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Review

The tumor microenvironment in hepatocellular carcinoma: Current status and therapeutic targets

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ABSTRACT

A growing body of literature highlights the cross-talk between tumor cells and the surrounding peritumoral stroma as a key modulator of the processes of hepatocarcinogenesis, epithelial mesenchymal transition (EMT), tumor invasion and metastasis. The tumor microenvironment can be broadly classified into cellular and non-cellular components. The major cellular components include hepatic stellate cells, fibroblasts, immune, and endothelial cells. These cell types produce the non-cellular components of the tumor stroma, including extracellular matrix (ECM) proteins, proteolytic enzymes, growth factors and inflammatory cytokines. The non-cellular component of the tumor stroma modulates hepatocellular carcinoma (HCC) biology by effects on cancer signaling pathways in tumor cells and on tumor invasion and metastasis. Global gene expression profiling of HCC has revealed that the tumor microenvironment is an important component in the biologic and prognostic classification of HCC. There are substantial efforts underway to develop novel drugs targeting tumor–stromal interactions.

In this review, we discuss the current knowledge about the role of the tumor microenvironment in pathogenesis of HCC, the role of the tumor microenvironment in the classification of HCC and efforts to develop treatments targeting the tumor microenvironment.

1. Introduction

Hepatocellular carcinoma (HCC) is the seventh most common malignancy and the third leading cause of cancer-related death worldwide [1]. Despite the recent advances in diagnosis and treatment of HCC, it remains a highly lethal disease. The main cause of death in HCC patients is tumor progression with metastasis. However, the underlying mechanisms of tumor initiation, progression and metastasis are still not fully understood [2].

The majority of HCC patients have an underlying chronic liver disease; and liver cirrhosis is the main risk factor for the development of HCC [3,4]. Chronic liver injury is associated with dysregulated growth of hepatocytes and results in the formation of regenerative nodules, dysplastic nodules, and HCC. Nitta et al. demonstrated that cirrhotic liver-derived hepatocytes (CLDHs) have a cellular signaling phenotype that indicates a change from a MAPK-independent cell survival pathway to a MAPK-dependent cell survival pathway. The CLDHs have increased vimentin and type 1 collagen expression, which are markers of mesenchymal cells, and morphologic features consistent with the epithelial-mesenchymal transition (EMT), a biologic process in which epithelial cells loose their phenotypic characteristics and acquire features typical of mesenchymal cells [5–7]. EMT is essential during embryonic development, tissue repair in the adult organism and cancer progression, and it is thought to be critical as a connection point between inflammation and the progression of degenerative fibrotic diseases and cancer [8].

Recent literature has highlighted the cross-talk between tumor cells and their surrounding microenvironments as well as a fundamental role of the tumor microenvironment in the pathogenesis of HCC. The tumor microenvironment plays a critical role in modulating the process of liver fibrosis, hepatocarcinogenesis, EMT, tumor invasion and metastasis. The tumor microenvironment largely consists of (1) cells such as hepatic stellate cells, fibroblasts, immune cells – including regulatory and cytotoxic T cells and tumor-associated macrophages, and endothelial cells, (2) growth factors including transforming growth factor β1 (TGF-β1) and platelet derived growth factor (PDGF), (3) proteolytic enzymes such as matrix metalloproteinases (MMPs) and tissue inhibitor

Abbreviations: ECM, extracellular matrix; FGF, fibroblast growth factor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HSPG, heparan sulfate proteoglycan; HS, heparan sulfate; IL, interleukin; MMP, matrix metalloproteinase; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor kappa B; PDGF, platelet-derived growth factor; STAT3, signal transducer and activator of transcription 3; Sulf1, sulfatase 1; Sulf2, sulfatase 2; TGF, transforming growth factor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

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of metalloproteinases (TIMPs), (4) extracellular matrix (ECM) proteins, and (5) inflammatory cytokines. In this review, we discuss the current understanding of each component of the tumor microenvironment and their roles in the pathogenesis of HCC. In addition, we examine current treatments targeting the tumor microenvironment as well as directions for future research.

2. Cells in the tumor microenvironment

2.1. Hepatic stellate cells (HSCs)

HSCs, which were once known as lipocytes, Ito cells, or perisinusoidal cells, are the major cell type responsible for collagen synthesis in the liver [9]. Hepatic stellate cells (HSC) are activated in response to liver damage and trans-differentiate into myofibroblast-like cells when liver injury is repeated, leading to the development of hepatic fibrosis [10,11]. HSCs undergo phenotypic transformation from quiescent, non-proliferating cells to proliferating, extracellular matrix (ECM) producing cells during the process of liver injury, which involves two steps. The initial phase is represented by the up-regulation of cytoskeletal protein expression including a-SMA, and the perpetuation phase is represented by the release of a multitude of cytokines, chemokines and growths factors [12–14]. Activated HSCs produce the extensive accumulation of ECM during liver fibrosis [11,15]. Activated HSCs also infiltrate the stroma of liver tumors and localize around tumor sinusoids, fibrous septa and capsules [16].

In addition to their role in development of liver fibrosis, activated HSCs promote HCC cell proliferation. Amann et al. demonstrated that the conditioned media collected from HSCs induce proliferation and migration of HCC cells cultured in monolayers and, moreover, they showed that in a 3-dimensional spheroid co-culture system, HSCs promote HCC growth and diminish the extent of central necrosis through the activation of NF kappa B and extracellular-regulated kinase (ERK) pathways [4]. Consistent with these findings, simultaneous *in vivo* implantation of HSCs and HCC cells into nude mice promoted tumor growth and invasiveness, and inhibited necrosis. PDGF, TGF-β1, MMP-9, JNK, insulin-like growth factor binding protein 5, cathepsins B and D, hepatitis B virus X protein, and HCV nonstructural proteins are all potent inducers of stellate cell activation, proliferation and collagen production, and therefore enhance liver fibrosis and hepatocarcinogenesis [5,11,17–23]. In contrast, adiponectin suppresses hepatic stellate cell activation and angiogenesis [24] (Fig. 1).

2.2. Cancer-associated fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) are the most prominent cell type within the tumor stroma of many cancers (most notably breast and pancreatic carcinoma) and play a critical role in tumor–stromal interactions [25–27]. They are activated by TGF-β and are responsible for the synthesis, deposition and remodeling of excessive ECM, such as various types of collagen. CAFs modulate the biological activities of HCC. Mazzocca and coworkers showed that HCC cell growth, intravasation and metastatic spread are dependent upon the presence of CAFs and HCC cells reciprocally stimulate proliferation of CAFs, suggesting a key role for CAFs in tumor–stromal interaction [28]. CAFs from different tumor types express several growth factors, including hepatocyte growth factor (HGF), members of the epidermal growth factor (EGF), fibroblast growth factor (FGF) and Wnt families, and cytokines, such as stromal-derived factor (SDF)-1α and IL-6 [27,29].

2.3. Lymphocytes and Kupffer cells

The immune response in the tumor and tumor microenvironment is an important regulator of progression in many cancers, including HCC. Fu et al. showed that CD4(+)CD25(+) regulatory T cells were more predominant than CD8+ T cells in HCC tissues compared with adjacent benign tissue. They also demonstrated that CD4(+)CD25(+) regulatory T cells impair cytotoxic CD8+ T cell proliferation, activation, degranulation, and production of granzyme A, granzyme B, and perforin. In line with these findings, several studies found that low intratumoral CD8+ T cell and high regulatory T cell numbers are associated with a worse prognosis in HCC patients [30,31]. In addition, dysfunctional regulation of the

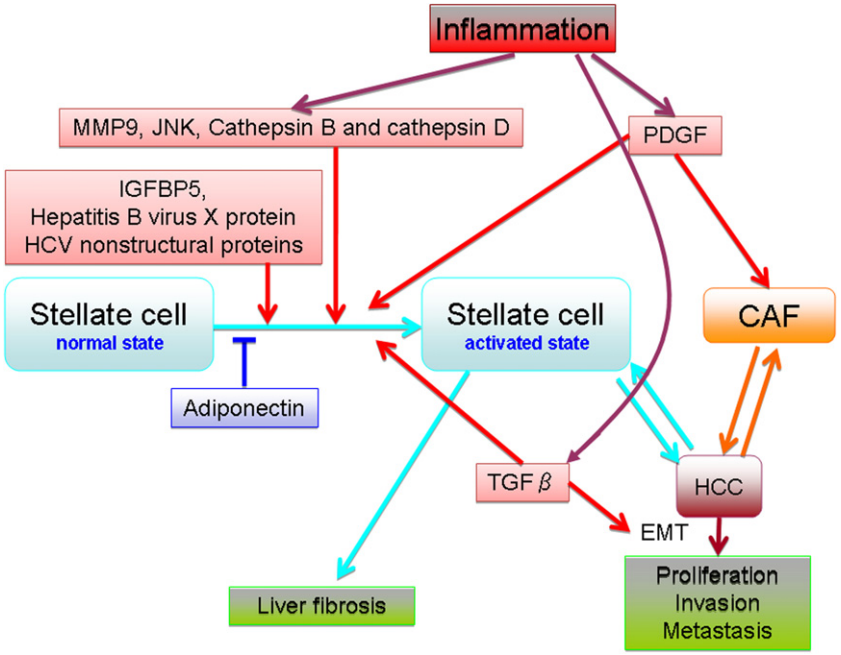

Fig. 1. Regulators of stellate cell activation and their roles in liver fibrosis and carcinogenesis. Stellate cells and CAF activated by several factors induce proliferation, invasion and metastasis in hepatocellular carcinoma. TGF-β plays a critical role in liver fibrosis and tumorigenesis through the epithelial-mesenchymal transition.