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Chronic Inflammation, Immune Escape and Oncogenesis In the Liver: A Unique Neighborhood for Novel Intersections

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Abstract

Sustained hepatic inflammation, driven by factors such as alcohol consumption, non-alcoholic fatty liver disease (NAFLD) and/or chronic viral hepatitis (Hepatitis B and C), results in damage to parenchyma, oxidative stress, and compensatory regeneration/proliferation. There is substantial evidence linking these inflammation-associated events with the increased incidence of hepatocellular carcinogenesis. While acute liver inflammation can play a vital and beneficial role in response to liver damage or acute infection, the effects of chronic liver inflammation, including liver fibrosis and cirrhosis, are sufficient in a fraction of individuals to initiate the process of transformation and the development of hepatocellular carcinoma (HCC). This review highlights immune-dependent mechanisms that may be associated with hepatocellular oncogenesis, including critical transformative events/pathways in the context of chronic inflammation and subverted tolerogenesis.

Keywords

hepatocellular; tolerance; innate; B-cells; senescence

Introduction

The liver is the largest internal organ and the primary regulator of systemic metabolism. Equally significant, the liver also functions as the primary lymphoid organ tasked with surveillance of the large and diverse antigen load inherent in the dietary intake(1). The hepatic immune system of the liver must not only be able to identify, detoxify and neutralize pathogens, it must also be able to tolerize the host to potentially damaging systemic immune responses against otherwise antigenic but beneficial nutritional components. The liver is anatomically situated to collect the blood flow directly from the gut after which it is directed through the architecturally unique, reduced-flow vasculature of the liver sinusoids, optimizing interaction with resident immune cells including lymphocytes, macrophages Kupffer cells (KC), NK/NKT and dendritic cells (2), while allowing establishment and enrichment of these otherwise mobile non-parenchymal cells (NPC). These factors combine to maintain a balance between the elimination of pathogenic components and tolerization of the local and systemic immune responses to non-pathogenic antigens. These same attributes also conspire to predispose the liver to pathologies that evolve from immune-mediated

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damage (hepatitis) and malignant redirection of tolerogenesis (neoplasia, persistent viral and microbial infections).

Dysregulated swelling and inflammation of the liver, defined as hepatitis, is characterized by the presence of excess inflammatory cells. When unresolved,, inflammatory components directly induce hepatic damage, often overwhelming the ability of the liver tissue to repair itself and leading to fibrosis and irreversible scar tissue formation called cirrhosis (3). Cirrhosis restructures liver tissue into nodules rich in both dying and replicating hepatocytes, compromising liver function and often leading to liver failure. This process typically occurs over decades driven by diverse etiologies including viral hepatitis, alcoholism or non-alcoholic fatty liver disease (NAFLD) and are associated with increased HCC risk (4). Intersection of hepatic immune-mediated processes including: oxidative damage (mutagenic), compensatory liver regeneration (mitogenic), and tolerogenesis to neo-antigens (tolerogenic), favors neoplastic transformation. Through the understanding of immune-dysregulation in hepatocellular carcinogenesis, key processes can be identified and beneficial interventions proposed. The following brief overview highlights some current aspects of the hepatocellular intersection of inflammation and carcinogenesis (Figure 1).

Oxidative stress and Kupffer cells

Within the diseased liver, free-radical production in the form of reactive oxygen and nitrogen species is initiated by cells of the immune system, including recruited neutrophils, monocytes and KC. These oxidizing components are normally balanced through redox regulation by antioxidant pathways (glutathione and superoxide dismutase) in healthy tissue. However, the robust immunologic milieu of the liver microenvironment can disrupt this balance during inflammation by deploying anti-pathogenic antioxidants. Hepatocytes sustain oxidative damage directly through chemical modification of proteins, lipids and nucleic acids. Additionally, apoptosis and necrosis programs can be triggered from alterations induced in mitochondrial permeability by the altered intracellular redox state (5). These combined processes collectively referred to as oxidative stress, further amplify the inflammatory response through the release of DAMPS (danger associated molecular patterns) and stress-related signaling molecules, culminating in chromosomal instability and oncogenic gene mutations. Furthermore, nitric oxide (NO) has been shown to protect virally- infected hepatocytes from apoptosis through activation of NF-κB and suppression of Th1 anti-tumor immune surveillance. Integration of diverse inflammatory signals initiated by oxidative stress, gut-derived microbes and endotoxins from the blood occurs through the modulation of pattern recognition receptors on resident KC. Although KCs can be involved in anti-tumor immunity, numerous human and mouse studies have recently uncovered their multi-faceted ability to contribute to promotion of liver tumorigenesis. In addition to the roles for antioxidants described above, chronic inflammation can also be mediated by KCs through the constant production of cytokines, including interleukin-6 (IL-6), TNF-α, and transforming growth factor-β (TGF-β). The contributions of these cytokines to chronic inflammation will be described below.

IL-6/STAT3 and TNF-α/NF-kB Axes

IL-6 and TNF-α, are active contributors to acute inflammatory responses(6). Expression of IL-6 and TNF-α is elevated in both liver cirrhosis and HCC (7). Although the mechanisms by which elevated IL-6 and TNF-α promote liver cancer are not clear, their signals regulate gene expression through the latent transcription factors STAT3 and NF-kB. NF-kB is the intracellular signaling effector for many pro-inflammatory cytokines including TNFα, IL-1, and TLRs. In many tumor types, NF-kB is highly activated and often addictive (inactivation leads to cell death or tumor remission) (8). In liver, NF-kB activation in non-parenchymal

cells is necessary for HCC tumor promotion (9) while its function in hepatocytes is pro-survival and induction of pro-tumor cytokines including IL-6 and TNFα (10). STAT3 can function as a driver oncogene and has been shown, along with IL-6, to be part of an epigenetic switch established during transformation (11). Overlap in the transcriptional regulatory network between inflammation and transformation has been demonstrated in other tumor models suggesting inflammation-driven signaling within pre-neoplastic cells can cooperate or contribute directly to oncogenic transformation (11). With regards to hepatocellular transformation, constitutive activating mutations in both gp130 (IL-6 signal transducer) and STAT3 have been demonstrated in more than 70% of inflammatory hepatocellular adenomas (12). Furthermore, isolated human HCC stem cells are marked by high expression of IL-6 and STAT3, but often also with loss of the type 2 TGF-β receptor (TGFBR2). This suggests that IL-6 autocrine signaling may be more important than maintaining TGF-β signaling in driving transformed stem cells toward HCC (13).

Dual functions of TGFβ/Smad

TGF-β plays a complex role depending on the context and stage of the "fibrosis-cirrhosis-HCC" process (9). A recent study revealed inactivation of TGF-β signaling through deletion of TGFBR2 reduced HCC formation caused by p53 loss in Albumin-cre transgenic mice (13). In contrast, Ozturk's group found that TGF-β treatment *in vitro* induced growth inhibition in well-differentiated HCC cell lines that have p53 mutations and express TGFBR2 (23). The results of their analysis of TGF-β expression in normal, cirrhotic, and HCC liver, using publicly available clinical data, showed that TGF-β was sharply increased in patients with liver cirrhosis, but was followed by a significant decrease in patients with early or advanced HCC. Furthermore, TGFBR2 is also reported to be down-regulated in 37–70% of patients with HCC (13). These paradoxical findings may be reconciled by recognizing that the roles of TGF-β in HCC tumorigenesis are inherently different at various stages of disease development. In cirrhotic liver, up-regulated TGF-β promotes the transformation and growth of neoplastic cells with existing p53 mutations while after tumor development, HCC cells may escape growth inhibition possibly by down-regulating their TGF-β receptors and "instructing" the microenvironment to shut down the expression of TGF-β. Stage-dependent TGF-β signaling is also influenced by differential cytokine-activated-kinase phosphorylation of Smad proteins. Normally TGF-β-mediated Smad3 signaling terminates hepatocyte proliferation during acute liver injury. Chronic pro-inflammatory cytokine exposure alters the phosphorylation state of Smad2/3 resulting in mitogenic (oncogenic) and fibrotic TGF-β signaling(14). The redundancy of these mechanisms in regulating TGF-β signaling underscores the necessity and importance of this pathway in hepatocellular oncogenesis.

LTβR signaling and oncogenesis

The tumor necrosis factor (TNF) super family (TNFSF) of cytokines consists of 29 members. In addition to the well documented pleiotropic roles of TNF-α in the liver, lymphotoxin (LT) α, along with LTβ and Light (TNFSF 14) have been implicated as drivers of hepatic stellate cell function/wound healing (15), liver regeneration (16) and hepatic carcinogenesis (17). These findings have evoked renewed interest in targeting LTβR in an attempt to thwart hepatocellular oncogenesis. Recent work from Haybaeck *et al.* has provided compelling evidence the inflammation resulting from LTαβ signaling is sufficient to drive HCC in the liver-specific AlbLTαβ murine model (17). Moreover the authors detail the increase in mRNA levels of LTβR ligands in liver samples derived from patients infected with HBV or HCV, as well as samples from patients with HCC, strengthening the link between LT signaling and HCC. While additional studies are needed to confirm the

pivotal role of the LTβR in HCC, strategies designed to block signaling via LTβR might be beneficial.

Oncogene-induced senescence/apoptosis and immune regulation

Activation of individual oncogenes modeling pre-malignant initiation, elicits distinct protective programs including senescence and apoptosis. These processes are dependent upon both cell-autonomous and cell-extrinsic mechanisms that function in concert to suppress and/or eliminate cells undergoing oncogenic stress. Senescent cells display characteristic secretomes that commonly include IL-6 and IL-8 to maintain the senescent state and promote immune surveillance of senescent cells. In liver, (oncogene-induced) senescent hepatocytes also secrete CTACK, IL-1α, leptin/leptin R, MCP1 and RANTES(18). Non-initiated bystander cells including immune cells can reinforce this program by also secreting pro-senescent cytokines. Apoptotic hepatocytes also release IL-1α which triggers KCs to orchestrate compensatory proliferation, essential to development of HCC in the diethylnitrosamine (DEN) model (19). Senescence, unlike apoptosis, does not result in cell elimination. Instead cells that undergo oncogene-induced senescence constitute a quiescent population of initiated premalignancies. The presence of these senescent cells provides the opportunity for escape or progression to malignancy through accumulated “second hits”. Interestingly, a recent report described an *in vivo* example of immune-surveillance of such oncogene-induced senescent cells(18). Kang *et al.* demonstrated $Nras^{G12V}$ oncogene-induced senescence in liver by examining senescence marker expression in oncogenic-$Nras^{G12V}$ transposon- and inactivated-Ras (effector loop signaling domain deletion) transposon- transduced livers. Oncogenic $Nras^{G12V}$ induced markers of senescence by 12 days, but by 60 days $Nras^{G12V}$-expressing cells were undetectable. Previously, an inducible p53-dependent model of HCC senescence showed the innate immune system was sufficient to mediate regression of established HCC tumors (20) suggesting a role for innate immune components in mediating surveillance of senescent cells. In Kang’s model, $Nras^{G12V}$ alone was not able to drive hepatic tumorigenesis in WT mice. Surprisingly $Nras^{G12V}$ did drive massive tumor development in CD4 $^{-/-}$ hosts, implicating a role for the adaptive immune system (T helper cells) in surveillance of cells undergoing oncogenic stress. Furthermore, the authors found Nras G12V peptide epitope specific Th cells indicating specificity for the driving oncogene. The authors also presented evidence that monocytes/macrophages function as effectors in clearing initiated cells but antibody-mediated depletion of neutrophils and NK cells showed only marginal or no effect. It has been noted that immunosuppressed individuals display a higher cancer rate, reviewed in (21), and in the case of hepatitis C, senescent hepatocytes build-up in infected livers of immunosuppressed patients (18). This is consistent with immune-mediated surveillance of senescence providing a major barrier to tumorigenesis by eliminating the reservoir of pre-malignant senescent cells that are primed for escape to transformation. However, additional studies, including single-cell analyses, are needed to formally exclude the possible pre-existence of senescence-resistant or otherwise transformed cells.

The Myc oncogene has been implicated in hepatocellular senescence (tumor regression), malignant progression and tumor-dependent immunoregulation. Overexpression of wild-type Myc is a feature in most HCC patients. Induction of Myc-driven genes also marks the transition from dysplastic nodules to “early” HCC (22) indicating that Myc may initiate or be an “effector” of transformation in HCC. Myc is the only oncogene (23) in its wild-type form that can induce high penetrance tumors with short latencies in most transgenic models. The Myc transgenic tumor model displays decreased latencies in response to hepatotoxins and hydrodynamic damage, implying Myc may collaborate with inflammation-driven signaling pathways (24). Interestingly, Myc is an addictive oncogene in models of lymphoma and HCC where tumor regression occurs when Myc is shut down. In lymphoma,

Myc evokes apoptosis which attracts and signals macrophages to secrete TGF-β inducing senescence. Just as CD4 T cells are required for successful surveillance of $Nras^{G12V}$ induced senescent cells, sustained tumor regression following Myc inactivation also depends upon CD4 T cells for, induction of senescence, collapse of angiogenesis, and long term suppression of minimal residual disease (25). Myc-driven transgenic HCC models also display the ability to modulate the adaptive immune response. Specifically, when Myc-OVA transgenic mice (Tet-Myc x OVA) bearing liver tumor were challenged by transferred OVA-specific T cells, transferred T cells penetrated the tumors in high numbers but were hyporesponsive as defined by *in vivo* cytotoxicity and IFN-γ production(26).

The microenvironment of the liver is unique in immune function and architecture

Multiple mechanisms govern tolerance development and immune function in the liver. First, an abundance of microbial-responsive antigen presenting cells (APC) highly express inhibitory programmed death ligands-1/2 (PDL-1/2) and IL10. Second, unique reduced flow architecture facilitates tolerance through T cell "trapping" combined with suppressive cytokines IL10 and TGF-β, enhancing CD8+ T cell apoptosis. Third, active recruitment and accumulation of suppressive MDSCs and Tregs during HCC progression further tips the immune balance toward suppression (Figure 2). These factors acting in concert form a bridge between cirrhosis and HCC development.

Chronic low level LPS drives IL10 synthesis, PDL1 and T cell Tolerance

Blood enters the liver via sinusoids supplied by the hepatic artery and portal vein. This blood from the intestine is rich in microbial-derived, TLR-activating factors that regulate innate immunity and IL10 synthesis. Lipopolysaccharide (LPS) activation of TLR4 on antigen-presenting cells, including KC, plasmacytoid DC (pDC) and myeloid DC (mDC), triggers the synthesis of multiple cytokines including TNF-α, IL-12, IL-18 and IL-10. (27) Coordinated down-regulation of this pro-inflammatory microenvironment is needed to prevent immune-mediated damage. The dynamic suppressive cytokine, IL-10, is essential for maintaining liver homoeostasis/tolerance. IL-10 modulates NK activity/function, induces T cell suppression (28), and polarizes adaptive Tregs (29) thereby suppressing the liver immune response and surveillance. Furthermore, the negative costimulatory receptor PD-1, expressed primarily on T cells and B cells, inhibits antigen-specific CD8+ T cell activation/ memory and inflammatory hepatitis (30). Expression of PD-1 ligands, PD-L1 and PD-L2 is elevated in steady state liver, with highest levels of expression on KC, infiltrating macrophages, LSECs, dendritic cells, and parenchymal cells (31). Increased expression of the inhibitory receptor, PD-1, and/or interactions with its ligands, have correlated with persistence of viruses (32), HCC aggressiveness and HCC recurrence following treatment (33). Therapies targeting the interaction of PD-1 with its ligands have shown promise in fighting chronic HCV infection and enhancing anti-tumor responses. Recently, Youngblood *et al.*, using epigenetic analysis of the Pdcd1 (PD-1 locus), suggested a unique regulatory program for PD-1 expression in antigen-specific T cells. The complete de-methylation of the Pdcd1 region coincided with sustained expression of PD-1 on exhausted CD8+ T cells in a setting of chronic viral infection (34). In contrast, acute infection was accompanied by only transient de-methylation of Pdcd1, followed by subsequent re-methylation upon differentiation into CD8+ memory cells (34). Franceschini et al. identified a role for PD-1 on T regulatory cells (Tregs) during chronic HCV infection. Infiltrating Tregs up-regulate PD-1 and upon ligation of PD-L1/2 inhibit TCR signaling and reduce IL2 signaling via inhibition of STAT5 phosphorylation, decreasing Treg proliferation (35). It is likely PD-1 can induce tumor specific CD8+ T cell apoptosis (33) and PD-1 signaling has been shown to

reduce IFNγ, TNF-α, and IL2 synthesis(30). Collectively, these studies suggest a pivotal role for the PD-1-PDL1/2 axis during initial viral escape and reduced tumor surveillance.

The liver is a preferred site of T cell accumulation and activation, due in part, to unique architectural features and the abundance of APC. Increased expression of adhesion molecules facilitates the trapping of activated T cells in sinusoids, where they may undergo apoptosis induced by FasL and Trail on KC or be phagocytosed (31). Moreover, T cells that recognize antigen in the liver are typically exposed to high levels of the suppressive cytokines IL-10 and TGF-β; further modulation occurs through PD1-PDL1/2 inhibitory signaling. This T cell tolerance likely explains the evolutionary need to have a substantial innate component, potentially explaining the abundance of pDC and NK cells.

Immune suppressors

The role and types of immunosuppressive cells that accumulate in chronic infection and HCC remain incompletely understood in the liver. Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells, which can expand and contribute to immune suppression during HCV infection and HCC (36, 37). The liver is a preferred site for homing and expansion of MDSCs (38). MDSCs can disrupt immune surveillance mechanisms including suppressing effector T cells (37), expanding Tregs (39), and impairing NK cell function (40). MDSC-derived ROS can induce oxidative stress and mediate T cell suppression during chronic HCV infection (36). The presence of intra-tumoral and circulating Tregs correlates with tumor progression (41), poor prognosis (42) and reoccurrence following surgical resection (43) of HCC. Tregs mediate immune suppression through functional modulation of tumor specific CD8+ and CD4+ T cells and by tumor specific accumulation mediated through chemokine receptor CCR4 (44). However, a recent report from Chen at el. identified a selective Treg recruitment pathway facilitated through CCR6-CCL20, selectively promoting HCC progression and predicting poor prognosis (45).

Innate surveillance

NK and NKT cells represent a major innate immune component in liver, constituting over 50% of hepatic lymphocytes in mice and humans (46). During chronic viral infection, suppression of NK cells can result in reduced surveillance. Recent evidence suggests that once HBV/HCV infections become persistent, NK cells lose their cytotoxic potential and ability to secrete IFNγ (47). NK cell function is also impaired in non-viral induced liver cirrhosis. In a carbon tetrachloride (CCl_4)-induced liver fibrosis model, NK cells suppress fibrosis by killing activated HSC early, but later, NK cells are inhibited by elevated levels of TGF-β and SOCS1(39) coinciding with the critical period for HCC development. In the c-Myc/Tgfα transgenic model, MHC-1 is down-regulated and Rae1 is upregulated on dysplastic hepatocytes targeting them for NK surveillance. However, malignant progression still succeeds presumably due to insufficient NK numbers (48). NKT cells significantly increased in c-Myc/Tgfα dysplastic liver as it progressed towards malignancy. Distinct NKT cell subsets can either promote ($CD4^+$ NKT) or inhibit (iNKT) liver cancer (49)(recently reviewed by Subleski *et al.* (50)) and can partially explain this observation. Bridging innate and adaptive immune systems, resident DCs are less functional in liver, but are still capable of priming anti-viral T cell responses sufficient for clearance. Upon viral escape, chronic liver inflammation renders liver DCs suppressed, as observed in chronically infected HCV patients showing a diminished ability to mature and prime T cell proliferation and induce IFN-γ (51). Interaction between HCV core protein and DCs in culture results in reduced frequency of pDC and direct inhibition of IFNα production(52). Core protein can also inhibit IL-12 production by DCs through an intracellular mechanism dependent on a

combination of TLR4 signaling and cross linking of the complement receptor (53) thereby contributing to a Th2 skewed microenvironment. HBV-infected patients have diminished pDC functions resulting in part from a specific HBV antigen (HBeAg) (54). These findings suggest persistent viral infection and chronic inflammation deprive DC's ability to prime T cell surveillance, augmenting hepatocellular carcinogenesis.

Dual role of B cells in hepatocellular carcinogenesis

Circulating B cells from cirrhotic patients have been reported to be hypo-responsive to *ex vivo* CD40/TLR9 stimulation, as characterized by LTα secretion, IgG production and T-cell allostimulation. A reduction in CD27+IgM+ memory B cells was also observed in cirrhotic patients (55). These changes support a reduced B cell-mediated anti-viral response allowing persistent viral infection, associated inflammation, and HCC development. In contrast, results from an inflammatory skin model of HPV16 squamous cell carcinomas (SCC) suggest a more direct, tumor promoting role for B cells, possibly via immunoglobulin accumulation. (56) Although controversial, increased levels of immunoglobulin in murine HCC models, serum from cirrhotic individuals and HCC lesions (57), all suggest a possible cause and effect linkage between the presence of immunoglobulin and HCC development. Previously, we established a murine *de novo* liver tumor model of adenoma and carcinoma by hydrodynamic injection of transposons containing myrAKT (AKT) and Δ90 β-catenin (β-CAT) (58). We observed hepatocellular development/progression to be largely dependent on B cells (Authors' unpublished observation). We also found that tumor infiltrating B cells express elevated levels of TNF-α (Authors' unpublished observation), suggesting that B cell-derived cytokines could be instrumental to tumor development/progression. Support for the role of TNF-α from B cells was recently detailed by Schioppa et al. who observed in B cell-specific TNF-α −/− mice a markedly reduced promotion of the HPV16 SCC model and a modulation of B10 cell activity (59). Collectively, these studies suggest a significant tumor-promoting role for B cells during carcinogenesis.

Perspective

Chronic liver injury, inflammatory pathway activation and oxidative stress intersect within the context of the uniquely tolerant liver microenvironment, thus facilitating hepatic oncogenesis. This review emphasizes the dynamic juncture of inflammation and oncogenesis, highlighting the critical players and immunological therapeutic targets, and suggesting areas for further research.

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Abbreviations

NAFLD	non-alcoholic fatty liver disease
HCC	hepatocellular carcinoma
KC	Kupffer cell
NK	natural killer
NPC	non-parechymal cell

NO	nitric oxide
IL-6	interleukin-6
TGF-β	transforming growth factor-b
TGFBR2	type 2 TGF-β receptor
TNF	tumor necrosis factor
TNFSF	tumor necrosis factor super family
LT	lymphotoxin
DEN	diethylnitrosamine
APC	antigen presenting cell
PDL-1/2	programmed death ligands-1/2
LPS	lipopolysaccharide
pDC	plasmacytoid dendritic cell
mDC	myeloid dendritic cell
Treg	T regulatory cell
MDSC	myeloid derived suppressor cell
CCl_4	carbon tetrachloride
HBeAg	hepatitis B protein-e-antigen
SCC	squamous cell carcinoma
SASP	senescence-associated secretory phenotype
DAMPS	danger-associated molecular patterns
CTL	cytotoxic T-lymphocyte

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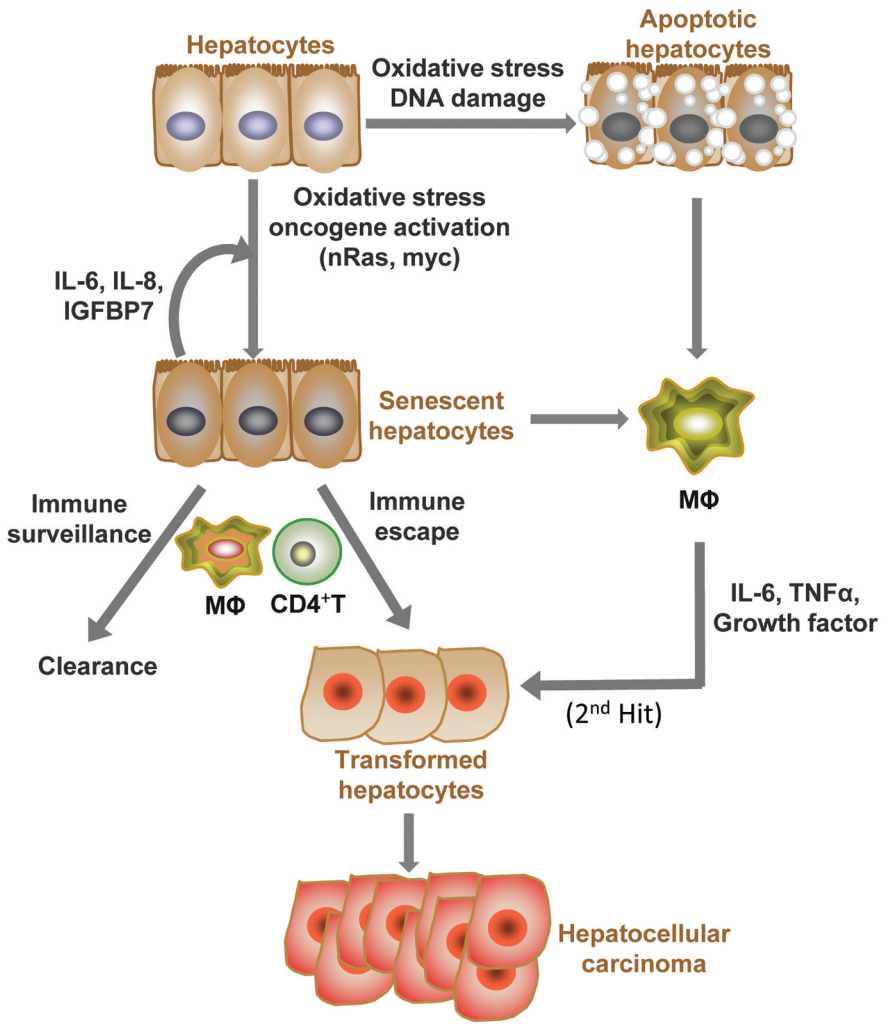

Figure 1. Avoiding hepatocellular oncogenesis through immune-mediated processes

Failsafe mechanisms to counter malignant transformation of hepatocytes involve both cell intrinsic and extrinsic components. In response to cellular damage, cell intrinsic checkpoint pathways arrest cell growth (senescence, autophagy) and/or initiate programs that result in clearance (apoptosis). In the liver, these programs elicit specific extrinsic responses by local immune cells. During the progression to hepatocellular transformation, oncogene activation can also induce both apoptosis and senescence. Both processes result in release of cytokines and chemokines (SASP, senescence associated secretory phenotype and DAMPS, damage associated molecular patterns) into the microenvironment to recruit and instruct immune system cells to reinforce these processes. Innate immune components such as NK and KC stand watch for signs of oncogene-induced hepatocellular stress such as NKG2D and DAMPS (IL-1α, HMGb1). KC also serve as the primary source for IL-6 in the orchestration of compensatory proliferation required for both hepatic regeneration and tumor progression. $CD4^+$ adaptive immune responses are mounted against oncogene induced senescent cells leading to clearance effected by macrophages. Oncogene-initiated cells may escape these mechanisms by acquiring additional or "2^{nd} hits" in these pathways leading to transformation.

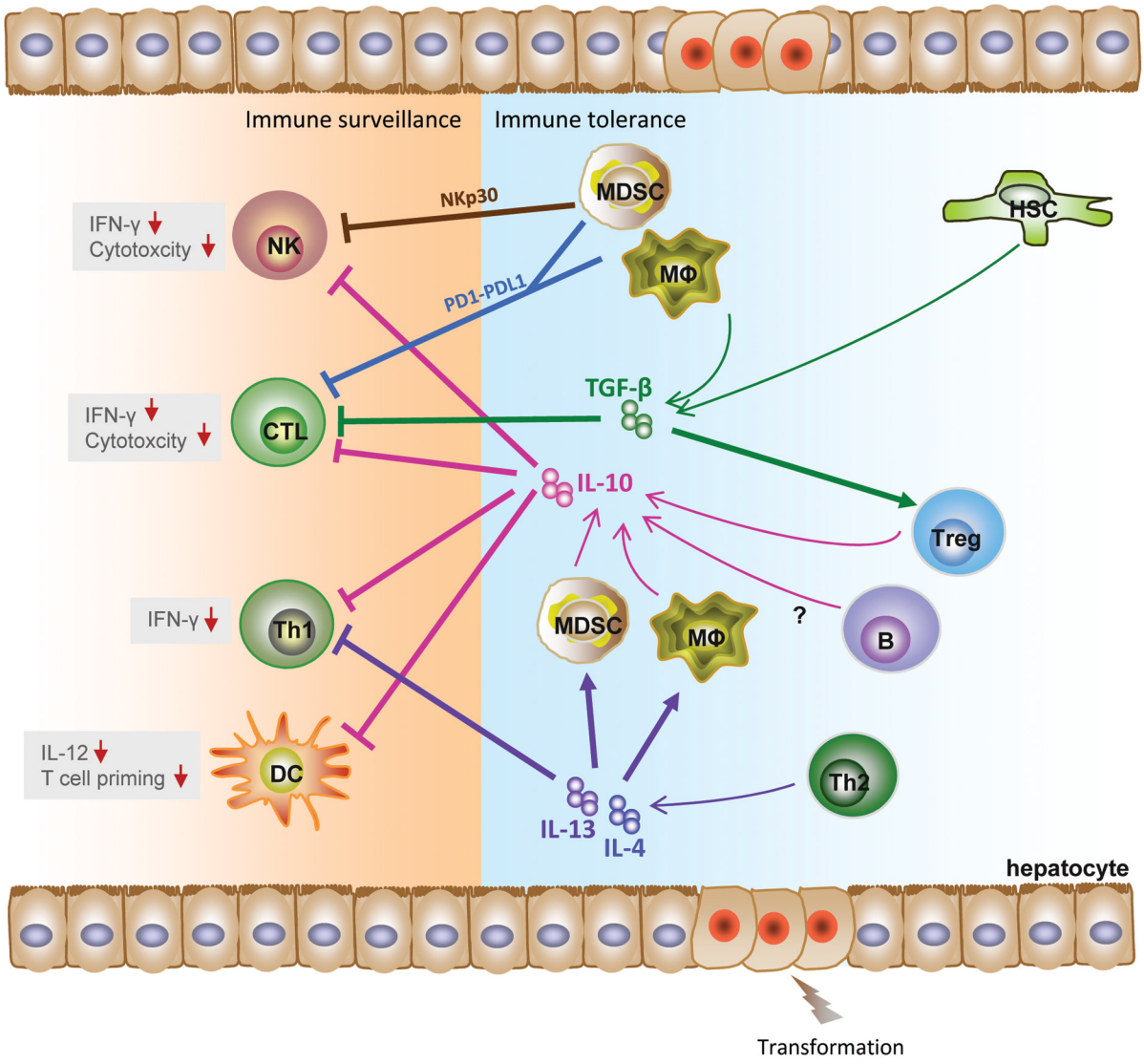

Figure 2. Malignant repurposing of immune surveillance and tolerance in HCC

Prolonged hepatitis induces alterations in TGF-β, IL-10, IL-13, and IL-4 and leads to an immune milieu permissive for hepatocellular transformation. IL-10, produced by MDSC, KC, B and Treg cells, downregulates the anti-tumor effect of NK, CTL, Th1 and DC cells. KC and HSC activation leads to elevated TGF-β levels stimulating Tregs and inhibiting CTLs. IL-13 and IL-4 produced by Th2 further inhibit Th1 cells MDSCs inhibit NK and CTL activity through NKp30 and PD-1. The concerted effect of these activities results in reduced immune sensitivity and increased tolerance.