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Review Article

Hepatocellular Carcinoma: The Impact of Compromised Host Immunity on Regulation of The Proliferative Response

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Hepatocellular carcinoma is the most common primary malignancy of the liver. 2014 produced 31,000 new cases of HCC in the United States, along with 24,000 deaths attributed to HCC [1]. In 2017, there is predicted to be a rise in both incidence and mortality associated with HCC or carcinoma of liver bile duct: an estimated 40, 700 new cases of hcc or biliary ductal cancers, would represent 2.4 % of all new cancer cases. Over 28,000 deaths in 2017 from HCC, or cholangiocarcinoma would account for 4.8% of all cancer deaths. There are over 60,000 individuals in the United States with diagnosis of hcc or intrahepatic cholangiocarcinoma.: Age adjusted rates: 8.6 per 100,000 men or women per year and number of deaths as 6.3 per 100,000 men and women based on 2010-2014. Approximately 1% of all men and women will develop primary liver cancer at some point in their lifetime (2012-2014 data). The estimated 5-year relative survival rate from 2007 to 2013 was 17 %, which was a significant improvement from past years. The 5-year relative survival estimate was 3% in 1975, increasing to 5.7 % in 1995 and to 16.8 % in 2005.

Worldwide over 700,000 new cases of hcc are diagnosed per year. HCC represents the second most common cause of cancer death in the world, with incidence to mortality ratio of 1.07. Worldwide, Asia and Africa represent the majority of cases which correlates with the relatively greater prevalence of hepatitis C virus (HCV) and hepatitis B virus (HBV) and HCV- or HBV-related cirrhosis which are major risk factors for development of HCC [2].

The increasing incidence of HCC in the United States as well as in other western regions, may be in part related to increasing prevalence of cirrhosis related to nonalcoholic steatohepatitis (NASH) along with alcoholic liver cirrhosis.

Hepatocellular carcinoma is staged by anatomy, liver function, and tumor activity (as measured by afp). The primary treatment for hcc is resection for patients with early staged tumors and normal liver function. Orthotopic liver transplant (OLT) is performed in patients with advanced stage liver disease with early staged tumors in that setting. The majority of cases, however, represent individuals with more advanced staged tumors-multifocal tumors. In addition, a significant proportion of patients present with tumors that have infiltrated the hepatic blood supply rendering them inoperable and ineligible for transplant.

Hepatocellular carcinoma in the setting of liver cirrhosis follows a multistep carcinogenesis model. The general principle is there is a chronic proliferative stimulus to hepatocytes. Transforming growth factor Beta (TGF B) expression and/or TGF B Receptor activation play an important part in the early stages of carcinogenesis in hepatocellular carcinoma. TGFB is a potent growth inhibitor of hepatocytes. However, TGF B expression is increased in HCC. In addition, TGF-Beta over expression in HCC corresponds to carcinogenesis, tumor progression (foot noted: malignant transformation by TGF-B pathway is due to mutations in the TGF -B receptor type I or Type II or smad protein-the intracellular mediator of transmitting the extracellularly initiated TGF-B signal to nuclear targets via control of transcription of selective genes). In malignant transformation, cells secrete TGF-Beta which acts to suppress antitumor immune responses, promote extracellular matrix production, and enhance angiogenesis [3]. This pro-proliferative activity reflects the normal role of TGF-beta in embryogenesis. During later phases hepatocellular carcinogenesis, there are mutations in p53, p16 and AKT signal pathway related molecules that develop. There is also evolution of surrounding stroma where there is an increase on stromal formation including proliferation of stromal fibroblasts. The stromal cell-hepatocellular cancer cell interactions may modulate a dual function of TGFB signaling in hepatocellular carcinogenesis.

Invasive hepatocellular carcinoma develops from a dysplastic, regenerative, nodule in a cirrhotic liver. The chronic inflammatory state that is a marker of cirrhosis is a perpetual stimulus to hepatocytes resulting in increased cell proliferation and cytokine production. There is a high incidence of regenerative nodules and dysplastic nodules, and the dysplastic nodule potentially can transform to malignancy. When a poorly differentiated hepatocellular carcinoma develops within a well differentiated nodule, this highlights the transition from early phase of hepatocellular carcinoma to a more histologically advanced state. This is also called a nodule-in-nodule lesion. Advanced hepatocellular carcinoma is more frequently associated with intra-hepatic metastases, portal vein invasion [4].

Molecular alterations contribute to stepwise progression to advanced hepatocellular carcinoma. TERT promoter mutations and mutations in the CTNNB1 gene represent some of the earlier genetic events in hepatocellular carcinogenesis in the background of liver cirrhosis [5,6]. Mutations in p53 are present in the more poorly differentiated hepatocellular carcinoma but none are found in the more well differentiated hcc nodule in the nodule -in-nodule model [7]. Higher grade tumors contain more frequently nuclear accumulation of mutated forms of p53. Hypermethylation of the p16 (CDKN2A) tumor suppressor gene-a cyclin dependent kinase inhibitor, results in loss of expression of p16. Lost expression of p16

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protein is more prevalent in advanced hepatocellular carcinoma compared to early hepatocellular carcinoma (40% vs 20%).

Conversely, heat shock protein 70 (hsp 70), and adenylate cyclase associated protein 2 are expressed in early hcc, but are expressed at high levels in advanced HCC, suggesting progressively increasing levels marking a stepwise progression from early to more advanced stages [8].

Hepatocellular carcinoma: A Successful Model of Immunity Evasion.

Immune tolerance and modulation of cellular cytotoxicity are essential to maintaining the liver microenvironment that is designed to accommodate exposure to vast amounts of antigens as a result of a dual circulatory system. The liver has the unique physiology that involves receiving a dual blood supply from the central venous and portal venous systems. The hepatic artery delivers oxygenated blood from the systemic circulation to the liver while nutrients, molecules including pathogen derived antigens reach the liver sinusoids via the portal vein [9]. The dual vascular system interfaces with hepatocytes at the sinusoidal space and specifically in the Space of Disse. The extensive exposure of the liver to gastro-intestinal pathogens makes it a major contributor to the host defense and in the management of self-tolerance [10]. The liver accounts for a significant proportion of effectors and regulators of immunity as in CD4-positive T cells, CD8-positive T cells (cytotoxic T cells, natural killer or NK cells) as well as regulatory T cells (T regs). Examination of the liver microenvironment as pertains to host immunity and immune tolerance has identified cellular components that modulate the intrahepatic immune response in distinct ways, and the derangement of these processes contribute potentially to defective anti-cancer immunity and escape of dysplastic or frankly malignant hepatocellular nodules from immune surveillance.

Liver sinusoidal endothelial cells (LSECs): endothelial cells that form the liver sinusoids, composed of a fenestrated cellular barrier that serves as a "filter" between sinusoidal blood and the hepatocytes. LSECs express toll-like receptors (?beta catenin/frizzled?) and both major histocompatibility complex (MHC) class 1 and class II molecules, which allows them to function as antigen presenting cells (APC). LSECS appear to contribute to immune tolerance/suppression by blunting otherwise robust and potentially harmful immune responses to bacterial related particles that constantly perfuse the liver sinusoids. They help create an environment that permits T cell proliferation but not T cell activation. They have suppressed expression of the CD80 and CD86 co-stimulatory molecules but have high levels of PD-L1, therefore abrogating their ability to activate CD4 positive (CD4+) or CD 8 positive T cells. MHC molecule expression is down regulated in LSECs in the presence of bacterial derived particles and bacterial/lipopolysaccharide induced soluble mediators transforming growth factor beta (TGFBeta), interleukin 10 (IL-10). LSECS via direct interaction /contact to dendritic cells (DC) decrease DC's activation of T cells [11]. Alongside LSEC's in the liver sinusoids are Kupffer cells (KCs)- liver stationed macrophages which also have low expression of MHC molecules and therefore join LSEC's in minimizing immune reactivity of T cells. In addition, Kupffer cells produce IL-10 and prostaglandins which are inhibitory cytokines. Kupffer cells can also selectively expand CD 25 positive /Forkhead box P3 (FoxP3) regulatory T cells (T regs) [12,13].

Immune reactivity in HCC is influenced and often defined by three features: T-cell exhaustion in chronic inflammation; immune evasion, and attenuated immune responses.

T cell exhaustion can be seen as the opposite of T cell activation or priming. In T cell riming: cancers or pathogens are eliminated by a 3-step process. First: cancer neoantigen (foreign antigen) is presented to the effector T cell in association with MHC molecule, forms a functional complex with the cognate CD4/CD8 + T cell receptor, this allows for immune recognition of non-self. To actually activate the T cell, a second signal is required that involves co-stimulatory receptors and their respective ligands located on the antigen presenting cell. Several Co stimulatory receptors involved in T cell activation are characterized: CD28-CD 80/86, ICOS-B7RP1, CD137-CD137L; OX40-OX40L, CD27-CD70. Finally, to sustain and/or complete the activation/priming of the T cell, a 3rd phase is required which involves signaling via soluble mediators-pro-inflammatory cytokines (IL-12, interferon-gamma {IFN-g}) which promote effector T cell proliferation and survival [14].

In contrast to the stimulatory signal conducted by these co-stimulatory receptors in conjunction with the foreign antigen-MHC complex, co-inhibitory receptors exist on the T cells which abrogate T cell activation, including: cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)-binding CD80/86, programmed death 1 (PD-1)-binding PD-L1, killer cell immunoglobulin Ig-like receptors (KIR)-binding MHC class I and class II molecules, lymphocyte-activation gene 3 (LAG3)-binding MHC class I and II molecules, and finally T cell immunoglobulin domain and mucin domain 3 (TIM-3)-binding galectin 9 [15] . This interaction is not sufficient to activate the T cell.

T cell exhaustion or inappropriate regulatory T cell activity develops as a result of derangements in any of the above three processes (antigen presentation-evasion; stimulatory signal, 3rd stimulatory signaling). Exhausted T cells are effector T cells capable of foreign antigen recognition but incapable of appropriate immune reaction. T cells having a chronic hypo responsive state express increased levels of co-inhibitory molecules such as CTLA-4, PD-1) and decreased levels of effector cytokines, resulting in compromised cellular cytotoxicity. T cell exhaustion develops in specific circumstances which share in common the presence of chronic inflammatory states. Examples include: viral hepatitis, autoimmune hepatitis, nonalcoholic fatty liver disease (insert Harding 19). Kupffer cells, liver sinusoidal endothelial cells (LSECs) and tumor associated leucocytes originating from inflamed livers demonstrate high levels of expression of PD-L1, correspondingly the effector lymphocytes also have increased levels of co-inhibitory molecules including PD-1 as well CTLA-4 and TIM-3 [16].

T cell exhaustion may have an adverse impact on the immune response to viral hepatitis with increased expression of PD-1 noted in lymphocytes derived from the livers of HBV infected hosts. T cell exhausted phenotype of CD8+ T cells notably as increased expression of additional co-inhibitory molecules besides PD-1, including CTLA-4 combined with suppressed expression of the co-stimulatory molecules CD28, CD 127. In a mouse model of HBV, anti-PD1 or anti-PD-L1 antibody rescues T cell activation and clearance of HBV virus. In vitro assays of effector T cells derived from HCV-infected hepatocytes required combined blockade of CTLA-4 and PD-1 in order to restore immune reactivity of the effector cells [17].

Evasion of Immune detection and defective immune responses in HCC:

Cytokine secretion patterns/ profiles can be altered in part by disturbing the balance between T helper subsets (TH1, TH2, etc.) and by increased secretion of soluble mediators characteristically involved in suppressing the T effector response. Each event may contribute to immunosuppressive state by restricting effector T cells responses to stimuli both pathogens as well as cancer neoantigens. Livers that are chronically inflamed have increased levels of IL-10 and TGF-Beta, each of which impairs effector T cell responses. Increased circulating

levels of TGF-Beta is associated with relatively worse prognosis in patients with HCC. TGF-Beta can promote tumor progression via increased neovascularization, inducing fibrosis, supporting metastasis in HCC. It also, however, can be immunosuppressive [18] where it can induce T reg polarization and differentiation [19,20], negatively regulating CD8+ T cells and increasing T cell exhaustion [21]. There is also an increased representation of TH2 derived cytokines (IL-4, IL-5, IL-8 and IL-10) and relative suppression of cytokines associated with TH 1 responses (IFN-g, IL-2, and IL-1). Patients with hepatocellular carcinoma in whom aberrant cytokine profiles are present (i.e. IL-4, IL-5, IL-8, and IL-10) have inferior outcomes and have more aggressive disease characteristics.

Hepatoma cells may have alterations in their antigen presentation capability which increases the likelihood of evading immune detection. Hepatocellular carcinoma cells can effectively "duck" from T cells as a result of defective antigen processing and or antigen presentation (the equivalent of a vehicle with a license plate smeared with dirt/debris which hampers complete vehicle identification). Reduced or deviant antigen expression on hepatoma cells can serve to cloak these cells from immune recognition and eradication. HCC specific antigens include: alpha fetoprotein (AFP), glypican 3, and New York esophageal squamous cell carcinoma 1 (NY-ESO-1), melanoma antigen gene A (MAGE-A), Wilms tumor 1, human telomerase reverse transcriptase which have been detected in patients with HCC [22,23,24]. These antigens promote cytotoxic T cell responses against HCC and have been detected at low levels in patients with HCC.

The ability of HCC-patient-derived lymphocytes to mount a CD8+ cytotoxic response to in vitro stimulation by tumor specific antigens (such as AFP, MAGE-A, glypican 3, and NY-ESO-1) correlated with improvement in survival. Tumor infiltrating lymphocytes from HCC patients that contain an increased ratio of activated CD8+T cells to inhibitory T regs served as a favorable prognostic factor for survival after curative hepatectomy in these patients.

Understanding cancer neoantigen recognition by effector T cells in HCC and its relationship to reactivity of cytotoxic T cells against hepatoma cells has provided an opportunity to develop therapies that address areas of defect or deficiency and allow restoration of a competent immune surveillance state. Current drug development has focused on the role of the co-stimulatory receptor and co-inhibitory molecules in the interactions among effector T cells, T regs and the antigen presenting cells found in the tumor microenvironment. Nivolumab is a fully human IgG4 monoclonal antibody against PD-1. Tremelimumab is a fully human IgG2 monoclonal antibody against CTLA-4 on the activated T cell. MEDIA4736 is a human IgG1 monoclonal antibody to PD-L1.

Immune suppressive signals against CD4/CD8+ T lymphocytes may come via regulatory T cells (T regs), dendritic cells, myeloid derived suppressor cells (MDSCs), tumor associated monocytes (Kupffer cell), and invariant natural killer T cells which regulate T cell response through T helper 2 cytokine production, prevents expansion of tumor antigen specific CD8+T cells.

Stellate cell infiltration suppresses immune response by suppressive cytokine production and via PD-L1 induction of T cell apoptosis [25]. Depletion of T regs, exhausted CD4+ T helper cells and MDSC's restored cytotoxic effector functions in the CD 8+ T cells (granzyme production, IFN-gamma producing CD4+ lymphocytes [26].

Recently, PDL1 ligand inhibition with novel therapies that up regulate MHC-1 targeted markers on the malignant cells has shown exceptional promise for various malignancies including melanoma, lung cancer, renal cell carcinoma, and sub-classes of colon cancer as well as in hepatocellular carcinoma. The mechanisms remain to be clarified in HCC. However, the impact of cirrhosis in creating the framework within which the host's immune system can benefit from these therapies may be critical in determining how effective these therapies can be [27]. Future study in the area of HCC will highlight how survival/proliferative and immune signaling pathways communicate in the background of cirrhosis compared other conditions and what may be the impact on the ability of driving the key pathogen etic events in the development and progression of HCC as well as influence the therapeutic approaches that are being actively studied to achieve control of this disease and thereby improve survival.

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