-angiogenic agents may offer dual benefits, disrupting the tumor's blood supply and enhancing immune responses [44,59].

One of the most promising aspects of TGF- β inhibition is its ability to reverse EMT, a process by which epithelial cancer cells gain mesenchymal properties, making them more invasive and resistant to therapy [36]. EMT is a crucial driver of metastasis in CRC, enabling tumor cells to spread to distant organs, such as the liver. TGF- β and reversing EMT, researchers hope to reduce the metastatic potential of CRC cells, making them more vulnerable to immune attack and increasing the efficacy of chemotherapy and immunotherapy [18]. Reversing EMT also has the potential to reduce the population of cancer stem-like cells, which are known to be particularly resistant to conventional treatments [20].

Despite the promising results from preclinical studies and early-phase clinical trials, there remain significant gaps in our understanding of how TGF- β promotes immune evasion and metastasis in CRC. Future research should focus on elucidating these complex interactions within the TME, including the potential for tumors to upregulate alternative immunosuppressive pathways in response to TGF- β inhibition [53,60]. For example, tumors may compensate by increasing PD-L1 expression or recruiting additional immunosuppressive cell populations, presenting new challenges for TGF- β -targeted therapies [3].

Researchers are investigating combination therapies that target multiple pathways within the TME to overcome these challenges. Combining TGF- β inhibitors with PI3K, MAPK or Wnt signaling pathway inhibitors could provide a more comprehensive approach to modulating the TME and enhancing the efficacy of ICIs [44,51]. In addition, exploring novel immunotherapeutic strategies, such as CAR-T-cells and cancer vaccines, in combination with TGF- β inhibitors, may further boost anti-tumor immunity and improve clinical outcomes for patients with CRC liver metastasis [13,25].

Identifying reliable biomarkers for predicting patient response to TGF- β inhibition remains a critical area of research. Biomarkers such as TGF- β expression levels, fibrosis within the tumor and the presence of immunosuppressive cells like Tregs and MDSCs could help identify patients most likely to benefit from TGF- β -targeted therapies [39,61]. The development of companion diagnostics to measure these biomarkers in real time could aid in personalizing treatment strategies, improving patient outcomes and minimizing unnecessary side effects [12].

Expanding on the existing knowledge surrounding TGF- β inhibition in CRC liver metastasis, additional scientific perspectives and gaps in the research deserve emphasis to create a more comprehensive overview. The role of TGF- β in the spatial dynamics of immune cell trafficking within the TME is a promising avenue for further exploration [19,58].

Research has shown that TGF- β signaling creates physical and biochemical barriers that restrict the entry and mobility of Cytotoxic T Lymphocytes (CTLs) and Natural Killer (NK) cells within tumor sites, especially in highly fibrotic environments such as liver metastases [62]. However, the precise mechanisms by which TGF- β signaling affect immune cell migration patterns and retention within the liver TME remain underexplored. A deeper understanding of how TGF- β controls immune cell spatial dynamics could guide the development of more effective therapeutic interventions targeting immune cell infiltration and retention in mCRC [24,58].

One crucial aspect not fully addressed in current research is the differential response of various immune cell subtypes to TGF- β inhibition in the liver TME. For instance, while CD8⁺ T-cells may benefit from TGF- β pathway inhibition, other immune cells, such as macrophages and dendritic cells, could react differently, potentially leading to unintended immune suppression or activation [50,63].

Recent studies indicate that TGF- β may polarize macrophages towards an M2-like, immunosuppressive phenotype, which promotes tumor progression. Investigating how TGF- β inhibition might reprogram these macrophages towards a more pro-inflammatory, anti-tumor M1-like phenotype in liver metastases could provide insights into improving immunotherapy outcomes and refining combination treatment strategies [47,64].

The influence of TGF- β on stromal cell populations, such as cancer-associated fibroblasts (CAFs), is another critical area for exploration. CAFs, activated by TGF- β signaling, contribute significantly to the fibrotic structure of the TME by producing extracellular matrix (ECM) components that act as physical barriers to immune cells. The dual roles of CAFs in promoting and inhibiting immune cell activities are still not fully understood [65].

Targeting CAFs with TGF- β could weaken the fibrotic stroma, allowing for greater immune penetration. Studies exploring the heterogeneity of CAFs and their specific responses to TGF- β inhibition might reveal how particular CAF subtypes could be selectively targeted to maximize immune cell access to the tumor core [39-41]. Furthermore, TGF- β 's role in angiogenesis within the TME is a complex process that is partially understood but not yet fully elucidated. TGF- β has been shown to promote abnormal vascularization, which results in a chaotic and inefficient vasculature within tumors, thereby impeding the delivery of therapeutic agents and immune cells [59,66].

The abnormal vasculature also creates regions of hypoxia within the tumor, contributing to tumor aggressiveness and resistance to treatment. Current therapies have only partially addressed this phenomenon [67]. Future studies should investigate how TGF- β inhibition may contribute to vascular normalization, potentially transforming the TME into one more accessible to immune cells and anti-cancer drugs [4].

TGF- β 's interaction with Cancer Stem Cells (CSCs) is another avenue for further research. CSCs possess unique self-renewal capabilities and are often associated with resistance to conventional therapies [26]. TGF- β signaling maintains CSC properties, promotes EMT and facilitates metastatic spread. As CSCs contribute to tumor recurrence and resistance, targeting TGF- β could help reduce CSC populations within tumors [68].

This strategy requires careful balance, as excessive inhibition may disrupt normal stem cell functions in healthy tissues. Research into selective TGF- β inhibition targeting CSCs while sparing normal stem cells could lead to innovative therapeutic approaches to control metastasis and prevent recurrence in mCRC [51,62].

The potential systemic consequences of TGF- β inhibition in CRC liver metastasis, particularly concerning immune regulation in non-tumoral tissues, remain under-investigated [19]. TGF- β plays a critical role in maintaining immune homeostasis throughout the body and its inhibition may lead to unintended effects, such as the worsening of autoimmune conditions or tissue-specific toxicities. For example, liver toxicity could result from off-target inhibition of TGF- β , particularly in patients with pre-existing liver conditions [35,69].

To address these potential side effects, advanced drug delivery systems, such as nanoparticle-based delivery, can be engineered to concentrate TGF- β inhibitors within the tumor site, minimizing systemic exposure. Further research is required to develop these technologies and determine the optimal dosing and targeting strategies to inhibit tumors while preserving systemic immune function effectively [27,44].

Emerging evidence suggests that TGF- β interacts with metabolic pathways within the TME, creating an environment that supports tumor cell survival and immune suppression. Metabolites such as lactate, produced through anaerobic glycolysis, are accumulated within the TME and contribute to its acidic and hypoxic nature, further suppressing immune cell function [18,66]. Since TGF- β signaling has been linked to metabolic reprogramming in tumor cells, exploring how metabolic inhibitors might synergize with TGF- β inhibition could open new avenues for therapy. Combining TGF- β inhibitors with drugs that target metabolic pathways may help normalize the TME, making it more conducive to immune cell activity and less hospitable to tumor cells [55-57].

The interplay between TGF- β and other immunosuppressive pathways, such as the PD-1/PD-L1 axis, presents a promising area for therapeutic development. Some studies suggest tumors may compensate for TGF- β pathway inhibition by upregulating PD-L1 expression or recruiting alternative immunosuppressive cell populations, thereby sustaining immune evasion [11,26]. Combined blockade of TGF- β and PD-1/PD-L1 pathways could provide a more comprehensive approach to counteract immune suppression in the TME, potentially leading to improved clinical outcomes for patients with CRC liver metastasis. However, more research is needed to elucidate these combined treatments' synergistic effects and identify the most effective therapeutic combinations for different patient subgroups [34,70].

Advances in omics technologies, such as single-cell RNA sequencing and spatial transcriptomics, have provided new insights into the cellular and molecular heterogeneity within the TME. These tools offer unprecedented resolution in studying the diverse cell populations influenced by TGF- β signaling in liver metastasis [56,60]. By analyzing the specific gene expression profiles of different immune and stromal cell types, researchers can identify novel biomarkers and therapeutic targets associated with TGF- β pathway activation [20]. Applying omics approaches to study TGF- β inhibition in mCRC could yield valuable data on the TME's evolving response to treatment and aid in developing more precise and personalized therapeutic strategies [12]. TGF- β inhibition holds excellent promise as a therapeutic strategy to enhance immunotherapy in CRC liver metastasis, several critical research areas remain unexplored [44]. The systemic and local effects of TGF- β inhibition, interactions with other pathways, stromal cell dynamics, metabolic adaptations and immune cell spatial dynamics all contribute to the complexity of the TME in mCRC [36].

To maximize the clinical impact of TGF- β inhibition, future studies should adopt a multi-faceted approach, combining omics data, advanced drug delivery systems and combination therapies targeting multiple pathways within the TME [52,61]. By addressing these gaps, researchers can optimize TGF- β inhibition as part of a comprehensive therapeutic strategy that could revolutionize treatment options for patients with metastatic CRC [13]. The potential systemic consequences of TGF- β inhibition

in CRC liver metastasis, particularly concerning immune regulation in non-tumoral tissues, remain under-investigated [22]. TGF- β plays a critical role in maintaining immune homeostasis throughout the body and its inhibition may lead to unintended effects, such as the worsening of autoimmune conditions or tissue-specific toxicities [59].

Liver toxicity could result from off-target inhibition of TGF- β , particularly in patients with pre-existing liver conditions. To address these potential side effects, advanced drug delivery systems, such as nanoparticle-based delivery, can be engineered to concentrate TGF- β inhibitors within the tumor site, minimizing systemic exposure. Further research is required to develop these technologies and determine the optimal dosing and targeting strategies to inhibit tumors while preserving systemic immune function effectively [32,46]. TGF- β interacts with metabolic pathways within the TME, creating an environment that supports tumor cell survival and immune suppression. Metabolites such as lactate, produced through anaerobic glycolysis, are accumulated within the TME and contribute to its acidic and hypoxic nature, further suppressing immune cell function [14,55]. Since TGF- β signaling has been linked to metabolic reprogramming in tumor cells, exploring how metabolic inhibitors might synergize with TGF- β inhibition could open new avenues for therapy. Combining TGF- β inhibitors with drugs that target metabolic pathways may help normalize the TME, making it more conducive to immune cell activity and less hospitable to tumor cells [54,66].

In addition, the interplay between TGF- β and other immunosuppressive pathways, such as the PD-1/PD-L1 axis, presents a promising area for therapeutic development. Some studies suggest tumors may compensate for TGF- β pathway inhibition by upregulating PD-L1 expression or recruiting alternative immunosuppressive cell populations, thereby sustaining immune evasion [33,41].

Combined blockade of TGF- β and PD-1/PD-L1 pathways could provide a more comprehensive approach to counteract immune suppression in the TME, potentially leading to improved clinical outcomes for patients with CRC liver metastasis. However, more research is needed to elucidate these combined treatments' synergistic effects and identify the most effective therapeutic combinations for different patient subgroups [4,27]. Finally, recent advances in omics technologies, such as single-cell RNA sequencing and spatial transcriptomics, have provided new insights into the cellular and molecular heterogeneity within the TME. These tools offer unprecedented resolution in studying the diverse cell populations influenced by TGF- β signaling in liver metastasis [28]. The specific gene expression profiles of different immune and stromal cell types, researchers can identify novel biomarkers and therapeutic targets associated with TGF- β pathway activation. Applying omics approaches to study TGF- β inhibition in mCRC could yield valuable data on the TME's evolving response to treatment and aid in developing more precise and personalized therapeutic strategies [49,63].

While TGF- β inhibition holds excellent promise as a therapeutic strategy to enhance immunotherapy in CRC liver metastasis, several critical research areas remain unexplored. The systemic and local effects of TGF- β inhibition, interactions with other pathways, stromal cell dynamics, metabolic adaptations and immune cell spatial dynamics all contribute to the complexity of the TME in mCRC [68,70]. To maximize the clinical impact of TGF- β inhibition, future studies should adopt a multi-faceted approach, combining omics data, advanced drug delivery systems and combination therapies targeting multiple pathways within the TME [2]. By addressing these gaps, researchers can optimize TGF- β inhibition as part of a comprehensive therapeutic strategy that could revolutionize treatment options for patients with metastatic CRC [46].

Conclusion

In conclusion, inhibiting TGF- β in CRC liver metastasis offers a novel and promising approach to enhancing immunotherapy. By targeting the immunosuppressive mechanisms driven by TGF- β within the TME, it may be possible to overcome the inherent resistance of MSS tumors to ICIs and improve clinical outcomes for patients with metastatic CRC. While significant challenges remain, including minimizing off-target effects and identifying reliable biomarkers for patient selection, the combination of TGF- β inhibitors with ICIs, chemotherapy, radiotherapy and other novel therapeutic modalities holds great potential for transforming the treatment landscape for mCRC. Future research should continue to optimize combination therapies, explore new drug delivery systems and elucidate the molecular mechanisms driving resistance to TGF- β inhibition. A deeper understanding of these processes will ultimately pave the way for more effective and personalized treatment strategies for patients with metastatic CRC.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

1. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGF β drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature*. 2018;554(7693):538-43.
2. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGF β attenuates tumour response to PD-L1 blockade by contributing to exclusion of T-cells. *Nature*. 2018;554(7693):544-8.
3. Park S, Oh JH, Park DJ, Zhang H, Noh M, Kim Y, et al. CU06-1004-induced vascular normalization improves immunotherapy by modulating tumor microenvironment via cytotoxic T-cells. *Front Immunol*. 2021;11:620166.
4. Huang S, Ding D, Lan T, He G, Ren J, Liang R, et al. Multifunctional nanodrug performs sonodynamic therapy and inhibits TGF- β to boost immune response against colorectal cancer and liver metastasis. *Acta Biomater*. 2023;164:538-52.
5. Case K, Tran L, Yang M, Zheng H, Kuhlreiber WM, Faustman DL. TNFR2 blockade alone or in combination with PD-1 blockade shows therapeutic efficacy in murine cancer models. *J Leukoc Biol*. 2020;107(6):981-91.
6. Tsukamoto H, Fujieda K, Miyashita A, Fukushima S, Ikeda T, Kubo Y, et al. Combined blockade of IL6 and PD-1/PD-L1 signaling abrogates mutual regulation of their immunosuppressive effects in the tumor microenvironment. *Cancer Res*. 2018;78(17):5011-22.
7. Dodagatta-Marri E, Meyer DS, Reeves MQ, Paniagua R, To MD, Binnewies M, et al. α -PD-1 therapy elevates Treg/Th balance and increases tumor cell pSmad3 targeted by α -TGF β antibody for durable rejection in carcinomas. *J Immunother Cancer*. 2019;7(1):62.
8. Ding D, Liang R, Li T, Lan T, Li Y, Huang S, et al. Nanodrug modified with engineered cell membrane targets CDKs to activate aPD-L1 immunotherapy against liver metastasis of immune-desert colon cancer. *J Control Release*. 2024;369:309-24.
9. Lin H, Wu Y, Chen J, Huang S, Wang Y. (-)-4-O-(4-O- β -D-glucopyranosylcaffeoyl) quinic acid inhibits the function of myeloid-derived suppressor cells to enhance anti-PD1 against colon cancer. *Pharm Res*. 2018;35(9):183.
10. Kim NH, Lee J, Kim SH, Kang SH, Bae S, Yu CH, et al. ALK5/VEGFR2 dual inhibitor TU2218 alone or with immune checkpoint inhibitors enhances immune-mediated antitumor effects. *Cancer Immunol Immunother*. 2024;73(10):190.
11. Pan PY, Zang Y, Weber K, Meseck ML, Chen SH. OX40 ligation enhances primary and memory cytotoxic T lymphocyte responses in immunotherapy for hepatic colon metastases. *Mol Ther*. 2002;6(4):528-36.
12. Yu G, Wu Y, Wang W, Xu J, Lv X, Cao X, et al. Low-dose decitabine enhances PD-1 blockade effect in colorectal cancer with microsatellite stability by re-modulating tumor microenvironment. *Cell Mol Immunol*. 2019;16(4):401-9.
13. Busenhardt P, Montalban-Arques A, Katkeviciute E, Morsy Y, Van Passen C, Hering L, et al. Inhibition of integrin $\alpha\beta6$ sparks T-cell antitumor response and enhances immune checkpoint blockade therapy in colorectal cancer. *J Immunother Cancer*. 2022;10(2).
14. Sun R, Limkin EJ, Vakalopoulou M, Dercle L, Champiat S, Han SR, et al. A radiomics approach to assess tumour-infiltrating CD8 cells and response to anti-PD-1 or anti-PD-L1 immunotherapy. *Lancet Oncol*. 2018;19(9):1180-91.
15. Principe DR, Park A, Dorman MJ, Kumar S, Viswakarma N, Rubin J, et al. TGF β blockade augments PD-1 inhibition to promote T-Cell-mediated regression of pancreatic cancer. *Mol Cancer Ther*. 2019;18(3):613-20.
16. O'Malley G, Treacy O, Lynch K, Naicker SD, Leonard NA, Lohan P, et al. Stromal cell PD-L1 inhibits CD8+ T-cell antitumor immune responses and promotes colon cancer. *Cancer Immunol Res*. 2018;6(11):1426-41.
17. Zboralski D, Hoehlig K, Eulberg D, Frömming A, Vater A. Increasing tumor-infiltrating T-cells through CXCL12 inhibition with NOX-A12 synergizes with PD-1 blockade. *Cancer Immunol Res*. 2017;5(11):950-6.
18. Shalapour S, Font-Burgada J, Di Caro G, Zhong Z, Sanchez-Lopez E, Dhar D, et al. Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature*. 2015;521(7550):94-8.
19. Takeda Y, Kataoka K, Yamagishi J, Ogawa S, Seya T, Matsumoto M. TLR3-specific adjuvant relieves innate resistance to PD-L1 blockade in tumor vaccine immunotherapy. *Cell Rep*. 2017;19(9):1874-87.
20. Wang H, Wang X, Xu L, Zhang J, Cao H. Analysis of transcriptomic features of microsatellite instability subtype colon cancer. *BMC Cancer*. 2019;19(1):605.
21. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov*. 2015;5(1):43-51.
22. Bald T, Smyth MJ. TGF β shuts the door on T-cells. *Br J Cancer*. 2018;119(1):1-3.
23. Grasselly C, Denis M, Bourguignon A, Talhi N, Mathe D, Tourette A, et al. The antitumor activity of combinations of cytotoxic chemotherapy and immune checkpoint inhibitors is model-dependent. *Front Immunol*. 2018;9:2100.
24. Oshima H, Nakayama M, Han TS, Naoi K, Ju X, Maeda Y, et al. Suppressing TGF β signaling in regenerating epithelia in an inflammatory microenvironment causes invasive intestinal cancer. *Cancer Res*. 2015;75(4):766-76.

25. Mace TA, Shakya R, Pitarresi JR, Swanson B, McQuinn CW, Loftus S, et al. IL-6 and PD-L1 antibody blockade combination therapy reduces tumour progression in murine models of pancreatic cancer. *Gut*. 2018;67(2):320-32.
26. Lazarus J, Maj T, Smith JJ, Perusina Lanfranca M, Rao A, D'Angelica MI, et al. Spatial and phenotypic immune profiling of metastatic colon cancer. *JCI Insight*. 2018;3(22).
27. Caja F, Vannucci L. TGF β : A player on multiple fronts in the tumor microenvironment. *J Immunotoxicol*. 2015;12(3):300-7.
28. Xiao Y, Freeman GJ. The microsatellite instable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. *Cancer Discov*. 2015;5(1):16-8.
29. Lu X, Horner JW, Paul E, Shang X, Troncso P, Deng P, et al. Effective combinatorial immunotherapy for castration-resistant prostate cancer. *Nature*. 2017;543(7647):728-32.
30. Foy SP, Mandl SJ, dela Cruz T, Cote JJ, Gordon EJ, Trent E, et al. Poxvirus-based active immunotherapy synergizes with CTLA-4 blockade to increase survival in a murine tumor model by improving the magnitude and quality of cytotoxic T-cells. *Cancer Immunol Immunother*. 2016;65(5):537-49.
31. Gallotta M, Assi H, Degagné É, Kannan SK, Coffman RL, Guiducci C. Inhaled TLR9 Agonist Renders Lung Tumors Permissive to PD-1 Blockade by Promoting Optimal CD4+ and CD8+ T-cell Interplay. *Cancer Res*. 2018;78(17):4943-56.
32. Tomé M, Pappalardo A, Soulet F, López JJ, Olaizola J, Leger Y, et al. Inactivation of Proprotein Convertases in T-cells Inhibits PD-1 Expression and Creates a Favorable Immune Microenvironment in Colorectal Cancer. *Cancer Res*. 2019;79(19):5008-21.
33. Lu C, Yang D, Klement JD, Oh IK, Savage NM, Waller JL, et al. SUV39H1 Represses the Expression of Cytotoxic T-Lymphocyte Effector Genes to Promote Colon Tumor Immune Evasion. *Cancer Immunol Res*. 2019;7(3):414-27.
34. De Henau O, Rausch M, Winkler D, Campesato LF, Liu C, Cymerman DH, et al. Overcoming resistance to checkpoint blockade therapy by targeting PI3K γ in myeloid cells. *Nature*. 2016;539(7629):443-7.
35. Le Flahec G, Badic B, Guibourg B, Doucet L, Bail JP, Marcorelles P, et al. Mismatch repair-deficient colorectal cancer: a model of immunogenic and immune cell-rich tumor despite nonsignificant programmed cell death ligand-1 expression in tumor cells. *Hum Pathol*. 2018; 72:135-43.
36. Wang D, Jiang W, Zhu F, Mao X, Agrawal S. Modulation of the tumor microenvironment by intratumoral administration of IMO-2125, a novel TLR9 agonist, for cancer immunotherapy. *Int J Oncol*. 2018;53(3):1193-203.
37. Vanpouille-Box C, Diamond JM, Pilonis KA, Zavadil J, Babb JS, Formenti SC, et al. TGF β is a master regulator of radiation therapy-induced antitumor immunity. *Cancer Res*. 2015;75(11):2232-42.
38. Lau J, Cheung J, Navarro A, Lianoglou S, Haley B, Totpal K, et al. Tumour and host T-cell PD-L1 is required to mediate suppression of anti-tumour immunity in mice. *Nat Commun*. 2017;8:14572.
39. Buss LA, Williams T, Hock B, Ang AD, Robinson BA, Currie MJ, et al. Effects of exercise and anti-PD-1 on the tumour microenvironment. *Immunol Lett*. 2021;239:60-71.
40. Jiao S, Subudhi SK, Aparicio A, Ge Z, Guan B, Miura Y, et al. Differences in tumor microenvironment dictate T helper lineage polarization and response to immune checkpoint therapy. *Cell*. 2019;179(5):1177-90.
41. Yang L. TGF β is a potent regulator of tumor microenvironment and host immune response, implication for therapy. *Curr Mol Med*. 2010;10(4):374-80.
42. Stalin J, Garrido-Urbani S, Heitz F, Szyndralewicz C, Jemelin S, Coquoz O, et al. Inhibition of host NOX1 blocks tumor growth and enhances checkpoint inhibitor-based immunotherapy. *Life Sci Alliance*. 2019;2(4).
43. TGF β Promotes Immune Evasion to Limit the Efficacy of Anti-PD-1/PD-L1. *Cancer Discov*. 2018;8(4):441-50.
44. Li HY, McSharry M, Bullock B, Nguyen TT, Kwak J, Poczobutt JM, et al. The tumor microenvironment regulates sensitivity of murine lung tumors to PD-1/PD-L1 antibody blockade. *Cancer Immunol Res*. 2017;5(9):767-77.
45. Conche C, Finkelmeier F, Pešić M, Nicolas AM, Böttger TW, Kennel KB, et al. Combining ferroptosis induction with MDSC blockade renders primary tumours and metastases in liver sensitive to immune checkpoint blockade. *Gut*. 2023;72(9):1774-82.
46. Wartewig T, Kurgys Z, Keppler S, Pechloff K, Hameister E, Trümper L, et al. PD-1 is a haploinsufficient suppressor of T-cell lymphomagenesis. *Nature*. 2017;552(7683):121-25.
47. Zaravinos A, Roufas C, Nagara M, de Lucas Moreno B, Oblovatskaya M, Efstathiades C, et al. Cytolytic activity correlates with the mutational burden and deregulated expression of immune checkpoints in colorectal cancer. *J Exp Clin Cancer Res*. 2019;38(1):364.
48. Holmgaard RB, Schaer DA, Li Y, Castaneda SP, Murphy MY, Xu X, et al. Targeting the TGF β pathway with galunisertib, a TGF β RI small molecule inhibitor, promotes anti-tumor immunity leading to durable, complete responses, as monotherapy and in combination with checkpoint blockade. *J Immunother Cancer*. 2018;6(1):47.
49. Zubeldia IG, Bleau AM, Redrado M, Serrano D, Agliano A, Gil-Puig C, et al. Epithelial to mesenchymal transition and cancer stem cell phenotypes leading to liver metastasis are abrogated by the novel TGF β 1-targeting peptides P17 and P144. *Exp Cell Res*. 2013;319(3):12-22.
50. Wu SP, Liao RQ, Tu HY, Wang WJ, Dong ZY, Huang SM, et al. Stromal PD-L1-positive regulatory T-cells and PD-1-positive CD8-positive T-cells define the response of different subsets of non-small cell lung cancer to PD-1/PD-L1 blockade immunotherapy. *J Thorac Oncol*. 2018;13(4):521-32.
51. Ludwig N, Wieteska L, Hinck CS, Yerneni SS, Azambuja JH, Bauer RJ, et al. Novel TGF β inhibitors ameliorate oral squamous cell

- carcinoma progression and improve the antitumor immune response of anti-PD-L1 immunotherapy. *Mol Cancer Ther.* 2021;20(6):1102-11.
52. Loftus PG, Watson L, Deedigan LM, Camarillo-Retamosa E, Dwyer RM, O'Flynn L, et al. Targeting stromal cell Syndecan-2 reduces breast tumour growth, metastasis and limits immune evasion. *Int J Cancer.* 2021;148(5):1245-59.
 53. Gallotta M, Degagné É, Kannan SK, Coffman RL, Guiducci C. Inhaled TLR9 agonist renders lung tumors permissive to PD-1 blockade by promoting optimal CD4⁺ and CD8⁺ T-cell interplay. *Cancer Res.* 2018;78(17):4943-56.
 54. Cao Z, Sun G, Zhang G, Huang C, Fu X, Zhang W. Galunisertib combined with PD-1 blockade enhances antitumor immunity in lung cancer. *Immunotherapy.* 2020;12(7):483-93.
 55. Tang J, Shalabi A, Hubbard-Lucey VM. Comprehensive analysis of the clinical immuno-oncology landscape. *Ann Oncol.* 2018;29(1):84-91.
 56. Woo SR, Corrales L, Gajewski TF. The STING pathway and the T-cell-inflamed tumor microenvironment. *Trends Immunol.* 2015;36(4):250-6.
 57. Tauriello DVF, Calon A, Lonardo E, Batlle E. Determinants of metastatic competency in colorectal cancer. *Mol Oncol.* 2017;11(1):97-119.
 58. Derynck R, Turley SJ, Akhurst RJ. TGF β biology in cancer progression and immunotherapy. *Nat Rev Clin Oncol.* 2021;18(1):9-34.
 59. Pei S, Huang M, Huang J, Zhu X, Wang H, Romano S, et al. BFAR coordinates TGF β signaling to modulate Th9-mediated cancer immunotherapy. *J Exp Med.* 2021;218(7).
 60. Fu X, Yang Y, Xie J, Pan X, Yang X, Du Z, et al. Subcutaneous inoculation position affects the immune environment in CT26 carcinomas. *Biochem Biophys Res Commun.* 2019;512(2):244-9.
 61. Luheshi NM, Coates-Ulrichsen J, Harper J, Mullins S, Sulikowski MG, Martin P, et al. Transformation of the tumour microenvironment by a CD40 agonist antibody correlates with improved responses to PD-L1 blockade in a mouse orthotopic pancreatic tumour model. *Oncotarget.* 2016;7(14):18508-20.
 62. Zboralski D, Hoehlig K, Eulberg D, Frömming A, Vater A. Increasing tumor-infiltrating T-cells through inhibition of CXCL12 with NOX-A12 synergizes with PD-1 blockade. *Cancer Immunol Res.* 2017;5(11):950-6.
 63. Shi W, Xu X, Liu H, Zhu S, Deng H, Zhang H. TGF- β signaling in T-cell immunity and epithelial-derived cancer: Targeting immune escape mechanism of colorectal cancer. *J Gastroenterol Hepatol.* 2024;39(1):50-8.
 64. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGF β attenuates tumour response to PD-L1 blockade by contributing to exclusion of T-cells. *Nature.* 2018;554(7693):544-8.
 65. Case K, Tran L, Yang M, Zheng H, Kuhtreiber WM, Faustman DL. TNFR2 blockade alone or in combination with PD-1 blockade shows therapeutic efficacy in murine cancer models. *J Leukoc Biol.* 2020;107(6):981-91.
 66. Wang H, Wang X, Xu L, Zhang J, Cao H. Analysis of transcriptomic features of microsatellite instability subtype colon cancer. *BMC Cancer.* 2019;19(1):605.
 67. Sun R, Limkin EJ, Vakalopoulou M, Dercle L, Champiat S, Han SR, et al. A radiomics approach to assess tumour-infiltrating CD8 cells and response to anti-PD-1 or anti-PD-L1 immunotherapy. *Lancet Oncol.* 2018;19(9):1180-91.
 68. Pan PY, Zang Y, Weber K, Meseck ML, Chen SH. OX40 ligation enhances primary and memory cytotoxic T-lymphocyte responses in immunotherapy for hepatic colon metastases. *Mol Ther.* 2002;6(4):528-36.
 69. Dodagatta-Marri E, Meyer DS, Reeves MQ, Paniagua R, To MD, Binnewies M, et al. α -PD-1 therapy elevates Treg/Th balance and increases tumor cell pSmad3 targeted by α -TGF β antibody for durable rejection in carcinomas. *J Immunother Cancer.* 2019;7(1):62.
 70. Huang S, Ding D, Lan T, He G, Ren J, Liang R, et al. Multifunctional nanodrug performs sonodynamic therapy and inhibits TGF- β to boost immune response against colorectal cancer and liver metastasis. *Acta Biomater.* 2023; 164:538-52.

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