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Invited Editorial

Tumour associated macrophages in hepatocellular carcinoma: pressing the metabolic switch to prevent T cell responses.

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Tumour associated macrophages in hepatocellular carcinoma: pressing the metabolic switch to prevent T cell responses.

Hepatocellular Carcinoma (HCC) is one of the leading causes of global cancer related deaths. The majority of patients present with advanced disease and current medical therapy only prolongs survival by a few months[1]. HCC occurs on the background of chronic liver disease in more than 90% of cases and is a paradigm for inflammation-induced cancer where the tumour microenvironment (TME) is characterised by ongoing metabolic stress and an immunosuppressive environment[2]. This has led to significant interest to assess if immunotherapy could be an effective approach to combatting this cancer. Immune checkpoint inhibitors (ICIs) have been at the forefront of immunotherapeutics for cancer with FDA approval for a range of tumours[3]. These approved agents target CTLA-4 or the PD-1/PD-L1 axis and blockade of these receptors removes the constraints on T cell activation and can unleash tumour specific immune responses. The action of these immune checkpoints are thought to occur predominantly at different sites of T cell activation, with CTLA-4 playing a role at priming of T cells (lymph nodes) and PD-1/PD-L1 inhibitory interaction occurring at the tumour site. Nevertheless, it is becoming clear that we need a better understanding of the biology and regulation of these molecules to improve the therapeutic outcomes with these drugs[4]. This is highlighted in recent ICI trials in HCC, demonstrating efficacy signals but with overall responses remaining low at 20%[5].

To improve the efficacy of ICIs, attention has shifted to the tumour microenvironment which contains a range of other cell types that are involved in cross talk with T cells including fibroblasts, endothelial cells and macrophages[6]. Macrophages play essential

roles in pathogen clearance, recognition of danger signals as well as important regulators of tissue remodelling and a significant body of work now confirms their role in mediating acute and chronic liver injury[7]. The tissue-resident macrophages of the liver, Kupffer cells, act as sentinels for danger signals released by tissue injury and this is followed by the recruitment of other monocyte-derived populations from the circulation by the CCL2/CCR2 axis. These populations have functional diversity in response to tissue microenvironmental signals contributing to both pro-inflammatory and fibrotic responses as well as resolution of inflammation and fibrosis regression[8-10]. In addition to liver injury and fibrosis, macrophages have also been noted to make up a major component of tumour stroma in hepatocellular carcinoma[11]. The majority of tumour-associated macrophages (TAMs) in general appear to be derived from peripheral blood monocytes with a small proportion being made up of a tissue-resident origin[12]. Though macrophages have functional diversity, many studies suggest that tumour-associated macrophages play an important role in promoting tumour progression, including in models of HCC[13]. There are several mechanisms by which macrophages may help tumour growth involving tumour angiogenesis and tumour cell invasion and metastasis[11]. In addition to supporting tumour growth, TAMs can also support tumour immune evasion via the promotion of an immunosuppressive phenotype, characterised by high levels of arginase 1, IL-10 and chemokines which promote the recruitment of regulatory T cells (e.g. CCL22)[14, 15]. Previous work in HCC demonstrates that TAMs also express high levels of the checkpoint receptor PD-L1[16]. To translate these findings to cancer therapy there is now an urgent need to understand and overcome these intrinsic macrophage regulatory pathways which promote the expression of these immunosuppressive molecules.

Immunometabolism has generated a great deal of interest as an approach to alter immune cell function. It is now clear that cellular metabolism as not solely a form of energy production and biosynthesis in immune cells but through a process of metabolic reprogramming can alter the function of these cells via transcriptional and post-transcriptional pathways. Previous work has shown that this is highly relevant to macrophage phenotype and function with pro-inflammatory macrophages or M1 (generated by LPS or interferon γ) having a different metabolic profile to alternatively activated M2 Macrophages (generated by IL-4)[17]. M1 macrophages are associated with metabolic profile characterised by aerobic glycolysis, whereas M2 macrophages are characterised by oxidative phosphorylation (OxPhos) which is generally associated with most cells in normoxic conditions to generate ATP and an intact Kreb's cycle. The differences between the metabolic features in these macrophage subsets can be explained by altered levels of critical enzymes that drives these pathways, for example M1 macrophages have increased levels of u-PFK2, an isoform of phosphofructokinase-2 that is highly active, promoting glycolysis[18]. In contrast, M2 macrophages express PFKFB1, an isoform that has limited effects in driving glycolysis[19]. Furthermore, whilst nutrients and oxygen levels are the classical drivers of metabolic pathways, in macrophages and dendritic cells the ligation of pattern recognition receptors by binding danger signals in the microenvironment makes a major contribution to regulating metabolic pathways[17].

In this issue, Chen *et al.* describe a new pathway involving recognition of danger signals by macrophages in the HCC micronenvironment that regulates their metabolism and has downstream effects on PD-L1 expression. Through a combination of gene expression analysis for glycolytic enzymes and seahorse extracellular flux studies, they demonstrate that peritumoural TAMS from human HCC samples were skewed towards a glycolytic

pathway compared to paired blood monocytes or non-tumour tissue populations. Previous studies from this group have shown that peritumoural TAMs in HCC upregulate the expression of PD-L1[16] and in this current study they provide evidence for glycolysis being a key driver of PD-L1 upregulation. They further demonstrate that PD-L1 upregulation on monocytes by tumour supernatant *in vitro* can be prevented by 2-deoxyglucose (2DG), a well-known inhibitor of glycolysis. Guided by their gene expression analysis and using a range of specific inhibitors and siRNA knockdown, the authors show that PFKFB3 was the critical glycolytic enzyme in regulating PD-L1 expression. Interestingly, populations of $CD68^{+}PFKFB3^{+}PD\text{-}L1^{+}$ monocyte populations were detectable in the peritumoural stroma of human HCC samples. This leads to the questions of what external factors in the tumour microenvironment support this metabolic reprogramming and, intrinsically, how does PFKFB3 drive a translational pathway that upregulates PDL1 expression? Chen *et al*. explore these questions by firstly identifying that the NF-κB pathway is upregulated in their *in vitro* model and that inhibition of PFKFB3 abrogates the pathway by preventing p65 translocation to the nucleus leading to prevention of PD-L1 upregulation. They go on to find that hyaluronan fragments, a matrix protein which has been shown to be secreted by several tumours into the microenvironment and correlates with outcome[20], is sensed by monocytes and upregulates the expression of PFKFB3 and PD-L1. It is important to point out that *in vivo* experiments in this study used a cell line tumour model with transfer of human monocytes with or without inhibition of PFKFB3 combined with autologous T cells and more robust clinically relevant models are required to confirm the functional contribution of this subset of CD68+PFKFB3+PD-L1+ TAMs in HCC progression.

Nevertheless, this study adds to the growing interest in targeting the metabolism of immune cells as a strategy for immunotherapy, but also highlights that macrophages cannot be clearly differentiated between anti-inflammatory and proinflammatory subtypes. TAMs are known to have immunosuppressive properties which are associated with alternatively activated (M2) macrophages that are characterised by the OxPhos pathway, yet in this study it is glycolysis which leads to high PD-L1 expression. Further work is required to assess if this $CD68^{+}PFKFB3^{+}PD\text{-}L1^{+}$ subtype of macrophages is found in all types of HCC, as this study predominantly derived tissue from Hepatitis B-associated HCC. From a therapeutic point of view, we also need better understanding of how safe and specific it is to target these metabolic pathways as an approach to alter TAM function.

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