IID Essay ICA

How could type 2 immunity-driven liver injury pathogenesis benefit treatment and diagnosis of hepatocellular carcinoma?

Student No. XXX

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Introduction

Hepatocellular carcinoma (HCC) is the most usual form of liver cancer and the fourth most common cause of cancer-related death worldwide (Villanueva, 2019). Usually arising at the end stage of chronic liver diseases (CLDs), HCC is a clear example of inflammation-related cancer. Viral infections, alcoholism, and obesity are common causes of CLDs, where non-resolving inflammation is known to promote and exacerbate malignancy (Bishayee, 2014; Yu *et al.*, 2018) Thus, it is crucial to understand the mechanisms underlying the abnormal immune responses that contribute to hepatocarcinogenesis.

Immune responses in the liver are orchestrated by both type 1 immunity (i1) and type 2 immunity (i2), which exhibit pro- and anti-inflammatory responses, respectively (Robinson *et al.*, 2016). The i1-i2 imbalance drives non-resolving inflammation in the liver, resulting in sequential development of fibrosis, cirrhosis, and eventually HCC (**Figure 1**), where there are common inflammatory cascades during the transition (Dondeti *et al.*, 2016). It is recently found that i2 is crucial for sustaining the non-resolving inflammation in liver fibrosis, whereas i1 activation proves protective against chronic injury (Hart *et al.*, 2017).

In light of the i2-driven pathology of liver fibrosis, this article aims to 1) appreciate anti-fibrotic treatment based on the pathology, 2) evaluate its applications in HCC, and 3) suggest further directions for therapeutic applications of i2 in HCC, with possible diagnostic and therapeutic strategies for HCC discussed in each section and summarised in the conclusion section.

Targeting cytokines in i1-i2 imbalance

The i1-i2 axis represents two opposing immune responses that shape tissue injury during the progression towards HCC (**Figure 1**). Distinct CD4+ T helper subpopulations, termed Th1 and Th2, can cause different immune responses patterns, which are characterised by different patterns of cytokines (Yamaguchi *et al.*, 2015). Th1 secrete Th1 cytokines, including interleukin (IL)-2, interferon (IF)-γ, and lymphotoxin-α and activate i1, inducing intense phagocytosis, while Th2 produce Th2 cytokines including IL-4, IL-5, IL-9 and IL-13 and activate i2, resulting in high antibody titres (Spellberg & Edwards Jr., 2001).

Hart *et al.* (2017) show that the serum levels of Th2 cytokines IL-4, IL-5 and IL-13 increase with the worsening of liver fibrosis in high-fat-fed mice (Gieseck *et al.*, 2018). By comparing liver injury in i1 and i2 activated mice, they further find that i1 activation instead protects the

liver from chronic injury (Hart *et al.*, 2017), which challenges the assumed key role of i1 in driving liver fibrosis (Dondeti *et al.*, 2016) and indicates liver fibrosis as an "overhealing wound." Townsend *et al.* (2019) further confirm i2 amplification in hepatic viral infection. Since fibrosis and sequent cirrhosis are found in more than 90% of HCC patients and drive hepatocarcinogenesis (Campbell *et al.*, 2005; Ghouri *et al.*, 2017). Therefore, different aetiologies of liver cancer share a common i2-driven pro-fibrotic pathway (**Figure 1**), which should be a promising diagnostic and therapeutic target.

Thus, potential clinical applications of Th2 cytokines have been actively investigated. For example, Hou *et al.* (2010) show elevated expression of IL-13Rα2, a receptor of Th2 cytokine IL-13 (Dondeti *et al.*, 2016), in cancer cell lines and tissues, which allows them to develop an effective targeted therapy using an IL-13 and diphtheria toxin fusion protein called DT389-hIL13-13E13K in liver cancer cell lines. However, further preclinical and clinical research is still needed. The association between HCC and Th2 cytokine IL-4 has been meta-analysed by Wu *et al.* (2015). It is found that *IL-4 -590C>T* polymorphism, which shows increased IL-4 expression (Saxena & Kaur, 2015), is associated with a higher risk for HCC, especially in the Asian population, yet further data and analyses are needed to support IL-4 polymorphism as a predictive biomarker. Moreover, as IL-4 signalling is enhanced in HCC (Guo *et al.*, 2017), whether existing or preclinical IL-4 targeted therapies could be used in HCC treatment requires further exploration (Bankaitis & Fingleton, 2015).

Likewise, increased i1 activation may also produce an anti-tumour effect due to restored i1-i2 imbalance (**Figure 1**). Previous studies show IFNα/β promotes differentiation into Th1 cell in human T cells and i1 activation (McNab *et al.*, 2015). Retrospective reviews also show a protective effect of IFNα/β against HCC among hepatitis C patients, independent of viral infection (Hsu *et al.*, 2015). Enomoto *et al.* (2017) further show IFNα can induce an anti-tumour effect *in vivo* but not *in vitro*. Thus, it is likely that IFNα could suppress tumour growth by initiating i1. However, despite reduced tumour recurrence, IFN treatment fails to reduce metastasis among melanoma patients in a phase III clinical trial (Parker *et al.*, 2016). During metastasis, cancer cells must pass a series of structural barriers formed by collagen (Jiang *et al.*, 2000), which is also a major component of liver fibrosis.

Therefore, it is likely that IFN treatment, like other i1-targeted treatment, enhances local immunosurveillance, but fails to restrict the cells with fibrosis (Garrido & Djouder, 2021), which questions the sole use of i1-targeted therapy and the role of fibrosis in HCC. In light of this, the role of i2 in metastasis and carcinogenesis will be further discussed.

Immunomodulation of cell differentiation in the liver

A key challenge for using anti-fibrotic therapies in HCC is the poorly understood links between liver injury and HCC. The liver is known to have a strong regeneration ability (Kung *et al.*, 2010), while different liver injury levels cause hepatocytes into either proliferation or senescence (Wang *et al.*, 2018). Upon acute liver injury, Th1 cytokine activation promotes liver regeneration, which is later resolved by Th2 cytokines (Markose *et al.*, 2018).

However, it has long been observed that chronic liver injury impairs immune function, which results in immunodeficiency and systemic inflammation (Albillos *et al.*, 2014; Zhang *et al.*, 2019). Specifically, liver-resident group 2 innate lymphoid cells (ILC2s) cooperates with Th2 cells to maintain the activation of M2 macrophages (**Figure 2**) (Bouchery *et al.*, 2015), which can develop into or recruit tumour-associated macrophages (TAMs). TAM accumulation predicts worse prognosis due to its role in metastasis, angiogenesis, and immune regulation (Lin *et al.*, 2019). Thus, a variety of treatment targeting TAMs has been proposed (**Figure 3**) to eliminate and convert M2 macrophages (Barry *et al.*, 2020). Additionally, approved anti-tumour treatment, such as PD-1/PD-L1 inhibition (Shi & Line, 2020), has also been found to mediate M2-M1 conversion.

Alternate activation of innate immune cells is closely associated with liver regeneration (Markose *et al.*, 2018; Garrido & Djouder, 2021). Specifically, liver-resident and recruited macrophages produce growth factors, especially TGF-β and PDGF, which activate hepatic stellate cells (HSCs), a kind of T cell-tolerant liver-resident antigen-presenting cells (Crispe, 2014). Then activated HSCs transdifferentiate into cancer-associated fibroblasts (CAFs), producing ECM and accelerating fibrosis (Barry *et al.*, 2020; Garrido & Djouder, 2021). In vitro co-culture of HSCs with HCC cells and in vivo co-implantation of HSC or myofibroblasts with tumour cells into mice confirm HSC activation's role in promoting cancer migration, growth and survival (Kang *et al.*, 2011; Barry *et al.*, 2020). Thus, i2 activation contributes to HCC onset and progression by promoting HSC activation through TAMs (**Figure 4A**). In light of this, HSCs can be a prognostic marker in HCC, while therapeutic strategies targeting HSC activation through TGF-β and PDGF are also under active research (Chan *et al.*, 2020).

A remaining question is the origin of HCC. Using genetic lineage tracing in an HCC mouse model, Tummala *et al.* (2017) show that HCC mostly originates from hepatocytes instead of hepatic progenitor cells, which primarily develop into benign lesions. With broad and deep-sequencing, Zhu *et al.* (2019) suggest that mutations implicated in HCC happen long before

tumour initiation. Furthermore, single-cell transcriptomics reveals highly diverse molecular landscapes of hepatocytes (Kho *et al.*, 2004; Henderson *et al.*, 2020), which may provide cell heterogeneity to undergo selection pressure due to chronic liver injury, as suggested by increased mutation loads with the course of liver injury (Zhu *et al.*, 2019).

Notably, distinct cirrhotic nodules have different disease involvements (**Figure 4B**), where most mutations promote survival, not tumorigenesis, which suggests a protective role of i2-driven fibrosis during early carcinogenesis, though the role of noncoding mutations remains to be elucidated (Müller *et al.*, 2019; Zhu *et al.*, 2019). Therefore, fibrosis may limit both immunosurveillance and tumour expansion, which should be critically evaluated when designing a new therapy. Also, in light of cell heterogeneity and fibrosis restriction undermining drug intake, targeted drug delivery to specific cells, e.g., HSCs and tumour cells in specific cirrhotic nodules, should be developed (Chen *et al.*, 2019).

Type 2 immunity: beyond liver immunity

As an immunological organ highly exposed to circulating antigens and endotoxins, the liver exhibits restricted immune responses, allowing itself to recover through liver regeneration; yet, the restricted responses, termed liver tolerance, cause incomplete antigen clearance (Crispe, 2014). The tolerance may well contribute to the cell heterogeneity within the liver, allowing cancer growth (Li & Tian, 2013). In fact, the liver is a common target of metastasis, during which cytokines in the liver promotes cancer cell expansion (Clark *et al.*, 2016), supporting the tumour-promoting role of liver immunity and suggesting inter-organ crosstalk.

Additional research shows the impact of i2 through neural regulation and metabolism, which also contributes to immunosuppression, which provides further targets in i2-driven pathogenesis of HCC. For example, it has recently been appreciated that gut microbiota play a role in sustaining tissue homeostasis in the gut-liver axis, whereas translocation of metabolites derived from gut microbiota to the liver will also interplay with liver-resident immune cells. Especially, certain species of the gut microbiota can sensitise patients' response to immune checkpoint inhibitors, which provides additional therapeutic insights beyond the liver (Ohtani & Kawada, 2019).

Another example is the role of neural regulation via neuropeptide neuromedin U (NMU). Cardoso et al. (2017) and Klose *et al.* (2017) reveal that neuronal signals can fast and potently induce i2 against helminth infection in the liver via ILC2 activation mediated by the

NMU. Wallrapp *et al.* (2017) further report the binding of NMU to its receptor NMUR2 activates ILC2 activation, making NMU/NMUR2 signalling a potential therapeutic. Besides, Li *et al.*, (2020) support NMU as a prognostic marker in HCC, where they also prove that worse prognostic is associated with increased M2 macrophages and Th2 cytokines.

Considering dose-dependents effect of NMU in metabolism and thermoregulation (Klose *et al.*, 2017; Abdullahi *et al.*, 2019) and its extensive roles in cancers, as reviewed by Przygodzka *et al.* (2019), it is worthwhile to explore neuro-immune communication, which may provide new therapeutic and diagnostic targets. For example, as Teranishi *et al.* (2018) show that NMU overproduction in the liver accelerates hepatic inflammation, whether liver-specific inhibition of NMU reduces inflammation is worth further studies.

Furthermore, accumulating evidence shows that neuronal guidance cues, such as netrin-1, are involved in promoting liver regeneration (Schlegel *et al.*, 2016), avoiding hepatocyte death (Lahlali *et al.*, 2016), inducing epithelial-mesenchymal transition (Schlegel *et al.*, 2016), etc. In renal ischemia-reperfusion injury, netrin-1 suppresses Th1/Th2/Th17 cytokines production, thus protecting against tissue injury (Tadagavadi *et al.*, 2010). Therefore, it is interesting to explore the neuroimmune communication, with verification in the liver, which may provide diagnostic and therapeutic insights (Kefeli *et al.*, 2017).

Conclusion

The expanded understanding of i2 has enabled new insights into HCC treatment. A balanced i1-i2 axis is vital to liver homeostasis. In contrast, dysregulated liver homeostasis skewing towards i2 profile cause a series compensating effects, which include molecular events such as cytokine release and activation, cellular activities such as macrophage polarisation, and tissue-level rearrangements such as fibrosis and cirrhosis, which eventually contributes to HCC, which provides significant therapeutic and diagnostic insights, including molecular targets including Th2 cytokines, cellular targets such as HSCs and an emphasis on drug delivery methods. Moreover, the extensive role of Th2 cytokines links HCC to more broad fields such as inter-organ crosstalk. However, it is impossible to all these contents, such as the gut-liver axis (Yang *et al.*, 2020), in this article. Undoubtedly, research into the role of i2 in liver cancer will eventually provide a more solid theoretical and practical basis for beneficial clinical applications and innovations in HCC treatment.

Figures & Tables

Figure 1. Th1 and Th2 cytokine in hepatocarcinogenesis, adapted from Gieseck *et al.* (2018). Regardless of the aetiologies, the CLD progression into HCC consists of three stages, including fibrosis, cirrhosis, and HCC, which takes about 20-40 years (Dhar *et al.*, 2020). Nonresolving inflammation, due to the imbalanced activation of i1 and i2, as illustrated above, disrupts normal tissue repair and regeneration, contributes to the progression, Regardless of the causes of CLDs. However, it should be noted that HCC could rarely happen without cirrhosis or fibrosis and that it also rarely happens in the fibrotic stage; however, cirrhosis is found in more than 90% of the HCC patients (Ghouri *et al.*, 2017), which suggests the universality of the three-stage progression discussed in this article.

Figure 2. Immune cells in liver fibrosis and HCC.

2A) Upon liver injury, alarmins, e.g., IL-33, IL-25, thymic stromal lymphopoietin (TSLP) move to activate ILC2 and Th2, which produces Th2 cytokines, resulting in eosinophil recruitment and pro-fibrotic M2 macrophage differentiation. Mediated by TGF-β, M2 macrophages further differentiate into fibroblasts, myofibroblasts, causing ECM deposition and sequent fibrosis, if there is not wound-healing resolution (Hams *et al.*, 2015).

2B) A number of cells are believed to be pro-tumour and anti-tumour factors, as shown in the figure. In addition to these cells, high liver immunotolerance in the liver, along with physical barriers of ECMs and active immune editing, favours tumour onset and growth. Not all therapeutic implications listed are mentioned in the main text, with details of therapies, such as cancer vaccines, reviewed by Wang *et al.* (2021).

Figure 3. TAMs in HCC

- **3A)** The continuum of macrophage polarisation states (Yamaguchi *et al.*, 2015) indicates that macrophage transdifferentiation is mediated by the centration of cytokines along the i1-i2 axis, where i1 is predominantly characterised by IFN-γ, TNF-α, poly(I:C), LPS, and i2 characterised by IL-4, IL-13, IL-33.
- **3B & 3C)** Therapeutic strategies targeting TAMs (Tian *et al.*, 2019). Proposed strategies include inhibition of monocyte recruitment, eliminating TAMs, M2-M1 conversion, neutralising M1 release products are proposed (3B), with examples listed in 3C.

Figure 4. Hepatocarcinogenesis mechanisms.

- **4A)** With immune crosstalk to initiate HSC differentiation into CAFs upon PDGF and TGF-β activation, activated CAFs promote tumour aggressiveness, hepato-carcinogenesis, angiogenesis, etc, impacting HCC onset (Zhang *et al.*, 2020).
- **4B)** Mutations arise during chronic liver injury. Zhu *et al.* (2019) suggest most mutations are adaptively beneficial, with cirrhotic nodules residing different colons (Müller *et al.*, 2019).

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