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

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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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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

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.

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

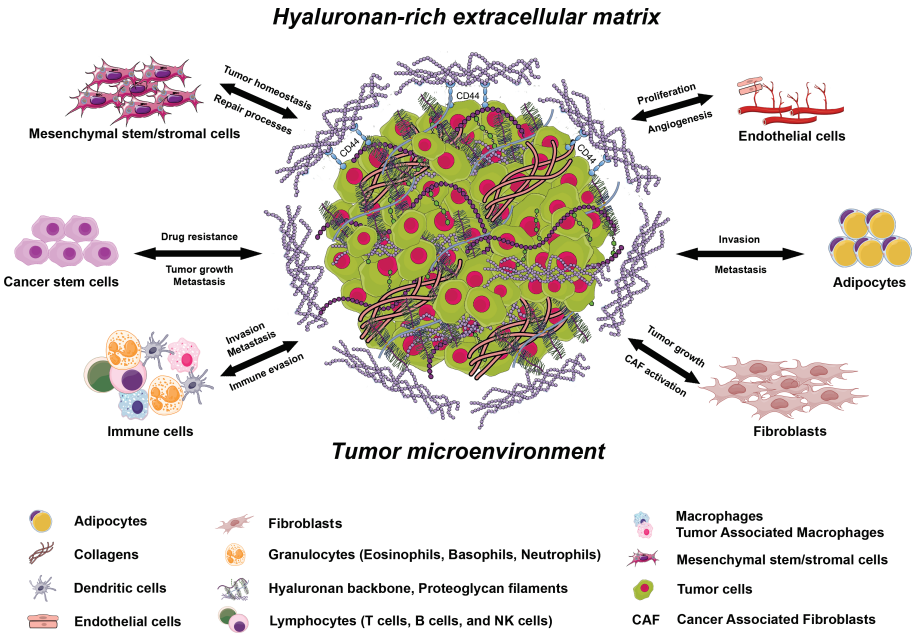

Figure 1 Schematic representation of the role of a HA-rich microenvironment in cancer progression. The TME is composed of non-tumor cells, such as fibroblasts, endothelial cells, MSCs, adipocytes, and infiltrating immune cells, and of non-cellular compartments, including secreted soluble factors and 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 $0.5x10^6$ to 10^7 Da, and to a lesser extent in a low molecular weight form, ranging from 10^4 to $0.5x10^6$ 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).

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 μm 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

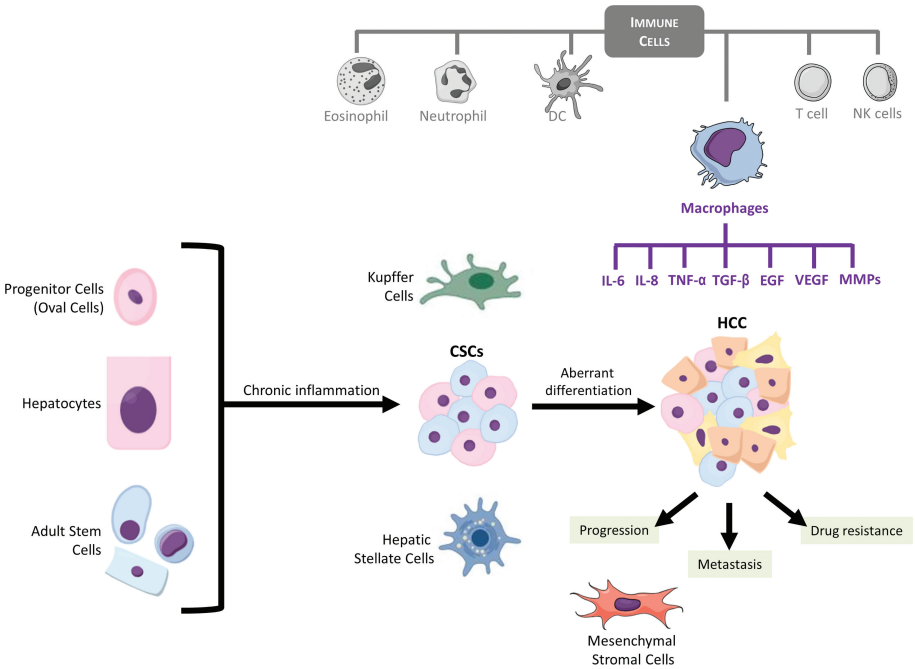

Figure 2 Stem and immune cells associated with tumor development. HCC is composed of a heterogeneous population of CSCs, which might derive from hepatocytes, progenitor cells (oval cells), or other adult stem cells, like bone marrow cells. CSCs have a very complex signaling network that includes crosstalk 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-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; 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/β-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$^{-/-}$ 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-β1), 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).

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 $EpCAM^+$ and $CD90^+$ cells are two independent subpopulations. $EpCAM^+$ cells have hepatic epithelial stem cell features and are associated with a high tumorigenic capacity, while $CD90^+$ 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 $CD133^+$ 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/β-catenin, BMI-1, TGF-β, 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-κB, 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-β1 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-β-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, TNFα, TGFβ, 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 $CD8^+$ 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-β 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).

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) reprograming 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 $CD133^{low}$ 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), β-catenin, and Myc expression, and upregulating p21WAF1/CIP1 (106, 107).

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.

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