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# The Role of the Tumor Microenvironment in the Development and Progression of Hepatocellular Carcinoma

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Doi: <http://dx.doi.org/10.15586/hepatocellularcarcinoma.2019.ch2>

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**Abstract:** There is growing evidence that supports the role of the tumor microenvironment in the development and progression of hepatocellular carcinoma. The tumor microenvironment is composed of cellular components, bioactive substances, and extracellular matrix comprising of proteins such as collagens, proteoglycans,

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In: *Hepatocellular Carcinoma*. Janina E.E. Tirnitz-Parker (Editor), Codon Publications, Brisbane, Australia. ISBN: 978-0-9944381-8-8. 2019; Doi: <http://dx.doi.org/10.15586/hepatocellularcarcinoma.2019>

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and the linear glycosaminoglycan hyaluronan. Hepatocellular carcinoma generally arises from fibrotic or cirrhotic liver, characterized by alteration in extracellular matrix components. In addition, non-tumoral cells such as mesenchymal stem/stromal cells (MSCs) are typically recruited to the injured or hypoxic area within the tumor. Besides the secretion of immunoregulatory proteins, growth factors, and cytokines, MSCs and hepatic stellate cells can also synthesize hyaluronan, amongst other components, which affects several tumor-associated processes. The tumor microenvironment also contains different types of immune cells. A key component in the genesis of hepatocellular carcinoma is the macrophages, as tumor-associated macrophages (TAM). This chapter provides an overview of the interaction of MSCs-hyaluronan-TAMs and tumor cells, and how this interaction potentially contributes to the development and progression of hepatocellular carcinoma.

**Keywords:** hepatocellular carcinoma; hyaluronic acid; macrophages; mesenchymal stem cells; tumor microenvironment

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## INTRODUCTION

The biology of a tumor can only be understood by studying different cell types within the tumor microenvironment (TME) (1). The interaction between tumor cells and the associated stroma plays a crucial role in the initiation and progression of a tumor (2). The heterogeneity of tumors is based not only on the genomic profile but also on their microenvironment composition (2). The microenvironment actively regulates tumor initiation, its progression, metastasis, and therapy response (3). The extracellular matrix (ECM), as part of the TME, is essential for asymmetric cell division and maintenance of tissue polarity; it may block or facilitate cell migration, determine the direction of cell–cell communication, and bind to growth factors to prevent their free diffusion (4). Changes in ECM support the development of hepatocellular carcinoma (HCC), and the complexity of TME and therapeutic failures may be explained, in part, by alterations of components of the ECM. The development of HCC is associated with prolonged inflammation caused by chronic virus infection, alcoholic exposure, or metabolic diseases. The inflammatory microenvironment facilitates the transformation of normal liver cells such as hepatocytes, stem, immune, and stellate cells by providing a suitable environment for the development and progression of a tumor (5, 6). HCC is a primary liver tumor that derives, in most cases, from hepatocytes and corresponds to approximately 90% of all liver cancers (7, 8). Since cholangiocarcinoma, hepatoblastoma, and angiosarcoma are less common than HCC, they are not discussed in this chapter.

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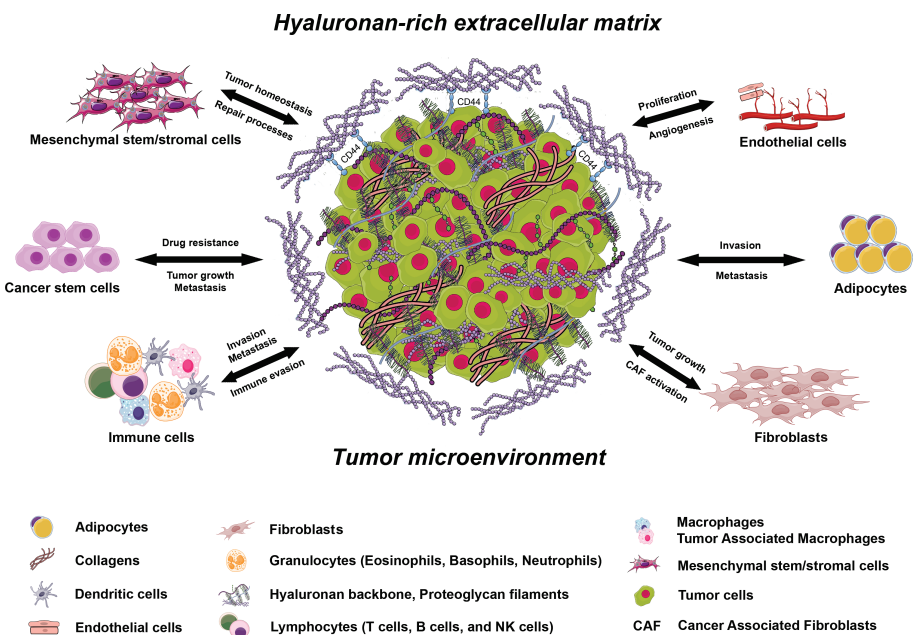
## THE TUMOR MICROENVIRONMENT

The TME is composed of non-cellular and cellular components (4). The ECM is the non-cellular component. The cellular component, apart from tumor cells, consists of a variety of cells including tumor-associated fibroblasts (TAFs),

angiogenic endothelial cells, bone marrow-derived cells, adipocytes, and cells of the immune system (Figure 1) (9). In HCC, hepatic stellate cells (HSCs) are also part of this cellular microenvironment (10). The bidirectional interaction between the tumor and its microenvironment greatly affects tumor initiation, progression, and drug resistance, and a better understanding of this interaction may enable the identification of novel targets for tumor therapy (11, 12).

## Non-cellular compartment

During embryonic development and organ homeostasis, the composition of ECM is tightly regulated. However, in diseases such as cancer, it is usually deregulated



**Figure 1** Schematic representation of the role of a HA-rich microenvironment in cancer progression. The TME is composed of non-tumor cells, such as fibroblasts, endothelial cells, MSCs, adipocytes, and infiltrating immune cells, and of non-cellular compartments, including secreted soluble factors and solid-state structural ECM. HA is an abundant component of the ECM that recruits and activates stromal cells to stimulate cell proliferation, migration, differentiation, angiogenesis, immune effects, and therapy resistance. HA induces intracellular signals through several receptors, mainly CD44, whose expression is associated with the characteristics of CSCs. Accumulation of HA in the tumor stroma drives the differentiation and activation of CAFs. CSCs are described as tumor initiators and are associated with tumor proliferation, drug resistance, and metastasis, whereas some cells such as MSCs can be integrated into the TME after recruitment and interact with tumor cells to promote tissue homeostasis and repair processes. The TME contains several types of immune cells including macrophages, neutrophils, dendritic cells, granulocytes, and lymphocytes. TAMs usually have a pro-tumoral action since they can promote tumor neovascularization and have an immunosuppressive action. CAF, cancer-associated fibroblasts; CSC, cancer stem cells; ECM, extracellular matrix; HA, hyaluronan; MSC, mesenchymal stem/stromal cells; TAM, tumor-associated macrophages; TME, tumor microenvironment.

and disorganized. Abnormal ECM alters the behavior of stromal cells and, as a consequence, supports and leads the generation of the TME (4). One of the components of ECM that is altered in tumors is the glycosaminoglycan hyaluronic acid (HA). HA is a linear molecule composed of disaccharide units of N-acetyl glucosamine and glucuronic acid; it is synthesized by hyaluronan synthases and degraded by hyaluronidases and glycosylphosphatidylinositol (GPI)-anchored hyaluronidase PH-20 (13, 14). Activities of these enzymes are shown to greatly influence tumor growth and metastasis (15). HA is overexpressed in both cirrhotic and liver tumor tissues, promoting tumor progression (16, 17). Several pieces of evidence indicate that HA inhibition by 4-methylumbelliferone (4-MU), a specific HA synthesis inhibitor, delays HCC growth (18, 19). Besides, the use of recombinant hyaluronidase as an adjuvant therapy in different types of cancer shows the complex relationship between hyaluronan synthases and hyaluronidases in maintaining HA expression (20). HA is an abundant component of the ECM that mediates cell proliferation, migration, and differentiation during inflammation and tumor development. Most malignant tumor tissues contain elevated levels of HA compared to their normal counterparts (21). Remarkably, HA levels rise in the serum of patients with liver injury, and it is proposed as a biomarker for high-score fibrosis and cirrhosis (16). HA is a ubiquitous molecule with high concentrations found in the synovial fluid, vitreous humor, skin, and umbilical cord. At homeostasis, HA is mostly present in a high molecular weight form, ranging from  $0.5 \times 10^6$  to  $10^7$  Da, and to a lesser extent in a low molecular weight form, ranging from  $10^4$  to  $0.5 \times 10^6$  Da. The low molecular weight form is mostly present in pathological conditions such as inflammation and cancer (22, 23). HA acts by inducing intracellular signals through several receptors: toll-like receptor 4, lymphatic vessel endothelial hyaluronan receptor 1, and receptor for hyaluronan-mediated motility (24, 25). The main receptor, CD44, is also considered a marker of cancer stem cells (CSC). It is encoded by the *CD44* gene, which is a large and highly conserved gene (20 exons, out of which 10 can undergo alternative splicing). It has been demonstrated that the interaction between HA and CD44 promotes tumor progression in different solid tumors, including HCC (14, 26).

Proteoglycans (PGs) are composed of at least one linear negatively charged polysaccharide chain, such as heparan sulfate, chondroitin sulfate, keratan/dermatan sulfate or heparin, that is covalently attached to a core protein (27). In healthy tissues, PGs are essential for structural scaffolding in the ECM, interactions with cytokines and growth factors and their receptors, and inducing cell signaling (28). During carcinogenesis, the expression of PG is markedly altered to promote cancer cell growth, survival, adhesion, migration, and angiogenesis (28).

## Cellular components

Several types of cells belonging to the TME have been described as key regulators of different aspects of the tumor process. CSCs are described as tumor initiators and are associated with tumor growth, drug resistance, and metastasis (29). HSCs are key cells in responding to the inflammatory state in the liver and are the principal cells that promote ECM remodeling (30), whereas MSCs can be attracted into the TME and, after recruitment, can interact with tumor cells to promote tumor modifications (12, 31). CSCs have a constant interaction with their specific

microenvironments called niches. CSC niches are formed by different cellular components and regulated by secreted factors such as cytokines and growth factors (12). CSCs exhibit the capacity for self-renewal, pluripotency, tumorigenicity, and resistance to therapy. Many cancer therapies eliminate most of the tumor cells but ultimately fail because they do not eliminate CSCs fully, which survive to regenerate new tumors. CSCs possess several intrinsic mechanisms of resistance to current chemotherapeutic drugs (1, 32, 33). They have a high-level expression of ATP-binding cassette (ABC) transporters, which are correlated with multidrug resistance. ABC transporters reduce the cellular accumulation of various types of therapeutic agents, and therefore, CSCs become more resistant to even higher doses of anti-tumor agents (34).

MSCs represent a heterogeneous population of multipotent progenitors first described in bone marrow but present in almost all vascularized organs. Due to their high plasticity, they show various functions according to the requirements of that particular tissue. These include, among others, homing to sites of tissue damage, the initiation of repair processes, and the regulation of tissue homeostasis. Tumor growth usually induces tissue remodeling, creating an inflammatory environment. Consequently, MSCs can be recruited to these tumor sites and activated to have repair and immunomodulation functions. Several factors such as interleukin (IL)-8, monocyte chemoattractant protein-1, growth-regulated oncogene, and autocrine motility factor, produced by the HCC, are known to attract and recruit MSCs (35, 36). It is known that MSCs can secrete several growth factors, cytokines, chemokines, and ECM components (37). Once within the tumor, direct and indirect interactions between MSCs, the ECM and cancer cells increase plasticity within the tumor tissue and its microenvironment.

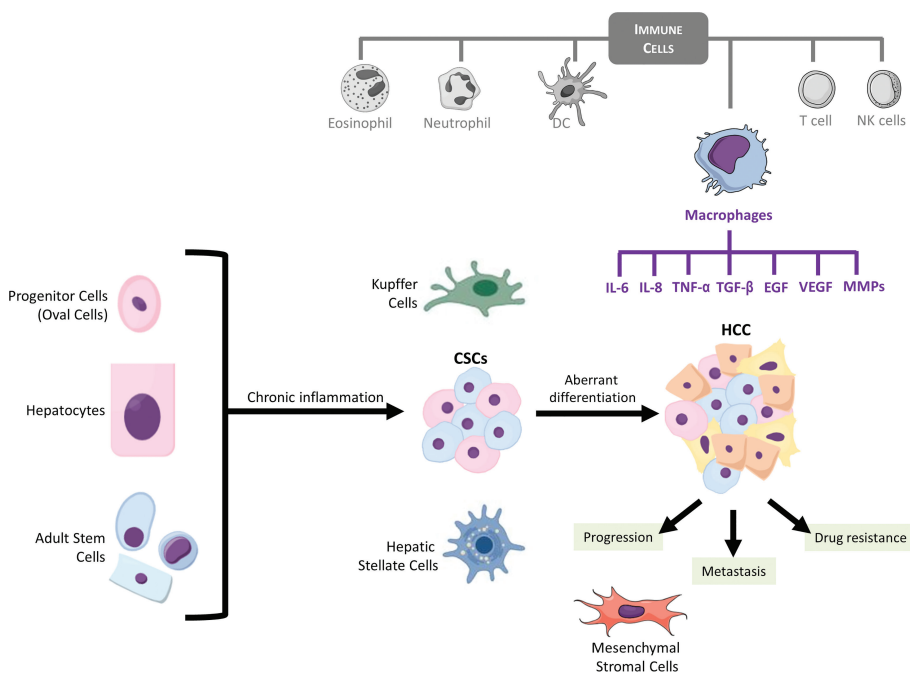
The TME also contains several types of immune cells such as macrophages, neutrophils, dendritic cells, T cells, regulatory T cells (Tregs), natural killer (NK) cells, and eosinophils (37). Studies have shown that changes in the number and function of these immune cells contribute to the development, tolerance, and progression of HCC (38–43). Macrophages are the major component of the immune infiltrate that is present in tumors (44, 45). Several studies indicate that tumor-associated macrophages (TAMs) usually have a pro-tumoral action, since they can stimulate angiogenesis, increase tumor cell invasion and motility, and have an immunosuppressive action (44, 45). In the case of HCC, TAMs, as infiltrated monocytes and resident Kupffer cells, are characterized as the most important immune cell type that promotes tumor invasion and metastasis (37).

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## THE TUMOR MICROENVIRONMENT IN HCC DEVELOPMENT

Hepatocarcinogenesis is a multifactorial process. Most HCC cases are associated with alcohol abuse, nonalcoholic steatohepatitis (NASH), and chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) inducing an inflammatory process followed by regeneration. Persistent hepatic injury and concurrent regeneration could produce an environment that eventually leads to the formation of hypoxia and inflammation, which are crucial features of HCC microenvironment (5, 6, 46). HCC has a heterogeneous population of CSC, which are considered to be tumor-initiating cells. It has been reported that 28–50% of HCC cells express

progenitor cell markers (47). Many potential origins of hepatic CSCs have been described. They may result from genetic and epigenetic modifications of hepatocytes, hepatic oval cells/liver progenitor cells (LPCs), or circulating bone marrow cells. These transformed cells, in combination with deregulated microenvironment, result in a distinct lineage of CSCs that have stem-like features (Figure 2). Some cell surface markers for CSCs include CD44, CD133, CD90, CD105, CD45, CD13, and epithelial cell adhesion molecule (EpCAM) (5). CSCs have a very complex signaling network that includes crosstalk with different non-tumoral cells. During tumor development, multiple immunosuppressive molecules are released from cancer cells, which subsequently contribute to the establishment of an immunosuppressive TME (5, 48). LPCs are small cells (7–10  $\mu\text{m}$  in diameter) with basophilic character. They have small ovoid nucleus and a high nuclear-cytoplasmic ratio. LPCs are heterogeneous, hardly detectable in healthy liver, but



**Figure 2** Stem and immune cells associated with tumor development. HCC is composed of a heterogeneous population of CSCs, which might derive from hepatocytes, progenitor cells (oval cells), or other adult stem cells, like bone marrow cells. CSCs have a very complex signaling network that includes crosstalk with different non-tumor cells, such as immune cells. The tumor microenvironment contains several types of non-tumor cells: macrophages, Kupffer cells, stellate cells, dendritic cells, T cells, Tregs, and NK cells. Changes in the number and function of these cells contribute to the development of immune tolerance and progression of HCC. Tumor-associated macrophages are characterized as the most important immune cell type that promotes tumor invasion and metastasis. Similar to cancer cells, macrophages such as Kupffer cells secrete several types of cytokines and factors crucial for HCC progression, metastasis, and drug resistance. CSC, cancer stem cells; EGF, epidermal growth factor; HCC, hepatocellular carcinoma; IL-6, interleukin 6; IL-8, interleukin 8; MMPs, matrix metalloproteinases; MSC, mesenchymal stem/stromal cells; NK, natural killer; TGF- $\beta$ , transforming growth factor- $\beta$ ; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor.

are activated in chronic liver injury. The origin of these cells is still debatable. The inhibition of LPCs correlates with reduced tumor development, and their activation and proliferation are linked to HCC development. In addition, they have been implicated in hepatocyte regeneration (49, 50).

The role of MSCs in tumor initiation is still controversial, particularly in HCC. In vitro evidence indicates that during MSC differentiation into hepatocytes, aberrant activation of Wnt/ $\beta$ -catenin is associated with a tumoral phenotype, involving increased proliferation, elevated proliferating cell nuclear antigen expression, cell cycle alteration, and spheroids formation (51). Another report suggests that MSCs may initiate HCC. The HCC cell line SK Hep-1 has been shown to display MSCs-like features and the capacity to differentiate into osteogenic and adipogenic lineages (52). Although these in vitro data indicate the potential role of MSCs in hepatocarcinogenesis, in vivo evidence to clarify this potential process is lacking.

Chronic inflammation is a risk factor for the development of tumors (53). HCC frequently arises in chronically inflamed liver. Sustained inflammation is characterized by a continuous activation of immune cells that release free radicals that can damage the DNA and cause a neoplastic transformation. The TAMs derived from Kupffer cells or circulating monocytes are recruited into the tumor tissues by chemokines and other factors secreted by tumor cells and the inflammatory cells present in the TME (37). TAM-derived cytokines and growth factors play a key role in the initiation of HCC. One of the most important TAM-derived cytokines is IL-6, which triggers pathways that promote proliferation and survival of hepatocytes, stimulating the initiation and development of HCC. It has been reported that IL-6<sup>-/-</sup> mice had lower incidence of HCC tumors and longer survival than wild-type mice (54).

The changes in ECM and its components allow the tumoral transformation of hepatocytes. It has been observed that patients with liver fibrosis and advanced cirrhosis present high levels of HA in serum (16). In an experimental model that mimics liver injury or fibrosis (18), HA was detected in injured/fibrotic liver but not in normal tissues. HA is synthesized by the synovial lining cells, HSCs, and MSCs during wound healing of the liver (16). HA is also associated with the stem cell niche. The ECM of this microenvironment is composed of HA among other components such as laminin, collagen, sulfated chondroitin-sulfate, and heparin-sulfate proteoglycans that maintain stemness (55). Liver injury induces the expression of HA; during the chronic process, HA elevation is continuous, allowing the interaction with the potential cancer stem cell marker CD44, which actively promotes tumor initiation (56). Lee et al. showed that HA-based multilayer films mimicked the stem cell niche and selected and enriched for liver CSCs (57). Besides, HA could be involved in HCC initiation, given its association with IL-6 expression. Particularly in cirrhotic liver, IL-6 is highly produced by Kupffer cells, and together with other inflammatory mediators, IL-6 has the ability to induce HSC trans-differentiation to myofibroblasts (58, 59). Moreover, IL-6 is essential for the expansion of mutated hepatocytes (60). It has been reported that IL-6 binds selectively to HA, suggesting that this retention and concentration near the site of secretion favor its paracrine and autocrine activities, contributing to tumor development. In addition, the inhibition of HA by 4-MU decreases IL-6 production in TME significantly, reducing tumor growth (18, 61). Recently, in a model of HBV-transgenic mice, the inhibition of HA by 4-MU was accompanied by a

reduction of CSC markers CD44, CD133, CD90, and EpCAM during hepatocarcinogenesis (62).

Other key players in cancer pathogenesis are PGs. Tumoral tissues have differential PG expression patterns, which are closely associated with their differentiation and biological behavior. Furthermore, during liver carcinogenesis, HSCs become activated; they proliferate and synthesize excess ECM proteins in most types of chronic liver diseases (63). Decorin is a member of the small leucine-rich proteoglycan (SLRP) gene family, containing a single chondroitin sulfate (CS) or dermatan sulfate chain, and is expressed by fibroblast and myofibroblasts (64). Syndecan molecules (syndecan-1, syndecan-2, syndecan-3, syndecan-4) are a major family of cell-surface heparin sulfate (HS) PGs. They mainly bear HS chains, although some members can be additionally substituted with CS chains (65, 66). In healthy liver, decorin levels are generally low. However, an increased decorin expression was observed in the connective tissue septa during fibrogenesis and in chronic liver injury (67). In this process, decorin colocalizes with high amounts of transforming growth factor beta 1 (TGF- $\beta$ 1), which is a key stimulator of fibrogenesis (68). In normal human liver, syndecan-1 is expressed in sinusoidal endothelial cells (69). As cirrhosis progresses, syndecan-1 expression is increased, and its localization extended to the entire hepatocyte membrane surface and expressed on the surface of biliary epithelial cells (70). Elevated syndecan-1 expression appears to be more closely associated with liver cirrhosis, rather than malignant transformation (65).

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## THE TUMOR MICROENVIRONMENT IN HCC PROGRESSION AND METASTASIS

HCC is known to harbor different populations of cancer cells with stem cell properties, which can be identified by different cell surface markers, such as EpCAM, CD44, CD90, and CD133. Some studies have shown that EpCAM<sup>+</sup> and CD90<sup>+</sup> cells are two independent subpopulations. EpCAM<sup>+</sup> cells have hepatic epithelial stem cell features and are associated with a high tumorigenic capacity, while CD90<sup>+</sup> cells have mesenchymal-vascular endothelial cell features and metastatic propensity. On the other hand, it has been shown in HCC cell lines that express CD133 participate in cell survival through the regulation of glucose uptake and autophagy. These studies suggest that CD133<sup>+</sup> CSCs could use autophagy to escape the selective pressure of nutrient deficiency and the hypoxic environment in HCC (71–73). CSCs originating from LPCs were found to have differential expression of a number of microRNAs (miRNAs). These miRNAs were mostly implicated in angiogenesis, post-transcriptional protein modification, and small molecule metabolism. Differential expression of miRNAs demonstrates crucial roles of LPCs during the progression of HCC (71, 73). Several signaling pathways, including Wnt/ $\beta$ -catenin, BMI-1, TGF- $\beta$ , Notch, and Hedgehog, are known to be stem cell regulators and to accelerate tumorigenesis. These, as well as some additional factors such as EpCAM, Lin28, or miR-181, interact with CSCs and enhance the progression of HCC (6, 71, 72). On the other hand, CSCs also benefit from other processes such as angiogenesis. In fact,



HCC is one of the most vascularized solid tumors with particular vascular anomalies (48, 72).

Once a tumor is established, MSCs can be recruited from a distant place of the same organ or peripheral tissues (e.g., bone marrow) into the TME. Studying the function of the recruited MSCs on the tumor development has been of great interest during the past decade. Studies that co-injected mice with exogenous MSCs (isolated from bone marrow, adipose tissue, or umbilical cord from healthy donors) and tumor cells produced equivocal results. While some reports indicated that MSCs promoted tumor development, others demonstrated that MSCs were able to inhibit tumor growth (74). The discrepancies of results could be related to several factors including the tumor type, the heterogeneity in MSC (source, donor age, culture conditions), and the timing at which MSCs are introduced into the TME. These discrepancies remain true for HCC as well. The first reports indicated that MSCs inhibited HCC growth *in vitro* and *in vivo* (75, 76). However, other results demonstrated either a pro-tumorigenic effect (77, 78) or a null effect of MSCs on HCC growth (35, 36, 79–82). The inhibition of tumor growth was associated with Wnt, NF- $\kappa$ B, and PI3-K/Akt signaling pathways (75, 83), whereas enhancement of microvessel density was observed in the case of tumor progression (77, 78). Not only MSCs but also their secretome affect HCC development. Conditioned medium from human fetal MSCs expressed insulin growth factor binding proteins that could bind to insulin-like growth factors (IGFs). This leads to reduced IGF-1R and PI3K/Akt activation and induces cell cycle arrest (84). Extracellular vesicles derived from human bone marrow-derived MSCs have also been demonstrated to inhibit HCC growth *in vitro* and *in vivo* (85, 86).

The role of MSCs in tumor metastasis has also been studied. Li et al. demonstrated in a subcutaneous model of HCC that MSC-treated mice exhibited larger tumors but a decreased number of lung metastases. This effect seemed to be related to TGF- $\beta$ 1 downregulation (87). Moreover, repeated inoculation of MSCs in a mouse model of high metastatic HCC resulted in an inhibitory effect on HCC growth at 3 weeks after MSC engraftment and downregulation of metastasis-related factors (88). It was also described that MSCs exposed to an inflammatory microenvironment promoted HCC metastasis through TGF- $\beta$ -induced epithelial-mesenchymal transition (EMT) in tumor cells (89). Efforts have been made to isolate and characterize MSCs from HCC tumors. Yan et al. isolated MSCs from human HCC tissues and demonstrated that the co-culture of these MSCs with HCC cells enhanced tumor formation and increased liver and lung metastasis. Tumor-associated MSCs produced several trophic factors including S100A4 that upregulated miR-155, leading to HCC proliferation and invasion (90). Similar data from Hernanda et al. indicated that conditioned medium from MSCs isolated from HCC tissues had trophic effects on the Huh7 hepatoma cell line *in vitro* and *in vivo* (91). It was also demonstrated that HCC-associated MSCs promoted EMT and liver tumorigenesis through the expression of a lncRNA-MUF (MSC-upregulated factor) in HCC tissue (92). These data suggest that MSCs can be educated by the tumor to favor its own growth. However, due to the heterogeneity of MSCs, and therefore the difficulty to investigate the endogenous MSCs, more studies are necessary to establish the precise role of these cells on tumor development.

The persistent inflammatory milieu not only promotes tumor development but also accelerates tumor progression, stimulates the formation of new blood

vessels, and remodels the ECM. Thus, TAMs are also considered as crucial players in tumor progression. In HCC, TAMs stimulate invasion, angiogenesis, and metastasis through the release of several mediators, including IL-6, IL-8, TNF $\alpha$ , TGF $\beta$ , EGF, VEGF, MMP-2, and MMP-9(93). These factors also promote EMT, which is a crucial event for tumor progression and metastasis (18, 22, 23, 37). In addition, infiltrating monocytes in HCC express high levels of programmed cell death-ligand 1 (PD-L1) that binds to PD-1 on CD8<sup>+</sup> T cells, suppressing its anti-tumoral cytotoxic activity (94).

The interaction of HA with its main receptor, CD44, promotes tumoral signaling involved in cell proliferation, invasion, chemoresistance, EMT, and angiogenesis (23). Hepatic HA accumulation may be linked to increased tumor tissue stiffness (95), which is associated with HCC development. HA was demonstrated to facilitate the aggressive phenotype of HCC cell lines, promoting cell proliferation, metastatic potential, and aerobic glycolysis switch in MHCC97H and HepG2 cells, both in vitro and in vivo (96).

PGs can regulate the bioavailability and activity of hormones, growth factors, cytokines, and their respective receptors which in turn can affect gene expression, tumor phenotype, tumor progression, and recurrence rates in specific tumor types (97). During angiogenesis, decorin induces endothelial cell sprouting and activates intracellular signal transduction pathways. Decorin interacts with several angiogenic growth factors, including VEGF, platelet-derived growth factor, fibroblast growth factor, IGF, connective tissue growth factor, and hepatocyte growth factor (98). In addition, decorin interacts with TGF- $\beta$  and neutralizes its activity, preventing the binding to its receptor, and therefore plays a significant role in tumor progression and angiogenesis (67). Decorin can also play a pro-angiogenic role by facilitating endothelial cell adhesion and migration on type I collagen (99).

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## TARGETING THE MICROENVIRONMENT TO INHIBIT TUMOR GROWTH

TAM-targeted therapies are usually aimed at: (i) eliminating TAMs, (ii) blocking the recruitment of circulating monocytes, and/or (iii) reprogramming TAMs to an anti-tumor phenotype. For example, it was reported that in mouse models of HCC, treatment with the tyrosine kinase inhibitor sorafenib reprogrammed TAMs and promoted the stimulatory activity of hepatic NK cells (100). Zoledronic acid was demonstrated to have an anti-tumor effect by targeting TAMs through phagocytosis by macrophages and induction of apoptosis (101). The therapy combining these two drugs, sorafenib and zoledronic acid, is currently being evaluated for the treatment of advanced HCC in phase II clinical trials (NCT01259193). Another strategy for targeting TAMs is inhibition of glypican-3, a proteoglycan that promotes the recruitment of macrophages into the tumor, by specific antibody (102). This strategy is currently in phase I clinical trials for advanced HCC (103). In addition, there are two more trials (NCT02723942 and NCT02395250) that use a similar strategy. So far, the most critical issue that TAM-targeted therapies need to overcome is the need to repolarize macrophages towards an anti-tumor behavior without causing any adverse events.

The abnormal metabolism of HA and its accumulation in the injured liver or an established tumor have led to the consideration that inhibition of HA synthesis may avoid tumor progression and metastasis. Several reports propose the use of 4-MU as an inhibitor of HA synthesis or the targeting of its receptor CD44 as anticancer treatments. The use of CD44 antisense oligonucleotide increased chemosensitivity to doxorubicin significantly and induced apoptosis and necrosis in HCC cell lines (104). The treatment of HCC cells with 4-MU significantly reduced tumor cell proliferation and induced apoptosis, without affecting normal hepatocytes. Systemic treatment with 4-MU resulted in the induction of necrosis and reduction in the number of tumor satellites in an orthotopic fibrosis/HCC mouse model. Mice treated with 4-MU had reduced levels of fibrosis and decreased the number of activated HSCs when compared with controls (18). This antitumor property could be associated with an inhibition of angiogenesis and decrease in IL-6 production (19). Furthermore, animal survival was increased when CD133<sup>low</sup> HCC cells, generated upon 4-MU treatment, were injected in a metastatic HCC model (105).

There is clear evidence that PG composition changes with liver cancer development. Thus, it could constitute targets for potential therapeutic agents and diagnostic biomarkers. Decorin represents a powerful tumor cell growth and migration inhibitor by modulating both tumor stroma deposition and cell signaling pathways (106). Soluble decorin acts as a tumor suppressor mainly by downregulating various receptor tyrosine kinases (such as EGFR, Met, IGFR, and VEGFR),  $\beta$ -catenin, and Myc expression, and upregulating p21WAF1/CIP1 (106, 107).

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## CONCLUSION

The HCC microenvironment is composed of several tumoral and non-tumoral cell types, and ECM components that are in continuous communication and interaction with each other. The cellular components include CSCs, LPCs, MSCs, and various populations of immune cells including TAMs. The major ECM components that are altered in HCC are GAGs such as hyaluronan, and PGs including decorin and syndecan. Their interactions make an important contribution to tumor progression by modulating tumor cell properties. The data generated in preclinical models and clinical trials targeting the TME, especially these molecules and cell types, show highly promising results; however, their clinical utility is yet to be ascertained. In addition, adverse events of such therapies need to be cautiously evaluated. A better knowledge of the microenvironment–tumor cell interactions could be useful and beneficial for the development of new therapeutic approaches for HCC.

**Conflict of Interest:** The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this manuscript.

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## REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell*. 2011;144(5):646–74. <http://dx.doi.org/10.1016/j.cell.2011.02.013>
2. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med*. 2013;19(11):1423–37. <http://dx.doi.org/10.1038/nm.3394>
3. Klemm F, Joyce JA. Microenvironmental regulation of therapeutic response in cancer. *Trends Cell Biol*. 2015;25(4):198–213. <http://dx.doi.org/10.1038/nm.3394>
4. Lu P, Weaver VM, Werb Z. The extracellular matrix: A dynamic niche in cancer progression. *J Cell Biol*. 2012;196(4):395–406. <http://dx.doi.org/10.1083/jcb.201102147>
5. Wang K, Sun D. Cancer stem cells of hepatocellular carcinoma. *Oncotarget*. 2018;9:23306–14. <http://dx.doi.org/10.18632/oncotarget.24623>
6. Nishida N, Kudo M. Oncogenic signal and tumor microenvironment in hepatocellular carcinoma. *Oncology*. 2017;93(Suppl 1):160–4. <http://dx.doi.org/10.1159/000481246>
7. European-Association-for-the-Study-of-the-Liver. EASL clinical practice guidelines: Management of hepatocellular carcinoma. *J Hepatol*. 2018;69(1):182–236.
8. Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol*. 2018;15(10):599–616. <http://dx.doi.org/10.1038/s41571-018-0073-4>
9. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. *J Cell Sci*. 2012;125(Pt 23):5591–6. <http://dx.doi.org/10.1242/jcs.116392>
10. Hernandez-Gea V, Toffanin S, Friedman SL, Llovet JM. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology*. 2013;144(3):512–27. <http://dx.doi.org/10.1053/j.gastro.2013.01.002>
11. Catalano V, Turdo A, Di Franco S, Dieli F, Todaro M, Stassi G. Tumor and its microenvironment: A synergistic interplay. *Semin Cancer Biol*. 2013;23(6 Pt B):522–32. <http://dx.doi.org/10.1016/j.semcancer.2013.08.007>
12. Skvortsov S, Skvortsova I, Tang DG, Dubrovskaya A. Prostate cancer stem cells: Current understanding. *Stem Cells*. 2018;36:1457–1474. <http://dx.doi.org/10.1002/stem.2859>
13. Lokeshwar VB, Mirza S, Jordan A. Targeting hyaluronic acid family for cancer chemoprevention and therapy. *Adv Cancer Res*. 2014;123:35–65. <http://dx.doi.org/10.1016/B978-0-12-800092-2.00002-2>
14. Toole BP. Hyaluronan: From extracellular glue to pericellular cue. *Nat Rev Cancer*. 2004;4(7):528–39. <http://dx.doi.org/10.1038/nrc1391>
15. Toole BP. Hyaluronan-Cd44 interactions in cancer: Paradoxes and possibilities. *Clin Cancer Res*. 2009;15(24):7462–8. <http://dx.doi.org/10.1158/1078-0432.CCR-09-0479>
16. Rostami S, Parsian H. Hyaluronic acid: From biochemical characteristics to its clinical translation in assessment of liver fibrosis. *Hepat Mon*. 2013;13(12):e13787. <http://dx.doi.org/10.5812/hepatmon.13787>
17. Mustonen AM, Salven A, Karja V, Rilla K, Matilainen J, Nieminen P. Hyaluronan histochemistry—a potential new tool to assess the progress of liver disease from simple steatosis to hepatocellular carcinoma. *Glycobiology*. 2019;29(4):298–306. <http://dx.doi.org/10.1093/glycob/cwz002>
18. Piccioni F, Malvicini M, Garcia MG, Rodriguez A, Atorrasagasti C, Kippes N, et al. Antitumor effects of hyaluronic acid inhibitor 4-methylumbelliferone in an orthotopic hepatocellular carcinoma model in mice. *Glycobiology*. 2012;22(3):400–10. <http://dx.doi.org/10.1093/glycob/cwr158>
19. Piccioni F, Fiore E, Bayo J, Atorrasagasti C, Peixoto E, Rizzo M, et al. 4-Methylumbelliferone inhibits hepatocellular carcinoma growths by decreasing Il-6 production and angiogenesis. *Glycobiology*. 2015;25(8):825–35. <http://dx.doi.org/10.1093/glycob/cwv023>
20. Khan N, Niazi ZR, Rehman FU, Akhtar A, Khan MM, Khan S, et al. Hyaluronidases: A therapeutic enzyme. *Protein Pept Lett*. 2018;25(7):663–76. <http://dx.doi.org/10.2174/0929866525666180629121823>
21. Boregowda RK, Appaiah HN, Siddaiah M, Kumarswamy SB, Sunila S, Thimmaiah KN, et al. Expression of hyaluronan in human tumor progression. *J Carcinog*. 2006;5:2. <http://dx.doi.org/10.1186/1477-3163-5-2>
22. Alaniz L, Garcia M, Rizzo M, Piccioni F, Mazzolini G. Altered hyaluronan biosynthesis and cancer progression: An immunological perspective. *Mini-Rev Med Chem*. 2009(9):1538–46. <http://dx.doi.org/10.2174/138955709790361485>

23. Spinelli FM, Vitale DL, Demarchi G, Cristina C, Alaniz L. The immunological effect of hyaluronan in tumor angiogenesis. *Clin Transl Immunol.* 2015;4(12):e52. <http://dx.doi.org/10.1038/cti.2015.35>
24. Bauer J, Rothley M, Schmaus A, Quagliata L, Ehret M, Biskup M, et al. Tgfbeta Counteracts lyve-1-mediated induction of lymphangiogenesis by small hyaluronan oligosaccharides. *J Mol Med (Berl).* 2018;96(2):199–209. <http://dx.doi.org/10.1007/s00109-017-1615-4>
25. Gurski LA, Xu X, Labrada LN, Nguyen NT, Xiao L, van Golen KL, et al. Hyaluronan (Ha) interacting proteins Rhamm and hyaluronidase impact prostate cancer cell behavior and invadopodia formation in 3d Ha-based hydrogels. *PLoS One.* 2012;7(11):e50075. <http://dx.doi.org/10.1371/journal.pone.0050075>
26. Thapa R, Wilson GD. The importance of Cd44 as a stem cell biomarker and therapeutic target in cancer. *Stem Cells Int.* 2016;2016:2087204. <http://dx.doi.org/10.1155/2016/2087204>
27. Kjellen L, Lindahl U. Proteoglycans: Structures and interactions. *Ann Rev Biochem.* 1991;60:443–75. <http://dx.doi.org/10.1146/annurev.bi.60.070191.002303>
28. Theocharis AD, Skandalis SS, Tzanakakis GN, Karamanos NK. Proteoglycans in health and disease: Novel roles for proteoglycans in malignancy and their pharmacological targeting. *FEBS J.* 2010;277(19):3904–23. <http://dx.doi.org/10.1111/j.1742-4658.2010.07800.x>
29. Rycaj K, Tang DG. Cell-of-origin of cancer versus cancer stem cells: Assays and interpretations. *Cancer Res.* 2015;75(19):4003–11. <http://dx.doi.org/10.1158/0008-5472.CAN-15-0798>
30. Zhang DY, Friedman SL. Fibrosis-dependent mechanisms of hepatocarcinogenesis. *Hepatology.* 2012;56(2):769–75. <http://dx.doi.org/10.1002/hep.25670>
31. Lindoso RS, Collino F, Vieyra A. Extracellular vesicles as regulators of tumor fate: crosstalk among cancer stem cells, tumor cells and mesenchymal stem cells. *Stem Cell Investig.* 2017;4:75. <http://dx.doi.org/10.21037/sci.2017.08.08>
32. Zinzi L, Contino M, Cantore M, Capparelli E, Leopoldo M, Colabufo NA. Abc transporters in Cscs membranes as a novel target for treating tumor relapse. *Front Pharmacol.* 2014;5:163. <http://dx.doi.org/10.3389/fphar.2014.00163>
33. Al Faraj A, Shaik AS, Al Sayed B, Halwani R, Al Jammaz I. Specific targeting and noninvasive imaging of breast cancer stem cells using single-walled carbon nanotubes as novel multimodality nanoprobe. *Nanomedicine.* 2016;11(1):31–46. <http://dx.doi.org/10.2217/nmm.15.182>
34. Singh VK, Saini A, Chandra R. The implications and future perspectives of nanomedicine for cancer stem cell targeted therapies. *Front Mol Biosci.* 2017;4:52. <http://dx.doi.org/10.3389/fmolb.2017.00052>
35. Bayo J, Fiore E, Aquino JB, Malvicini M, Rizzo M, Peixoto E, et al. Increased migration of human mesenchymal stromal cells by autocrine motility factor (AMF) resulted in enhanced recruitment towards hepatocellular carcinoma. *PLoS One.* 2014;9(4):e95171.
36. Bayo J, Real A, Fiore EJ, Malvicini M, Sganga L, Bolontrade M, et al. Il-8, Gro and Mcp-1 produced by hepatocellular carcinoma microenvironment determine the migratory capacity of human bone marrow-derived mesenchymal stromal cells without affecting tumor aggressiveness. *Oncotarget.* 2017;8(46):80235–48. <http://dx.doi.org/10.18632/oncotarget.10288>
37. Yan L, Xu F, Dai CL. Relationship between epithelial-to-mesenchymal transition and the inflammatory microenvironment of hepatocellular carcinoma. *J Exp Clin Cancer Res.* 2018;37(1):203. <http://dx.doi.org/10.1186/s13046-018-0887-z>
38. Ninomiya T, Akbar S, Masumoto T, Horiike N, Onji M. Dendritic cells with immature phenotype and defective function in the peripheral blood from patients with hepatocellular carcinoma. *J Hepatol.* 1999;31(2):323–31. [http://dx.doi.org/10.1016/S0168-8278\(99\)80231-1](http://dx.doi.org/10.1016/S0168-8278(99)80231-1)
39. Zhu XD, Zhang JB, Zhuang PY, Zhu HG, Zhang W, Xiong YQ, et al. High expression of macrophage colony-stimulating factor in peritumoral liver tissue is associated with poor survival after curative resection of hepatocellular carcinoma. *J Clin Oncol.* 2008;26(16):2707–16. <http://dx.doi.org/10.1200/JCO.2007.15.6521>
40. Schrader J, Iredale JP. The inflammatory microenvironment of HCC—The plot becomes complex. *J Hepatol.* 2011;54(5):853–5. <http://dx.doi.org/10.1016/j.jhep.2010.12.014>
41. Cai L, Zhang Z, Zhou L, Wang H, Fu J, Zhang S, et al. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. *Clin Immunol.* 2008;129(3):428–37. <http://dx.doi.org/10.1016/j.clim.2008.08.012>

42. Wu K, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of Cd8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res.* 2009;69(20):8067–75. <http://dx.doi.org/10.1158/0008-5472.CAN-09-0901>
43. Chen KJ, Lin SZ, Zhou L, Xie HY, Zhou WH, Taki-Eldin A, et al. Selective recruitment of regulatory T cell through Ccr6-Ccl20 in hepatocellular carcinoma fosters tumor progression and predicts poor prognosis. *PLoS One.* 2011;6(9):e24671. <http://dx.doi.org/10.1371/journal.pone.0024671>
44. Noy R, Pollard JW. Tumor-associated macrophages: From mechanisms to therapy. *Immunity.* 2014;41(1):49–61. <http://dx.doi.org/10.1016/j.immuni.2014.06.010>
45. Mantovani A, Allavena P. The interaction of anticancer therapies with tumor-associated macrophages. *J Exp Med.* 2015;212(4):435–45. <http://dx.doi.org/10.18632/oncotarget.8515>
46. You Y, Zheng Q, Dong Y, Xie X, Wang Y, Wu S, et al. Matrix stiffness-mediated effects on stemness characteristics occurring in HCC cells. *Oncotarget.* 2016;7(22):32221–31. <http://dx.doi.org/10.18632/oncotarget.8515>
47. Mishra L, Banker T, Murray J, Byers S, Thenappan A, He AR, et al. Liver stem cells and hepatocellular carcinoma. *Hepatology.* 2009;49(1):318–29. <http://dx.doi.org/10.1002/hep.22704>
48. Tahmasebi Birgani M, Carloni V. Tumor microenvironment, a paradigm in hepatocellular carcinoma progression and therapy. *Int J Mol Sci.* 2017;18(2):405. <http://dx.doi.org/10.3390/ijms18020405>
49. Köhn-Gaone J, Gogoi-Tiwari J, Ramm GA, Olynyk JK, Tirmitz-Parker JEE. The role of liver progenitor cells during liver regeneration, fibrogenesis, and carcinogenesis. *Am J Physiol Gastrointest Liver Physiol* 2016;310:G143–G54. <http://dx.doi.org/10.1152/ajpgi.00215.2015>
50. Forbes SJ, Raven A. Hepatic progenitors in liver regeneration. *J Hepatol.* 2018;69(6):1394–5. <http://dx.doi.org/10.1016/j.jhep.2018.03.004>
51. Herencia C, Martinez-Moreno JM, Herrera C, Corrales F, Santiago-Mora R, Espejo I, et al. Nuclear translocation of beta-catenin during mesenchymal stem cells differentiation into hepatocytes is associated with a tumoral phenotype. *PLoS One.* 2012;7(4):e34656. <http://dx.doi.org/10.1371/journal.pone.0034656>
52. Eun JR, Jung YJ, Zhang Y, Zhang Y, Tschudy-Seney B, Ramsamooj R, et al. Hepatoma SK Hep-1 cells exhibit characteristics of oncogenic mesenchymal stem cells with highly metastatic capacity. *PLoS One.* 2014;9(10):e110744. <http://dx.doi.org/10.1371/journal.pone.0110744>
53. Balkwill F, Mantovani A. Inflammation and cancer: Back to virchow? *Lancet.* 2001;357(9255):539–45. [http://dx.doi.org/10.1016/S0140-6736\(00\)04046-0](http://dx.doi.org/10.1016/S0140-6736(00)04046-0)
54. Naugler W, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy A, et al. Gender disparity in liver cancer due to sex differences in Myd88-dependent Il-6 production. *Science.* 2007;317:121–4. <http://dx.doi.org/10.1126/science.1140485>
55. Wang Y, Yao HL, Cui CB, Wauthier E, Barbier C, Costello MJ, et al. Paracrine signals from mesenchymal cell populations govern the expansion and differentiation of human hepatic stem cells to adult liver fates. *Hepatology.* 2010;52(4):1443–54. <http://dx.doi.org/10.1002/hep.23829>
56. Bourguignon LY, Shiina M, Li JJ. Hyaluronan-Cd44 interaction promotes oncogenic signaling, MicroRNA functions, chemoresistance, and radiation resistance in cancer stem cells leading to tumor progression. *Adv Cancer Res.* 2014;123:255–75. <http://dx.doi.org/10.1016/B978-0-12-800092-2.00010-1>
57. Lee IC, Chuang CC, Wu YC. Niche mimicking for selection and enrichment of liver cancer stem cells by hyaluronic acid-based multilayer films. *ACS Appl Mater Interfaces.* 2015;7(40):22188–95. <http://dx.doi.org/10.1021/acsami.5b04436>
58. Bataller R, Brenner DA. Liver fibrosis. *J Clin Investig.* 2005;115(2):209–18. <http://dx.doi.org/10.1172/JCI24282>
59. Hammerich L, Tacke F. Role of gamma-delta T cells in liver inflammation and fibrosis. *World J Gastrointest Pathophysiol.* 2014;5(2):107–13. <http://dx.doi.org/10.4291/wjgp.v5.i2.107>
60. He G, Karin M. NF-KappaB and STAT3—Key players in liver inflammation and cancer. *Cell Res.* 2011;21(1):159–68. <http://dx.doi.org/10.1038/cr.2010.183>
61. Vincent T, Jourdan M, Sy MS, Klein B, Mechti N. Hyaluronic acid induces survival and proliferation of human myeloma cells through an interleukin-6-mediated pathway involving the phosphorylation of retinoblastoma protein. *J Biol Chem.* 2001;276(18):14728–36. <http://dx.doi.org/10.1074/jbc.M003965200>

62. Sukowati CHC, Anfuso B, Fiore E, Ie SI, Raseni A, Vascotto F, et al. Hyaluronic acid inhibition by 4-methylumbelliferone reduces the expression of cancer stem cells markers during hepatocarcinogenesis. *Sci Rep.* 2019;9(1):4026. <http://dx.doi.org/10.1038/s41598-019-40436-6>
63. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol.* 2017;14(7):397–411. <http://dx.doi.org/10.1038/nrgastro.2017.38>
64. Schaefer L, Iozzo RV. Biological functions of the small leucine-rich proteoglycans: From genetics to signal transduction. *J Biol Chem.* 2008;283(31):21305–9. <http://dx.doi.org/10.1074/jbc.R800020200>
65. Baghy K, Tatrai P, Regos E, Kovalszky I. Proteoglycans in liver cancer. *World J Gastroenterol.* 2016;22(1):379–93. <http://dx.doi.org/10.3748/wjg.v22.i1.379>
66. Tumova S, Woods A, Couchman J. Heparan sulfate proteoglycans on the cell surface: versatile coordinators of cellular functions. *Int J Biochem Cell Biol.* 2000;32(3):269–88. [http://dx.doi.org/10.1016/S1357-2725\(99\)00116-8](http://dx.doi.org/10.1016/S1357-2725(99)00116-8)
67. Baghy K, Iozzo RV, Kovalszky I. Decorin-Tgfbeta axis in hepatic fibrosis and cirrhosis. *J Histochem Cytochem.* 2012;60(4):262–8. <http://dx.doi.org/10.1369/0022155412438104>
68. Dudás J, Kovalszky I, Gallai M, Nagy J, Schaff Z, Kniittel T, et al. Expression of decorin, transforming growth factor-beta1, tissue inhibitor metalloproteinase 1 and 2, and type Iv collagenases in chronic hepatitis. *Am J Clin Pathol.* 2001;115(5):725–35. <http://dx.doi.org/10.1309/J8CD-E9C8-X4NG-GTVG>
69. Roskams T, Moshage H, De Vos R, Guido D, Yap P, Desmet V. Heparan sulfate proteoglycan expression in normal human liver. *Hepatology.* 1995;21(4):950–8. <http://dx.doi.org/10.1002/hep.1840210410>
70. Tatrai P, Egedi K, Somoracz A, van Kuppevelt TH, Ten Dam G, Lyon M, et al. Quantitative and qualitative alterations of heparan sulfate in fibrogenic liver diseases and hepatocellular cancer. *J Histochem Cytochem.* 2010;58(5):429–41. <http://dx.doi.org/10.1369/jhc.2010.955161>
71. Wang K, Sun AD. Cancer stem cells of hepatocellular carcinoma. *Oncotarget.* 2018;9(33):23306–14. <http://dx.doi.org/10.18632/oncotarget.24623>
72. Yao H, Liu N, Lin MC, Zheng J. Positive feedback loop between cancer stem cells and angiogenesis in hepatocellular carcinoma. *Cancer Lett.* 2016;379(2):213–19. <http://dx.doi.org/10.1016/j.canlet.2016.03.014>
73. Anfuso B, El-Khobar KE, Sukowati CH, Tiribelli C. The multiple origin of cancer stem cells in hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol.* 2015;39(Suppl 1):S92–7. <http://dx.doi.org/10.1016/j.clinre.2015.05.011>
74. Klopp AH, Gupta A, Spaeth E, Andreeff M, Marini F, 3rd. Concise review: Dissecting a discrepancy in the literature: Do mesenchymal stem cells support or suppress tumor growth? *Stem Cells.* 2011;29(1):11–19. <http://dx.doi.org/10.1002/stem.559>
75. Qiao L, Xu Z, Zhao T, Zhao Z, Shi M, Zhao RC, et al. Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model. *Cell Res.* 2008;18(4):500–7. <http://dx.doi.org/10.1038/cr.2008.40>
76. Lu YR, Yuan Y, Wang XJ, Wei LL, Chen YN, Cong C, et al. The growth inhibitory effect of mesenchymal stem cells on tumor cells in vitro and in vivo. *Cancer Biol Ther.* 2008;7(2):245–51. <http://dx.doi.org/10.4161/cbt.7.2.5296>
77. Niess H, Bao Q, Conrad C, Zischek C, Notohamiprodjo M, Schwab F, et al. Selective targeting of genetically engineered mesenchymal stem cells to tumor stroma microenvironments using tissue-specific suicide gene expression suppresses growth of hepatocellular carcinoma. *Ann Surg.* 2011;254(5):767–74; discussion 74–5. <http://dx.doi.org/10.1097/SLA.0b013e3182368c4f>
78. Gong P, Wang Y, Wang Y, Jin S, Luo H, Zhang J, et al. Effect of bone marrow mesenchymal stem cells on hepatocellular carcinoma in microcirculation. *Tumour Biol.* 2013;34(4):2161–8. <http://dx.doi.org/10.1007/s13277-013-0749-4>
79. Chen XC, Wang R, Zhao X, Wei YQ, Hu M, Wang YS, et al. Prophylaxis against carcinogenesis in three kinds of unestablished tumor models via Il12-gene-engineered MSCs. *Carcinogenesis.* 2006;27(12):2434–41. <http://dx.doi.org/10.1093/carcin/bgl069>
80. Chen X, Lin X, Zhao J, Shi W, Zhang H, Wang Y, et al. A tumor-selective biotherapy with prolonged impact on established metastases based on cytokine gene-engineered MSCs. *Mol Ther.* 2008;16(4):749–56. <http://dx.doi.org/10.1038/mt.2008.3>

81. Gao Y, Yao A, Zhang W, Lu S, Yu Y, Deng L, et al. Human mesenchymal stem cells overexpressing pigment epithelium-derived factor inhibit hepatocellular carcinoma in nude mice. *Oncogene*. 2010;29(19):2784–94. <http://dx.doi.org/10.1038/onc.2010.38>
82. Garcia MG, Bayo J, Bolontrade MF, Sganga L, Malvicini M, Alaniz L, et al. Hepatocellular carcinoma cells and their fibrotic microenvironment modulate bone marrow-derived mesenchymal stromal cell migration in vitro and in vivo. *Mol Pharm*. 2011;8(5):1538–48. <http://dx.doi.org/10.1021/mp200137c>
83. Qiao L, Zhao TJ, Wang FZ, Shan CL, Ye LH, Zhang XD. NF-KappaB Downregulation may be involved in the depression of tumor cell proliferation mediated by human mesenchymal stem cells. *Acta Pharmacol Sin*. 2008;29(3):333–40. <http://dx.doi.org/10.1111/j.1745-7254.2008.00751.x>
84. Yulyana Y, Ho IA, Sia KC, Newman JP, Toh XY, Endaya BB, et al. Paracrine factors of human fetal MSCs inhibit liver cancer growth through reduced activation of Igf-1r/Pi3k/Akt signaling. *Mol Ther*. 2015;23(4):746–56. <http://dx.doi.org/10.1038/mt.2015.13>
85. Bruno S, Collino F, Deregibus MC, Grange C, Tetta C, Camussi G. Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth. *Stem Cells Dev*. 2013;22(5):758–71. <http://dx.doi.org/10.1089/scd.2012.0304>
86. Ko SF, Yip HK, Zhen YY, Lee CC, Lee CC, Huang CC, et al. Adipose-derived mesenchymal stem cell exosomes suppress hepatocellular carcinoma growth in a rat model: Apparent diffusion coefficient, natural killer T-cell responses, and histopathological features. *Stem Cells Int*. 2015;2015:853506. <http://dx.doi.org/10.1155/2015/853506>
87. Li GC, Ye QH, Xue YH, Sun HJ, Zhou HJ, Ren N, et al. Human mesenchymal stem cells inhibit metastasis of a hepatocellular carcinoma model using the Mhcc97-H cell line. *Cancer Sci*. 2010;101(12):2546–53. <http://dx.doi.org/10.1111/j.1349-7006.2010.01738.x>
88. Li T, Song B, Du X, Wei Z, Huo T. Effect of bone-marrow-derived mesenchymal stem cells on high-potential hepatocellular carcinoma in mouse models: An intervention study. *Eur J Med Res*. 2013;18:34. <http://dx.doi.org/10.1186/2047-783X-18-34>
89. Jing Y, Han Z, Liu Y, Sun K, Zhang S, Jiang G, et al. Mesenchymal stem cells in inflammation micro-environment accelerates hepatocellular carcinoma metastasis by inducing epithelial-mesenchymal transition. *PLoS One*. 2012;7(8):e43272. <http://dx.doi.org/10.1371/journal.pone.0043272>
90. Yan XL, Jia YL, Chen L, Zeng Q, Zhou JN, Fu CJ, et al. Hepatocellular carcinoma-associated mesenchymal stem cells promote hepatocarcinoma progression: Role of the S100a4-Mir155-Socs1-Mmp9 axis. *Hepatology*. 2013;57(6):2274–86. <http://dx.doi.org/10.1002/hep.26257>
91. Hernanda PY, Pedroza-Gonzalez A, van der Laan LJ, Broker ME, Hoogduijn MJ, Ijzermans JN, et al. Tumor promotion through the mesenchymal stem cell compartment in human hepatocellular carcinoma. *Carcinogenesis*. 2013;34(10):2330–40. <http://dx.doi.org/10.1093/carcin/bgt210>
92. Yan X, Zhang D, Wu W, Wu S, Qian J, Hao Y, et al. Mesenchymal stem cells promote hepatocarcinogenesis via Lncrna-Muf interaction with Anxa2 and Mir-34a. *Cancer Res*. 2017;77(23):6704–16. <http://dx.doi.org/10.1158/0008-5472.CAN-17-1915>
93. Ju C, Tacke F. Hepatic macrophages in homeostasis and liver diseases: From pathogenesis to novel therapeutic strategies. *Cell Mol Immunol*. 2016;13(3):316–27. <http://dx.doi.org/10.1038/cmi.2015.104>
94. Kuang DM, Zhao Q, Peng C, Xu J, Zhang JP, Wu C, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through Pd-L1. *J Exp Med*. 2009;206(6):1327–37. <http://dx.doi.org/10.1084/jem.20082173>
95. Kharishvili G, Simkova D, Bouchalova K, Gachechiladze M, Narsia N, Bouchal J. The role of cancer-associated fibroblasts, solid stress and other microenvironmental factors in tumor progression and therapy resistance. *Cancer Cell International*. 2014;(14):41 doi: <http://10.1186/1475-2867-14-41>.
96. Li J-H, Wang Y-C, Qin C-D, Yao R-R, Zhang R, Wang Y, et al. Over expression of hyaluronan promotes progression of HCC via CD44-mediated pyruvate Kinase M2 nuclear translocation. *Am J Cancer Res*. 2016;6(2):509–21.
97. Nikitovic D, Berdiaki A, Spyridaki I, Krasanakis T, Tsatsakis A, Tzanakakis GN. Proteoglycans-biomarkers and targets in cancer therapy. *Front endocrinol (Lausanne)*. 2018;9:69. <http://dx.doi.org/10.3389/fendo.2018.00069>



98. Jarvelainen H, Sainio A, Wight TN. Pivotal role for Decorin in angiogenesis. *Matrix Biol.* 2015;43:15–26. <http://dx.doi.org/10.1016/j.matbio.2015.01.023>
99. Fiedler LR, Schonherr E, Waddington R, Niland S, Seidler DG, Aeschlimann D, et al. Decorin regulates endothelial cell motility on collagen I through activation of insulin-like growth factor I receptor and modulation of alpha2beta1 integrin activity. *J Biol Chem.* 2008;283(25):17406–15. <http://dx.doi.org/10.1074/jbc.M710025200>
100. Sprinzl MF, Reisinger F, Puschnik A, Ringelhan M, Ackermann K, Hartmann D, et al. Sorafenib perpetuates cellular anticancer effector functions by modulating the crosstalk between macrophages and natural killer cells. *Hepatology.* 2013;57(6):2358–68. <http://dx.doi.org/10.1002/hep.26328>
101. Coscia M, Quaglino E, Iezzi M, Curcio C, Pantaleoni F, Riganti C, et al. Zoledronic acid repolarizes tumour-associated macrophages and inhibits mammary carcinogenesis by targeting the mevalonate pathway. *J Cell Mol Med.* 2010;14(12):2803–15. <http://dx.doi.org/10.1111/j.1582-4934.2009.00926.x>
102. Degroote H, Van Dierendonck A, Geerts A, Van Vlierberghe H, Devisscher L. Preclinical and clinical therapeutic strategies affecting tumor-associated macrophages in hepatocellular carcinoma. *J Immunol Res.* 2018;2018:7819520. <http://dx.doi.org/10.1155/2018/7819520>
103. Ikeda M, Ohkawa S, Okusaka T, Mitsunaga S, Kobayashi S, Morizane C, et al. Japanese Phase I study of Gc33, a humanized antibody against glypican-3 for advanced hepatocellular carcinoma. *Cancer Sci.* 2014;105(4):455–62. <http://dx.doi.org/10.1111/cas.12368>
104. Xie Z, Choong PF, Poon LF, Zhou J, Khng J, Jasinghe VJ, et al. Inhibition of Cd44 expression in hepatocellular carcinoma cells enhances apoptosis, chemosensitivity, and reduces tumorigenesis and invasion. *Cancer Chemother Pharmacol.* 2008;62(6):949–57. <http://dx.doi.org/10.1007/s00280-008-0684-z>
105. Rodriguez MM, Fiore E, Bayo J, Atorrasagasti C, Garcia M, Onorato A, et al. 4mu Decreases Cd47 expression on hepatic cancer stem cells and primes a potent antitumor T cell response induced by interleukin-12. *Mol Ther.* 2018;26(12):2738–50. <http://dx.doi.org/10.1016/j.ymthe.2018.09.012>
106. Baghy K, Horvath Z, Regos E, Kiss K, Schaff Z, Iozzo RV, et al. Decorin interferes with platelet-derived growth factor receptor signaling in experimental hepatocarcinogenesis. *FEBS J.* 2013;280(10):2150–64. <http://dx.doi.org/10.1111/febs.12215>
107. Santra M, Mann D, Mercer E, Skorski T, Calabretta B, RV I. Ectopic expression of decorin protein core causes a generalized growth suppression in neoplastic cells of various histogenetic origin and requires endogenous P21, an inhibitor of cyclin-dependent kinases. *J Clin Invest.* 1997;100(1):149–57. <http://dx.doi.org/10.1172/JCI119507>