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Stellate cells and the development of liver cancer; therapeutic potential of targeting the stroma

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Key Points

- Liver carcinogenesis represents a paradigm to study the role of the microenvironment in cancer given that most tumors develop in a background of liver fibrosis/cirrhosis
- Tumor cell microenvironment remodeling is associated with tumor progression
- HSC play an important role in liver carcinogenesis as key modulators of fibrosis and tumor cell microenvironment

- Targeting HSC and the crosstalk between tumor and stromal cells may represent a promising therapeutic strategy

Summary

Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the most common types of primary tumors in the liver. Although major advances have been made in understanding the cellular and molecular mechanisms underlying liver carcinogenesis, HCC and ICC are still deadly cancers worldwide waiting for innovative therapeutic options. Growing evidences from the literature highlight the critical role of the tumor cell microenvironment in the pathogenesis of cancer diseases. Thus, targeting the microenvironment, particularly the crosstalk between tumor cells and stromal cells, has emerged as a promising therapeutic strategy. This strategy would be particularly relevant for liver cancers which frequently develop in a setting of chronic inflammation and microenvironment remodeling associated with hepatic fibrosis and cirrhosis, such processes in which hepatic stellate cells (HSC) greatly contribute. This review brings a genomic point of view on the alterations of the cellular microenvironment in liver cancers, particularly the stromal tissue within tumor nodules, emphasizing the importance of the crosstalk between tumor cells and stromal cells, notably activated HSC, in tumor onset and progression. Furthermore, potential therapeutic modalities of targeting the stroma and HSC are discussed.

Introduction

Growing evidences from genetic, genomic and cell-biology indicate that tumorigenesis is determined not only by malignant cells but also by their microenvironment [1]. The microenvironment is a complex and dynamic system involving extracellular matrix (ECM) components, soluble factors, and stromal cells, whose distribution and composition vary in space and time [1-3]. Under physiological condition, the microenvironment serves as an important barrier to epithelial cell transformation, notably by maintaining cell polarity and by controlling cell proliferation [1-3]. In response to emerging epithelial cancerous lesions, the microenvironment experiences important changes (e.g. recruitment/activation of stromal cells, ECM remodeling) which contribute to cancer initiation and progression and influence the therapeutic response [1-3]. Thus, targeting the tumor microenvironment is now viewed as a promising strategy to treat cancer in a variety of organs including liver [3-4]. Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the two major types of primary tumors in the liver. Alteration of the microenvironment is a hallmark of liver carcinogenesis given that more than 80% of tumors arise in a setting of chronic inflammation associated with liver fibrosis and/or cirrhosis, such conditions which constitute real precancerous stages [4]. As key drivers of liver fibrosis and ECM remodeling [5], activated hepatic stellate cells

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(HSC) might represent attractive targets in the design of innovative therapeutic strategies against the stroma in liver carcinogenesis.

The tumor microenvironment in liver carcinogenesis

Tumor microenvironment includes cellular and non-cellular components. In the liver, HSC, fibroblasts, myofibroblasts as well as immune and endothelial cells represent the main cell types of the hepatocyte microenvironment [4]. In addition to ECM macromolecules (e.g. collagens, proteoglycans), non-cellular components include diverse soluble factors such as cytokines and growth factors that can be stored into the ECM and orchestrate the interplays between tumor cells and the microenvironment [1-4].

Combination of cell and molecular biology, biochemistry, histopathology and gene to gene approaches greatly contributed to identify pathways deregulated in the tumor stroma and their impact on tumor cells [1]. Recently, genomic approaches revisited the field allowing an exhaustive and integrated view of these deregulations at a pangenomic scale [6]. Even more, by combining laser microdissection and gene expression profiling, stromal gene signatures linked to clinical outcomes were established in cancer [6]. This approach was proven to be fruitful for deciphering the molecular mechanisms underlying the alterations of the tumor microenvironment, with the ultimate goal of designing new therapeutic approaches, and identifying novel diagnostic and prognostic biomarkers [6]. In liver, few studies addressed this issue at a genomic scale. In HCC, gene expression profiling highlighted the importance of Th1 and Th2 cytokines in the

surrounding non-tumor tissue of HCC associated with metastasis [7]. A metastasis-inclined microenvironment signature was characterized by an increase in Th2 cytokines and a decrease in Th1 cytokines [7]. This study suggested that the dynamics of the immune microenvironment in the non-tumor tissue adjacent to the tumor nodules may result in the establishment of a suitable niche to promote tumor cell dissemination. Genomic studies also provided molecular insights into the pathogenesis of ICC which is usually characterized by a dense desmoplastic stroma [8]. From these studies clinically relevant ICC subgroups were highlighted and new therapeutic opportunities were proposed, based on *BRAF/KRAS* mutations and activation of oncogenic pathways (e.g. receptor tyrosine kinases) [8]. Recently, we identified specific alterations of the stroma in ICC through an unsupervised analysis of microdissected human tumors [9]. Thus, a signature which included genes related to cell cycle, ECM and transforming growth factor beta (TGFβ) pathways was shown to significantly discriminate the tumor stroma from fibrous tissue isolated in the adjacent non tumor liver. A clear correlation between genomic changes in the stroma and the aggressiveness of ICC was also demonstrated. Notably, high stromal expressions of osteopontin and TGFβ were identified as poor prognosis factors [9]. Besides promising biomarkers, osteopontin and TGFβ represent potential therapeutic targets given their proven role in driving the oncogenic process, from early stages of tumor development to late invasive stages. Indeed, gain and loss of function studies demonstrated osteopontin to be crucial for tumor cell growth and metastasis, notably by mechanisms involving apoptosis escape, angiogenesis, and ECM degradation [10]. Activation of the TGFβ signaling was found in HCC [11], ICC [9] and combined HCC-ICC [12]. Importantly, studies using engineered mice demonstrated

that the modulation of the TGFβ pathway in stromal fibroblasts influence the oncogenic potential of the adjacent epithelial cells [2]. These results imply that genomic alterations in stromal cells may result in tumor initiation and progression.

HSC in tumor onset and progression

The activation of HSC and subsequent phenotypic changes towards a myofibroblast-like phenotype is a key event in liver fibrosis [5]. Gene expression studies showed that activated HSC markedly express genes involved in fibrogenesis and fibrolysis, inflammation and apoptosis [13]. HSC activation is controlled by numerous factors and signaling pathways (e.g. TGFβ, platelet-derived growth factors (PDGF), hedgehog, notch, microRNAs) [5]. Recently, the hedgehog signaling was shown to control the fate of HSC, thus opening new opportunities for a therapeutic targeting of HSC [14]. The nuclear factor-kappa B (NF-κB) pathway has been also reported to contribute to HSC activation and survival, and more largely to be a central factor in the progression of hepatic diseases, linking liver injury, inflammation, fibrosis and HCC (reviewed in [15]).

Besides a major role in fibrogenesis, HSC exhibit biological functions that influence the onset and the progression of HCC [5]. Thus, HSC can induce phenotypic changes in cancer cells, notably through the production of growth factors and cytokines in favor of tumor cell proliferation (e.g. hepatocyte growth factor, interleukin-6 (IL-6)) [5,16]. Migration and proliferation of HCC cells are also modulated by ECM components produced by activated HSC, including basement membrane components, e.g. laminin-5 [17]. Interestingly, HSC were reported as key players in liver tumorigenesis associated

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with the gut-liver axis [18]. Thus, the gut microbiome was shown to induce inflammatory and fibrogenic responses, and HSC activation leading to the production of epiregulin, a mitogenic factor for hepatocytes [18]. Cellular and molecular approaches demonstrated that a bidirectional crosstalk exists between HSC-derived myofibroblasts and tumor hepatocytes [5]. Early studies showed that the exposition of HSC to conditioned media derived from HCC tumor cells resulted in HSC activation, migration and expression of pro-angiogenic factors such as vascular endothelial growth factor alpha (VEGFA) [5]. Recently, we reported that the crosstalk between hepatoma cells and activated HSC also increased the expression of proinflammatory cytokines and chemokines (e.g. IL-6, IL-8), and modified the phenotype of hepatoma cells toward motile cells, together with the generation of a permissive pro-angiogenic microenvironment, characterized by the overexpression of VEGFA and matrix metallopeptidase 9 (MMP9) in HSC [19]. Interestingly, integrative genomics demonstrated that a gene signature of this crosstalk was predictive of a poor prognosis and metastasis propensity in human HCC [19]. In addition to pro-inflammatory cytokines, TGFβ was reported to be central in driving the pro-tumorigenic effects of activated HSC on the progression of transformed hepatocytes [20]. Interplay between HSC and cancer cells has been also reported in ICC, notably through the SDF1/CXCR4 axis which activation was shown to influence both cancer cell survival and metastasis [20]. In addition to early stages of tumor development, HSC are involved in late tumor stages, notably by producing factors that directly participate in the formation of a pro-metastatic microenvironment [16]. Such factors in favor of the metastatic growth of tumor cells include inducers of the epithelial to mesenchymal cell transition (EMT), modulators of ECM synthesis and degradation, pro-angiogenic and

immune-suppression factors (e.g. TGFβ, ECM proteins, MMP, VEGFA) [16]. HSC can produce immune-regulatory cytokines (e.g. MCP1, RANTES, CCL21) to promote chemotaxis, adherence and activation of inflammatory cells [5,22]. Paracrine interactions of HSC with endothelial cells have been reported, as well as the secretion of VEGFA and angiopoietins by HSC favoring a pro-angiogenic microenvironment [22].

Therapeutic targeting of tumor microenvironment and HSC

Targeting the microenvironment is emerging as a promising strategy given the well-recognized role of the stroma in carcinogenesis [3]. Examples of drugs targeting the microenvironment include anti-angiogenic and anti-proliferative agents such as bevacizumab, an inhibitor of VEGFA, cetuximab, an inhibitor of EGFR, and sorafenib, an inhibitor of multiple protein kinase receptors (e.g. EGFR, VEGFR, PDGFR) [4]. Interestingly, sorafenib, which is currently the only drug recommended for advanced HCC, was shown to reduce the proliferation of transformed hepatocytes but also to attenuate liver fibrosis, notably by reducing HSC proliferation and ECM accumulation [23]. This observation opens an opportunity to target not only the tumor cells but also their microenvironment. One can expect that a better understanding of the cellular and molecular mechanisms underlying the process of HSC activation/proliferation, and ECM remodeling associated with tumor onset and progression may open opportunities to design new lines of therapeutic approaches. Therapeutic targeting of HSC has been investigated with interest given the key role HSC in fibrosis and HCC [22]. Induction of cellular apoptosis or senescence and reversion of activated HSC toward a quiescent

state have been proposed as mechanisms to clear activated HSC [22]. As example, selective induction of HSC apoptosis may be achieved by nanoparticules or gene transfer systems designed to inhibit NF-κB transcription factor which controls HSC activation and proliferation [15]. Boosting specific immune cell populations may represent another innovative strategy to control HSC. Recently, hepatic $\gamma\delta$ T-cells have been shown to promote the apoptosis of HSC through a mechanisms involving Fas-ligand [24]. NK cells have been also shown to selectively kill early or senescent activated HSC and to produce anti-fibrotic cytokines such as IFNγ [22]. Among immune effectors which exhibit anti-fibrotic potentials (e.g. IFNγ, IL-22, IL-10), IL22 was reported as a potent inducer of HSC senescence [22].

Targeting the crosstalk between tumors cells and their microenvironment may also represent a promising therapeutic strategy. Exploring this possibility, we recently applied an integrative functional genomics approach to identify molecules that could interfere with the crosstalk between hepatocytes and HSC [19]. Thus, by using a connectivity map algorithm we were able to connect a gene signature of the hepatocyte-HSC crosstalk with trichostatin A, an inhibitor of histone deacetylases. Accordingly, the effects of the crosstalk on tumor cell migration and endothelial cell angiogenesis were reversed in presence of trichostatin A, suggesting that epigenetic modulators may be clinically relevant [19]. Soluble factors may be also targeted to modulate the communication between the tumor cells and the microenvironment. In that regard, TGFβ may represent a promising target given that this cytokine is involved in multiple stages of liver carcinogenesis. TGFβ exhibits both oncogenic and tumor suppressive properties depending on tumor stage [11,25]. As mentioned above, at preneoplastic

stages TGFβ acts as a potent inducer of HSC activation and fibrogenesis but also exhibits growth-inhibitory and apoptosis-inducing effects, and in advanced stages TGFβ promotes HCC/ICC tumor cell invasion, notably as an inducer of EMT [11,25]. Thus, therapeutic targeting of the TGFβ pathway should be appropriately designed to inhibit its oncogenic properties while retaining its cytostatic effects. Targeting the TGFβ signaling by using small molecule inhibitors (e.g. LY2109761) or neutralizing antibodies has shown promising results in experimental HCC mouse models, including inhibition of cell invasion and abrogation of neo-angiogenesis [25]. Inhibition of the TGFβ signaling was also reported to be effective in blocking tumor-stroma crosstalk and tumor progression in HCC [25]. Thus, appropriately interfering with the crosstalk between cancer cells and their microenvironment may open new therapeutic opportunities in liver cancers (Figure 1).

Conclusive remark

Advances in understanding the molecular mechanisms involved in the crosstalk between tumor cells and their microenvironment at the pangenomic scale open the path to design new lines of therapeutic approach and associated biomarkers. Recent findings highlight that future adapted therapeutics would target both the tumor cells and the microenvironment by favorably modulating specific regulators in cellular pathways, and widespread mechanisms related to the neoplastic stroma evolution.

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Figure legend.

Figure 1. Therapeutic potential of targeting HSC and the stroma in liver cancer. Depicted are the interplays between tumors cells and components of the microenvironment, notably HSC, which influence tumor progression by creating a permissive environment and by perverting the phenotype of tumor cells. Each of these interactions, symbolized by arrows, could be targeted for therapeutic purpose. Epigenetic modulation of the crosstalk between tumor cells and their microenvironment may also open new therapeutic opportunities in liver cancers.
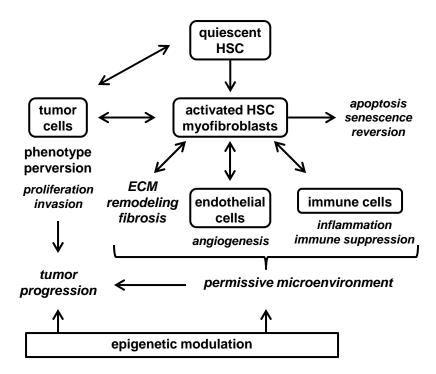
Figure 1