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Reprogramming Tumour-Associated Macrophages in Hepatocellular Carcinoma: From Molecular Mechanisms to Immunotherapeutic Strategies

Xinyu Song

School of Pharmacy, Shenzhen University Medical School, Shenzhen, 518060, China

ABSTRACT

Hepatocellular carcinoma (HCC) is still one of the top causes of cancer-related mortalities worldwide, and immunosuppressive tumour microenvironment (TME) is a key obstacle for effective therapy. Tumour-associated macrophages (TAMs) are one of the most abundant immune cells in the HCC TME and display a pro-tumourigenic character, which is related with immunosuppression, angiogenesis and resistance to systemic therapy. Reprogramming TAMs from M2-like pro-tumourigenic to an anti-tumour M1-like state has appeared as a viable therapeutic strategy. Herein we discuss the molecular pathways involved in TAM polarisation in HCC, including the CSF1R, PI3Kγ and NF-κB/STAT3 signalling pathways and review the therapeutic approaches targeting TAM re-programming including CSF1R inhibition, TLR agonists, combination with immune checkpoint blockade, CAR-macrophage engineering and nanoparticle-mediated delivery. We also highlight the clinical challenges of TAM-targeted therapy such as phenotypic plasticity and off-target toxicity, and markers for patient stratification.

KEYWORDS

Tumour-associated macrophages; Hepatocellular carcinoma; Macrophage reprogramming; M1/M2 polarisation; Tumour microenvironment; CSF1R; Immune checkpoint blockade; CAR-macrophage

1. INTRODUCTION

1.1. Research Background

Hepatocellular carcinoma (HCC) is the most common primary tumour of the liver and the third most common cause of cancer death globally. Less than 20% of people with advanced disease will survive five years. Over 900,000 new cases are diagnosed every year. Systemic therapy for more than a decade is limited to sorafenib as the primary first-line agent, partly due to the highly immunosuppressive nature of HCC TME leading to immune escape and therapeutic resistance in cancer cells. Given the inflammatory milieu of chronic hepatitis and cirrhosis that characterises the tissue background, the TME of HCC is in comparison to other solid tumours, uniquely immunosuppressive.

TAMs are among the most common immune cells in the HCC TME and their density is negatively linked to patient survival and response to therapy. In HCC, TAMs are mostly polarised to an M2-like, alternatively activated phenotype, which inhibits cytotoxic T cell function, enhances angiogenesis and drives tumour invasion and dissemination, instead of acting as anti-tumor effectors. The concept of functional plasticity of TAMs and their reprogramming from pro-tumor to anti-tumor states by appropriate therapies has opened a new therapeutic paradigm in HCC immunotherapy.

1.2. Research Questions and Contributions

In this review, we ask three questions: (RQ1) What are the molecular processes by which TAMs enhance progression of HCC? (RQ2) Which signalling mechanisms regulate TAM polarisation and are potential therapeutic targets? (RQ3) What are the immunotherapeutic techniques targeting TAM reprogramming with the most clinico-therapeutic potential for HCC and the major limitations of these strategies? We incorporate findings from molecular biology, pre-clinical models and clinical trials to give a comprehensive framework for understanding and treating TAMs in HCC.

2. THEORETICAL AND MECHANISTIC FRAMEWORK

2.1. TAM Origin and Phenotypic Plasticity in HCC

TAMs in HCC stem from two sources: circulating monocytes recruited through the CCL2/CCR2 axis and tumour-educated liver-resident Kupffer cells. These populations have diverse transcription profiles and recruited monocyte-derived TAMs are generally more flexible than reprogrammed Kupffer cells. Single-cell RNA sequencing (scRNA-seq) studies have shown that TAMs are very heterogeneous in the HCC TME and have identified functionally distinct subpopulations with heterogeneous immunosuppressive, angiogenic and phagocytic gene programs [1, 2].

The M1/M2 polarisation model is a simplification of the continuum of macrophage activation states, but it provides a useful paradigm for studies of TAM activity in HCC. Mantovani et al. gave the conceptual underpinning for reprogramming-based approaches, defining the plasticity of macrophage polarisation and bidirectional interaction with lymphocyte subsets as important features of macrophage biology [14]. M1-polarized macrophages, which are usually activated by interferon-γ, release IL-12, TNF-α and reactive oxygen species, which are all beneficial for CD8+ T cell activation . IL-4-, IL-13- and tumour-derived signal-driven M2-polarized macrophages release IL-10, TGF-β and VEGF that suppress adaptive immunity and increase angiogenesis. In HCC, hypoxia, lactate and CSF1 are potent enough to shift TAMs into M2-like, pro-tumourigenic states [3].

2.2. Molecular Mechanisms of TAM-Mediated Tumour Promotion

TAMs promote HCC development through several interconnected processes. The immunosuppression is principally mediated by the production of IL-10 and TGF-β which directly limit the proliferation and the effector activity of CD8+ cytotoxic T lymphocytes and the over-expression of PD-L1 on the TAM surface which transmits inhibitory signals to T cells expressing PD-1 [4]. Besides direct inhibition of T cells, TAMs also reshape the immunosuppressive architecture of TME by recruiting regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) via the production of CCL22 and other chemotactic molecules, thus establishing a self-reinforcing immunosuppressive network. Huang et al. first characterised the molecular link between myeloid suppressor cells and Treg production in tumor-bearing rats and established the biological foundation of the immunosuppressive network by which TAMs control the HCC TME [15]. VEGF-A, MMP-9 and basic FGF increase angiogenesis, whereas EGF-dependent signalling improves HCC cell motility and MMP-mediated remodelling of the extracellular matrix promotes carcinoma invasion and metastasis [3, 4].

TAMs are also important mediators of resistance to systemic therapy in HCC. Resistance to sorafenib, the previous standard of therapy, has been related with TAM-derived hepatocyte growth factor (HGF) that activates the c-Met receptor tyrosine kinase on HCC cells and bypasses the VEGFR/PDGFR signalling blocked by sorafenib. This process was directly demonstrated in HCC tissues and models by Zhu and colleagues, who revealed that simultaneous c-Met/VEGFR inhibition overcomes TAM-mediated sorafenib resistance [11]. This underscores the need for incorporation of TAM-targeting approaches into combination regimens.

2.3. Key Signalling Pathways Governing TAM Polarisation

The CSF1/CSF1R signalling axis is the most developed treatment mechanism that affects TAM recruitment, survival and differentiation in HCC. CSF1R is expressed in monocytes and macrophages and its ligands CSF1 and IL-34 are heavily released from HCC cells and the tumour stroma, providing a chemotactic gradient that induces monocyte recruitment and M2 polarisation [5]. Pharmacological blockade of CSF1R with small-molecule inhibitors such as pexidartinib (PLX3397) results in depletion of pro-tumourigenic TAMs and reprogramming of residual macrophages towards an immunostimulatory phenotype, while increasing CD8+ T cell infiltration through relief of macrophage-mediated immunosuppression [5].

The PI3Kγ signalling axis is a second prominent modulator of the immunosuppressive TAM polarisation. Kaneda et al. [6] shown that PI3Kγ controls a key switch in TAMs from immune activation to immune suppression. Activation of PI3Kγ induces M2-like polarisation via the mTOR/S6K pathway and suppression of NF-κB-driven M1-associated gene expression. Preclinical models have shown that genetic or pharmacological loss of PI3Kγ shifts TAMs towards an M1-like phenotype, boosts intratumoral CD8+ T cell infiltration and activity and enhances the efficacy of anti-PD-1 checkpoint inhibition. All these considerations have made the suppression of PI3Kγ an ideal partner for combination immunotherapy [6]. M2 polarisation is further regulated by other transcription factors, STAT3 and STAT6 downstream of IL-10, IL-4 and IL-13 receptor signalling . Established TAM traits are stabilised by epigenetic processes such as histone H3K27 methylation and DNA methylation at polarisation-associated gene loci. Metabolic reprogramming provides another layer of TAM regulation. Leone and Powell have shown how the nutritional and metabolite environment within the TME, including lactate, succinate and glutamine, determines macrophage immunological activity and represents a targetable dimension of TAM reprogramming [13].

3. IMMUNOTHERAPEUTIC STRATEGIES TARGETING TAM REPROGRAMMING

3.1. Blocking TAM Recruitment and CSF1R-Mediated Survival

One of the most clinically advanced approaches is targeting TAMs with the use of CSF1R inhibitors to reduce pro-tumourigenic TAMs and to limit recruitment of monocyte precursors to the TME. In 2019, the US Food and Drug Administration approved pexidartinib, the first CSF1R inhibitor, for tenosynovial giant cell tumour. Preclinical studies in HCC and other solid tumour models have revealed that CSF1R blockade resulted in a substantial decrease in TAM density, a shift in the phenotype of the surviving macrophages to an M1-like phenotype and increased infiltration of CD8+ T cells. Alternatively, CCR2 antagonism targets the upstream chemokine receptor responsible for recruitment of monocytes into tumours via the CCL2/CCR2 axis. Early-phase clinical data show tolerability and early signs of pharmacodynamic activity in reducing circulating inflammatory monocytes [1, 4].

3.2. Repolarisation: M2-to-M1 Switching Strategies

Direct pharmacological repolarisation of pro-tumourigenic TAMs into anti-tumour phenotypes, without depletion of the macrophage compartment, offers the advantage of preserving the phagocytic and antigen presentation functions of macrophages, while removing their immunosuppressive activities. The many repolarisation strategies have been comprehensively reviewed by Anfray et al., who classify them by mechanism and discuss their pros and cons within the frame of combination immunotherapy [12]. TLR agonists such as CpG oligodeoxynucleotides (TLR9), Poly I:C (TLR3) and MPLA (TLR4) can be given intra-tumourally or systemically [3] and induce M1 repolarisation by activation of NF-κB. CD40 agonist antibodies activate macrophages and dendritic cells through

CD40L–CD40 interactions, which reprogram immunosuppressive TAMs into cytotoxic effectors that can directly kill cancer cells. CD40 agonists in combination with chemotherapy and checkpoint inhibition have shown signals of benefit in pancreatic and colorectal malignancies, with ongoing study in HCC in clinical studies. Inhibition of PI3Kγ is a mechanistically driven approach to repolarisation with a strong preclinical rationale for combination with immune checkpoint blockade [6] as mentioned above.

3.3. Combination with Immune Checkpoint Blockade

A solid molecular rationale exists for combining TAM reprogramming with PD-1/PD-L1 immune checkpoint blocking (ICB). Using intravital imaging, Arlauckas and colleagues showed that TAMs physically tear off anti-PD-1 antibodies from the surface of CD8+ T cells within minutes, by means of FcγR-dependent capture, directly implicating TAMs in ICB resistance and advocating TAM-targeted co-therapy to rescue anti-PD-1 efficacy [7]. The combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) has been established as the standard of care for first-line treatment of unresectable HCC following the IMbrave150 phase III trial showing a significant improvement in overall survival compared with sorafenib (median OS 19.2 vs 13.4 months; HR 0.66) [8]. The T+A regimen has been successful in part because of the VEGF blockade with bevacizumab that has an effect on the TME (by decreasing VEGF-driven immunosuppression and TAM recruitment), thus adding an immunological component to the classic anti-angiogenic mechanism [8].

Preclinical studies combining CSF1R inhibitors with anti-PD-1 antibodies demonstrate synergistic effectiveness, with TAM repolarisation improving T cell infiltration potentiated by checkpoint blocking - prompting ongoing clinical trials of this combination in HCC and other solid tumours [5, 8].

3.4. Novel Approaches: CAR-Macrophages and Nanoparticle Delivery

Chimeric antigen receptor-engineered macrophages (CAR-M) are the most physically innovative strategy to target TAMs, engineering macrophages to express antigen-specific chimeric receptors, thus reprograming them from passive suppressors to active anti-tumour effectors. Preclinical investigations have demonstrated that GPC3 targeted CAR-M cells provide potent anti-cancer activity against HCC with antigen specific phagocytosis, production of pro-inflammatory M1 cytokines and direct tumour cell killing in 3D HCC spheroid models [9]. Carisma Therapeutics has entered into a collaboration with Moderna to develop an in vivo CAR-M approach for HCC using lipid nanoparticles to deliver anti-GPC3 CAR mRNA to endogenous myeloid cells, a potentially off-the-shelf approach that circumvents the manufacturing challenges of ex vivo cell therapy [9].

Nanoparticle-mediated delivery of repolarising drugs to TAMs leverages the phagocytic capacity of macrophages to selectively deliver intracellularly small chemicals, nucleic acids and cytokine modulators promoting M1 polarisation without systemic toxic effects. This strategy is exciting because it allows the delivery of agents such as PI3Kγ inhibitors, HDAC inhibitors, and mRNA encoding pro-inflammatory transcription factors that would be poorly tolerated or insufficiently targeted in systemic formulations and can lead to effective TAM reprogramming within the HCC TME [6, 9].

4. CLINICAL TRANSLATION AND CHALLENGES

4.1. Biomarkers for TAM-Targeted Therapy

The clinical translation of TAM-targeting treatments requires validated indicators to select patients and to monitor response. The density of CD68+ macrophages and the ratio of CD163+/CD68+ M2: total macrophages in tumour tissues have been established as independent predictive indicators in

HCC. Chen et al. performed a meta-analysis to validate the substantial correlation between high intratumoral TAM infiltration and poor overall survival and disease-free survival in HCC, confirming the predictive importance of TAM density in diverse patient populations [10]. Moreover, the CD163+CD206+ TAM signature has been associated with sorafenib resistance and possibly ICB resistance in HCC. Circulating monocyte subsets, especially the CD14+CD16+ non-classical monocyte population, have been proposed as readily accessible pharmacodynamic markers of CSF1R inhibitor activity, allowing longterm monitoring of target engagement without repeated tumour biopsies [5]. The integration of multi-omic data from scRNA-seq, bulk transcriptomics, and proteomics is rapidly being used to derive TAM subpopulation signatures with predictive value for various TAM-targeting medications, moving towards a precision oncology paradigm for TAM-directed therapy in HCC [2, 3].

4.2. Clinical Challenges and Resistance Mechanisms

Although the preclinical justification for TAM-targeting in HCC is intriguing, significant clinical hurdles remain to be addressed. The phenotypic flexibility of TAMs, their ability to quickly revert back to immunosuppressive phenotypes following initial reprogramming in response to continuous tumour-derived signals, poses a key obstacle to achieving sustained therapeutic responses with repolarisation techniques [1, 3]. In patients with HCC and underlying cirrhosis and inadequate hepatic reserve, lifting systemic CSF1R inhibition raises significant concerns for off-target toxicities, including implications on bone homeostasis, neuropathology and liver function. The complexity of tumour heterogeneity and diversity of TAM subpopulations, which differ in origin, transcriptional state and function, make the development of uniform therapeutic strategies difficult and underscore that successful TAM targeting in HCC will require spatially and temporally adaptive strategies guided by molecular profiling of individual patient TMEs [2, 4].

5. DISCUSSION AND CONCLUSION

5.1. Synthesis: TAM Reprogramming as a Therapeutic Paradigm

The molecular and clinical evidence presented here supports the notion that TAM reprogramming is a scientifically established and therapeutically actionable paradigm for immunotherapy of HCC. Mechanistic understanding of CSF1R, PI3Kγ and NF-κB/STAT3 pathways, along with innovative approaches to delivery such as CAR-M engineering and nanoparticle-mediated repolarisation and the clinical validation afforded by the T+A regimen place TAM targeting as a fundamental pillar of next-generation immunotherapy for HCC.

5.2. Future Directions

The most promising near-term avenues include clinical testing of CSF1R inhibitor and anti-PD-1/PD-L1 combinations with biomarker-enriched patient selection, clinical translation of GPC3-targeted CAR-M approaches and identification of spatially-resolved TAM subpopulation signatures predictive of response to specific reprogramming strategies. Combination therapies that simultaneously target TAM recruitment, repolarise residual immunosuppressive TAMs and enhance T cell effector activity through ICB are likely to elicit the most sustained anti-tumor responses.

5.3. Limitations and Concluding Remarks

This study summarises published preclinical and clinical research and does not present new experimental data. The translational gap between mouse HCC models and human disease, especially the hepatic immune milieu moulded by underlying viral hepatitis or cirrhosis, remains a major constraint for interpretation of preclinical data. However, the convergent evidence from numerous

independent research programs reviewed here provides good grounds for optimism that TAM reprogramming may deliver clinically relevant advancements in HCC immunotherapy in the future decade.

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