[Frontiers in Bioscience, Landmark, 20, 1056-1067, June 1, 2015]

Role of microRNAs in hepatocellular carcinoma

Lin-Na Liu[1], Dan-Dan Li[1], Hui-Xiong Xu[1], Shu-Guang Zheng[1], Xiao-Ping Zhang[2]

[1]Department of Medical Ultrasound, Shanghai Tenth People's Hospital, Tongji University School of Medicine, 200072, Shanghai, China, [2]Department of Nuclear Medicine, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, PR China

TABLE OF CONTENTS

1. ABSTRACT

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer related death worldwide. HCC develops through a multistep process that involves genetic and epigenetic changes. In addition to genetic and epigenetic mechanisms, recent studies have shown that microRNAs (miRNAs) play essential roles in hepatocellular carcinogenesis through the post-transcriptional regulation of tumor associated-genes. In this review, we summarize the role of miRNAs in HCC and its microenvironment, and discuss the implications for HCC therapy.

2. INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer and the third leading cause of cancer-related death worldwide (1). In 2008, approximately 748,300 patients were diagnosed with liver cancer and 695,900 liver-cancer-related deaths occurred worldwide, of which HCC accounted for 70%–85%. HCC is a late complication of chronic liver disease, and is often associated with cirrhosis. The main risk factors for the development of HCC are infection with hepatitis B virus and/or hepatitis C virus, both of which account for 80% of HCC cases. Other risk factors include vinyl chloride, foodstuffs contaminated with aflatoxin B1heavy alcohol intake nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, autoimmune hepatitis and hemochromatosis (2). Hepatocarcinogenesis is a complex and multi-step process resulting from a combination of epigenetic and genetic alterations, such as the activation of cellular oncogenes and/or the inactivation of tumor suppressor genes, and the dysregulation of multiple signal transduction pathways. The major pathways involved in hepatocarcinogenesis include Wnt/β-catenin, p53, Rb, mitogen-activated protein kinase (MAPK), Janus kinase (JAK)/signal transducer and activator of transcription (STAT), phosphatidylinositol 3-kinase (PI3K)/AKT, Hedgehog and growth factors such as epidermal growth factor (EGF) and transforming growth factor-β (TGF-β) (3). As high degree of malignant and prognosis, detection of HCC at an early stage may be important to generate more optional therapeutic strategies and decrease the mortality of this disease by using advanced imaging techniques and molecular biomakers (4,5).

The tumor microenvironment is composed of fibroblasts, endothelial cells, pericytes, immune cells, cancer stem cells, and the surrounding extracellular matrix (ECM) (6). These cell types can produce the non-cellular components of the tumor stroma, including ECM proteins, proteolytic enzymes, growth factors and inflammatory cytokines. An increasing body of literature has provided evidence that the cross-talk between tumor cells and their surrounding microenvironments plays a critical

role in modulating the process of hepatocarcinogenesis, invasion, angiogenesis and metastasis (7).

MicroRNAs (miRNAs) are a class of small, non-coding, single-stranded RNAs that suppress gene expression post-transcriptionally primarily through sequence-specific interaction with the 3′-untranslated regions (3′-UTRs) of cognate mRNA targets (8). Previous studies have highlighted that miRNAs play critical roles during the progression of cancer, such as promoting sustained proliferation, resistance to cell death, angiogenesis and the acquisition of invasive phenotypes (9). In this review, we summarize the biogenesis of miRNAs, their functions and mechanisms in HCC and its microenvironment as well as the implications for HCC therapy.

3. CHARACTERISTICS OF MICRORNAS

Since the discovery of the first miRNA lin-4 in the nematode Caenorhabditis elegans (10,11), 30424 mature miRNAs have been identified in 24521 miRNA loci from 206 species according to the latest miRBase Sequence Database (12), many of them with unknown functions. Based on their locations in the genome, miRNAs can be classified as intergenic, intronic, and exonic miRNAs. As independent transcription units, intergenic miRNAs are transcribed from their own transcriptional units in the intergenic regions of the genome (13). Intronic and exonic miRNAs are located within the introns and exons of host genes (protein-coding or non-protein coding genes), respectively, and hence share common regulatory mechanisms and expression patterns with their host genes (14,15). Approximately half of all miRNAs are encoded by polycistronic transcription units that generate multiple miRNAs. In human cancer, miRNA genes are frequently located at fragile sites, as well as in minimal regions of loss of heterozygosity, minimal regions of amplification (minimal amplicons), or common breakpoint regions, suggesting that miRNAs may play an important role during the progression of human cancer (16). The first report about the function of miRNAs in cancer established that a miRNA cluster located at chromosome 13 (miR-15a/miR-16-1) is frequently deleted or downregulated in chronic lymphocytic leukemia (CLL), and miR-15a/miR-16-1 induces apoptopsis in leukemic cells by targeting the oncogene Bcl-2 (17,18). Since then, numerous studies have described miRNAs that are involved in human cancers including HCC.

4. BIOGENESIS OF MICRORNAS

In the cell nucleus, miRNA genes are initially transcribed by RNA polymerase II as primary miRNAs (pri-miRNAs), which contain a 5′-7-methylguanosine cap, one or several stem loop hairpin structures and a 3′-poly-A tail (19). The hairpin of the pri-miRNA is recognized by the nuclear RNAse-III enzyme Drosha and its obligate RNA-binding protein partner DGCR8 and is cleaved to an approximately 70 nt double-stranded RNA hairpin intermediate (pre-miRNA) by the Drosha-DGCR8 complex (20). Pre-miRNAs are exported from the nucleus to the cytoplasm by exportin 5. Once in the cytoplasm, pre-miRNAs undergo further processing by Dicer, an RNAse III enzyme, and yield imperfect miRNA-miRNA* duplexes (21,22). The miRNA strand becomes a mature miRNA, while most often the miRNA* strand is degraded. The mature miRNA is incorporated into the RNA induced silencing complex (RISC), which is comprised of Dicer, the double-stranded RNA binding factor , and Argonaut protein 2 (23). The miRNA within RISC can bind to the 3′-UTRs of target mRNAs through complementary base pairing, resulting in translational inhibition or mRNA cleavage (24).

5. BIOLOGICAL FUNCTIONS OF MIRNAS IN HCC

5.1. MicroRNAs in proliferation

Increased cell proliferation is a common feature of malignancy. Cell cycle regulators, which include cyclins, cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CDKIs), are strongly implicated in the development and progression of human cancers including HCC (25). Upon mitogenic stimulation, intracellular levels of D-type cyclins (D1, D2 and D3) increase, resulting in the formation and nuclear localization of cyclin D-cyclin-dependent kinase 4 (CDK4) and cyclin D-CDK6 complexes (26). The CDK complexes can phosphorylate Rb and release it from the E2F transcription factor. Activation of E2F transcription factors induces the transcription of G1-S target genes, including the gene encoding cyclin E. This leads to the accumulation of mitogen-independent E-type cyclins, which associate with CDK2 to further phosphorylate Rb, inducing G1-S gene expression and driving cell cycle entry (27).

The role of miRNAs in the control of cell proliferation in HCC is well established. Recent studies showed that they contribute to hepatocellular carcinogenesis by perturbing critical cell cycle regulatory pathways. As a tissue-specific miRNA, miR-122 accounts for 70% of all hepatic miRNAs (28). Silencing of miR-122 is an early event during hepatocarcinogenesis associated with nonalcoholic steatohepatitis (29), and miR-122a is downregulated in approximately 70% of HCCs and in all HCC-derived cell lines (30). Overexpression of miR-122 inhibits the growth of hepatoma cells by targeting cyclin G1 and E2F1 (31,32). In addition to its function as a cell cycle regulator, miR-122 inhibits cell proliferation by directly targeting oncogenes such as AKT3 and TCF-4 (28,33). MiR-520b, miR-193b and miR-195 cause cell cycle arrest at G1 phase by directly targeting cyclin D1 in human HCC cells (34-36).

On the other hand, the expression of negative regulators of the cell cycle can be downregulated by miRNAs in HCC. An important class of cell cycle inhibitors, CDK inhibitors, can be categorized into two families, namely the p16 family (p15, p16, p18 and p19) and the p21 family (p21, p27, p28 and p57) (37). The tumor suppressor p21 (Cip1) is a major transcriptional target of the p53 protein and is necessary for cell cycle arrest. In HCC, miR-423 promotes cell growth and regulates G1/S transition by targeting p21Cip1/Waf1 (38). p27Kip1, a target of miR-221, is frequently down-regulated in HCC (39). Upregulation miR-221 and downregulation of p27Kip1 are significantly associated with tumorigenesis (40).

5.2. MicroRNAs in apoptosis

Apoptosis or programmed cell death is essential for organ development and tissue homeostasis. Aberrant regulation of apoptosis is linked to multiple human cancers including HCC. Apoptotic programs occur through two pathways: a mitochondrial-dependent pathway (also known as the intrinsic pathway) and a death receptor-dependent pathway (also known as the extrinsic pathway) (41).

The stimuli that initiate the intrinsic pathway can induce permeabilization of the outer mitochondrial membrane and the release of cytochrome c (cyt c) into the cytoplasm. Once released, cyt c binds to the caspase adaptor Apaf-1, changing its conformation (42). Via the adaptor molecule, Apaf-1, cyt-c and caspase-9 form a complex, which, in turn, activates downstream effector caspases and triggers a caspase cascade that includes caspases-3, 6 and 7, leading to DNA fragmentation and cell death (43). The extrinsic apoptosis pathway is induced by the binding of death ligands to their appropriate death receptors (DRs) on the cell surface. To date, six DRs have been identified: TNFR1 (TNFRSF1A), Fas (also known as CD95, APO-1 or TNFRSF6), DR3 (TNFRSF12), DR4 (also known as TRAILR1 or TNFRSF10A), DR5 (also known as TRAILR2 or TNFRSF10B) and DR6 (TNFRSF21) (44). The most important ligand-death receptor system is composed of TNF-TNFR1 and the Fas ligand FasL.

To date, most of the apoptosis-associated miRNAs that have been identified in HCC belong to the intrinsic pathway. The Bcl-2 (B-cell leukemia/lymphoma 2) family of proteins, which play an important role in controlling the intrinsic pathway, is composed of anti-apoptotic proteins (including Bcl-2, Bcl-XL, Mcl-1, Bfl-1/A1, Bcl-W, Bcl-G) and pro-apoptotic proteins (including Bax, Bak, Bok, Bad, Bid, Bik, Bim, Bcl-Xs, Krk, Mtd, Nip3, Nix, Noxa, Bcl-B) (45). Bcl-2 proteins localize or translocate to the mitochondrial membrane and modulate apoptosis by altering the inner and/or outer membrane permeability, leading to or preventing the release of cyt c. Many miRNAs that regulate apoptosis by targeting Bcl-2 have been identified in HCC. The miR-15a/miR-16 cluster, which is located at the 13q14 chromosome, induces apoptosis by targeting the oncogene Bcl-2 (18). A recent study showed that the hepatitis B virus inhibits apoptosis of hepatoma cells by sponging the miRNA-15a/16 cluster and upregulating the expression of Bcl-2 (46). Moreover, other miRNAs, such as miR-34a, miR-125b and miR-29, have also been shown to directly target Bcl-2 and promote hepatoma cell apoptosis (47-49). In addition to Bcl-2, miR-215b also promotes apoptosis by downregulating the expression of Mcl-1, Bcl-w and IL-6R in HCC (50).

MiRNAs can also regulate apoptosis of HCC by targeting other apoptosis related signaling pathways. The tumor suppressor miR-122 induces cell apoptosis in HCC by directly targeting the Wnt/β-catenin pathway (51). MiR-26a, which is frequently downregulated in HCC tissues, can promote apoptosis by targeting the interleukin-6-Stat3 pathway in human HCC (52).

5.3. MicroRNAs in invasion and metastasis

Metastasis is a complex multi-step process that involves the recruitment of blood vessels through the secretion of angiogenic factors and an increase in cell motility and invasion caused by the secretion of matrix metalloproteinases or epithelial-mesenchymal transition (EMT). The invasive tumor cells pass through the blood vessels (intravasation) and enter the circulatory system. The circulating tumor cells evade the immune system and avoid anoikis during their dissemination and extravasation, and reach an appropriate colonization site in a distant organ, where they undergo metastatic growth (53).

EMT is a pivotal cellular program in which cells lose cell-cell and cell-matrix contacts, gain invasive ability and become motile mesenchymal cells. These processes are stimulated by extracellular cytokines, such as TGF-β, HGF, FGF and EGF, or intracellular EMT-transcription factors, such as ZEB1, ZEB2, Snail1, Slug and Twist (54). Many miRNAs act as crucial regulators of the EMT process and metastasis in HCC.

MiR-21 is highly expressed in HCC tumors and cell lines, and increased miR-21 expression levels are correlated with poor prognosis in patients with HCC (55,56). Overexpression of miR-21 promotes cancer progression, invasion and metastasis by regulating the activity of PTEN and the hSulf-1-mediated AKT and ERK pathways in HCC (57). MiR-10b is dysregulated in some types of cancer and plays an important role in invasion and metastasis (58,59). Li *et al*. showed that miR-10b is highly expressed in metastatic HCC tissues and cell lines. Overexpression of miR-10b promotes invasion and metastasis of HCC by targeting CADM1 (60).

Many miRNAs play a significant role in suppressing invasion and metastasis in HCC. The tumor

suppressor p53 prevents EMT by downregulating the expression of ZEB1 and ZEB2, and this modulation is mediated by the upregulation of miR-200 and miR-192 family members (61). In addition, miR-141 suppresses both the growth and motility of HCC cells by targeting ZEB2 (62). The upregulation of miR-148a in HCC was first reported by Yuan *et al*., and anti-miR-148a suppressed cell proliferation, cell cycle progression, cell migration, anchorage independent growth in soft agar and subcutaneous tumor formation in SCID mice. However, subsequent studies showed that miR-148a is silenced by HBx or hypermethylation in HCC, and that overexpression of miR-148a in hepatoma cells reduced growth, EMT, invasion, and metastasis by targeting HPIP and repressing the AKT/ERK/FOXO4/ATF5 pathway (63,64). Zhang *et al*. showed that miR-148a is significantly decreased in HCC tissues, and restoration of miR-148a expression significantly repressed the migration and pulmonary metastasis of hepatoma cells by targeting c-met (65). The opposite phenotype in miR-148a may result from the use of different systems. The AKT signaling pathway is also a functional target of miR-612 and miR-7. Overexpression of miR-612 or miR-7 is associated with human hepatoma cell invasion and metastasis (66,67).

6. THE ROLE OF MICRORNAS IN THE TUMOR MICROENVIRONMENT

6.1. Impact of microRNAs on angiogenesis

Similar to normal tissues, tumor cells require nutrients and oxygen to support their growth. The microenvironment of solid human tumors is characterized by heterogeneity in oxygenation (68), and the tumor-associated neovasculature, which is generated by the process of angiogenesis, meets these needs. Tumor angiogenesis is a complex process that is regulated by many factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), angiopoietin-1 (Ang1) and Ang2, and FGF. Antiangiogenic factors include TSP-1, endostatin, angiostatin, calreticulin and interferon (69).

VEGF is a potent stimulator of angiogenesis under both physiological and pathological conditions and is highly expressed in most solid tumors, including HCC. VEGF family proteins, which function as ligands for the VEGF tyrosine kinase receptor superfamily, include VEGF-A, -B, -C,-D, -E and -F, with splice variants of VEGF-A resulting in several different isoforms (70). In HCC, miR-26a expression is inversely correlated with VEGF-A expression. Overexpression of miR-26a inhibits the expression of VEGF-A and angiogenesis *in vivo* and *in vitro*. The anti-angiogenic effect of miR-26a is mediated mainly though the regulation of the PI3K/AKT/HIF/VEGF-A pathway (71). However, miR-26a also targets the hepatocyte growth factor-c-Met pathway, thus suppressing VEGF-A production to promote angiogenesis in HCC (72). In addition to miR-26a, miR-195 and miR-503 suppress angiogenesis in HCC by directly inhibiting the expression of VEGF (71,73,74).

MiRNAs can also regulate angiogenesis by targeting angiogenesis related genes. In a genome-wide search for deregulated miRNAs in human HCC, Shih *et al*. showed that miR-214, which is upregulated in other human cancers, was uniquely downregulated in human HCC. Downregulation of miR-214 was associated with increased tumor recurrence and poor clinical outcomes. MiR-214 suppresses angiogenesis by targeting HDGF (75). MiR-29b, which is downregulated in HCC tumor tissues, suppresses tumor angiogenesis, invasion, and metastasis by regulating matrix metalloproteinase 2 expression (76). MiR-125b plays an anti-angiogenic role in HCC by inhibiting PIGF (77).

In addition to their cell autonomous functions, the non-cell-autonomous roles of angiogenesis-related miRNAs have been described in HCC. Endothelial cells (ECs) are critical for angiogenesis. By co-culturing a highly metastatic human HCC cell line (HCCLM3) with HUVECs, Zhu *et al*. showed that HCCLM3 cells enhanced the angiogenic activity of HUVECs by upregulating miR-146a expression. Further study confirmed that miR-146a promotes the angiogenic activity of HUVECs by directly suppressing the expression of BRCA1 and in turn upregulating the expression of PDGFRA (78).

6.2. Regulation of tumor cell immunophenotype by microRNAs

Although the presence of immune infiltrates of variable content in human solid tumors has long been established, the prognostic value of these components remains controversial. Accumulating data suggest that local immune infiltrates strongly influence tumor biology through various factors (79). Immune cells include T and B lymphocytes, natural killer (NK) cells, NK-T cells, dendritic cells (DCs), macrophages, neutrophils, eosinophils and mast cells (80). CD4+CD25+ regulatory T cells (Tregs) are a minor but functionally unique population of T cells that play a significant role in immune homeostasis, immune tolerance and the control of autoimmunity. In contrast to CD8+ CTLs, which generally exert a suppressive effect on tumor growth, Tregs have a positive effect on tumor growth through the suppression of antitumor immune cells. The accumulation of Tregs concurrent with a significantly reduced infiltration of CD8(+) T cells was observed in tumor regions compared with non-tumor regions in patients with HCC (81). An increase in tumor-infiltrating Tregs was found to be associated with poor overall survival in patients with HCC (82). CCL-22, which is secreted by macrophages and dendritic cells upon stimulation with microbial products, recruits Treg cells to promote tumor growth and metastasis by modulating the immune response (83). miR-34a is downregulated by TGF-β and suppresses the expression of CCL-22 in HCC cells, whereas overexpression of miR-34a

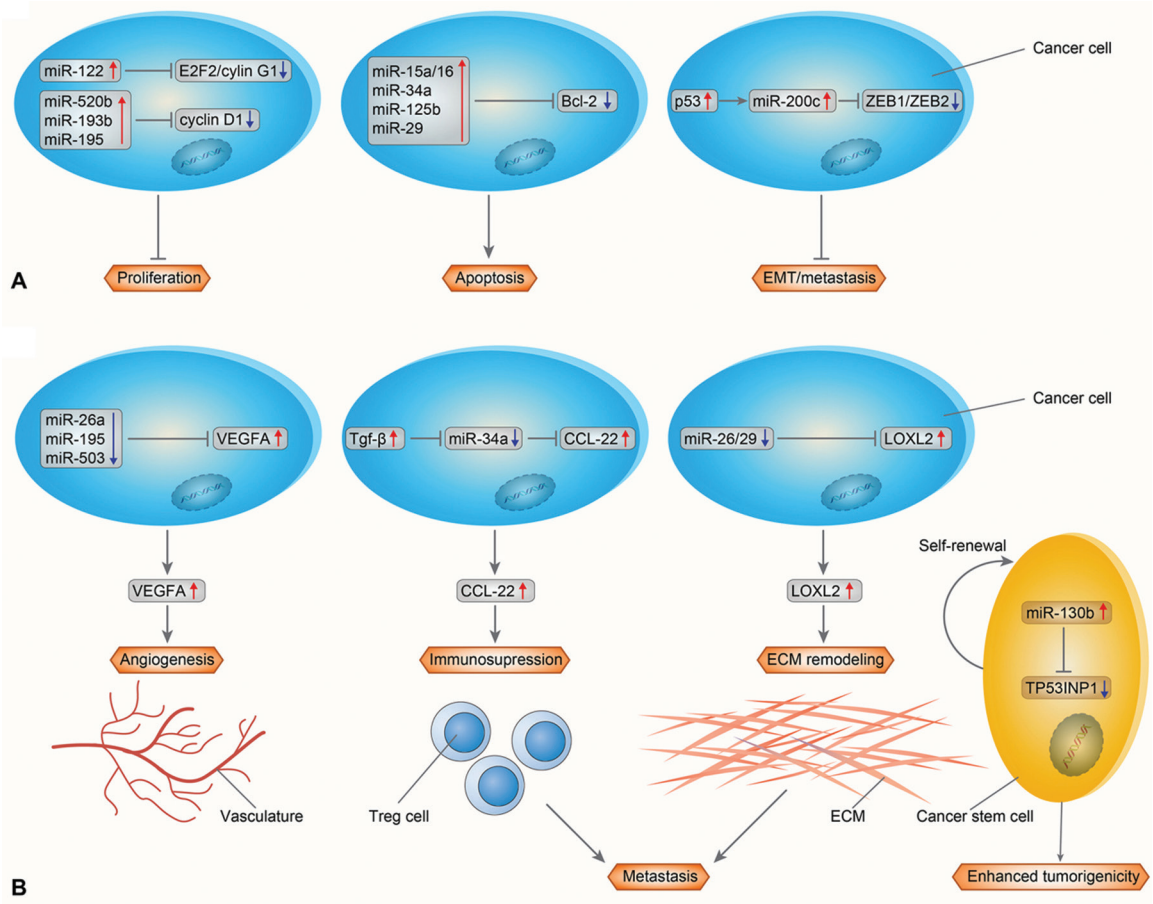

Figure 1. The functions of miRNAs in HCC and its microenvironment. (A) Many miRNAs regulate proliferation, apoptosis and metastasis by targeting their related genes in HCC. (B) Roles of different miRNAs in the regulation of the tumor microenvironment. The effect of miRNAs on the tumor microenvironment is mediated by the regulation of cancer-activating cytokines and chemokines in HCC, or by the direct induction of phenotypic changes to promote HCC progression in stroma cells.

decreases the migratory activity of Tregs *in vitro* and the accumulation of Tregs *in vivo*, resulting in an anti-metastatic effect (84). Foxp3, a crucial transcription factor in Tregs, is upregulated in HCC activated-Tregs. Chen *et al*. showed that miR-182-5p, miR-214-3p, miR-129-5p and miR-30b-5p are upregulated in HCC-activated Tregs compared to normal Tregs (85). These data suggest that miRNAs play important roles in the process of Treg cell infiltration in HCC.

6.3. Modulation of the extracellular matrix by microRNAs

The ECM is composed of approximately 300 proteins, including fibrous proteins, glycoproteins, and proteoglycans (86). These components make up both the basement membrane and the interstitial matrix. During tumor progression, changes in the composition of the ECM strongly influence tumor and stromal cell properties, such as proliferation and motility. Collagens are the most abundant proteins in the ECM and provide a structural support for cells. Collagens can promote cell migration and proliferation in HCC. Let-7g is a tumor suppressor miRNA that is significantly associated with poor survival in HCC. Overexpression of let-7g inhibits HCC cell migration and growth by downregulating COL1A2 (87).

Lysyl oxidase (LOX) is a secreted copper-dependent amine oxidase that catalyzes the covalent cross-linking of the component side chains of collagen and elastin (88). The Lox family includes five members: LOX, LOX-like 1 (LOXL1), LOX-like 2 (LOXL2), LOX-like 3 (LOXL3), and LOX-like 4 (LOXL4) (89). LOXL2 is significantly overexpressed in tumor tissues and the sera of HCC patients. It remodels collagen to promote HCC cell adhesion in the tumor microenvironment and metastatic niche formation, and it is downregulated by miR-26/29 in HCC (90).

6.4. The role of microRNAs in liver cancer stem cells

Cancer stem cells (CSC), a subpopulation of tumor cells possessing stem cell properties such as

self-renewal and differentiation, play an important role in sustaining tumor formation and growth (91). CSCs, which also have undefined characteristics, consist of a small population within tumors that is highly tumorigenic, metastatic, chemotherapy and radiation resistant and responsible for tumor relapse after therapy (92). In HCC, several CSC markers have been identified, such as epithelial cell adhesion molecule (EpCAM), CD133, CD90, CD44, CD24 and CD13 (93). Previous studies have shown that several signaling pathways, such as TGF-β, Wnt/β-catenin, NOTCH and Hedgehog, are involved in stem cell renewal, differentiation and survival in HCC. The functional role of miRNAs in hepatic CSCs has also been reported. CD133 accounts for approximately 1.3.%–13.6.% of cells in human primary HCC. Ma *et al*. used a SYBR Green-based qPCR miRNA array to show that miR-130b is overexpressed in CD133(+) tumor initiating cells (TICs) compared to CD133- cells. Overexpression of miR-130b in CD133- cells increased resistance to chemotherapeutic agents, enhanced tumorigenicity *in vivo*, and increased the potential for self-renewal. Conversely, antagonizing miR-130b in CD133+ TICs had the opposite effect. miR-130b was shown to regulate CD133+ liver TICs in part by targeting TP53INP1 (94). MiR-150 is upregulated in CD133- subpopulations from human primary HCC cells, and induces cell cycle arrest and apoptosis in CD133+ cells by targeting the transcription factor c-Myb (95). Highly invasive epithelial cell adhesion molecule (EpCAM) (+) HCC cells from alpha-fetoprotein (AFP) (+) tumors have the ability to self-renew, differentiate, and initiate aggressive tumors *in vivo*. Ji *et al*. used a global microarray-based miRNA profiling approach to show that conserved miR-181 family members were upregulated in EpCAM (+) HCCs and in EpCAM (+) HCC cells isolated from AFP(+) tumors. Inhibition of miR-181 reduced EpCAM (+) HCC cell quantity and tumor initiating ability, and exogenous miR-181 expression enriched the EpCAM (+) HCC cell population (96).

7. CONCLUSIONS AND THERAPEUTIC PERSPECTIVES

Traditionally, the curative treatment of HCC has involved surgical resection, liver transplantation, or local ablation (97-100), drug treatment for the more advanced stages of HCC has also been attempted in clinical trials (101). Preliminary research indicates that HCC is characterized by global dysregulation of miRNA expression in comparison to the corresponding normal tissues. Increasing evidence suggests that miRNAs, which function as either oncogenes or tumor suppressors, play an important role in the initiation and progression of HCC. The deregulation of one single miRNA is sufficient to trigger global alterations of genetic programs implicated in cell proliferation, differentiation, survival or invasiveness. Therefore, interfering with the function of miRNAs is a promising potential treatment strategy for HCC.

Many *in vitro* and *in vivo* studies have demonstrated the effects of miRNA treatments, such as impairing cell proliferation, invasion and angiogenesis through the introduction of suppressive miRNAs, or increasing apoptosis through the inhibition of oncogenic miRNAs in HCC. miR-122, a specific tumor suppressor miRNA in the liver, is downregulated in HCC. LNP-DP1, which is a cationic lipid nanoparticle formulation, was developed as a vehicle for miRNA delivery. Hsu *et al*. showed that intratumor injection of LNP-DP1 encapsulated miR-122 mimics resulted in approximately 50% growth suppression of HCC xenografts within 30 days (102). The compound Rubone was shown to specifically upregulate miR-34a in HCC cells and to inhibit tumor growth in a mouse xenograft model of HCC, suggesting strong anti-HCC activity (103). By using adeno-associated virus delivery system, Kota J *et al*. showed that systemic administration of miR-26a inhibited hepatoma cell proliferation and induced tumor cell apoptosis in and *in vivo* mouse model (104).

However, the potential side effects should be carefully considered and examined before translating these treatment strategies into the clinic. Hundreds of miRNAs have been reported to be involved in the initiation and progression of tumors. However, ensuring that an effective dose of miRNAs reaches the appropriate target cells and maintaining a sufficient concentration within cells for miRNA exerting its function remain important issues (105). The slowdown of miRNA diffusion in solid tumors caused by higher interstitial fluid pressure and the complex ECM play a significant role in hindering the movement of miRNAs to their target cancer cells (106). In addition, the blood-brain-barrier represents a problem for miRNA therapy involving central nervous system cancer (107). Another problem is off-target effects. Since miRNAs can have multiple downstream targets involved in different signaling pathways via imperfect pairing with 3′-UTRs, unwanted gene silencing of tumor suppressor genes can occur. Such off-target gene inhibition may cause potential toxicities and reduce therapeutic effects.

8. ACKNOWLEDGEMENTS

Lin-Na Liu and Dan-Dan Li are the co-first authors. Hui-Xiong Xu and Xiaoping Zhang are both the co-corresponding authors. This work was supported in part by Grant 14441900900 from Science and Technology Commission of Shanghai Municipality, Grant 81302332, 81301299, 81301229, 81371570 from the National Natural Science Foundation of China, and Grant 2012045 from Shanghai Municipal Human Resources and Social Security Bureau.

9. REFERENCES

1. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular

carcinogenesis. *Gastroenterology* 132(7), 2557-76 (2007)
DOI: 10.1053/j.gastro.2007.04.061

2. Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. *Nat Rev Gastroenterol Hepatol* 7(8), 448-58 (2010)
DOI: 10.1038/nrgastro.2010.100
3. Zender L, Villanueva A, Tovar V, Sia D, Chiang DY, Llovet JM. Cancer gene discovery in hepatocellular carcinoma. *J Hepatol* 52(6), 921-9 (2010)
DOI: 10.1016/j.jhep.2009.12.034
4. Xu HX, Lu MD, Liu LN. Discrimination between neoplastic and non-neoplastic lesions in cirrhotic liver using contrast-enhanced ultrasound. *Br J Radiol* 85(1018), 1376-84 (2012)
DOI: 10.1259/bjr/19932596
5. Zheng SG, Xu HX, Liu LN. Parametric imaging with contrast-enhanced ultrasound: usefulness for characterization of dynamic effects of microvascularization for hepatocellular carcinoma and focal nodular hyperplasia. *Clin Hemorheol Microcirc* 55(3), 375-89 (2013)
Doi not found.
6. Suzuki HI, Katsura A, Matsuyama H, Miyazono K. MicroRNA regulons in tumor microenvironment. *Oncogene* [Epub ahead of print] (2014)
DOI: 10.1038/onc.2014.254
7. Yang JD, Nakamura I, Roberts LR. The tumor microenvironment in hepatocellular carcinoma: current status and therapeutic targets. *Semin Cancer Biol* 21(1): 35-43 (2011)
DOI: 10.1016/j.semcancer.2010.10.007
8. Zhang H, Li Y, Lai M. The microRNA network and tumor metastasis. *Oncogene* 29(7), 937-48 (2010)
DOI: 10.1038/onc.2009.406
9. Jansson MD, Lund AH. MicroRNA and cancer. Molecular oncology 6(6), 590-610 (2012)
DOI: 10.1016/j.molonc.2012.09.006
10. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75(5), 843-54 (1993)
DOI: 10.1016/0092-8674(93)90529-Y
11. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell* 75(5), 855-62 (1993)
DOI: 10.1016/0092-8674(93)90530-4
12. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res* 42(Database issue), D68-73 (2014)
DOI: 10.1093/nar/gkt1181
13. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J* 21(17), 4663-70 (2002)
DOI: 10.1093/emboj/cdf476
14. Ramalingam P, Palanichamy JK, Singh A. Biogenesis of intronic miRNAs located in clusters by independent transcription and alternative splicing. *RNA* 20(1), 76-87 (2014)
DOI: 10.1261/rna.041814.113
15. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. *Genome Res* 14(10A), 1902-10 (2004)
DOI: 10.1101/gr.2722704
16. Calin GA, Sevignani C, Dumitru CD. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A* 101(9), 2999-3004 (2004)
DOI: 10.1073/pnas.0307323101
17. Calin GA, Dumitru CD, Shimizu M. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 99(24), 15524-9 (2002)
DOI: 10.1073/pnas.242606799
18. Cimmino A, Calin GA, Fabbri M. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A* 102(39), 13944-9 (2005)
DOI: 10.1073/pnas.0506654102
19. Lee Y, Kim M, Han J. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 23(20), 4051-60 (2004)
DOI: 10.1038/sj.emboj.7600385
20. Schickel R, Boyerinas B, Park SM, Peter ME. MicroRNAs: key players in the immune system, differentiation, tumorigenesis and cell death. *Oncogene* 27(45), 5959-74 (2008)
DOI: 10.1038/onc.2008.274

21. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. *Science* 303(5654), 95-8 (2004) DOI: 10.1126/science.1090599

22. Chendrimada TP, Gregory RI, Kumaraswamy E. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* 436(7051), 740-4 (2005) DOI: 10.1038/nature03868

23. Liu N, Olson EN. MicroRNA regulatory networks in cardiovascular development. *Dev Cell* 18(4), 510-25 (2010) DOI: 10.1016/j.devcel.2010.03.010

24. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat Rev Genet* 12(2), 99-110 (2011) DOI: 10.1038/nrg2936

25. Matsuda Y, Ichida T. p16 and p27 are functionally correlated during the progress of hepatocarcinogenesis. *Med Mol Morphol* 39(4), 169-75 (2006) DOI: 10.1007/s00795-006-0339-2

26. Steinman RA. Cell cycle regulators and hematopoiesis. *Oncogene* 21(21), 3403-13 (2002) DOI: 10.1038/sj.onc.1205325

27. Bertoli C, Skotheim JM, de Bruin RA. Control of cell cycle transcription during G1 and S phases. *Nat Rev Mol Cell Biol* 14(8), 518-28 (2013) DOI: 10.1038/nrm3629

28. Nassirpour R, Mehta PP, Yin MJ. miR-122 regulates tumorigenesis in hepatocellular carcinoma by targeting AKT3. *PLoS One* 8(11), e79655 (2013) DOI: 10.1371/journal.pone.0079655

29. Takaki Y, Saito Y, Takasugi A. Silencing of microRNA-122 is an early event during hepatocarcinogenesis from nonalcoholic steatohepatitis. *Cancer Sci* (2014) DOI: 10.1111/cas.12498

30. Chang J, Nicolas E, Marks D. miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1. *RNA Biol* 1(2), 106-13 (2004) DOI: 10.4161/rna.1.2.1066

31. Gramantieri L, Ferracin M, Fornari F. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res* 67(13), 6092-9 (2007) DOI: 10.1158/0008-5472.CAN-06-4607

32. Wang B, Hsu SH, Wang X. Reciprocal regulation of microRNA-122 and c-Myc in hepatocellular cancer: role of E2F1 and transcription factor dimerization partner 2. *Hepatology* 59(2), 555-66 (2014) DOI: 10.1002/hep.26712

33. Fan CG, Wang CM, Tian C. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. *Oncol Rep* 26(5), 1281-6 (2011) Doi not found.

34. Xu T, Zhu Y, Xiong Y, Ge YY, Yun JP, Zhuang SM. MicroRNA-195 suppresses tumorigenicity and regulates G1/S transition of human hepatocellular carcinoma cells. *Hepatology* 50(1), 113-21 (2009) DOI: 10.1002/hep.22919

35. Zhang W, Kong G, Zhang J, Wang T, Ye L, Zhang X. MicroRNA-520b inhibits growth of hepatoma cells by targeting MEKK2 and cyclin D1. *PLoS One* 7(2), e31450 (2012) DOI: 10.1371/journal.pone.0031450

36. Xu C, Liu S, Fu H. MicroRNA-193b regulates proliferation, migration and invasion in human hepatocellular carcinoma cells. *Eur J Cancer* 46(15), 2828-36 (2010) DOI: 10.1016/j.ejca.2010.06.127

37. Wu WK, Lee CW, Cho CH. MicroRNA dysregulation in gastric cancer: a new player enters the game. *Oncogene* 29(43), 5761-71 (2010) DOI: 10.1038/onc.2010.352

38. Lin J, Huang S, Wu S. MicroRNA-423 promotes cell growth and regulates G(1)/S transition by targeting p21Cip1/Waf1 in hepatocellular carcinoma. *Carcinogenesis* 32(11), 1641-7 (2011) DOI: 10.1093/carcin/bgr199

39. Fu X, Wang Q, Chen J. Clinical significance of miR-221 and its inverse correlation with p27Kip(1) in hepatocellular carcinoma. *Mol Biol Rep* 38(5), 3029-35 (2011) DOI: 10.1007/s11033-010-9969-5

40. Fornari F, Gramantieri L, Ferracin M. MiR-221 controls CDKN1C/p57 and CDKN1B/

p27 expression in human hepatocellular carcinoma. *Oncogene* 27(43), 5651-61 (2008) DOI: 10.1038/onc.2008.178

41. Lorenzo HK, Susin SA. Therapeutic potential of AIF-mediated caspase-independent programmed cell death. *Drug Resist Updat* 10(6), 235-55 (2007) DOI: 10.1016/j.drup.2007.11.001
42. Iannolo G, Conticello C, Memeo L, De Maria R. Apoptosis in normal and cancer stem cells. *Crit Rev Oncol Hematol* 66(1), 42-51 (2008) DOI: 10.1016/j.critrevonc.2007.09.004
43. Geng YJ. Molecular signal transduction in vascular cell apoptosis. *Cell Res* 11(4), 253-64 (2001) DOI: 10.1038/sj.cr.7290094
44. Gonzalvez F, Ashkenazi A. New insights into apoptosis signaling by Apo2L/TRAIL. *Oncogene* 29(34), 4752-65 (2010) DOI: 10.1038/onc.2010.221
45. Qiao L, Wong BC. Targeting apoptosis as an approach for gastrointestinal cancer therapy. *Drug Resist Updat* 12(3), 55-64 (2009) DOI: 10.1016/j.drup.2009.02.002
46. Liu N, Zhang J, Jiao T. Hepatitis B virus inhibits apoptosis of hepatoma cells by sponging the MicroRNA 15a/16 cluster. *J Virol* 87(24), 13370-8 (2013) DOI: 10.1128/JVI.02130-13
47. Yang F, Li QJ, Gong ZB. MicroRNA-34a targets Bcl-2 and sensitizes human hepatocellular carcinoma cells to sorafenib treatment. *Technol Cancer Res Treat* 13(1), 77-86 (2014) Doi not found.
48. Zhao A, Zeng Q, Xie X. MicroRNA-125b induces cancer cell apoptosis through suppression of Bcl-2 expression. *J Genet Genomics* 39(1), 29-35 (2012) DOI: 10.1016/j.jgg.2011.12.003
49. Xiong Y, Fang JH, Yun JP. Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. *Hepatology* 51(3), 836-45 (2010) Doi not found.
50. Gong J, Zhang JP, Li B. MicroRNA-125b promotes apoptosis by regulating the expression of Mcl-1, Bcl-w and IL-6R. *Oncogene* 32(25), 3071-9 (2013) DOI: 10.1038/onc.2012.318
51. Xu J, Zhu X, Wu L. MicroRNA-122 suppresses cell proliferation and induces cell apoptosis in hepatocellular carcinoma by directly targeting Wnt/beta-catenin pathway. *Liver Int* 32(5), 752-60 (2012) DOI: 10.1111/j.1478-3231.2011.02750.x
52. Yang X, Liang L, Zhang XF. MicroRNA-26a suppresses tumor growth and metastasis of human hepatocellular carcinoma by targeting interleukin-6-Stat3 pathway. *Hepatology* 58(1), 158-70 (2013) DOI: 10.1002/hep.26305
53. Nguyen DX, Massague J. Genetic determinants of cancer metastasis. *Nat Rev Genet* 8(5), 341-52 (2007) DOI: 10.1038/nrg2101
54. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 15(3), 178-96 (2014) DOI: 10.1038/nrm3758
55. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 133(2), 647-58 (2007) DOI: 10.1053/j.gastro.2007.05.022
56. Wang WY, Zhang HF, Wang L. miR-21 expression predicts prognosis in hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol* (2014) Doi not found.
57. Bao L, Yan Y, Xu C. MicroRNA-21 suppresses PTEN and hSulf-1 expression and promotes hepatocellular carcinoma progression through AKT/ERK pathways. *Cancer Lett* 337(2), 226-36 (2013) DOI: 10.1016/j.canlet.2013.05.007
58. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 449(7163), 682-8 (2007) DOI: 10.1038/nature06174
59. Tian Y, Luo A, Cai Y. MicroRNA-10b promotes migration and invasion through KLF4 in human esophageal cancer cell lines. *J Biol Chem* 285(11), 7986-94 (2010) DOI: 10.1074/jbc.M109.062877
60. Li QJ, Zhou L, Yang F. MicroRNA-10b promotes migration and invasion through

CADM1 in human hepatocellular carcinoma cells. *Tumour Biol* 33(5), 1455-65 (2012)
DOI: 10.1007/s13277-012-0396-1

61. Kim T, Veronese A, Pichiorri F. p53 regulates epithelial-mesenchymal transition through microRNAs targeting ZEB1 and ZEB2. *J Exp Med* 208(5), 875-83 (2011)
DOI: 10.1084/jem.20110235

62. Wu SM, Ai HW, Zhang DY. miR-141 targets ZEB2 to suppress HCC progression. *Tumour* Biol (2014)
DOI: 10.1007/s13277-014-2299-9

63. Yuan K, Lian Z, Sun B, Clayton MM, Ng IO, Feitelson MA. Role of miR-148a in hepatitis B associated hepatocellular carcinoma. *PLoS One* 7(4), e35331 (2012)
DOI: 10.1371/journal.pone.0035331

64. Xu X, Fan Z, Kang L. Hepatitis B virus X protein represses miRNA-148a to enhance tumorigenesis. *J Clin Invest* 123(2), 630-45 (2013)
Doi not found.

65. Zhang JP, Zeng C, Xu L, Gong J, Fang JH, Zhuang SM. MicroRNA-148a suppresses the epithelial-mesenchymal transition and metastasis of hepatoma cells by targeting Met/Snail signaling. *Oncogene* 33(31), 4069-76 (2014)
DOI: 10.1038/onc.2013.369

66. Tao ZH, Wan JL, Zeng LY. miR-612 suppresses the invasive-metastatic cascade in hepatocellular carcinoma. *J Exp Med* 210(4), 789-803 (2013)
DOI: 10.1084/jem.20120153

67. Fang Y, Xue JL, Shen Q, Chen J, Tian L. MicroRNA-7 inhibits tumor growth and metastasis by targeting the phosphoinositide 3-kinase/Akt pathway in hepatocellular carcinoma. *Hepatology* 55(6), 1852-62 (2012)
DOI: 10.1002/hep.25576

68. Tonini T, Rossi F, Claudio PP. Molecular basis of angiogenesis and cancer. *Oncogene* 22(42), 6549-56 (2003)
DOI: 10.1038/sj.onc.1206816

69. Giordano G, Febbraro A, Venditti M. Targeting angiogenesis and tumor microenvironment in metastatic colorectal cancer: role of aflibercept. *Gastroenterol Res Pract* 2014, 526178 (2014)
DOI: 10.1155/2014/526178

70. Patil AS, Sable RB, Kothari RM: Occurrence, biochemical profile of vascular endothelial growth factor (VEGF) isoforms and their functions in endochondral ossification. *J Cell Physiol* 227(4), 1298-308 (2012)
DOI: 10.1002/jcp.22846

71. Chai ZT, Kong J, Zhu XD. MicroRNA-26a inhibits angiogenesis by down-regulating VEGFA through the PIK3C2alpha/Akt/HIF-1alpha pathway in hepatocellular carcinoma. *PLoS One* 8(10), e77957 (2013)
DOI: 10.1371/journal.pone.0077957

72. Yang X, Zhang XF, Lu X. MicroRNA-26a suppresses angiogenesis in human hepatocellular carcinoma by targeting hepatocyte growth factor-cMet pathway. *Hepatology* 59(5), 1874-85 (2014)
DOI: 10.1002/hep.26941

73. Wang R, Zhao N, Li S. MicroRNA-195 suppresses angiogenesis and metastasis of hepatocellular carcinoma by inhibiting the expression of VEGF, VAV2, and CDC42. *Hepatology* 58(2), 642-53 (2013)
DOI: 10.1002/hep.26373

74. Zhou B, Ma R, Si W. MicroRNA-503 targets FGF2 and VEGFA and inhibits tumor angiogenesis and growth. *Cancer Lett* 333(2), 159-69 (2013)
DOI: 10.1016/j.canlet.2013.01.028

75. Shih TC, Tien YJ, Wen CJ. MicroRNA-214 downregulation contributes to tumor angiogenesis by inducing secretion of the hepatoma-derived growth factor in human hepatoma. *J Hepatol* 57(3), 584-91 (2012)
DOI: 10.1016/j.jhep.2012.04.031

76. Fang JH, Zhou HC, Zeng C. MicroRNA-29b suppresses tumor angiogenesis, invasion, and metastasis by regulating matrix metalloproteinase 2 expression. *Hepatology* 54(5), 1729-40 (2011)
DOI: 10.1002/hep.24577

77. Alpini G, Glaser SS, Zhang JP. Regulation of placenta growth factor by microRNA-125b in hepatocellular cancer. *J Hepatol* 55(6), 1339-45 (2011)
DOI: 10.1016/j.jhep.2011.04.015

78. Zhu K, Pan Q, Zhang X. MiR-146a enhances angiogenic activity of endothelial cells in hepatocellular carcinoma by promoting PDGFRA expression. *Carcinogenesis* 34(9), 2071-9 (2013)

DOI: 10.1093/carcin/bgt160

79. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* 21(3), 309-22 (2012)
DOI: 10.1016/j.ccr.2012.02.022

80. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer* 12(4), 298-306 (2012)
DOI: 10.1038/nrc3245

81. Fu J, Xu D, Liu Z. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology* 132(7), 2328-39 (2007)
DOI: 10.1053/j.gastro.2007.03.102

82. Wang F, Jing X, Li G. Foxp3+ regulatory T cells are associated with the natural history of chronic hepatitis B and poor prognosis of hepatocellular carcinoma. *Liver Int* 32(4), 644-55 (2012)
DOI: 10.1111/j.1478-3231.2011.02675.x

83. Curiel TJ, Coukos G, Zou L. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 10(9), 942-9 (2004)
DOI: 10.1038/nm1093

84. Yang P, Li QJ, Feng Y. TGF-beta-miR-34a-CCL22 signaling-induced Treg cell recruitment promotes venous metastases of HBV-positive hepatocellular carcinoma. *Cancer Cell* 22(3), 291-303 (2012)
DOI: 10.1016/j.ccr.2012.07.023

85. Chen L, Ma H, Hu H. Special role of Foxp3 for the specifically altered microRNAs in Regulatory T cells of HCC patients. *BMC Cancer* 14, 489 (2014)
DOI: 10.1186/1471-2407-14-489

86. Gilkes DM, Semenza GL, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nat Rev Cancer* 14(6), 430-9 (2014)
DOI: 10.1038/nrc3726

87. Ji J, Zhao L, Budhu A. Let-7g targets collagen type I alpha2 and inhibits cell migration in hepatocellular carcinoma. *J Hepatol* 52(5), 690-7 (2010)
DOI: 10.1016/j.jhep.2009.12.025

88. Cox TR, Erler JT. Lysyl oxidase in colorectal cancer. *Am J Physiol Gastrointest Liver Physiol* 305(10), G659-66 (2013)
DOI: 10.1152/ajpgi.00425.2012

89. Nishioka T, Eustace A, West C. Lysyl oxidase: from basic science to future cancer treatment. *Cell Struct Funct* 37(1), 75-80 (2012)
DOI: 10.1247/csf.11015

90. Wong CC, Tse AP, Huang YP. Lysyl oxidase-like 2 is critical to tumor microenvironment and metastatic niche formation in hepatocellular carcinoma. *Hepatology* 60(5), 1645-58 (2014)
DOI: 10.1002/hep.27320

91. Soltanian S, Matin MM. Cancer stem cells and cancer therapy. *Tumour Biol* 32(3), 425-40 (2011)
DOI: 10.1007/s13277-011-0155-8

92. Ma S. Biology and clinical implications of CD133(+) liver cancer stem cells. *Exp Cell Res* 319(2), 126-32 (2013)
DOI: 10.1016/j.yexcr.2012.09.007

93. Yamashita T, Wang XW. Cancer stem cells in the development of liver cancer. *J Clin Invest* 123(5), 1911-8 (2013)
DOI: 10.1172/JCI66024

94. Ma S, Tang KH, Chan YP. miR-130b Promotes CD133(+) liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. *Cell Stem Cell* 7(6), 694-707 (2010)
DOI: 10.1016/j.stem.2010.11.010

95. Zhang J, Luo N, Luo Y, Peng Z, Zhang T, Li S. microRNA-150 inhibits human CD133-positive liver cancer stem cells through negative regulation of the transcription factor c-Myb. *Int J Oncol* 40(3), 747-56 (2012)
Doi not found.

96. Ji J, Yamashita T, Budhu A. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology* 50(2), 472-80 (2009)
DOI: 10.1002/hep.22989

97. Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 63(5), 844-55 (2014)
DOI: 10.1136/gutjnl-2013-306627

98. Yin XY, Xie XY, Lu MD. Percutaneous thermal ablation of medium and large hepatocellular carcinoma: long-term outcome and prognostic factors. *Cancer* 115(9), 1914-23 (2009)

DOI: 10.1002/cncr.24196

99. Yin XY, Xie XY, Lu MD. Percutaneous ablative therapies of recurrent hepatocellular carcinoma after hepatectomy: proposal of a prognostic model. *Ann Surg Oncol* 19(13), 4300-6 (2012)
DOI: 10.1245/s10434-012-2433-0

100. Zheng SG, Xu HX, Lu MD. Role of contrast-enhanced ultrasound in follow-up assessment after ablation for hepatocellular carcinoma. World journal of gastroenterology WJG 19(6), 855-65 (2013)
Doi not found.

101. Cheng AL, Kang YK, Chen Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 10(1), 25-34 (2009)
DOI: 10.1016/S1470-2045(08)70285-7

102. Hsu SH, Yu B, Wang X. Cationic lipid nanoparticles for therapeutic delivery of siRNA and miRNA to murine liver tumor. *Nanomedicine* 9(8), 1169-80 (2013)
DOI: 10.1016/j.nano.2013.05.007

103. Xiao Z, Li CH, Chan SL. A small molecule modulator of the tumor suppressor miRNA-34a inhibits the growth of hepatocellular carcinoma. *Cancer Res* 74(21), 6236-47 (2014)
DOI: 10.1158/0008-5472.CAN-14-0855

104. Kota J, Chivukula RR, O'Donnell KA. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 137(6), 1005-17 (2009)
DOI: 10.1016/j.cell.2009.04.021

105. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. *Nat Rev Drug Discov* 13(8), 622-38 (2014)
DOI: 10.1038/nrd4359

106. Li C, Li L, Keates AC. Targeting cancer gene therapy with magnetic nanoparticles. *Oncotarget* 3(4), 365-70 (2012)
Doi not found.

107. Caffo M, Barresi V, Caruso G. Innovative therapeutic strategies in the treatment of brain metastases. *Int J Mol Sci* 14(1), 2135-74 (2013)
DOI: 10.3390/ijms14012135

Abbreviations: TGF-β, transforming growth factor-β; ECM, extracellular matrix; 3′UTRs, 3′-untranslated regions; pre-miRNA, precursor-miRNA; Apaf-1, apoptotic protease-activating factor-1; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; PDGF, platelet-derived growth factor; Ang1, Angiopoietin-1; Ang2, angiopoietin-2; FGF, basic fibroblast growth factor; EGF, epidermal growth factor, HDGF; hepatoma-derived growth factor; PIGF, placenta growth factor

Key Words: HCC, microenvironment, miRNA, therapy

Send correspondence to: Hui-Xiong Xu, Department of Medical Ultrasound, Shanghai Tenth People's Hospital, Tenth People's Hospital of Tongji University, Shanghai 200072, China, Tel: 021-66300588; Fax: 021-66300588; E-mail: huixiong_xu@163.com