The role of non-coding RNAs in the tumor microenvironment of hepatocellular carcinoma

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Review

The role of noncoding RNAs in the tumor microenvironment of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the leading fatal malignancy worldwide. The tumor microenvironment (TME) can affect the survival, proliferation, migration, and even dormancy of cancer cells. Hypoxia is an important component of the TME, and hypoxia-inducible factor-1α (HIF-1α) is the most important transcriptional regulator. Noncoding RNAs (ncRNAs), including microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs), comprise a large part of the human transcriptome and play an important role in regulating the tumorigenesis of HCC. This review discusses the role of ncRNAs in hepatocarcinogenesis, epithelial-mesenchymal transition (EMT), and angiogenesis in a hypoxic microenvironment, as well as the interactions between ncRNAs and key components of the TME. It further discusses their use as biomarkers and the potential clinical value of drugs, as well as the challenges faced in the future.

Keywords: noncoding RNAs; HIF-1α; hepatocellular carcinoma; tumor microenvironment.
1. Introduction

Liver cancer is a fatal malignant tumor. According to CA: A Cancer Journal for Clinicians, the incidence of liver cancer ranks fifth among men, seventh among women, and second among malignant tumors in China. Therefore, finding a treatment for liver cancer has become increasingly important[1]. Liver cancer can be divided into primary liver cancer and metastatic liver cancer, with the most common being hepatocellular carcinoma (HCC), accounting for 75% – 85% of primary liver cancers.

Noncoding RNAs (ncRNAs) are a class of RNA molecules that do not encode proteins but have important regulatory functions. According to their length and source, ncRNAs include microRNAs (miRNAs), long chain noncoding RNAs (lncRNAs), circular RNAs (circRNAs), and tRNA-derived fragments (tRFs). Yong et al. reviewed the use of endogenous expression of miRNAs to silence target genes to achieve therapeutic approaches for HCC in 2007. With advances in bioinformatics and next-generation sequencing technologies, many ncRNAs were found to play important roles in crosstalk between HCC cells and the tumor microenvironment (TME)[3]. TME is a small microenvironment around tumor cells, including fibroblasts, immune cells, blood vessels, inflammatory cells, various signaling molecules and extracellular matrix (ECM). The interaction between the tumor and TME plays a key role in tumorigenesis and progression [4]. The oxygen concentration in the tissue is blocked, resulting in a low oxygen concentration in the TME, which is called hypoxia[5]. Hypoxia is a typical feature of the TME and a sign of cancer. To adapt to the hypoxic environment, cancer cells acquire invasiveness, metastasis and resistance to chemotherapy and radiotherapy, which together constitute a lethal cancer phenotype[6].

Recent studies also suggested possible therapeutic strategies to target HCC metabolism by regulating the expression of specific ncRNAs[7, 8]. NcRNAs are considered biomarkers and therapeutic targets for the treatment of cancers, including HCC. This review focuses on the regulatory role of ncRNAs in the TME of HCC.

2. TME and Tumorigenesis

2.1 Hypoxia inducible factors (HIF) and TME

HIF-1 transcription factor activation is one of the widely studied pathways in the TME. HIF-1 has two subunits, an oxygen-sensitive α-subunit (HIF-1α) and a constitutively expressed β-subunit (HIF-1β)[9]. Among them, HIF-1α is a major regulator of hypoxia signal transduction and is widely expressed[10]. The α subunits degrade rapidly under normal oxygen conditions but remain stable during hypoxia[11]. The β subunit was constitutively expressed, not regulated by intracellular oxygen concentration, and had no transcriptional activity alone. Only the heterodimer of HIF-1α and HIF-1β subunits was active[12]. Under normal oxygen conditions, HIF-1α undergoes hydroxylation of conservative proline residues 402 and 564 by the prolyl hydroxylase domain (PHD)[13]. Subsequently, von protein (pVHL) mediates HIF-1α ubiquitination, followed by degradation by the proteasome[14].
Under anoxic conditions, the hydroxylation and acetylation of HIF-1α were inhibited, thereby stabilizing HIF-1α and allowing it to form a dimer with HIF-1β. The dimer then combines with CREB-binding protein (CBP)/p300, forming a transcription initiation complex and activating target genes[15]. In recognition of their contributions to the study of hypoxia, William G. Kaelin, Peter J. Ratcliffe, and Gregg L. Semenza were awarded the 2019 Nobel Prize in Physiology or Medicine.

Saurabh et al. reviewed the role of HIF in tumor progression, specifically as a fuel for cancer progression[9]. Chen et al. reviewed that intratumoral hypoxia had a major impact on the development and progression of breast cancer (BC)[16]. Instead of exerting a limited regional impact, hypoxia created a positive macroenvironment for BC. HIF-1 was broadly induced under BC hypoxia, activating the transcription of multiple oncoproteins. Zhang et al. showed that HIF mediated a cascade of molecular events that enabled cancer cells to adapt and multiply[17]. In summary, hypoxia was beneficial to cancer cell growth in the TME. In most tumors within the TME, the hypoxic environment leads to the production of HIF-1α[18]. In an anoxic environment, activated HIF-1α increased the activity of Snail and Twist, which reduced the expression of E-cadherin and promoted EMT[19]. Goyette and his colleagues[20] found that interference with AXL (a member of the TAM receptor tyrosine kinase family) reduced HIF-1α levels during hypoxia, thereby altering the hypoxia response. This led to a reduction in the production of key cytokines involved in hypoxia-induced EMT, invasion, and macrophage behavior, ultimately enhancing the antitumour microenvironment and immunotherapy response. Mut homologue 6 (MSH6) is an overexpressed oncogene in glioblastoma (GBM). The expression of β-lactamase forms an anoxic TME in GBM, thus promoting EMT, proliferation, migration, invasion and angiogenesis and ultimately promoting the development of GBM[21]. Collectively, the pivotal role of HIF-1α in driving tumorigenesis and promoting tumor progression under hypoxic conditions has been extensively elucidated. Consequently, HIF-1α has emerged as a promising candidate for targeted cancer therapy, holding immense potential in the realm of oncological interventions.

2.2 HIF in HCC

Tumor hypoxia is a unique and critical environmental factor for tumor cells to survive under insufficient oxygen supply by modifying tumor cell metabolism through hypoxia, but normal cells cannot survive in the hypoxic TME. However, hypoxia could induce genomic changes, enabling tumor cells to adapt to the adverse microenvironment characterized by hypoxia and malnutrition so that living cell subsets with the genetic mechanism required for malignant tumor progression could survive[22, 23]. HCC is a highly metabolized tumor that consumes more oxygen than surrounding normal tissues[12]. In recent years, the in-depth study of the hypoxic microenvironment has provided new ideas for the treatment and possible prevention strategies of liver cancer[24, 25]. Zheng and his colleagues[26] found that HIF-1α overexpression was associated with poor overall survival (OS) and disease-free survival (DFS). HIF-1α is mainly involved in promoting tumor proliferation[27], migration, invasion and angiogenesis[28], as well as EMT[29], glycolysis
regulation[30] and lipid metabolism[31], involving various signaling pathways (Table 1).

HIF-1α, through the IL-8/nuclear factor kappa B (NF-κB) axis, promoted the migration and invasion of hepatoma cells. In addition, HIF-1α-activated TM4SF1-AS1 plays an important role in promoting the proliferation, migration and invasion of liver cancer cells by enhancing the expression of TM4SF1[32]. HIF-1α-induced EMT is a key process related to metastasis. Ma et al. found that activated hepatic stellate cells promoted the upregulation of transglutaminase 2 (TGM2) in HCC cells through inflammatory signals, leading to HIF-1α accumulation. This results in a pseudohypoxic state and promotes EMT in HCC cells[29]. Reprogramming of lipid metabolism has become a sign of cancer. Recently, it was reported that HIF-1α was related to this process. Fatty acid binding protein 5 (FABP5) promoted HIF-1α synthesis and disrupted the FIH/HIF-1α interaction, enhancing HIF-1α's ability to promote lipid accumulation and cell proliferation in HCC cells[31].

2.3 ncRNAs and TME

Additional studies have shown that ncRNAs participate in intercellular communication[39] and regulate the activation, proliferation and cytokine secretion of tumor immune cells[40], thus affecting tumor invasion, metastasis and immune escape. Many ncRNAs have been found to play an important role between HCC cells and the TME. HIF-1α is involved in the regulation of the hypoxia response and could be used as the central hub for regulating multiple cancer markers. Therefore, exploring the interaction between ncRNAs and HIF-1α has become a promising target for anticancer therapy.

It was reported that more than 1000 target genes were affected by HIF-1α regulation to mediate the hypoxia-induced phenotype[6]. ncRNAs regulated by hypoxia signals are called hypoxia-responsive ncRNAs (HRNs). According to their interaction with the HIF-1α complex, HRNs can be divided into those participating in HIF-1α-mediated direct regulation and those participating in HIF-1α-mediated indirect regulation[14]. miRNA was the most studied subgroup of ncRNAs. Hypoxia-responsive miRNAs (HRMs) show promising carcinogenic or tumor suppressive functions in the occurrence and development of cancer[41]. LncRNA expression could be altered by hypoxia, which in turn regulates HIF-1α activity through a variety of mechanisms, such as chromatin modification, RNA stability and protein stability, and regulation of the transcriptional activity of HIF-1α[42]. It has also been shown that lncRNAs can act as competing endogenous RNAs (ceRNAs) for miRNAs to regulate the expression of related mRNAs at the posttranscriptional level[43], including HIF-1α mRNA. The mechanisms of ncRNA regulation of HIF-1α expression are summarized in Table 2.

3. NF-κB signaling regulates the TME of HCC
The NF-κB family is involved in a large number of biological processes, including inflammation, the immune response, and the regulation of cellular homeostasis\[58, 59\]. These biological phenomena are closely related to the occurrence, progression, and metastasis of various malignant tumors. Additionally, NF-κB plays multiple roles in the TME, including promoting tumor cell growth and invasion, suppressing immune surveillance, and promoting angiogenesis\[60, 61\]. Therefore, NF-κB signaling is not only an important regulatory pathway of inflammation and immunity but also a key factor in malignancy (Figure 1). The NF-κB transcription factor family consists of five major subunits, including Rel (c-Rel), RelB, p65 (RelA, NF-κB3), p50 (NF-κB1) and p52 (NF-κB2), all of which have an N-terminal fragment containing approximately 300 amino acid residues, known as the Rel homeodomain (RHD). Among them, p65, c-Rel and RelB had c-terminal transactivation domains (TADs), which conferred the ability to activate NF-κB and actively regulate its expression. Although p50 and p52 lacked transcriptional activation domains, their homodimers inhibited transcription. The correlation between activation of the NF-κB pathway and hypoxic conditions, particularly in relation to HIF, has been shown to be a major mediator of the hypoxic response that promotes cancer progression \[62, 63\]. Additionally, the NF-κB signalling pathway is one of the most important signalling pathways involved in physiological and pathological conditions. It is always quiescent in normal tissues and activated in a variety of inflammatory diseases and tumors\[64\]. Growing evidence suggests that dysregulated NF-κB signaling enhances cancer cell proliferation and metastasis and mediates radio- and chemoresistance \[65-67\].

Sustained activation of NF-κB is responsible for tumorigenesis, metastasis, tumor evasion, resistance to apoptosis, angiogenesis and proliferation in HCC \[68\]. NcRNAs have been found to regulate the NF-κB signaling pathway in different settings, and our laboratory has also reviewed the regulatory role and clinical significance of ncRNAs in NF-κB signaling in cancer \[69\].

3.1 Proliferation and apoptosis

Xie et al \[70\] found that lncRNA-PDIA3P1 was upregulated in HCC, and its higher levels were associated with recurrence and survival rates in human HCC. Additionally, upregulation of PDIA3P1 was significantly associated with elevated tumor necrosis factor receptor-associated factor 6 (TRAF6), p-p65, and NF-κB downstream anti-apoptotic genes in human HCC tissues. Mechanistically, PDIA3P1 bound to miR-125a/b and miR-124 and thereby deregulated their inhibitory effects on TRAF6, activating the NF-κB signaling pathway to confer chemoresistance \[70\]. Yang et al.\[71\] investigated the regulatory role of miR-20a on NF-κB in Huh7 HCC cells and its effect on the sensitivity of Huh7 cells to chemotherapeutic drugs. It was found that miR-20a activated the NF-κB signaling pathway and decreased the expression of apoptosis-related proteins by upregulating the expression of the proteins Livin and Survivin, which attenuated the sensitivity of cells to chemotherapeutic drugs and reduced the level of apoptosis \[71\]. The expression of miR-26b was significantly downregulated in human liver cancer tissues compared to paraneoplastic
tissues. By forcibly upregulating miR-26b, it was found to inhibit cell proliferation and induce apoptosis, exerting an anticancer effect. Next, upregulation of miR-26b could significantly inhibit the NF-κB pathway and thus suppress tumor growth in human HCC[72]. Wen et al. found that the expression of miR-27b in HCC was lower than that in adjacent nontumor tissue (ANT)[73]. The decreased expression of hsa-miR-27b was associated with poor survival among HCC patients. They demonstrated that miR-27b acted as an inhibitor of the NF-κB pathway in HCC by targeting transforming growth factor-activated kinase-binding protein 3 (TAB3). Furthermore, miR-27b significantly inhibited HCC cell proliferation.

3.2 Epithelial-mesenchymal transition (EMT), migration and invasion

The expression of miR-605-3p was downregulated in HCC tissues compared to paraneoplastic tissues, and the OS and DFS rates were lower in HCC patients with low miR-605-3p expression than in those with high miR-605-3p expression. Additionally, immunofluorescence and protein blotting analysis revealed that miR-605-3p inhibited EMT and attenuated the activation of NF-κB signaling in HCC cells, thus exerting its oncogenic function [74]. It was demonstrated that lncRNA fragment cancer susceptibility candidate 2 (CASC2) was downregulated in human HCC tissues and HCC cell lines compared to paraneoplastic tissues and the normal hepatocyte line LO2. By downregulating its expression, it was found to significantly promote migration and invasion of HCC cells. Mechanistically, CASC2 was found to regulate hepatocellular carcinogenesis by targeting miR-362-5p and thereby inhibiting the NF-κB pathway [75]. To investigate the role of lncRNA and NF-κB in the regulation of cancer metastasis, Chen and colleagues [76] identified the lncRNA that interacts with NF-κB, NKILA, which was found to be downregulated in HCC tissues and cell lines, and its reduced levels were associated with poor prognosis in HCC patients. In addition, NKILA inhibited the migration and invasion of HCC cells in vitro and in vivo. Mechanistically, NKILA blocks the Slug/EMT pathway by inhibiting the phosphorylation of IκBα, p65 nuclear translocation and NF-κB activation [76].

4. Regulation of the TME by ncRNAs in HCC

4.1 Role of ncRNAs in TME homeostasis

4.1.1 NcRNAs regulate cancer-associated fibroblasts (CAFs) in the TME of HCC

Fibroblasts were identified as the predominant cell population in solid tumors and were stimulated by various factors secreted by tumor cells or immune cells, leading to their transformation into CAFs. CAFs, recognized as a distinct subset of activated fibroblasts within the TME[77], play a crucial role in tumor growth, proliferation, and metastasis as one of the most abundant and critical components of the tumor mesenchyme. Studies have demonstrated the impact of CAFs on the malignant progression, metastasis, drug resistance, and recurrence of HCC[78]. Through a comparison between primary cultured CAFs and noncancerous fibroblasts (NF) obtained from resected HCC specimens of the same patient, it was observed that CAFs significantly enhanced HCC cell proliferation, migration, and invasion. The upregulation of CXCL11 in HCC tissues and CAFs has been reported, and CXCL11...
secreted by CAFs was found to promote HCC cell proliferation and migration. To explore the specific mechanism, Liu et al. identified that LINC00152 exerted a positive regulatory effect on CXCL11 expression in CAFs through direct binding to miR-11-205p. This regulatory axis influences the proliferation and migration abilities of HCC cells in vitro and the growth of HCC tumors in vivo [79]. A growing body of evidence suggests that cellular interactions between cancer cells and surrounding stromal cells within the TME play a crucial role in modulating cancer progression and treatment response [80, 81]. CAFs were identified as key contributors to the promotion of human cancer growth, invasion, metastasis, and therapy resistance through exosome-mediated cellular communication [80]. It was found that exosomal LINC TUG1 derived from CAFs promoted the migration, invasion, and glycolysis of HepG2 cells. However, these effects were attenuated by miR-524. Mechanistically, SIX1 was identified as a target gene of miR-524, and the inhibition of SIX1 abolished the promoting effects of miR-524-5p inhibitors on migration, invasion, and glycolysis[82]. Qi et al. reported that CAFs exerted oncogenic effects on HCC cells through the transfer of exosomes carrying miR-20a-5p[83]. Mechanistically, LIM domain and actin-binding 1 (LIMA1) was identified as a tumor suppressor in HCC, and miR-20a-5p was found to act as an oncogene in HCC. Furthermore, it was observed that miR-20a-5p was present in CAF-derived exosomes that were transferred from CAFs to HCC cells, leading to the suppression of LIMA1 expression[83]. CAF-derived extracellular vesicles (EVs) were shown to promote tumor progression through the delivery of miRNA. Zhang et al. found that CAF-derived EVs could promote the proliferation, migration, invasion potential, and resistance to sorafenib in HCC cells [84]. Specifically, miR-1228-3p carried by CAF-EVs was found to enhance the chemoresistance of HCC by activating the placenta-associated 8 (PLAC8)-mediated PI3K/AKT pathway[84]. CAFs have been recognized for their contribution to tumor progression, with miRNAs playing a crucial role in regulating the tumor-promoting properties of CAFs. In a review conducted by Zheng et al., the dysregulated expression of miRNAs in HCC-CAFs and their oncogenic characteristics were examined. The study revealed that miR-101-3p and miR-490-3p were downregulated in HCC-CAFs, and their common target gene was identified as TGFBR1 [85]. The downregulation of miR-101-3p and miR-490-3p, along with the upregulation of TGFBR1, were found to be associated with a poor clinical prognosis in HCC patients. Furthermore, increased expression of TGFBR1 was correlated with the infiltration of immunosuppressive immune cells such as MDSCs, M2 macrophages, and Treg cells[85].

4.1.2 NcRNAs affect extracellular matrix (ECM) remodelling

The ECM is recognized as one of the most crucial components of the TME and consists of protein components, including collagen, fibronectin, glycosaminoglycans, and proteoglycans. It serves as a significant tissue barrier against tumor invasion and metastasis [60]. The ECM exhibits a highly dynamic network structure, and matrix metalloproteinases (MMPs) play a vital role in the remodelling and turnover of the ECM. MMPs act as key regulators in multiple tumor pathological processes[86, 87]. NcRNAs were reported to participate in ECM remodelling by regulating the
expression of MMPs[88, 89]. Wang et al. discovered that aspirin decreased the level of the tumor suppressor miRNA let-7g by inhibiting the lncRNA LMCD1-AS1, which acted as a sponge. Consequently, this inhibition enhanced the targeting of let-7g on its target gene, prolyl 4-hydroxylase (P4H), thereby exerting inhibitory effects on tumor growth in HCC and collagen deposition. These findings revealed a novel role and regulatory mechanism of aspirin in inhibiting HCC through the disruption of abnormal collagen deposition[90]. Excessive accumulation of ECM can lead to hepatic fibrosis (HF), where hepatic stellate cells (HSCs) are the main cells involved. In a study by Xu et al., miRNA-708 regulated HSC activation and enhanced ECM accumulation by directly targeting transmembrane protein 88 (TMEM88). These findings provide a potential target for future research on the process of liver fibrosis[91]. In a study conducted by Wang et al., miR-22-3p and miR-29a-3p acted as fibrosis inhibitors and synergistically inhibited hepatic fibrosis (HF). Serine/threonine kinase 3 (AKT3) was identified as the common target gene of these two miRNAs[92]. This study provided new insights into the regulation of AKT3 expression in HF and opened up new possibilities for miRNA-based therapeutic regimens for HF. Similarly, Zhang et al. found that the expression of the long noncoding RNA SNHG16 was significantly increased in HCC tissues and cell lines and was associated with poor prognosis in HCC patients[93]. Mechanistically, SNHG16 promoted the malignant behavior of HCC cells by activating the ECM-receptor interaction pathway[93]. ECM remodelling requires the concerted action of multiple proteolytic enzymes and their endogenous inhibitors, among which tissue inhibitor of metalloproteinases 2 (TIMP2) plays an important role. Alan and colleagues found that TIMP2 was frequently and significantly downregulated in human HCC, and this downregulation was associated with aggressive tumor behavior and poorer patient prognosis[94]. Mechanistically, TIMP2 suppression in a hypoxic environment was induced through a regulatory feedback circuit consisting of HIF-1α, miR-210 and HIF-3α[94]. Cao et al. found that abnormal expression of miR-324-5p in HCC cells was involved in cell migration and invasion. Overexpression of miR-324-5p reduced the expression of E26 transformation-specific 1 (ETS1) and specificity protein 1 (SP1) and potentially inhibited ECM degradation by suppressing MMP2 and MMP9 in HCC[95]. Therefore, miR-324-5p could be considered a potential new target for the treatment of invasive HCC.

4.2 NcRNAs and angiogenesis in HCC

Angiogenesis plays a pivotal role in the pathophysiology of cancer and is intricately regulated by diverse components within the TME[96]. A large amount of vascularization was observed in rapidly growing tumors at an early stage, which was the significance of tumors in tumor treatment proposed by Judah Folkman[97]. Excessive proliferation of tumor cells leads to an increase in oxygen consumption, and when the tumor mass exceeds the blood supply, the tumor becomes hypoxic. Hypoxia induces the production of angiogenic factors, leading to enhanced angiogenesis[98]. Hypoxia-induced HIF-1α was stable and promoted the upregulation of several angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), and platelet-derived growth factor (PDGF)[99].
Liu et al. identified that miR-138-5p targeted HIF-1α and regulated the expression of HIF-1α and vascular endothelial growth factor A (VEGFA), thereby inhibiting angiogenesis in HCC[47]. Overall, miRNAs were identified to be involved in different stages of tumor progression, and some of them even played an important role in regulating multiple cancer features, making them promising targets for cancer therapy that deserve further exploration.

Angiogenesis is essential for the occurrence, progression and metastasis of HCC. To investigate the biological function of the long-chain noncoding RNA nuclear paraspeckle assembly transcript 1 (NEAT1) in HCC, Guo et al.[100] detected elevated levels of NEAT1 and reduced levels of miR-125a-5p in HCC tissues and cells. The dual luciferase reporter gene assay showed that NEAT1 bound to miR-125a-5p, which in turn bound to VEGF. NEAT1 enhanced VEGF angiogenesis in HCC by regulating competitive endogenous RNA (ceRNA) for miR-125a-5p in the AKT/mTOR and ERK pathways. Fei and coworkers[101] detected that upregulation of MYLK-AS1 was associated with enhanced angiogenesis and tumor progression in HCC tumor tissues and cell lines. MYLK-AS1 acted as a ceRNA that regulated angiogenesis in HCC, and miR-424-5p was identified as a direct target of MYLK-AS1. Mechanistically, MYLK-AS1 promoted tumor progression and angiogenesis by targeting the miR-424-5p/E2F7 axis and activating the VEGFR-2 signaling pathway in HCC. Oncogenic MALAT1 exerted an antiangiogenic effect in HCC by sponging miR-3064-5p as a competitive ceRNA with its attenuated inhibitory effect on the FOXA1/CD24/Src pathway[102]. These findings suggest that angiogenesis plays an important role in rapid tumor growth and metastasis [97]. Wu et al. [103] identified a downregulated circRNA, circ_0004018, in HCC by RT-qPCR. Through a series of functional assays, we found that overexpression of circ_0004018 significantly inhibited angiogenesis in HCC. Mechanistically, circ_0004018, activated by estrogen receptor 1 (ESR1), inhibited angiogenesis in HCC by binding to FUS and stabilizing TIMP2 expression.

4.3 NcRNAs and immune modulation in the TME

In the TME, immune cells are the most abundant cellular component and have been the target of interest due to their potent cytotoxicity[104-106]. Macrophages are one of the major components of the innate immune system and are responsible for pathogen clearance and antigen presentation. Tumor-associated macrophages (TAMs), the most abundant immune cells in the TME, are critical for cancer initiation and progression [105]. Based on various stimuli, macrophages were acknowledged to undergo polarization into either the M1 phenotype characterized by antitumour activity or the M2 phenotype characterized by pro-tumour activity[60]. M2-like polarized TAMs represent a predominant subset of infiltrating immune cells in HCC, demonstrating substantiated evidence of profound immunosuppressive properties and protumoral effects[107]. Yu et al found that exosomal miR-21-5p derived from HCC cells directly targeted the ras homolog family member B (RhoB) 3’-untranslated region (UTR), downregulating RhoB levels, which weakened mitogen-activated protein kinase (MAPK) axis signaling pathways and induced macrophage M2 polarization[108]. Kupffer cells (KCs) have been recognized for their crucial role in
HCC through intricate communication with various immune cell populations, thereby exerting a protective effect against HCC development. Liu et al. found that the polarization of KCs towards the M2 phenotype was a pivotal factor contributing to the pathogenesis of HCC in AKT/Ras mice[109]. Notably, the dysregulation of miRNA-206 was observed to promote the M1 polarization of KCs, thereby facilitating the augmented infiltration of CD8 T cells and exerting a protective effect against HCC progression[109]. These significant findings underscored the potential of miRNA-206 as a promising immunotherapeutic intervention for HCC. A previous study demonstrated that miRNA-15a/16-1 exhibited the capacity to mitigate immune suppression by interfering with C-C motif chemokine 22 (CCL22)-mediated intercellular communication between KCs and regulatory T cells (Tregs)[110]. This modulation of the KC-Treg interaction highlights miRNA-15a/16-1 as a prospective immunotherapeutic approach for HCC. CD8$^+$ T cell dysfunction is a critical factor in HCC immune escape. Hu et al. discovered upregulated expression of circCCAR1 in HCC samples and cell lines, promoting HCC growth and development in vitro and in vivo[111]. The circCCAR1/miR-127-5p/Wilms tumor 1-associated protein (WTAP) feedback loop enhanced HCC proliferation and metastasis. Exosomal circCCAR1 from HCC cells impaired activated CD8$^+$ T cells by stabilizing PD8 protein, suggesting therapeutic potential in targeting exosomal circCCAR1 or cell division cycle and apoptosis regulator 1 (CCAR1) for improving HCC immunotherapy[111]. Zhang et al. identified a significant upregulation of LINC01132 expression in HCC with a concurrent association with poorer OS in HCC patients[112]. Functionally, LINC01132 overexpression was found to exert promotive effects on HCC cell growth, proliferation, invasion, and metastasis. Mechanistically, silencing of LINC01132 resulted in CD8$^+$ T cell infiltration, implicating its role in modulating the tumor immune microenvironment[112]. Moreover, the combined approach of LINC01132 knockdown and anti-PDL1 treatment demonstrated enhanced antitumour immunity, highlighting the potential of this novel therapeutic combination for HCC.

In summary, ncRNAs play multiple roles in the TME, which could promote or inhibit the immune system and angiogenesis, increase the permeability of endothelial cells, promote cancer metastasis, and cause ECM remodelling, which together support tumor progression. The communication between cells and the TME mediated by ncRNAs is shown in Figure 2.

5. Conclusion and Prospects

Hepatocarcinogenesis is a multifactorial process in which ongoing liver injury and concurrent regeneration might produce an environment that ultimately leads to hypoxia and inflammation, which are key features of the liver TME[113, 114]. Under hypoxic conditions, HIF-1α is an important transcription factor that mediates the effects of hypoxia on the adaptive regulation of tumor cells and the TME[115]. The feasibility of using HIF-1α as a therapeutic target has been demonstrated in a number of studies, suggesting that interventions that alter HIF-1α activity by direct or indirect
means could be effective in the treatment of HCC. In their review, Shant et al. provided a comprehensive analysis of diverse potential novel therapeutic agents for HCC treatment[23]. These agents include hypoxia-activated prodrugs, HIF inhibitors, nanomaterials, antisense oligonucleotides, and natural compounds, all of which specifically target the HIF/hypoxia signaling pathway in HCC. Their findings underscored the promising potential of HIFs as effective therapeutic targets in the management of HCC.

NcRNAs have emerged as key regulators of posttranscriptional activation in cancer. miRNAs, among the most extensively studied, were found to be significantly dysregulated in HCC, thereby promoting tumor progression [116]. Furthermore, alongside miRNAs, other ncRNAs, such as lncRNAs that predominantly function as miRNA sponges, were implicated in modulating sorafenib resistance by regulating EMT and stemness in HCC[117]. No studies have reported a role for circRNAs in sorafenib resistance; however, circRNAs are involved in regulating the stemness characteristics of HCC cells [118]. Several ncRNA biomarkers or therapeutic targets could be highly specific for a single liver disease, considering the tissue-specific expression of ncRNAs, thus enabling rapid diagnosis and improved management of HCC. These findings provide new insights into ncRNA-mediated interactions between the HCC microenvironment, metabolism and tumor cell state, thereby further enhancing our understanding of ncRNA-mediated cell state transitions in sorafenib resistance.

Due to the important role of the HIF pathway in conferring survival and resistance to cancer cells, the search for HIF inhibitors is critical to overcome the chemotherapy resistance that is observed in many cancers. Over the years, direct and indirect HIF inhibitors have been identified and evaluated in clinical trials at various stages[119]. Equally promising were miRNA mimics (agomiRs) that supplemented tumor suppressor miRNAs and/or miRNA inhibitors (antagomiRs) targeting oncomiR-dependent tumor sites. Some of these inhibitors demonstrated promising response rates in patients, although many were still in early clinical trials. However, challenges persisted concerning the specificity, stability, and short half-life of the target molecules. Hence, it is imperative that we amplify our endeavors to surmount the challenges hindering the translation of our present knowledge into clinical applications. By doing so, we can broaden our horizons and unlock new therapeutic strategies against HCC.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References


25. Ocana MC, Martinez-Poveda B, Quesada AR, and Medina MA, Metabolism within the


microRNA-142-3p restrains the progression of hepatocellular carcinoma.


60. Ma Z, Shuai Y, Gao X, Wen X, and Ji J, *Circular RNAs in the tumour*


68. Mohan CD, Bharathkumar H, Dukanya, Rangappa S, Shanmugam MK,


Legends:
Figure 1. Regulation of NF-κB signaling by ncRNA in the HCC TME

Figure 2. The communication mediated by ncRNAs between tumour cells and the TME
For Peer Review

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Table 1 Role and potential mechanism of HIF-1α in HCC

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<th>Function</th>
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<td>Migration</td>
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<td>Invasion</td>
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<td>PFKFB3/HIF-1α feedback loop</td>
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<td>Cancer</td>
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Highlights:

Noncoding RNAs, including miRNAs, lncRNAs, and circRNAs, constitute a large portion of the human transcriptome and play important roles in regulating HCC tumorigenesis. Here, we discuss the role of ncRNAs in hepatocarcinogenesis, epithelial-mesenchymal transition (EMT), and angiogenesis in hypoxic microenvironments, as well as the interactions between ncRNAs and key components of the TME.

(1) NcRNA regulates cancer-associated fibroblasts (CAF) in HCC TME

(2) NcRNA affects extracellular matrix (ECM) remodeling

(3) NcRNA affects angiogenesis in HCC
Cancer-Associated Fibroblasts (CAFs) → non-coaing RNA → Tumor cells → non-coaing RNA → Angiogenesis

Extracellular Matrix (ECM) → non-coaing RNA → Tumor cells → non-coaing RNA → Immune cells

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