Role of microRNAs in hepatocellular carcinoma

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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 B1, heavy 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 biomarkers (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
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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 apoptosis 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 DGC8R8 and is cleaved to an approximately 70 nt double-stranded RNA hairpin intermediate (pre-miRNA) by the Drosha-DGC8 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 Argonait 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).
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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 caspsases 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...
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6.2. Modulation of regulatory T cell trafficking by microRNAs

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 oncopgenes 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 Rubene 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.

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**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

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