

The Role of miRNA-21 in the Metastasis of Hepatocellular Carcinoma as a Therapeutic Target

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ABSTRACT

Hepatocellular carcinoma (HCC) is a crucial health concern worldwide, representing a leading cause of cancer-related mortality and the most common form of primary liver cancer. The aggressive nature of HCC is mainly due to its high intention for invasion and metastasis, processes that are regulated by a complex network of genetic and molecular pathways. Among the critical regulators of these processes is microRNA-21 (miR-21), a small non-coding RNA that has been implicated in various oncogenic activities. This review provides a comprehensive analysis of the role of miR-21 in promoting HCC metastasis progression, with a particular focus on its interaction with key signaling pathways, including the PTEN/PI3K/AKT, PDCD4/AP-1, RECK/MMP, and TIMP-3 axes. By targeting tumor suppressors, miR-21 facilitates epithelial-to-mesenchymal transition (EMT), invasion, and metastasis of HCC cells. Understanding the molecular mechanisms regulated by miR-21 not only sheds light on the pathogenesis of HCC but also highlights possible therapeutic targets for combating this aggressive cancer.

Keywords: Hepatocellular carcinoma; Cancer; Epigenetics; miR-21

INTRODUCTION

Cancer is a collection of illnesses distinguished by the unregulated proliferation and division of abnormal cells with the capability of invading surrounding tissues and spreading via the lymphatic system and bloodstream to other body organs, a process known as metastasis¹. This uncontrolled growth arises when cells bypass the normal regulatory mechanisms that govern cell division and death, leading to the formation of tumors. Genetic mutations and epigenetic alterations often transform normal cells into cancerous cells. These

changes can result from various factors, including environmental carcinogens, radiation, and viral infections²⁻⁴. Several types of primary liver cancer exist, such as intrahepatic cholangiocarcinoma, hepatocellular carcinoma, hepatoblastoma, and fibrolamellar carcinoma, depending on where the cancer originates⁵⁻⁸.

HCC is the first common liver cancer caused by genetic changes in liver cells or hepatocytes. In 2018, Hepatocellular carcinoma (HCC) accounted for the third-highest number of cancer-related deaths globally, with over 780,000 fatalities recorded.

Annually, around 800,000 new HCC cases are diagnosed⁹. As one of the main characteristics of this disease, we can point to its high progression among all cancers, and on the other hand, the survival rate of this cancer is extremely low^{10, 11}.

Hepatocellular carcinoma is characterized by three types of clinical symptoms, which can occur in 90 to 95 percent of people with the disease: Upper and right-side abdominal pain, palpable bumps, and weight loss. Liver enlargement and abnormality are also evident, as well as weakness, fatigue, indigestion, anorexia, shortness of breath, vomiting, enlarged spleen, fever, and jaundice. Once in advanced and final stages, patients exhibit ascites, peripheral edema, and hepatic encephalopathy¹².

Various risk factors contribute to HCC formation in patients, one of which is a chronic infection with hepatitis B, which establishes its compromising effects through HBV-associated immune pathogenesis¹³. Similarly, HCV is capable of inducing HCC through chromosomal changes in fibrotic liver cells, causing tumors. HCV also destroys DNA during cirrhosis, and it causes chronic inflammation, which switches the signaling pathway of TGF- β from inhibiting tumorigenesis to fibrosis and cancerous hepatocytes¹⁴. Furthermore, consuming alcoholic beverages serves as a risk factor for HCC. Alcohol's metabolites are in charge of toxic effects on the liver, affecting intracellular signaling pathways and controlling gene expression, which all cause fat accumulation, fibrogenesis in liver cells, and innate and adaptive immunity activation¹⁵. Fungal infections such as *Aspergillus Flavus* bring about toxic product formation in the human body. Aflatoxin B1 is one of the most tumorigenic poisons, and it is capable of leading to HCC complications through the inhibition of cell growth and immune system changes¹⁶. Moreover, Recent research has shown that 2.38-13% of people suffering from HCC were due to nonalcoholic fatty liver disease that causes HCC through inducing liver cirrhosis¹⁷. Besides, obesity is another factor affecting HCC^{18, 19}. The occurrence of fatty liver disease in industrialized countries is closely related to a person's diet. Diets high in vegetables, white meat, and fish can lower the risk of liver cancer, whereas diets high in red meat products increase the risk^{20, 21}. In addition to

diet, there is a robust and significant link between diabetes mellitus and HCC in the way that hyperinsulinemia, hyperglycemia, activation of insulin-like growth factor pathways, and insulin resistance have been implicated in HCC emergence²². Furthermore, smoking can increase inflammatory and pro-inflammatory products such as TNF- α , IL-1, and IL-6, which are able to damage liver cells. Smoking significantly suppresses the P53 protein, a tumor suppressor factor²³. Studies have also indicated the foremost role of age in HCC occurrence in a way that people of higher ages are more susceptible to getting HCC than younger ones²⁴.

Beyond these established risk factors, increasing attention has been directed toward epigenetic regulators, particularly microRNAs (miRNAs). MiRNAs are small, non-coding RNAs that post-transcriptionally regulate gene expression by binding to target messenger RNAs. They are essential for a wide range of biological processes, including cell proliferation, differentiation, apoptosis, and stress responses. Dysregulation of specific miRNAs has been strongly implicated in cancer development and progression, where they can act as either oncogenes or tumor suppressors.

Among these, miR-21 has emerged as one of the most consistently upregulated oncogenic miRNAs in HCC. MiR-21 contributes to the aggressive phenotype of HCC by targeting multiple tumor suppressor genes and driving processes such as epithelial-to-mesenchymal transition (EMT), invasion, and metastasis. Given the lack of effective treatment options and the high mortality rate of HCC, understanding the molecular mechanisms of miR-21 offers new opportunities for therapeutic intervention. Therefore, this review aims to comprehensively examine the role of miR-21 in invasion, migration, and metastasis of HCC through its effects on key cellular pathways.

MicroRNA-21

Cellular RNAs can be broadly classified into messenger RNAs (mRNAs), which encode proteins, and non-coding RNAs, which do not. Among non-coding RNAs, miRNAs represent a well-studied class of small, endogenous RNAs that regulate gene

expression at the post-transcriptional level. MiRNAs typically bind to complementary sequences in the 3' untranslated region (UTR) of target mRNAs, leading either to mRNA degradation or translational repression²⁵. Through this mechanism, miRNAs regulate approximately 30% of human genes, influencing diverse biological pathways. They play essential roles in tumorigenesis, metabolic reprogramming, and the altered energy requirements of rapidly dividing tumor cells^{26,27}. Accordingly, dysregulation of miRNAs can contribute to uncontrolled cell proliferation and cancer development, while normal miRNA function is also crucial for maintaining cellular homeostasis²⁸⁻³¹.

Among these, microRNA-21 (miR-21) is one of the most extensively studied oncogenic miRNAs. The *MIR21* gene, located on chromosome 17q23.2 in humans, encodes miR-21, which is highly conserved across species. MiR-21 regulates multiple cellular processes, including differentiation, apoptosis, and proliferation, by binding to the 3' UTRs of specific mRNAs. Beyond its role in normal physiology, miR-21 exhibits anti-apoptotic properties, enhances cell survival under stress, and modulates immune responses. It also contributes to developmental processes by influencing tissue differentiation and organogenesis³².

However, aberrant upregulation of miR-21 is strongly associated with a variety of diseases, particularly cancer, where it functions as an oncogene by downregulating multiple tumor suppressor genes^{32,33}. Dysregulated expression of miR-21 has been reported in HCC, as well as in breast, lung, colorectal cancers, and multiple myeloma. In these malignancies, miR-21 promotes cancer progression by regulating pathways involved in cell cycle control, apoptosis resistance, invasion, and migration. Notable targets of miR-21 include Phosphatase and Tensin Homolog (PTEN), Tropomyosin 1 (TPM1), Programmed Cell Death Protein 4 (PDCD4), B-cell lymphoma 2 (BCL2), and various Matrix Metalloproteinases (MMPs)³⁴⁻³⁶.

In the context of HCC, genomic studies have consistently identified miR-21 overexpression as a driver of tumor progression^{37, 38}. By inhibiting tumor suppressors such as PDCD4 and KLF5, miR-21 facilitates EMT, invasion, and metastatic

dissemination of HCC cells³⁹. Collectively, these findings highlight miR-21 as a central regulator of hepatocarcinogenesis and an important candidate for therapeutic targeting.

Invasion, migration, and metastasis of cancer cells

Despite advances in cancer diagnosis and therapy, metastasis remains the primary cause of cancer-related mortality worldwide⁴⁰⁻⁴². Metastasis is defined as the dissemination of tumor cells from the primary site to distant organs, where they establish secondary tumors. This process underlies nearly 90% of cancer deaths and represents one of the most malignant hallmarks of cancer⁴³. Targeting metastasis remains a major clinical challenge because it depends on complex interactions between tumor cells and their surrounding microenvironment⁴⁴.

In HCC, local invasion, the initial step of metastasis, is observed in a considerable proportion of patients^{45, 46}. Once HCC cells acquire invasive capacity, they can infiltrate adjacent tissues, enter the bloodstream or lymphatic system, and subsequently colonize distant organs. Figure 1 illustrates both the major risk factors contributing to HCC development and the central role of miR-21 in facilitating invasion and metastasis. To reduce the morbidity and mortality associated with HCC, it is crucial to identify and inhibit the molecular drivers of invasion and metastatic spread. The following subsections describe the mechanisms by which miR-21 promotes these processes through regulation of multiple signaling pathways.

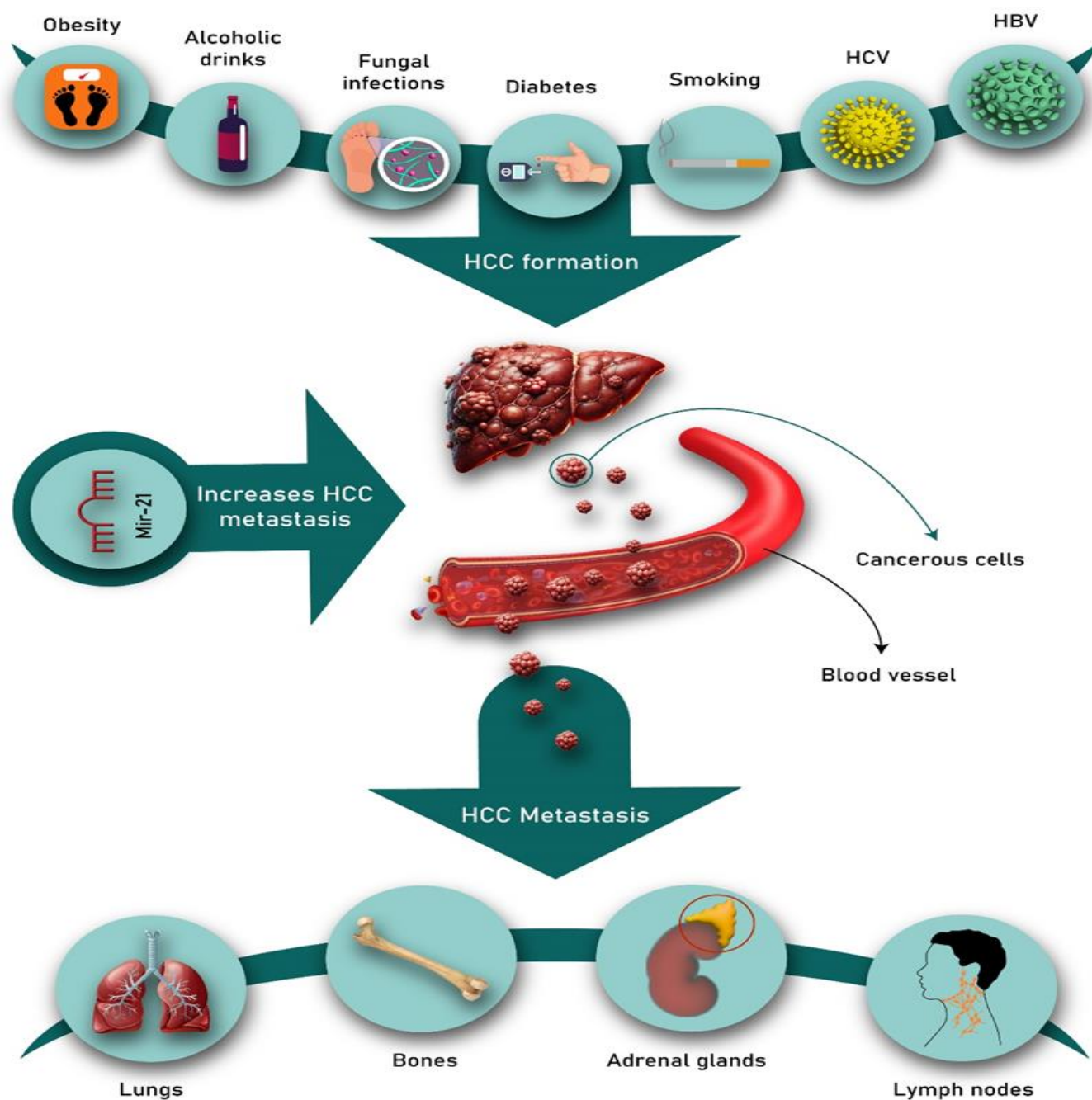


Figure 1. Several factors contribute to HCC formation, including obesity, drinking alcoholic beverages, fungal infections, diabetes, smoking, HCV, and HBV. Besides the potential of HCC to spread out in the body, miR-21 acts as a contributor to cancerous cell migration through blood vessels, ultimately having them spread to other body organs, encompassing lungs, bones, adrenal glands, and lymph nodes, forming secondary tumors.

The role of miR-21/PDCD4/c-jun/AP-1/MMP-2, 9-axis in HCC metastasis

To understand the role of miR-21 in HCC metastasis, it is first necessary to consider the contribution of MMPs. These proteolytic enzymes degrade components of the extracellular matrix (ECM), thereby facilitating tumor cell invasion into surrounding tissues and entry into the bloodstream^{47,48}. MMPs are also involved in angiogenesis, apoptosis, and inflammation^{49,50}. Among the 25 known MMPs, gelatinases (MMP-2 and MMP-9) are of particular importance. They degrade type IV collagen, fibronectin, and other ECM proteins, promoting tumor invasion⁵¹. Excessive activity of MMP-2 and MMP-9 disrupts normal cellular pathways, accelerates tumor progression, and supports angiogenesis through the induction of pro-angiogenic factors such as tumor necrosis factor- α (TNF- α) and vascular endothelial growth factor (VEGF)^{52, 53}.

The expression of MMP-2 and MMP-9 is tightly regulated by intracellular signaling pathways. One critical regulator is Programmed Cell Death Protein 4 (PDCD4), a tumor suppressor that inhibits the activity of the transcription factor AP-1. Under normal conditions, PDCD4 prevents AP-1-mediated upregulation of MMPs, thereby limiting invasion and metastasis. However, when miR-21 is overexpressed, it directly targets and suppresses PDCD4, removing this inhibitory control⁵⁴. The result is increased AP-1 activity, which in turn elevates the transcription of MMP-2 and MMP-9, driving invasion and angiogenesis.

Beyond this linear regulation, a feedback loop amplifies the oncogenic effects of miR-21. Activated AP-1 can further enhance miR-21 expression, creating a self-reinforcing cycle in which miR-21 suppresses PDCD4, AP-1 activity rises, and MMP expression continues to increase^{34,55}. This positive feedback loop accelerates tumor progression, angiogenesis, and metastasis in HCC.

Taken together, the miR-21/PDCD4/AP-1/MMP axis plays a pivotal role in promoting HCC invasion and metastasis. Importantly, this pathway exemplifies how miR-21 amplifies oncogenic signaling by targeting tumor suppressors, thereby creating a

cycle of sustained MMP activation and aggressive tumor behavior.

The KLF5-inhibitory effect of miR-21 on HCC metastasis

Kruppel-like transcription factors (KLFs) are a family of transcriptional regulators with diverse roles in cancer. KLF5, in particular, has been reported to function as a tumor suppressor in certain contexts. It regulates cell growth, cell cycle progression, and angiogenesis, and its overexpression has been associated with improved prognosis in HCC⁵⁶. Importantly, miR-21 directly targets KLF5 by binding to its 3'-UTR, leading to reduced expression and loss of tumor-suppressive activity. Wang et al.³⁹ demonstrated that miR-21 overexpression in HCC tissues and cell lines enhanced the invasive potential of cancer cells through KLF5 inhibition. Conversely, restoring KLF5 expression counteracted miR-21's pro-invasive effects, highlighting the central role of the miR-21/KLF5 axis in suppressing or promoting HCC progression.

However, other studies suggest that KLF5 may act as a tumor promoter in HCC. An et al.⁵⁷ reported that higher KLF5 levels correlated with poorer clinical outcomes in patients. Mechanistically, KLF5 has been shown to activate the PI3K/AKT/Snail pathway and induce EMT, a process in which epithelial cells acquire mesenchymal characteristics, thereby increasing their invasive and migratory potential⁵⁷⁻⁵⁹. KLF5 also enhances the expression of mesenchymal markers (Vimentin, N-Cadherin, Snail) while reducing epithelial markers (E-cadherin), and it can stimulate MMP-2 and MMP-9 expression, further promoting invasion. Intriguingly, Wang et al.³⁹ also reported evidence that miR-21 may inhibit tumor growth by downregulating KLF5, suggesting a context-dependent dual role.

Taken together, these findings indicate that miR-21 exerts complex effects on HCC by modulating KLF5. While some data support KLF5 as a tumor suppressor inhibited by miR-21, other studies implicate KLF5 as an oncogenic factor. This duality highlights the need for further research to clarify the precise contribution of the miR-21/KLF5 axis in HCC progression.

The role of miR-21/PTEN/PI3K/AKT/EMT pathway in HCC metastasis

One of the most vital signaling pathways hyper-activated in highly proliferative cancers is the Phosphatidylinositol 3-kinase/Activated Protein Kinase B (PI3K/AKT) pathway. It normally regulates EMT, which is principally responsible for the transient transformation of epithelial cells into mesenchymal phenotypes⁶⁰. The transition from Epithelial to mesenchymal phenotype can be mediated by AKT, which has been shown to enhance the expression of Snail, a mesenchymal marker⁶¹ contributing to metastasis⁶². AKT becomes constitutively activated, leading to enhanced cell survival and proliferation, contributing to tumor progression^{63,64}, once PTEN, one of the most important tumor suppressor genes⁶⁵, is mutated or inactivated, as commonly seen in various cancers. AKT activation enhances glucose uptake and metabolism, which is crucial for the energy demands of rapidly proliferating cancer cells^{63,66}. PTEN is able to negatively regulate PI3K, which undertakes the role of promoting cell cycle progression and preventing apoptosis, thereby supporting tumor growth^{67,68}. Hence, higher levels of activated PI3K following partial or complete inactivation of PTEN are detectable in various cancers and have tumor cells increased and spread out⁶¹.

MiR-21, a widely-known regulator of PTEN, attenuates its function, inducing AKT activation and higher EMT function. It also causes a raised level of PI3K, resulting in tumor growth. Thus, miR-21 brings about the metastasis of HCC cells through the PI3K/AKT/PTEN pathway⁶¹. Additional signaling pathways have shown an association with miR-21 in the induction of cancer cells' metastasis, such as RHOB/PI3K/AKT/NF- κ B/MMP pathway. Several studies have directly examined the association between miR-21 and PTEN in HCC. Meng et al. first demonstrated that inhibition of miR-21 in HCC cells restored PTEN expression, leading to decreased proliferation, migration, and invasion⁶⁹. More recently, He et al. reported that in sorafenib-resistant HCC cells, miR-21 was upregulated, resulting in reduced PTEN levels and activated AKT signaling; inhibition of miR-21 re-sensitized these cells to sorafenib by restoring autophagy through the

PTEN/AKT pathway⁷⁰. In addition, exosomal miR-21 has been shown to contribute to tumor progression by transferring oncogenic signals between cells: Cao et al. found that exosomal miR-21 downregulated PTEN and its pseudogene PTENp1, increased p-AKT, and enhanced proliferation and migration of HCC cells⁷¹. Collectively, these findings support the critical role of the miR-21/PTEN/PI3K/AKT axis in promoting EMT and metastasis, and further highlight its potential as a therapeutic target in HCC.

The role of miR-21/RHOB/PI3K/AKT/NF- κ B/MMP pathway in HCC metastasis

RhoB, known as the Ras homolog gene family member B, belongs to the Rho family of small GTPases, which are essential regulators of a variety of cellular processes, including cell morphology, adhesion, and migration. It regulates actin organization and vesicle transport⁷². The overexpression of RhoB inhibits tumor formation and assists in metastasis prevention by repressing the Ras/PI3K/AKT pathway, which plays a foremost role in cell migration⁷³. This pathway is able to induce Nuclear Factor Kappa B (NF- κ B) activation, contributing to hepatocyte survival and protection against cell death. The continuous NF- κ B activation is strongly correlated with unfavorable prognostic features and properties of stemness in HCC⁷⁴. Notably, NF- κ B activation induces MMP expression, further enhancing invasion and metastasis^{75,76}. This mechanism complements the previously described role of MMPs in HCC progression^{75,76}. PI3K/AKT can also indirectly induce MMP production through NF- κ B at a post-transcriptional level, and in the case of RhoB overexpression, this pathway is restricted⁷³. Intriguingly, RhoB was identified by Connolly et al. in 2010 as miR-21's target⁷⁷. Hence, researchers found that the decrease in RhoB expression caused by miR-21 contributes to the amplification of various in vitro cell characteristics linked to the heightened metastatic capability of the cells^{73,77,78}.

Several studies beyond the current discussion have directly explored the functional interplay between miR-21 and RhoB across different biological contexts. In cancer cell lines (Huh-7, HepG2, and MDA-MB-231), overexpression of miR-21 promotes metastatic traits, such as enhanced invasion and

migration, by directly targeting RhoB, thereby suppressing its tumor-suppressive functions⁷⁹. In endothelial cells, miR-21 has been shown to inhibit angiogenic processes: it reduces RhoB expression and impairs migration and tubulogenesis, acting via a conserved binding site within the RhoB 3'UTR⁸⁰. Additionally, in models of pulmonary hypertension, miR-21 negatively regulates RhoB and Rho-kinase activity; mice lacking miR-21 display elevated RhoB levels and pronounced pulmonary hypertensive manifestation⁸¹.

Putting the evidence together, miR-21 has been evidenced to raise HCC metastasis and invasion by preventing RhoB activation. However, h-sulf-1/MAPK/ERK/ZEB1/EMT and PTEN/MAPK/ERK pathways are other well-known pathways that miR-21 affects in the process of HCC aggravation and metastasis.

The role of miR-21/h-sulf-1/MAPK/ERK/ZEB1/EMT and miR-21/PTEN/MAPK/ERK pathways in HCC metastasis

Heparin-degrading Endosulfatase 1 (hSulf-1) negatively regulates growth factor signaling by desulfating and hydrolyzing the sulfate ester bonds of cell surface heparan sulfate proteoglycans (HSPGs), which are one of the main components of the extracellular matrix (ECM). N-acetyl glucosamine residues of HSPG coated on the cell surface must be sulfated to trigger the heparin-binding growth factor signaling. This desulfation interrupts the binding ability of heparin-binding growth factors to their specific receptors, thereby inhibiting cell proliferation and migration. Various cancers, comprising ovarian, breast, and HCC, show low levels of hSulf-1, which is involved in invasion, tumor growth, angiogenesis, and carcinogenesis (82-84). Interestingly, miR-21's level has an inverse association with hSulf-1, and the decrease in hSulf-1 by miR-21 causes higher connections of heparin-binding growth factors, including extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) to their receptors and cell proliferation and migration⁸⁴. The MAPK/ERK pathway takes decisive responsibility for the HCC progression. It regulates cell differentiation, survival, and proliferation, which are essential for tumor

growth and metastasis^{85, 86}. After its activation by the indirect effect of miR-21 reducing hSulf-1, it promotes the EMT process by enhancing the zinc finger E-box binding homeobox 1 (ZEB1), a transcription factor that serves as an oncogene (87). Consequently, an increase in the translation of mesenchymal markers occurs, causing enhanced invasion of tumor cells. Moreover, Bao et al. that miR-21 regulates HCC cellular proliferation, invasion, migration, and tumor extension by suppressing the expression of PTEN separately in HCC cells, as the primary mechanism by which the MAPK/ERK pathway is activated to help cancerous cells spread⁸⁴.

Therefore, the invasion and spread of HCC are attributed to miR-21 by the abovementioned mechanisms and pathways. Thus, targeting these mechanisms can put them in the focus of finding proper treatments for HCC. Additionally, other pathways have been found to aggravate HCC by metastasis induction, including the RECK/MMP pathway under the authority of miR-21.

The role of miR-21/RECK/MMP pathway in HCC metastasis

The reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is a glycoprotein located on the cellular membrane. This glycoprotein is produced less in cancerous cells than in normal ones. By inhibiting RECK, miR-21 indirectly elevates MMP expression and activity, thereby supporting ECM degradation and metastatic dissemination^{88, 89}. Studies have disclosed RECK protein as a miR-21 target. It has been demonstrated in a survey by Zhou et al. that miR-21, in addition to inhibiting PDCD4 and PTEN, can also increase the HCC cells metastasis via the RECK protein inhibition, thereby increasing the activity and expression of the MMPs⁸⁸. Subsequently, MMPs enable the breakdown of the ECM and help HCC cells' metastasis. The explained mechanisms so far are not the only ways miR-21 induces metastasis in HCC patients. TIMP-3 is another protein miR-21 influences to establish its aggravating effects on HCC patients.

The TIMP-3-inhibitory effect of miR-21 on HCC metastasis

As previously mentioned, MMP enzymes, especially MMP-2 and 9, exert metastasis-promoting activities. Researchers have identified two types of endogenous regulators or inhibitors of MMP enzymes: general and specific inhibitors. The general inhibitor of these enzymes is alpha-2-macroglobulin, which inhibits these enzymes in serum and body fluids. There is another group called Tissue Inhibitors of Matrix Metalloproteinases (TIMPs), which are more specific. All MMPs, especially MMP-9 and MMP-2, are adjusted and controlled by TIMPs^{90, 91}. TIMP-1, 2, and 4 are among the secretory proteins secreted by cells, and TIMP-3 is among the extracellular matrix-anchored proteins⁴⁸. The high level of production and secretion of TIMPs reduces tumor cell metastasis through MMP inhibition, thereby preventing the excessive degradation of the ECM⁹². Besides inhibiting MMP enzymes, TIMP-3 also prevents Vascular Endothelial Growth Factor (VEGF) from connecting to Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2), which

consequently prevents angiogenesis and, ultimately, cancer cell metastasis⁹³. A study was published in 2020 in which, for the first time, Li et al. showed that TIMP-3 is one of the targets of miR-21 and, after being targeted, causes the migration and, subsequently, metastasis of HCC cells⁹⁴. Targeting the miR-21/TIMP-3 axis can probably present a therapeutic approach for limiting HCC progression and metastasis. However, it necessitates additional surveys to determine the exact relationship between miR-21 and TIMP-3.

Considering the studies' results reviewed in our study, miR-21's ability to target multiple tumor suppressor proteins, as mentioned previously, underscores its pivotal role in orchestrating the molecular events that lead to HCC progression (Figure 2). Therapeutic strategies aimed at inhibiting miR-21 or restoring tumor inhibitors' expression could potentially disrupt these pathological processes, thereby reducing the metastatic potential of HCC (Table 1).

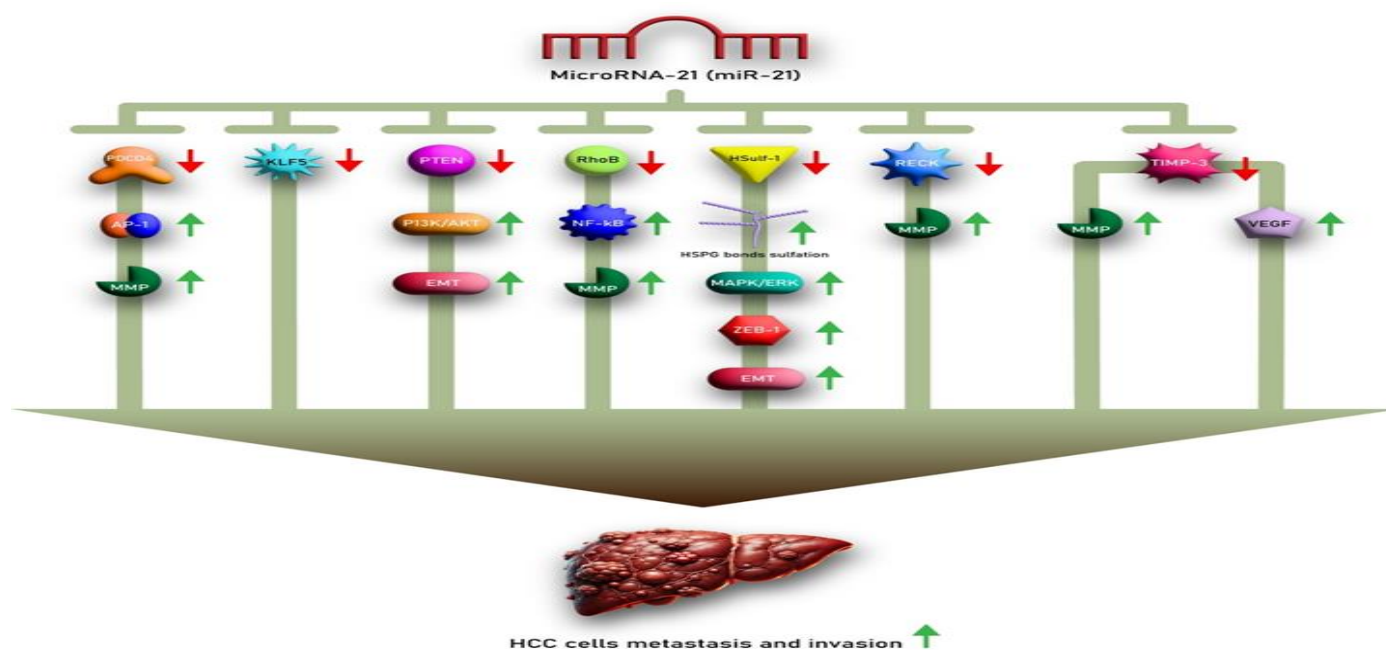


Figure 2. miR-21-associated signaling pathways enrolled in HCC metastasis. A) The inhibitory impact of miR-21 on PDCD4 causes AP-1 activation, and MMP arises as a result. B) Also, KLF5 reduces once miR-21 increases. C) MiR-21 lessens the amount of activated PTEN, further promoting the P13K/AKT pathway, leading to EMT activation. D) RhoB acts as a tumor suppressor reduced by the activity of miR-21, causing NF-κB activation and MMP enhancement. E) the reduction in hSulf-1 following the miR-21 enhancement causes lower desulfation and higher sulfation of HSPG bonds, resulting in MAPK/ERK activation, which mediates ZEB-1 activation and eventually EMT promotion. F) Reck decreases under the impact of miR-21, causing escalated levels of MMPs. G) Lower TIMP-3 amounts after being inhibited by miR-21 heighten MMPs and VEGF. A, B, C, D, E, F, and G processes ultimately lead to increased HCC metastasis.

Table 1. miR-21 direct targets in HCC and downstream consequences

Target (miR-21 direct)	Immediate downstream / pathway nodes	Primary phenotypic effects in HCC	Evidences	References
PDCD4	↓ PDCD4 → ↑ AP-1 (c-Jun) → ↑ MMP-2, MMP-9	Invasion, angiogenesis, metastasis	HCC tissues & cell lines; AP-1 ↔ miR-21 feedback loop	(34, 54, 55)
KLF5	↓ KLF5; context-dependent effects on PI3K/AKT/Snail, MMP-2/9	Invasion/migration; context may vary	HCC tissues & cell lines; mixed tumor-suppressive vs oncogenic reports	(39, 57, 59)
PTEN	↓ PTEN → ↑ PI3K/AKT → ↑ EMT; autophagy suppression; exosomal transfer	EMT, proliferation, invasion/migration; sorafenib resistance	HCC cell lines & tissues; sorafenib-resistant models; exosomal miR-21	(61, 69-71)
RhoB	↓ RhoB → ↑ PI3K/AKT → ↑ NF-κB → ↑ MMP-2/9	Invasion, migration; pro-metastatic traits	HCC cell lines; supportive endothelial/in vivo context for RhoB regulation	(73, 77, 80, 81)
hSulf-1	↓ hSulf-1 → ↑ MAPK/ERK → ↑ ZEB1 → ↑ EMT	Proliferation, migration, EMT	HCC models (cellular and in vivo)	(82-84, 86)
RECK	↓ RECK → ↑ MMP-14/MMP-9/MMP-2/MT1-MMP	ECM degradation, invasion/migration; prognostic implications	HCC cell studies; pathway schematic	(88, 89)
TIMP-3	↓ TIMP-3 → ↑ MMP activity; ↑ VEGF-VEGFR-2 signaling	ECM degradation, angiogenesis, metastasis	HCC cell studies; first report of miR-21 → TIMP-3 in 2020	(93, 94)

Therapeutic Implications of Targeting miR-21

MiR-21 represents a promising therapeutic target in HCC because of its central role in silencing tumor suppressors (e.g., PTEN, PDCD4, TIMP-3) and promoting invasion, migration, and metastasis. Preclinical studies using antisense oligonucleotides (anti-miR-21) have shown robust suppression of HCC growth in vitro and in vivo, with increased apoptosis, reduced clonogenicity, and impaired migration⁹⁵. Locked nucleic acid (LNA)-modified inhibitors, which exhibit high nuclease resistance and binding affinity, further enhance miR-21 knockdown efficiency⁹⁶. Similar strategies targeting other oncogenic miRNAs, such as miR-221, have produced strong anti-tumor effects and survival benefits in HCC models⁹⁷, supporting the clinical feasibility of oligonucleotide-based therapy.

Efficient delivery remains a major challenge. Systemic administration of chemically modified antisense oligos can achieve high liver accumulation, but tumor-selective targeting is needed to reduce off-target effects⁹⁷. Nanocarrier systems, including lipid nanoparticles, polymeric micelles, and exosome-based vehicles, have been developed to improve stability, circulation time, and tumor uptake⁹⁸. These carriers can be engineered to release their miR-21 inhibitors in response to tumor-specific cues (e.g., acidic pH or high glutathione levels), minimizing systemic toxicity. Co-delivery

approaches, demonstrated in other cancers, use sequential release of miR-21 inhibitors followed by chemotherapy to maximize therapeutic synergy⁹⁹. Emerging technologies such as CRISPR/Cas systems offer the possibility of permanently disrupting miR-21 biogenesis, although these methods remain at an early stage and require careful safety evaluation⁸. Small-molecule modulators of miRNA processing may also provide indirect ways to downregulate miR-21³². Overall, combining direct miR-21 inhibition with targeted delivery and conventional therapies—such as kinase inhibitors or immune checkpoint blockade—may provide a precision medicine strategy to control HCC progression and metastasis. Further work should focus on optimizing delivery vehicles, validating long-term safety, and conducting early-phase clinical trials to translate these preclinical advances into effective patient therapies. miR-21 is widely expressed in normal tissues and participates in physiological processes such as wound healing, fibrosis, and immune regulation; therefore, its systemic inhibition carries the risk of off-target effects, including impaired tissue repair, altered immune responses, and hepatotoxicity. Moreover, partial complementarity of antisense oligonucleotides to other miRNAs or mRNAs may trigger unintended gene silencing, complicating interpretation of therapeutic outcomes. Adaptive resistance is another concern, as tumor cells may

compensate for miR-21 inhibition by upregulating other oncogenic miRNAs. These issues highlight the importance of developing tumor-selective delivery systems, conducting detailed toxicology studies, and designing combination therapies that mitigate escape mechanisms. Only through addressing these challenges can miR-21 inhibition be translated from a promising concept into a safe and effective treatment for HCC¹⁰⁰.

CONCLUSION

MiR-21 has emerged as a pivotal factor in the progression of HCC, influencing multiple signaling pathways that drive tumor invasion, migration, and metastasis. By targeting critical tumor suppressor proteins such as PDCD4, PTEN, KLF6, RhoB, RECK, and TIMP-3, miR-21 facilitates the activation of cell growth-inducing factors and the EMT, thereby promoting the aggressive phenotype characteristic of HCC. The intricate regulatory network orchestrated by miR-21 underscores its significance as a biomarker for HCC prognosis and potential therapeutic target. The insights gained from this review emphasize the need for continued research into miR-21's role in HCC, particularly in the context of developing targeted therapies that can effectively disrupt its oncogenic functions. Such therapeutic strategies could significantly improve clinical outcomes by inhibiting the metastatic spread of HCC and enhancing the efficacy of existing treatments. As our understanding of miR-21 and its associated pathways deepens, there is potential to translate these findings into clinical applications, offering new hope for patients with this challenging and deadly disease.

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CONFLICT OF INTEREST

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