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Molecular Hepatic Carcinogenesis: Impact of Inflammation

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Abstract

Hepatocellular cancer (HCC) represents one of the most rapidly spreading cancers in the world. Most HCC develops in cirrhotic livers after prolonged inflammation, supporting the hypothesis that inflammation contributes to cancer development. Increasing evidence suggests that inflammatory cell recruitment and activation is an important contributor to promoting cancerous malformation in hepatocytes. Intracellular signaling pathways involved in classical inflammatory pathway activation can be altered in parenchymal cells, hepatocytes, in the liver to promote HCC development. Inflammation is triggered by pathogen-derived or endogenous danger-associated molecular patterns via pattern recognition receptors. Activation of the pattern recognition receptors triggers downstream signaling cascades to induce proinflammatory cytokine production, release of reactive oxygen species and modulate cellular responses. Many of these inflammatory mediators have adverse effects on DNA repair and induce DNA methylation, both of which are important elements in HCC development. This review summarizes the key points and discusses recent findings related to the role of inflammation in cancer and HCC development.

Keywords

Inflammation; Innate immunity; Hepatocellular cancer; Carcinogenesis; Cytokine; Hepatitis C virus; Hepatitis B virus; NF- κ B; Cirrhosis

Introduction

Hepatocellular cancer (HCC) represents an increasingly common complication of chronic hepatitis caused by hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease, non-alcoholic steatohepatitis and other causes of cirrhosis. The yearly incidence exceeds half a million new HCC cases worldwide, among which 20,000 are found in the United States. The overall mortality from HCC increased in the past two decades, particularly among whites and Hispanics between 45 and 60 years of age, and its 5-year survival rate is less than 12%. HCC is the fifth and seventh most common cancer among men and women, respectively, and men show higher prevalence compared to women [1].

Chronic liver disease, particularly cirrhosis, predisposes to the development of HCC. Among the most frequent risk factors for HCC development are chronic HBV infection even without cirrhosis and cirrhosis due to chronic HCV infection, alcoholic liver disease, non-alcoholic

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steatohepatitis or hemochromatosis. Chronic inflammation results in progressive liver disease, fibrosis and over time can lead to liver cirrhosis in most patients. Although the mechanisms are yet to be understood, the cirrhotic liver tissue environment supports the development of HCC [2]. Indeed, 80–90% of the HCC cases appear in cirrhotic patients [1]. The pathogenesis of HCC involves multistep molecular events. These include chronic liver inflammation, fibrosis and cirrhosis that in combination with mutations in p53 and/or aberrant DNA methylation can give rise to dysplastic cells. A compound change in p53 mutation and reduced p21 expression can lead to early HCC, which over time can lead to advanced and metastatic HCC [3, 4].

Mechanisms of Carcinogenesis in HCC

Different concepts of oncogenesis have been proposed to explain the development of HCC. These include the multistep carcinogenesis model, the cancer platform concept of cell proliferation coupled with aberrations in tumor suppressor genes and inhibition of apoptosis, and finally the cancer stem cell concept [2, 3]. Key characteristics of the cancer cell phenotype in HCC include growth factor-independent proliferation, resistance to growth inhibition, resistance to apoptosis, limitless replication, sustained angiogenesis, invasion and metastasis [3]. Molecular pathways involved in hepatocarcinogenesis involve tumor suppressor pathways, oncogenic pathways, growth factors and their receptors, intracellular signaling pathways, and cell cycle regulatory pathways [2–4]. Of the growth factor signaling pathways, fibroblast growth factor, transforming growth factor- α , epithelial growth factor (EGF), hepatocyte growth factor, vascular endothelial growth factor (VEGF), platelet-derived growth factor, insulin-like growth factor, and Wnt/ β -catenin pathways have been found to be altered [3–5].

One of the distinguishing features of dysplastic and malignant cells compared to normal cells is their proliferation independent of growth factors. Growth factors that usually promote homeostasis or liver regeneration, including EGF, transforming growth factor- α , hepatocyte growth factor, insulin-like growth factor, fibroblast growth factor, platelet-derived growth factor and VEGF activation are often present and overactivated in dysplastic nodules and HCC. EGF-receptor ligands, such as transforming growth factor- α , EGF and amphiregulin promote HCC cell proliferation [3, 4, 6]. Oncogenic mutations were identified in several key oncogenic pathways in HCC including β -catenin/wnt signaling as well as in its downstream targets c-myc and cyclin D1. In addition, expression of the Wnt pathway regulators, Axin 1 and Axin 2 are also altered in HCC [3]. Studies revealed that in 30–40% of HCC, Wnt abnormalities are present, most commonly β -catenin, Axin1 or Axin 2 mutations. Inactivation of the tumor suppressor adenomatous polyposis gene induces HCC in mice through activation of the Wnt pathway [7]. In human HCC, frizzled7 was up-regulated in HBV-related HCC and the HCV core protein was shown to induce Wnt1 expression [5].

Parallel with oncogene mutations, tumor suppressor gene expression and function are also altered in HCC. Potential mechanisms for inactivation of tumor suppressor genes include deletion, mutation and epigenetic modulation such as hypermethylation. For example, the tumor suppressor gene p53 is inactivated in 50% of HCCs and other cell cycle regulators

such as p73, p16, p21, Krueppel-like factor 6, phosphatase and tensin homolog and breast cancer type 2 susceptibility protein (BRCA2) are also affected [3].

Another key feature of carcinogenesis in HCC is resistance to apoptosis. Mutations during carcinogenesis can result in inhibition of oncogene-induced apoptosis, which makes cells resistant to apoptosis and contribute to unrestrained proliferation. Rapid cell proliferation is an oxy- gen- and energy-consuming process, thus sustained angiogenesis in the tumor mass aims to provide delivery of nutrients to sustain tumor growth. In the rapidly growing tumor tissue hypoxia activates hypoxia-inducible factors (HIF), nuclear regulatory proteins that induce ex- pression of target genes such as the proangiogenic factor VEGF. VEGF, fibroblast growth factor and plateletde- rived growth factor stimulate endothelial growth and vessel formation and increase the permeability of tumor microvessels [8].

HCC Pathogenesis in HBV and HCV Infection

According to the WHO, over 2 billion people are infected with HBV worldwide and about 150 million of them have chronic HBV infection. HCV infection is estimated to affect about 170 million people worldwide and 70–80% of the infections result in chronic HCV disease. Both chronic HCV and HBV significantly increases the risk of liver cancer. About 80% of individuals with HCC have cirrhosis, and most of the HCC cases without cirrhosis are related to HBV infection [1].

The HBV proteins, HBV X core and pre-S/S protein have oncogenic activities. HBV integrates into the host DNA and plays a primary role in noncirrhotic HCC. HBV disease activity also contributes as HCC development is 3.9 times higher in individuals with detectable DNA than in those with undetectable HBV DNA [9]. This data support the current practice guidelines to de- crease HBV viral levels in patients with chronic HVB infection. Unlike HBV, HCV does not incorporate into the host genome and it is associated with an increased risk of HCC development in patients with cirrhosis. Recent animal studies have linked HCC development and HCV protein expression with inflammation. Mice that expressed HCV NS5A protein and received repeated stimulation with the TLR4 ligand lipopolysaccharide (LPS) were prone to develop HCC. The same researchers showed that HCC was attenuated in mice that lacked the expression of TLR4, providing evidence for a link between inflammatory pathways and HCC development in the presence of HCV protein [10]. This could be relevant to chronic HCVinfected patients where sustained low level inflammation is present as a result of increased serum endotoxin levels and HCV viral proteins [11].

Pathways Promoting Inflammation in the Liver

The contribution of inflammation to carcinogenesis has received major attention in hepatocarcinogenesis. Several lines of evidence support the hypothesis that the tissue environment with chronic inflammation contributes to dysplasia and development of HCC. This is particularly relevant to chronic liver diseases such as alcoholic liver disease, non-alcoholic steatohepatitis, Wilson's disease and autoimmune hepatitis where prolonged low-grade inflammation over decades contributes not only to the development of fibrosis and

cirrhosis, but also to HCC. In chronic viral hepatitis, the sustained inflammation acts in concert with some of the direct effects of viral proteins in promoting HCC [2, 3].

Inflammation in the liver is a response of the innate immune system to pathogen-derived (PAMPs) or endogenous danger-associated molecular patterns (DAMPs)/ signals. Danger signals from pathogens and injured self are recognized by evolutionarily preserved pattern recognition receptor systems (PRRs) that include Toll-like receptors (TLRs), helicase receptors, Nod-like receptors and some other nonspecific receptor systems. Ligand recognition by these receptors generally induces a core innate immune response in the form of proinflammatory cytokines and/or type I IFN production [12] (fig. 1).

Even in the absence of infection, gut-derived PAMPs such as bacterial LPS can translocate from the intestine to the liver via the portal circulation. Indeed, both human studies and experimental animal models have demonstrated that in liver cirrhosis, regardless of the etiology of the chronic liver disease, there are increased levels of LPS in the portal circulation [13]. LPS is the most potent ligand for activation of TLR-4 to induce downstream signaling that induces NF- κ B activation and production of proinflammatory cytokines such as TNF- α , IL-6 and IL-1 [12]. It is yet to be determined if in addition to LPS, other PAMPs from the gut microbiota contribute to inflammation in chronic liver diseases. Early studies demonstrated that liver cirrhosis and portal hypertension are associated with increased translocation of bacteria to the mesenteric lymph nodes, suggesting both LPS and other microbial products and/or live microbes can translocate. Portal hypertension in general predisposes to gut 'leakiness' and translocation of gut microbial components to the portal circulation and, thus, the liver. Therefore, it is tempting to speculate that with progression of liver disease, the amount and complexity of gut-derived PAMPs increases and this will likely elicit increased proinflammatory cell activation and cytokine production in the liver.

In addition to gut-derived PAMPs, damaged hepatocytes in chronic hepatitis release a variety of endogenous danger signals that can activate PRRs to promote inflammation. The exact nature and the composition of these DAMPs from damaged hepatocytes is yet to be delineated, but release of contents of the intracellular matrix, such as the high-mobility group proteins (HMGB1), uric acid, cellular DNA, potassium efflux, etc., can induce and/or contribute to activation of pro-inflammatory cytokines via PRRs. TLR-induced inflammation can be further amplified via activation of the inflammasome. Inflammasomes are multiprotein complexes that are composed of Nod-like receptors, an adapter molecule and procaspase-1 [14]. Upon activation of the Nod-like receptors by an endogenous ligand, the inflammasome complex through an enzymatic process activates caspase-1 that cleaves pre-IL-1 β or pre-IL-18 into the active secretable form. PRRs are expressed in the liver not only by immune cells, but also by other liver cells including hepatocytes, sinusoidal endothelial cells, biliary epithelial cells and stellate cells. Many of these cells respond directly to PAMPs and DAMPs during an inflammatory stimulus with chemokine or cytokine production, or other more specific changes in their cellular responses [15].

In addition to the direct stimulatory effects of PAMPs and DAMPs on immune cells, damaged liver parenchymal cells, hepatocytes, also provide danger signals for activation of inflammatory cells. For example, in non-alcoholic fatty liver disease, hepatocytes stimulated

by saturated fatty acids and LPS release mediators that activate IL-1 production in liver mononuclear cells [15]. This is just one of the many scenarios where damaged hepatocytes trigger ongoing inflammation in the liver. The repertoire of the immune cell types that contribute to inflammation in the liver is also broad. In addition to the liver resident macrophages, Kupffer cells, recruitment of new immune and inflammatory cells rapidly happens from the bone marrow in response to liver damage. Neutrophil leukocytes and inflammatory macrophage subsets produce large amounts of reactive oxygen radicals (ROS) at the site of inflammation that can damage surrounding parenchymal cells [16].

Impact of Inflammation on HCC Development

HCC is a heterogeneous cancer at the molecular level that arises on the background of persistent liver injury, inflammation and compensatory hepatocyte proliferation, which are all characteristics of liver cirrhosis and chronic hepatitis. In induction of proinflammatory cytokines, engagement of TLRs by their respective ligands induces downstream activation of NF- κ B that upon translocation to the nucleus binds to the promoter region of target genes including proinflammatory cytokine and chemokine genes resulting in their activation. The NF- κ B-induced proinflammatory cytokine genes include TNF- α , IL-1, IL-6, IL-12 and a broad spectrum of chemokines [12]. Together, these cytokines and chemokines contribute to local inflammation in the liver as well as systemic inflammation, thereby changing the tissue milieu for hepatocyte regeneration and fibrosis. NF- κ B activation is also a major regulator of cell survival and proliferation after partial hepatectomy [6, 16].

NF- κ B seems to have a dual role in HCC development. In early stages of tumor development, the cytoprotective effect of NF- κ B is desirable because it prevents hepatocyte death. In late stages of hepatocarcinogenesis, NF- κ B supports malignant cell survival. In nonparenchymal cells in all stages, NF- κ B activation leads to persistent activation of the inflammatory cascade and other NF- κ B-dependent genes that promote cancer cell survival such as HIF and VEGF [3, 8, 16]. In addition to its proinflammatory action, TNF- α is a cytotoxic cytokine that can induce death of activated hepatocytes and other target cells. However, NF- κ B activation protects hepatocytes from TNF- α -induced apoptosis [17].

NF-KB Activation at the Crossroads of Inflammation and Cancer in the Liver

The classical inflammatory antiapoptotic inhibitor of κ B kinase (IKKb) and NF- κ B signaling pathway plays a crucial role in liver injury and inflammation, and its autonomous activation has been shown in various tumor samples. In normal tissue, harmful signals activate the IKKb/NF- κ B pathway, which further results in inflammatory cytokine production, regeneration and antiapoptotic signaling. If this well-regulated transcription factor becomes autoactivated, it might enhance the proliferation of tumor cells, protect them from apoptosis and provide the cancerous cells with continuous inflammatory micro-environment. Multiple studies demonstrated that the contribution of NF- κ B activity to HCC is complex. For example, inhibition of NF- κ B through expression of a nondegradable variant of I κ B α blocks the development of HCC in a mouse model and enhances the apoptosis of malignant hepatocytes. Kupffer cell activation due to NF- κ B-based cytokine production by

hepatocytes will increase growth factors and IL-6 in an NF- κ B-dependent manner which in turn can expand mutated cells. Studies have shown that IL-6 is elevated in cirrhosis and HCC, and correlates with the progression rate from viral hepatitis to HCC. It has been suggested that the elevated IL-6 promotes HCC development partially via signal transducer and activator of transcription 3 (STAT3) activation. Activated STAT3 is found in approximately two thirds of HCC samples and these tumors are more aggressive [16]. Although the IKKb/NF- κ B pathway may promote tumor growth, it also has been shown that lack of IKKb results in ROS accumulation which in turn promotes hepatocyte death partially due to c-Jun N-terminal kinase activation and induces compensatory proliferation in the liver. The proliferating cells are more susceptible to mutations; therefore, this could be an explanation why in chemically induced hepatocarcinogenesis researchers have seen increased tumor formation in mice lacking IKKb [16]. Therefore both up- or downregulation of this critical inflammatory pathway may enhance carcinogenesis.

Cancer-Related Inflammation

In general, cancer-related inflammation can be divided into two types. First, inflammation during the precancerous phase, and second, inflammation present in the cancer itself. It has been well established in different tissue models that chronic inflammation contributes to increased cancer risk and the liver is no exception. Inflammatory mediators and cells contribute to the genetic and epigenetic instability of the affected cells in the tissue. In addition, cytokines and mediators from inflammatory cells such as adhesion molecules and tissue metalloproteases largely contribute to cancer cell migration and invasion locally as well as during metastasis formation [3, 6, 8, 9].

Prolonged inflammation can increase the cancer risk by accumulating genetic and epigenetic damage. Inflammation-related signaling inhibits DNA repair mechanisms via ROS production. DNA methylation, a key epigenetic damage, contributes to aberrant histone modification and microRNA expression that are well established aberrations leading to cancerous transformation [3, 6, 8, 9, 16, 18].

Inflammation-Related Immune Alterations in Cancer

In the presence of normal immunity, host cells with cancerous malformation are recognized by the immune system as ‘non-self’ and are eliminated by either direct cytotoxicity from natural killer and natural killer T cells or by adaptive immune responses involving cytotoxic T cells. Initiation of an adaptive immune response to cancerous cells, however, requires intact antigen-presenting cell function that is profoundly altered in the presence of inflammation. Dendritic cells, the main antigen-presenting cell type of the immune system, appear to be functionally ‘immature’ in the liver, which itself may contribute to a defective elimination of transformed cells in the liver microenvironment. The presence of inflammation due to inflammatory and immunomodulatory cytokines can further interfere with the pivotal function of liver dendritic cells in recognizing early cancerous cells and inducing an appropriate CD8 T cell response to aid tumor elimination [19].

Inflammation as a Target for Disease Modulation in HCC

While our understanding on the importance of inflammation in HCC development is expanding, the direct clinical applicability of these findings is yet to be understood. Identification of specific signaling molecules and pathways (such as NF- κ B) provides potential therapeutic targets for further evaluation. For example, sorafenib, a RAF/MEK/ERK inhibitor that inhibits tumorigenesis and induces tumor cell apoptosis is now in clinical use with some effectiveness in HCC. Future studies will be needed to explore evolving targets in the inflammatory pathways for intervention in HCC and related liver disease [20].

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Fig. 1.
Danger signals induce inflammation.