Review article

Deciphering the multifaceted role of microRNAs in hepatocellular carcinoma: Integrating literature review and bioinformatics analysis for therapeutic insights

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ABSTRACT

Hepatocellular carcinoma (HCC) poses a significant global health challenge, necessitating innovative therapeutic strategies. MicroRNAs (miRNAs) have emerged as pivotal regulators of HCC pathogenesis, influencing key processes such as self-renewal, angiogenesis, glycolysis, autophagy, and metastasis. This article integrates findings from a comprehensive literature review and bioinformatics analysis to elucidate the role of miRNAs in HCC. We discuss how dysregulation of miRNAs can drive HCC initiation, progression, and metastasis by modulating various signaling pathways and target genes. Moreover, leveraging high-throughput technology and bioinformatics tools, we identify key miRNAs involved in multiple cancer hallmarks, offering insights into potential combinatorial therapeutic strategies. Through our analysis considering p-values and signaling pathways associated with key features, we unveil miRNAs with simultaneous roles across critical cancer characteristics, providing a basis for the development of high-performance biomarkers. The microRNAs, miR-34a-5p, miR-373-3p, miR-21-5p, miR-214-5p, miR-195-5p, miR-139-5p were identified to be shared microRNAs in stemness, angiogenesis, glycolysis, autophagy, EMT, and metastasis of HCC. However, challenges such as miRNA stability and delivery hinder the translation of miRNA-based therapeutics into clinical practice. This review underscores the importance of further research to overcome existing barriers and realize the full potential of miRNA-based interventions for HCC management.

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1. Introduction

According to the World Health Organization (WHO), hepatocellular carcinoma (HCC) is the leading cause of liver cancer mortality, comprising about 80 % of all liver cancers worldwide. Projections also indicate that more than one million individuals globally will die from liver cancer by 2030, underscoring a significant public health crisis [1]. In patients with cirrhosis and chronic hepatitis are serum alpha-fetoprotein (AFP) test and liver ultrasound (US) are recommended for early screening of HCC. Abdominal US is a routine procedure that is used due to its convenience, cost-effectiveness, minimal radiation exposure, and non-invasiveness. However, one of the disadvantages of US is that the accuracy of it depends on the technician or radiologist who performs the scan. In addition to determining disease progression, pathological grading and prognosis, serum AFP plays an important role in screening and early diagnosis of HCC (2). Liver biopsy, which is the only method for distinguishing between the growth of malignant and benign liver cancer. The biopsy method has limitations in small and large tumors. In small tumors, there are almost 40 % false negative results. Complications such as intrahepatic or intraperitoneal bleeding have been reported in larger tumors. It may be difficult to definitively diagnose HCC through a small histological sample, so other cytological smears are required, each of which has a specific complication. These limitations have led to the use of alternative methods such as non-invasive imaging techniques (3). Imaging methods of the liver in order to determine the different stages of liver cancer, including a CT scan of the liver, are performed using a contrast-enhancing substance, and its disadvantage is that it exposes the person to ionizing radiation. magnetic resonance imaging (MRI), which, like a CT scan, can image many areas of the chest and abdomen, and since no ionizing radiation is involved, it is less dangerous (4, 5). Another method of imaging cancer, including HCC, is PET imaging. This technique is a type of molecular imaging that takes place after injecting radioactive materials into the patient's body. This method is very useful for detecting and measuring metabolic changes at cellular levels, for detecting lesions and initial stages of cancer, metastasis and recurrence of cancer, as well as for monitoring treatment after chemotherapy. The risk of this method is insignificant, and the patients are exposed to radiation, and due to the low dose, they do not suffer from any special complications (6). Considering the limitations of HCC detection techniques and the high costs of some methods, alternative methods can help to solve these problems and limitations. miRNAs as diagnostic, prognostic and predictive biomarkers can be used as Non-invasive serological markers of HCC should be used. Identification of changes in the level of miRNA that exist in stable circulation in the serum and plasma of patients with HCC can be determined as HCC biomarkers and play an important role in timely diagnosis and optimal treatment of HCC (7–9).

Despite advances in HCC treatment strategies, including surgical interventions (tumor removal or liver transplantation) [2], radiotherapy [3], and chemotherapy [4], the five-year survival rate in advanced stages remains low due to late diagnosis, drug resistance, and high frequency of recurrence [5]. The late manifestation of symptoms often leads to delayed diagnosis, limiting the effectiveness of current treatments [6]. Moreover, the limited understanding of the molecular machinery driving cancer progression has resulted in suboptimal outcomes. Therefore, elucidating the pathogenesis and key processes underlying HCC is crucial for developing new diagnostic and prognostic biomarkers, as well as effective biodrugs [7].

Among various biomolecules, microRNAs (miRNAs) have gained significant attention for their roles in cancer diagnosis [8], treatment [9,10], and prognosis [11]. These evolutionarily conserved, endogenous, non-coding RNAs regulate gene expression post-transcriptionally [11]. Genome-wide expression analyses using microarray and qPCR techniques have revealed dysregulated miRNAs critical in cancer formation and development [12]. These natural oligonucleotides finely modulate biological processes such as autophagy [13], apoptosis [14], angiogenesis [15] glycolysis [16], epithelial-mesenchymal transition (EMT) [17], and metastasis [18], which are integral to HCC progression [19–22].

Considering the potential of miRNA-based therapies as a novel approach to cancer treatment, several candidates have shown promise for HCC control [23]. Phase I clinical trials targeting HCC with miRNA-based therapies, such as the miR-34a mimic formulated in liposomal carrier, MRX34, have demonstrated encouraging results [24]. Similarly, the miR-193a-3p mimic formulated in a lipid nanoparticle named INT-1B3 has shown success in Phase I trials across various cancers including HCC [25–27]. Moreover, ongoing research endeavors, such as the development of lipid nanoparticle-formulated tumor suppressor microRNAs, signify a promising frontier in HCC treatment (https://interna-technologies.com). Future trials are warranted to identify optimal miRNA candidates to address the multifaceted challenges posed by HCC.

Herein, we will examine miRNAs dysregulated in six pivotal processes underlying HCC, namely autophagy, apoptosis, glycolysis, angiogenesis, EMT, and metastasis. Leveraging *in silico* analysis of datasets and a meticulous evaluation of the existing literature, we aim to shed light on shared miRNA (hubs) activities across the above-mentioned crucial features of HCC pathogenesis. Our exploration underscores the potential of harnessing hub miRNAs comment to these six processes as a strategic approach to enhance the vulnerability of HCC and inform pivotal treatment strategies.

2. The complexity and heterogeneity of HCC

Several factors play a role in the occurrence of liver cancer among them, viral infections, metabolic factors, genetic/epigenetic changes, environmental/lifestyle changes, tumor microenvironment, and immune responses are more frequent.

Among the environmental risk factors, chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are directly involved in the occurrence of HCC. As a powerful piece of evidence for this, we observe the highest rate of HCC occurrence in sub-Saharan Africa and Southeast Asia, where HBV infection is highly prevalent [28–30]. Additionally, viral infections by HBV and HCV lead to the exhaustion of hepatitis virus-specific T cells, which is associated with persistent antigen stimulation, one of the underlying factors in the development of liver tumors [31,32]. Molecular and cellular events, associated with hepatitis virus infection, revealed that

chromatin instability, intracellular DNA rearrangements, and mutations stimulate the activation of chronic inflammatory signals in liver cells, and are considered among the main causes of primary liver cancer and liver cancer malignancy [33].

In addition, there is a range of metabolic factors that are closely related to HCC occurrence. Among them, alcohol consumption is the primary cause of liver cirrhosis, which in turn is one of the independent risk factors for primary liver cancer in humans [34]. The process of carcinogenesis in patients with alcoholic liver disease (ALD) also provides the basis for liver cancer due to changes in metabolic pathways, increased production of reactive oxygen species (ROS), and increased inflammation [35]. Moreover, excessive caloric intake and physical inactivity lead to the most common liver disease worldwide i.e. non-alcoholic fatty liver disease (NAFLD), which can develop into non-alcoholic steatohepatitis (NASH) [36]. In NAFLD patients, the accumulation of fat in the liver causes damage to liver cells and contributes to the inflammation inherent in NASH. In hepatocytes, lipotoxicity induces metabolic reprogramming, which results in the accumulation of potentially toxic metabolites with an inflammatory microenvironment and DNA damage. Oxidative stress and DNA damage lead to liver cancer, as well [37,38].

Epidemiological studies have reported smoking and aflatoxin exposure as two factors that increase the risk of HCC [39]. Aflatoxin B1 (AFB1), is a mycotoxin found in contaminated food and is predominantly found in Africa and Asia. The C > A genetic mutations and R249S-specific mutation in tumor suppressor TP53 are associated with HCC [40,41]. Exposure to genotoxic pollutants can also lead to single nucleotide polymorphisms (SNPs) due to changes in the genes of the glutathione S-transferase enzyme, which is involved in carcinogenic detoxification. These genetic changes make people more prone to HCC [42,43].

Genetic background is also considered another risk factor for liver cancer. These factors have been studied in rodent models, which have confirmed the identification of chromosomal locations affecting genetic predisposition to liver carcinogenesis caused by chemicals [44,45]. Patients with HCC experience high levels of heterogeneity within the cancerous tissue of the liver, where cells are phenotypically different [46,47]. In most cases, HCC patients in the early stages of the disease relapse even after treatment. Many studies have reported that a high proportion of these patients have clones distinct from the primary tumor; recurrence is due to the presence of intrahepatic micrometastases or residual tumors, contributing to recurrence because of tumor heterogeneity.

In addition to liver damage, HCC is associated with additional tumor heterogeneity regarding the host microbiome and the specific mechanism of liver damage [46,48,49]. Studies show that the microenvironmental factors of the host's intestinal microbiota also play a significant role in liver tumorigenesis [50].

HCC heterogeneity directly affects the response to treatment of HCC patients. Peritumor microenvironmental factors that activate the same oncogenic pathways influence the liver tumor phenotype and play a role in the development of liver malignancies and HCC heterogeneity [51].

Another factor in HCC heterogeneity is the immune system, which is unique in each person due to their genome and expression patterns, thus leading to heterogeneity among HCC patients. The tumor immune microenvironment has become an effective first-line treatment option against HCC patients. Studies of interactions between immune cells, tumor cells, and non-immune stromal cells have revealed heterogeneous patterns in different individuals, suggesting clinicopathological significance [52–54]. For example, genes related to interferon (IFN) and cytotoxic T cells are classified as HCC "immune active class" which is associated with a better prognosis for HCC [55,56].

Moreover, several genetic changes occur as internal factors in the initial stages of HCC. HCC rarely arises from monogenic syndromes. Many HCC-causing gene polymorphisms that result in CLD are closely related [51]. There is a correlation between environmental and genetic (micro) factors. For example, between HBV infection and mutations in TP53, polymorphisms of phospholipase domain-like protein 3 (PNPLA3) are associated with chronic liver disorders and NAFLD [57–59]. Additionally, variants of member 2 of superfamily 6 (TM6SF2) are linked to NAFLD and are correlated with increased steatosis, cirrhosis, and NASH [60,61]. Among other cases, it has been observed in HCC patients that there are mutations in the CTNNB1 gene encoding the β-catenin protein [62]. In addition, genetic changes vary depending on the stage of cancer and the underlying cause of HCC. Mutations in CTNNB1 that normally precede telomerase reverse transcriptase (TERT) promoter mutations induce proliferation in hepatocytes. While in most cases, TERT promoter mutations are observed in the cirrhotic liver [60,62,63]. Moreover, changes in some genes CCND1, FGF3, FGF4, and FGF19 related to fibroblast growth factors, and mutations and deletions of Cyclin-dependent kinase inhibitor 2A (CDKN2A) and TP53 occur in advanced stages of liver cancer [62]. Despite the identification of risk factors in genetic alterations, there is still remarkable heterogeneity in diverse molecular signatures in patients with HCC. Mutation in telomerase, hepatic differentiation genes, oxidative stress, a mutation in telomerase, the MAP kinase, cell cycle, PI3K/AKT/mTOR pathway, β pathway (TGFβ), chromatin remodeling, JAK/STAT pathway, telomerase enzyme subunit from genetic changes in each of these factors and acts as pathways that trigger liver inflammation [51,62,63]. The WNT/b-catenin pathway is known as one of the most common oncogenic pathways driving HCC [64].

Besides genetic changes, epigenetic mutations are other predisposing factors for HCC [65,66]. These modifications play a role in the regulation of gene transcription. Aberrant expression, changes in epigenetic information, and the activity of epigenetic enzymes play an essential role in the emergence of HCC [66,67].

3. Biogenesis and maturation of miRNA

MicroRNAs (miRNAs) are evolutionarily conserved, non-coding ribonucleic acids approximately twenty-two nucleotides in length. They function as post-transcriptional regulators by binding to the 3′ untranslated region of mRNA, thereby inhibiting mRNA translation. Notably, a single miRNA can modulate the expression of over 200 mRNAs. impacting cellular, physiological, and pathological processes [68].

The biogenesis of miRNAs begins in the nucleus with the transcription of primary miRNA (pri-miRNA) by RNA polymerases II, in a process known as the atypical miRNA biogenesis pathway (Mirtron pathway). The pri-miRNA, typically 500 to 3000 nucleotides long,

undergoes cleavage by the nuclear enzyme Drosha (Rnas3) and its cofactor DGCR8 (DiGeorge syndrome critical region 8), producing a 70–100 nucleotide hairpin-structured precursors (pre-miRNA) [69]. Exportin-5 and Ran-GTP mediate the transport of pre-miRNA to the cytoplasm, where the Dicer endonuclease processes it into a double-stranded miRNA of approximately twenty-two nucleotides in length [70]. This double-stranded miRNA is subsequently loaded onto Argonaute (AGO) protein and unwinds. The guide strand of the miRNA duplex is retained by the RNA-induced silencing complex (RISC), which binds to the target mRNA and inhibits its translation [71] (Fig. 1).

4. MicroRNA-mediated regulatory mechanisms in HCC progression

In the liver, miRNAs play crucial roles in maintaining homeostasis and regulating tissue regeneration. They control various processes including cholesterol and fatty acid synthesis, systemic iron regulation, detoxification, hepatocyte proliferation, or apoptosis. Consequently, miRNA dysregulation can lead to liver complications such as alcoholic liver toxicity (ALT), hepatic fibrosis, and cirrhosis [72].

Emerging evidence indicates that miRNAs contribute to HCC development and progression by modulating mediators in ontogenesis-related signaling pathways [73]. They regulate numerous biological processes, such as cellular endoribonuclease activity [74], GTPase activator activity [75], endoplasmic reticulum (ER) to Golgi transport system [76], phosphoprotein binding [77], mitotic spindle pole [78], protein SUMOylation [79], protein tyrosine phosphatase activity [80], fibroblast growth factor receptor signaling [81] and BMP signaling [82].

Notably, aberrant miRNA expression disrupts critical pathways including, the mammalian target of rapamycin (mTOR), Wnt, JAK/STAT, and MAPK signaling pathways, which are central to HCC pathogenesis [83]. These dysregulated miRNAs affect various cellular functions such as glucose metabolism (under the control of Akt-mTOR and JAK/STAT pathways) [84], gluconeogenesis, and c-Myc activation (under the control of miR-IL6-STAT3 signaling pathways) [85,86], and increased glycolysis in HCC (under the control of Akt-mTOR pathway) [87].

The JAK/STAT pathway, involved in stem cell maintenance and inflammatory responses, is often over-activated in HCC due to decreased levels of regulatory miRNAs, contributing to metastasis [83]. Wnt and MAPK pathways, crucial for cell growth and differentiation, also regulate cell migration, autophagy, and apoptosis, with Wnt/β-catenin signaling specifically influencing EMT in HCC [83,88]. The dysregulation of miRNAs in these pathways is a significant factor in HCC development and progression [83], highlighting their potential as targets for innovative and effective HCC therapies.
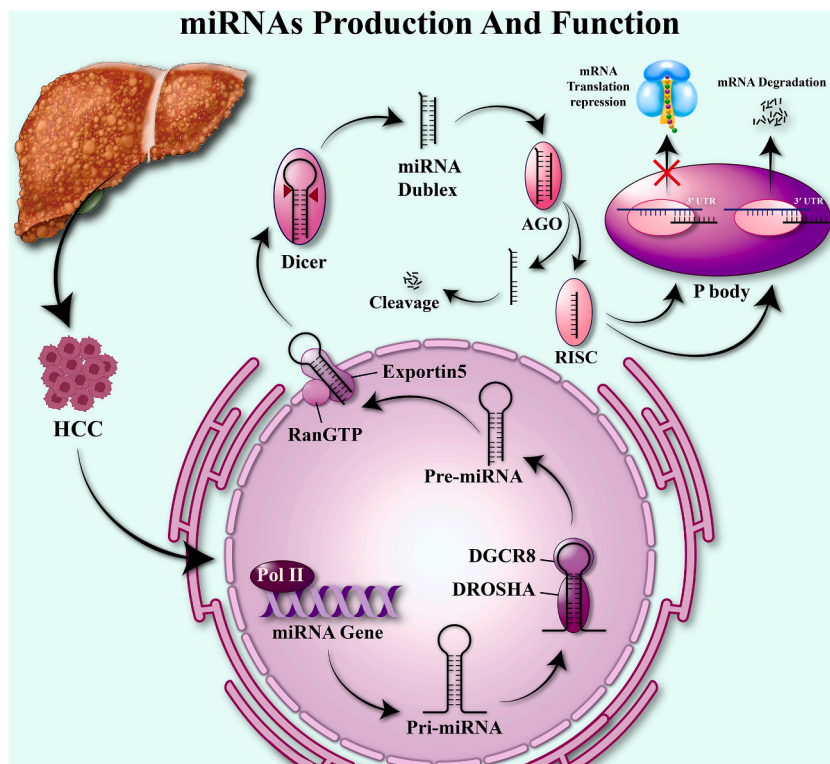

Fig. 1. Transcription and processing of microRNA.

5. Autophagy dysregulation in HCC

Autophagy is a catabolic process involving the sequestration of organelles and cellular components into autophagosomes, followed by degradation through fusion with lysosomes [89]. This process is crucial for energy homeostasis, stress responses, organelle elimination, and tissue remodeling [90]. Dysregulated autophagy can impact cell survival, normal cell death, genomic stability, metabolic stress, and tumorigenesis [91]. In cancer, autophagy plays dual roles as both a tumor suppressor and a tumor promoter, influencing cancer cell development, proliferation, and drug resistance [92].

The autophagy process is tightly regulated by a series of proteins, notably mTORC1 complex. Dysregulation of mTOR is associated with cell proliferation, stress response, and cancer progression [93]. mTOR operates through two complexes (mTORC1 and mTORC2), each with distinct functions and localization [94]. Activated mTORC1 inhibits autophagy by phosphorylating autophagy-related proteins (ATGs), while under conditions such as starvation and organelle damage, mTORC1 inhibition induces autophagy. mTORC1 activity is regulated by AMP-activated protein kinase (AMPK), where increased AMPK levels can trigger autophagy by inhibiting mTORC1 [95].

Recent research suggests that autophagy biomarkers have potential as prognostic indicators in HCC and may inform new therapeutic approaches. However, the precise role of autophagy in HCC remains unclear with evidence supporting both tumorigenic [96]
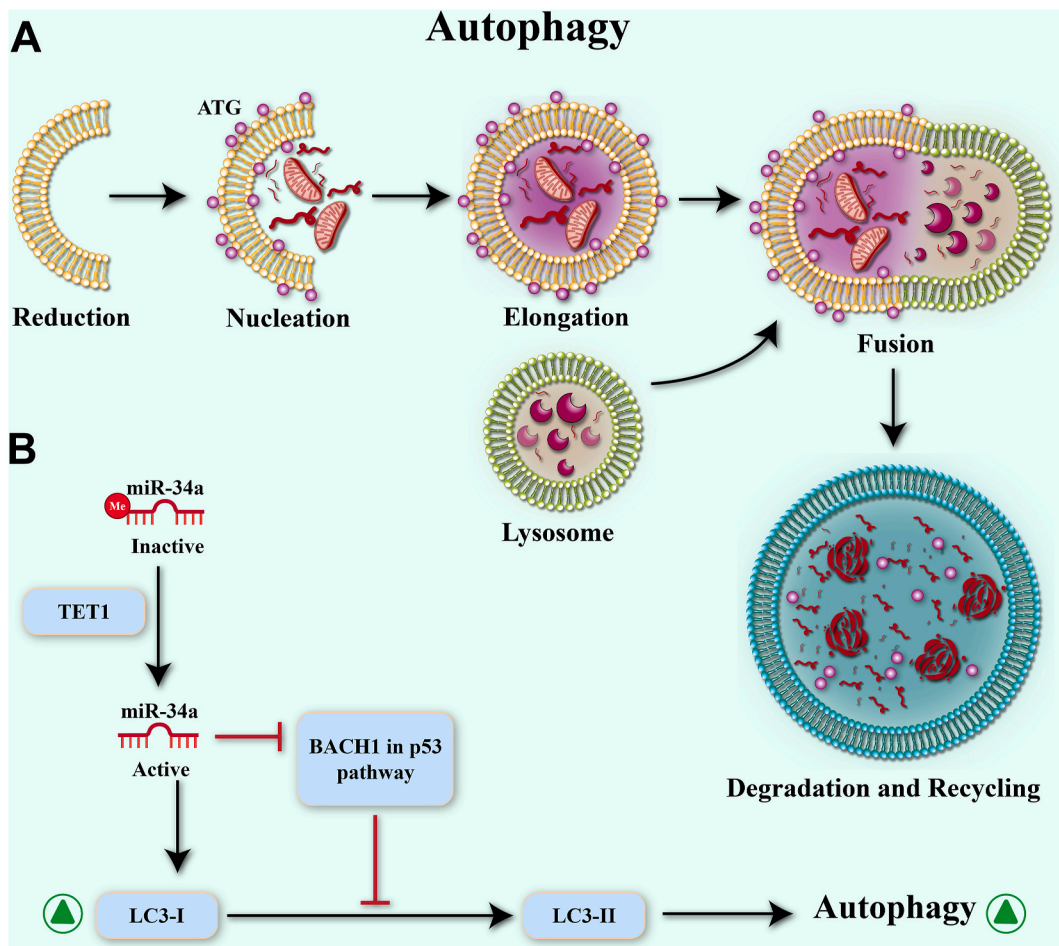

Fig. 2. Functions of miRNAs in HCC Autophagy A. Autophagy maintains cellular homeostasis by degrading misfolded or damaged proteins as well as removing aged or malfunctioning organelles. Following the induction of autophagy, the pre-autophagosome occurs, which is a cup-shaped structure separated from the ER membrane and formed. The next step, the nuclear ATG complex, consists of a set of autophagy-related proteins that gradually begin to engulf damaged cellular proteins and components. The next stage is elongation, which starts the complete separation of membrane and autophagosome formation. After the formation of the autophagosome, it merges with the lysosome, and the contents inside the autophagosome are destroyed by lysosomal hydrolases.
B. MicroRNAs are key regulators of autophagy. TET1 activates miR-34 through demethylation. miR-34 regulates autophagy through the miR-34a/BACH1/p53 axis. BACH1 is the target of the miR-34a gene in the p53 pathway, inhibiting BACH1 expression and increasing LC3-I expression leads to the cytoplasmic form of I3-LC being cleaved to the autophagic membrane form LC3- II is converted and then it leads to stimulation of autophagy formation steps.

and tumor-suppressive functions [96] reviewed elsewhere [97].

miRNAs significantly regulate key proteins in the mTOR pathway, affecting various stages of autophagy, from upstream signaling to autolysosomal degradation [98]. For example, miR-7 targets the PI3K/Akt pathway, including mTOR, p70S6K, and PIK3CD, in HCC cells [99]. Moreover, miR-34 modulates autophagy through TET1 and miR-34a/BACH1/p53 axis. Specifically, BACH1, a target of the miR-34a gene in the p53 pathway, is regulated by TET1, which demethylates miR-34a. Activation of the p53 pathway by miRNA-34a inhibitor counteracts the effect of TET1 resulting in decreased autophagosome formation [100] (Fig. 2).

Evidence shows that miRNA dysregulation contributes to autophagy-mediated cancer formation [101], invasion [102], and metastasis [102], and affects responses to chemotherapy or radiotherapy [103]. Therefore, considering the importance of autophagy for cancer biology, the study of autophagy-regulating miRNA in cancer will allow a better understanding of malignancies and lead to the development of novel disease markers and therapeutic targets. Table 1 summarizes some of the autophagy-regulating miRNAs. Among these miRNAs, miR142-3p has emerged as a novel autophagy-regulating miRNA. involved in sorafenib resistance, which is a systemic agent for the treatment of advanced HCC [96]. Therefore, co-targeting miR-142-3p is recognized as a new therapeutic strategy [104]. Similarly, miR-30a, which is significantly downregulated in HCC, targets autophagy-related proteins Beclin 1 and Atg5. This regulation is associated with vascular invasion, metastatic potential, and recurrence, indicating miR-30a′s potential as a therapeutic and diagnostic target [105].

6. Apoptosis regulatory mechanisms in HCC

Apoptosis is known as an orchestrated mechanism via which cells are programmed to die after receiving particular stimuli. This process is characterized by a series of manifestations, including morphologic modifications, such as nuclear fragmentation, chromatin condensation, and reduction of cell bulk [106]. Additionally, biochemical changes such as caspase activation, perturbations in protein networks, DNA degradation, and changes in membrane surfaces allow the apoptotic cell to be recognized and inundated by phagocytic cells [107,108].

Dysregulation of apoptosis contributes to cancer development. Thus, targeting apoptotic processes is an essential approach in designing antitumor medications that are capable of killing cancer cells. Central to this process are caspases; initiator caspases (e.g., caspase-8 and -9) activate executioner caspases (such as caspase-3), leading to the cleavage of cellular substrates and cell destruction [109].

MiRNAs are key regulators of apoptosis, influencing various pathways, including intrinsic (e.g., Bcl-2 and Mcl-1), extrinsic (e.g., TRAIL and Fas), p53-induced, endoplasmic reticulum-induced pathways, and necroptosis (Hajizadeh et al., 2023). Understanding the specific expression patterns of anti-apoptotic miRNA clusters in different cancer types can reveal novel therapeutic opportunities [104].

While the mTOR pathway is the most well-characterized miRNA-regulated pathway in HCC, it is proven that miRNA expression levels also influence Wnt, JAK/STAT, or MAPK pathways [110,111]. For instance, miR-520c-3p negatively correlates with GPC3 protein levels (a key protein in the Wnt signaling pathway) in HCC, suggesting its potential as a therapeutic target [112]. In HCC cell lines, like HepG2 and SMMC-7721, lower expression of miR-644a compared to normal hepatocytes (L-O2) was observed [113]. It is also shown that overexpression of miR-644a can promote apoptosis by downregulating the Heat Shock Factor 1 (HSF1) which is among

Table 1
Autophagy-regulating miRNAs in Hepatocellular carcinoma.

MicroRNAs Type	Suppression or induction	Mechanism of action	Study type	Potent Application	Ref
miR-142-3p	suppression	Targets ATG5/ATG16L1	*in vitro/in vivo*	Therapeutic	(31)
miR-223	suppression	Targets FOXO3a	*in vitro/in vivo*	Therapeutic	(36)
miR-651-3p	suppression	Targets ATG3-Mediated Cell Autophagy	*In vitro*	Therapeutic	(37)
miR-185	induction	via AKT signaling	*in vitro*	Therapeutic	(38)
miR-30a	suppression	Targets Beclin 1 and Atg5	*in vitro/in vivo*	Therapeutic	(35)
miR-26a/b	suppression	Through activation of the AMPK Signaling Pathway via Rab10	*in vitro/in vivo*	Therapeutic	(39)
miR-519d	induction	Through activation of the AMPK signaling pathway via Rab10	*in vitro/in vivo*	Therapeutic	(40)
miR-541	suppression	Inhibition of ATG 2A or RAB1B	*in vitro/in vivo*	Therapeutic/ prognostic	(41)
miR-25	induction	Targets FBXW7	*In vitro*	Therapeutic	(42)
miR-34a	suppression	Targets BTB domain and CNC homology 1 (BACH1)	*in vitro/in vivo*	Therapeutic/ Prognostic	(34)
miR-423-5p	induction	Targets ATG7	*in vitro/in vivo*	Therapeutic/ Prognostic	(43)
miR-7	induction	Targets m-TOR	*In vitro*	Therapeutic	(44)
miR-21	suppression	Inhibiting autophagy via the PTEN/Akt pathway	*in vitro/in vivo*	Therapeutic	(45)

the activators of p53-dependent apoptosis [113]. Similarly, miR-644a expression was found to be lower in HCC tissues compared to adjacent non-cancerous tissue [113]. Also, analyzing the prognostic significance of miR-644a expression in HCC patients based on their 5-year survival ratebyKaplan-Meier survival curves showed that HCC patients with low miR-644a expression were associated with lower overall survival [113] (Fig. 3).

Conversely, miR-224 was found to exert an anti-apoptotic effect in HCC cells by regulating the expression of apoptosis-promoting genes such as CDC42, CDH1, PAK2, BCL-2, and MAPK1 genes in Hek293 and Huh7 cell lines. Hence, it is evident that apoptosis regulation leads to tumor cell proliferation, migration, and invasion in HCC [114]. Therefore, miRNAs that regulate apoptosis of cancer cells hold potential as therapeutic and diagnostic targets in HCC disease (Table 2).

7. Exploring EMT's impact on HCC metastasis

Epithelial-mesenchymal transition (EMT) is a multifaced process directed by EMT-activating transcription factors (TFs) such as SNAIL, TWIST, and ZEB families [115]. These EMT-TFs dynamically regulate various phases of cancer progression from initiation to metastasis, and confer resistance to therapeutic regimens [116]. During gastrulation, fibroblast growth factor (FGF) signaling triggers activation of the EMT-transcription factor (TF) Snail (SNAI1), which in turn transcriptionally suppresses E-cadherin, promoting EMT [117]. EMT can be induced by several biofactors such as transforming growth factor beta (TGF-β), growth factors that function through tyrosine kinase receptors (RTKs), like platelet-derived growth factor (PDGF) and FGF receptors (FGFRs), and the proteins nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Wnt, hedgehog, and Notch proteins [118,119].

Numerous miRNAs have been identified that directly target the EMT components and processes, as well as those capable of reversing EMT targeting associated signaling pathways [119]. Notably, potent inducers of EMT, such as TGF-β, can coordinate both fibrogenesis and carcinogenesis in HCC. For instance, miR-4458 interferes with the TGF-β signaling pathway by directly targeting
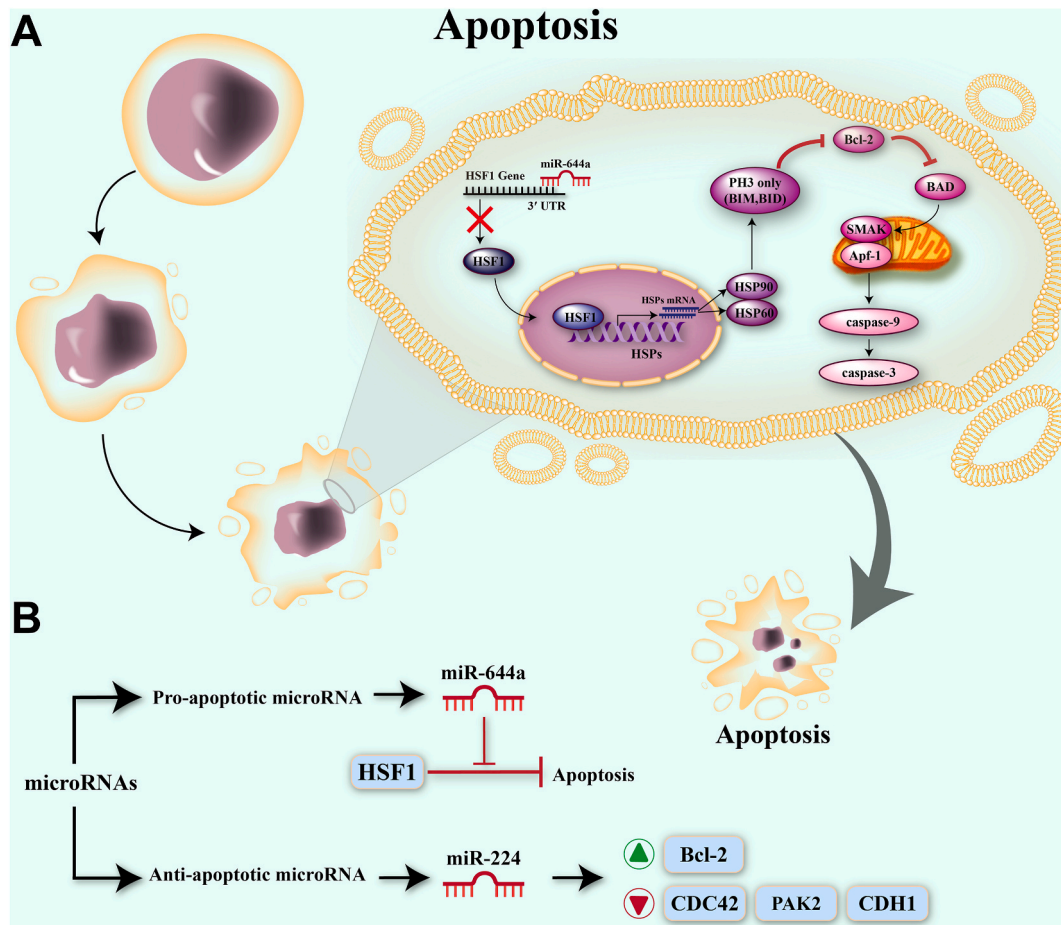

Fig. 3. MicroRNAs in HCC apoptosis. A. The miR-644a, as a promoter of apoptosis, suppresses HSF1 expression by binding to the 3′-UTR of HSF1, and the suppression of HSF1 expression leads to increased expression of BH3-only family proteins including BID, BAD, BIM, SMAC, Apaf-1 and cleaved caspases-3 and -9. **B.** miR-224 targets genes whose products are involved in cell apoptosis, and therefore, increased expression of miR-224 downregulates apoptosis-promoting genes (CDC42, CDH1, and PAK2) and increases the expression of the anti-apoptotic gene BCL-2.

Table 2
Apoptosis-regulating miRNAs in Hepatocellular carcinoma.

microRNAs type	Induction or suppression	Mechanism of action	Study type	Potent Application	Ref.
miR-26b	Induction	Targeting TAK1 and Table 3	*in vitro*	Therapeutic	(54)
miR-196b	Induction	Targeting IGF2BP1	*in vitro*	Therapeutic	(55)
miR-221	Suppression	Inhibition of Caspase-3–Mediated Apoptosis	*in vivo/in vitro*	Therapeutic/ Prognostic	(56)
miR-29	Induction	Targeting Mcl-1 and Bcl-2	*in vitro/in vivo*	Therapeutic/ Prognostic	(50)
miR-429	Induction	Directly targeted NOTCH1	*in vitro*	Diagnosis	(57)
miR-211-5p	Induction	Targeting ZEB2	*in vitro*	Diagnostic/ Therapeutic	(58)
miR-542-3p	Induction	Directly targeting surviving	*in vitro*	Diagnostic/ Therapeutic	(59)
miR-133a	Induction	Inhibiting FOSL2 through TGF-β/Smad3 signaling pathway	*in vitro/in vivo*	Therapeutic	(60)
miR-383	Induction	Targeting IL-17 via STAT3 signaling pathway	*in vitro*	Diagnostic/ Therapeutic	(61)
miR-16-2	Induction	Targeting Bcl-2 and cyclin D1	*in vitro/in vivo*	Therapeutic	(62)
miR-16	Induction	by MC-LR	*in vitro*	Therapeutic	(63)
miR-33b	Induction	Targeting Fli-1-mediated Notch1 pathway	*in vitro/in vivo*	Therapeutic	(64)
miR-373-3p	Induction	Inhibiting TFAP4/PI3K/AKT pathway	*in vitro/in vivo*	Prognostic/ Therapeutic	(65)
miR-375	Induction	Targets AEG-1	*in vitro/in vivo*	Therapeutic	(66)
miR-199a/b-3p	Suppression	Targeting PDCD4	*in vitro/in vivo*	Therapeutic	(67)
miR-34a	Suppression	Targeting CCL22	*in vitro/in vivo*	Therapeutic	(68)
miR-9-5p	Suppression	Suppressing PDK4	*in vitro*	Therapeutic	(69)
miR-3163	Induction	Targets ADAM-17	*in vitro/in vivo*	Therapeutic	(70)
miR-135a	Suppression	Targeting forkhead box O1	*in vitro*	Therapeutic	(71)
mir-183	Suppression	Downregulation of PDCD4 expression	*In vitro*	Diagnostic	(72)
miR-224	Suppression	Targeting CDC42, CDH1, PAK2, and BCL-2	*In vitro*	Diagnostic	(53)
miR-195-5p	Induction	Targeting PHF19	*in vitro/in vivo*	Therapeutic	(73)
miR-139-5p	Induction	Targeting SPOCK1	*in vitro/in vivo*	Therapeutic	(74)

TGFB receptor1 on the EMT process, following the invasion and migration of HCC cells [120,121].

EMT is pivotal in cancer metastasis—the spread of tumor cells from the original site to distant body parts—posing a major risk in cancer progression. Metastasis involves local invasion, intravasation into the bloodstream, transport to new sites, and colonization [122]. In HCC, the risk of metastasis is relatively high due to the large number of blood vessels and rich vascularization in the liver. Key stages of metastasis include extracellular matrix (ECM) degradation, EMT, and vascular invasion [123].

The tumor microenvironment (TME) of HCC, which is a mixture of ECM proteins, tumor stromal cells, growth factors, immune cells, hepatic stellate cells (HSCs), fibroblasts, endothelial cells (ECs), proteolytic enzymes and inflammatory cytokines, HSCs and tumor-associated macrophages (TAMs), significantly influences tumor development and is responsible for invasion and angiogenesis. ECs also facilitate metastasis through proliferation and neovascularization [124]. Cancer cells exhibit phenotypic plasticity to adapt to the evolving TME with EMT being a critical driver exploited by cancer cells to transition from an epithelial to a motile aggressive mesenchymal state, facilitating dissemination from the primary tumor [125]. This process is reversible through mesenchymal-to-epithelial transition (MET), allowing cancer cells to regain characteristics for colonization at distant sites [126].

EMT is mainly controlled at the transcriptional level by a series of transcription factors (e.g. Snail, Zeb1, and Twist) and miRNAs (e. g. the miR-200 family) that coordinately repress epithelial genes and stimulate mesenchymal genes [127]. The steps of EMT involve a mixture of molecules regulating the microenvironment to accommodate cellular expansion to stimulate either tissue expansion in pathological or physiological perspectives. These changes are based on temporal and spatial signals, taken into account by the construction of secretory enzymes to damage the ECM while cell development remains, ECM deprivation by matrix metalloproteinases (MMPs) is a strong sample of this step of EMT. In addition, TGF-β modulates MMPs and downstream Smad signaling to enhance these deprivation events. The steps of EMT in cancer are the same when cells intravasate into the vascular and/or lymphatic structures, extravasate at the minor site, and macrometastasis advance all through progressive disease [128]. These phenotypic modifications demonstrate the cancer cell elasticity essential for tumor emission.

miRNAs play critical roles in regulating EMT and metastasis. For instance, downregulation of miR-1296 expression in HCC tissues and cell lines correlates with increased metastasis and tumor recurrence tumors. Overexpression of miR-1296 inhibits HCC LM3 cell migration, invasion, and EMT by negatively regulating SRPK1 via the PI3K/AKT pathway. Hypoxia downregulates miR-1296, correlating with advanced tumor-node-metastasis (TNM) stage, venous invasion, and multiple tumor nodes. Low miR-1296 expression is linked to reduced overall survival (OS) and disease-free survival (DFS) in HCC patients, suggesting its potential as a prognostic biomarker [129].

MiR-345 also acts as an inhibitor of the EMT and metastasis process in HCC cells by targeting IRF1 and the mTOR/STAT3/AKT pathway and its downstream targets, including Slug, Snail, and Twist in the IRF1-mediated EMT process, highlighting the potential properties of miR-345 in the diagnosis and treatment of HCC [130] (Fig. 4).
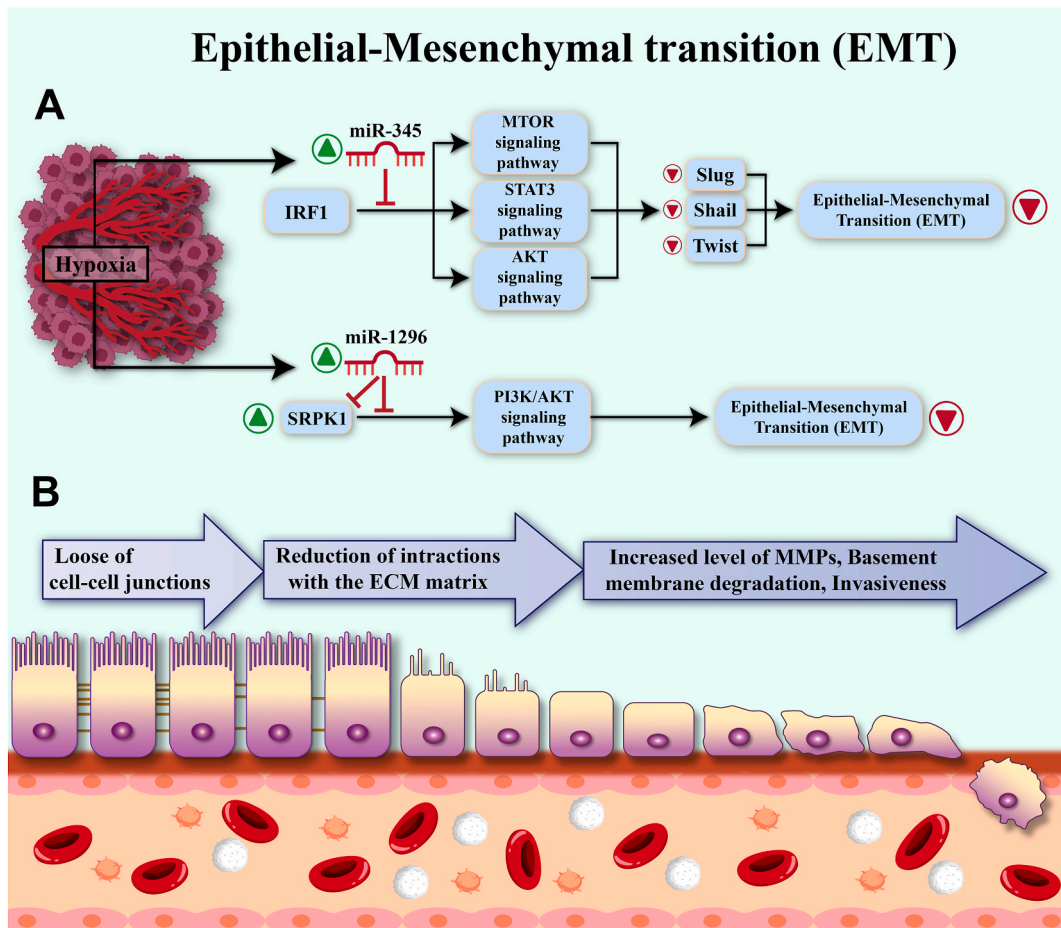
Fig. 4. MicroRNAs in EMT process. A. In the lower parts of epithelial and endothelial tissues, there is a thin layer of extracellular matrix called basement membrane (BM). Cancer cells must invade the basement membrane of the extracellular matrix to metastasize. Laminins are the main components of the ECM, which are involved in the epithelial-mesenchymal transition (EMT) of the basement membrane (BM) of cancer cells. Cells with (EMT) characteristics are degraded through the matrix metalloproteinase (MMP)-associated pathway. The increased expression of MMPs leads to increased destruction of the basement membrane and the entry of cells with EMT characteristics into the blood circulation, which leads to invasion and then metastasis.
B. Increasing miR-345 by targeting IRF1 mRNA and inhibiting the activation of the mTOR/STAT3/AKT triple pathway and reducing the expression of its downstream targets such as Slug, Snail, and Twist reduce EMT in HCC cells. Up-regulation of miR-1296 inhibits hypoxia-promoting effects on metastasis and EMT of HCC cells by targeting SRPK1 gene expression and inhibiting PI3K/AKT signaling pathway activation which both miR-345 and miR-1296 could be used to control the EMT progression.

In addition, ADAM10 induces an E-cadherin/β-catenin signaling pathway, which is regulated by miR-655-3p, resulting in the reduction of intracellular β-catenin signaling and metastasis induction [131] (Fig. 5). Collectively, we can conclude that miRNAs are important in tumorigenesis including EMT and metastasis process (Tables 3 and 6), and their levels could be potent cell-free biomarkers to evaluate prognosis, and response to current therapies and even be introduced as a therapeutic option.

8. Glycolytic dysregulation in HCC

Metabolic reprogramming is a hallmark of cancer cells [132]. Despite adequate oxygen levels, tumor cells tend to generate energy from aerobic glycolysis, known as the Warburg effect, rather than mitochondrial oxidative phosphorylation, which often results in increased glucose uptake, ATP accumulation, and lactate formation in tumor cells [133]. Though glycolysis yields less ATP compared to oxidative phosphorylation, the Warburg effect confers benefits to cell growth not only by supplying carbon sources essential for rapid cell proliferation but also by decreasing the production of reactive oxygen species (ROS) [134]. Normal isolated hepatocytes do not generate energy from aerobic glycolysis under non-hypoxic environments. However, in HCC, extensive reprogramming of metabolic pathways occurs [134,135]. Understanding these metabolic changes is crucial for developing novel therapeutic targets for HCC.

MicroRNAs are involved in the degradation of important mRNAs regulating cancer cell metabolism by targeting protein-coding
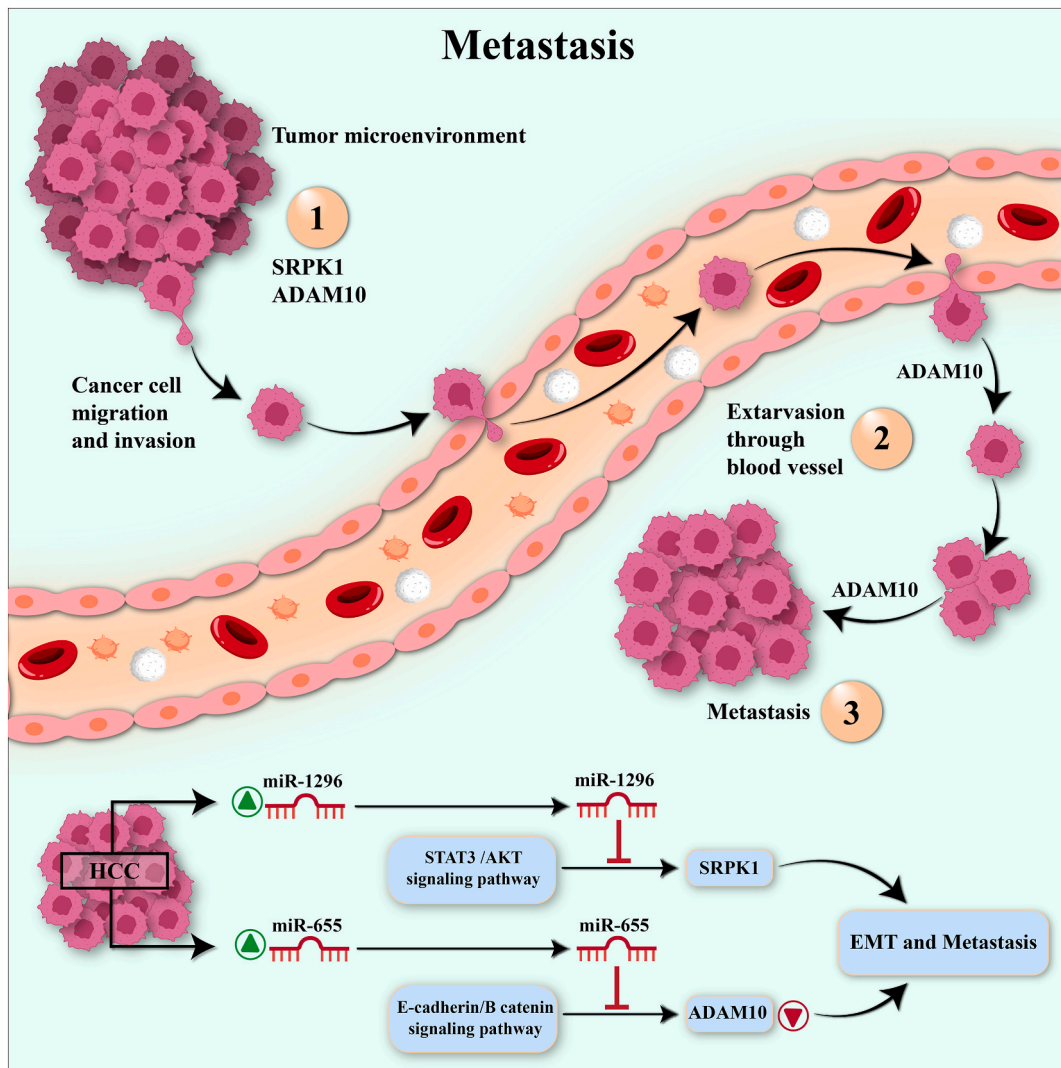
Fig. 5. Role of miRNAs in metastasis of HCC. . 1. To create metastasis, first, cancer cells are separated from the tissue mass with the origin of cancer by ADAM proteins, a family of secretory and membrane metalloproteinases, and SRPK1, which is a splicing factor of SRSF protein kinase 1. They cause the movement and migration of cells removed from tumor tissue in the tumor microenvironment of blood vessels 2. Tumor cells pass through the vessels reach the surrounding healthy tissues and create the characteristics of cancer cells in healthy tissues. 3. Metastasis occurs in distant and nearby tissues.

Up-regulation of miR-1296 inhibits hypoxia-promoting effects on metastasis and EMT of HCC cells by targeting SRPK1 gene expression and inhibiting PI3K/AKT signaling pathway activation. Also, Mir-655 inhibits metastasis in HCC cells by directly targeting ADAM10 inhibiting the β-catenin pathway, and downregulating E-cadherin protein expression.

transcripts that are directly inhibiting key molecules (kinases/transporters or enzymes) of metabolic processes and transporters and also regulating various oncogenic signaling pathways or tumor suppressors including c-Myc, AMPK and AKT and p53 signaling pathway [136,137]. The molecular mechanisms of the Warburg effect in cancer cells were considered as an example to clarify miRNA regulation in energy metabolism (Table 4). For example, in the primary stage of glucose metabolism, glucose can be transported across the plasma membrane by GLUT3 or GLUT4, and miR-133 or miR-195-5p are among the microRNAs active in this process [138,139].

Recently, miR-3662 was found to be upregulated during hematopoietic differentiation, but downregulated in acute myeloid leukemia [140]. Increased miR-3662 expression was also observed in lung adenocarcinoma [141]. miR-3662 was found to be frequently downregulated in HCC tissues and cell lines. After treatment with the hypoxia mimetic CoCl2, miR-3662 regulated the Warburg effect and HCC progression by decreasing hypoxia-inducible factor 1α (HIF-1α) and repressed the activation of ERK and JNK signaling pathways [142] (Fig. 6). These findings suggest a mechanistic role for miR-3662/HIF-1α as a potential therapeutic strategy in HCC. In addition, miR-183-5p targets PTEN to activate Akt/mTOR signaling and also increases the expression of genes related to glycolysis, including PKM2, HK2, LDHA, GLUT1, and others [143] (Fig. 6). Conversely, miR-196a and miR-196b suppress glycolysis by targeting SOCS2, thereby inhibiting the JAK/STAT pathway. Their downregulation is associated with enhanced glycolytic activity, increased

Table 3
EMT-regulating miRNAs in Hepatocellular carcinoma.

MicroRNA	suppression or induction	Mechanism of action	Study type	Potent application	Ref.
miR-23b	Suppression	Targets Pyk2	*in vitro*	prognostic and Therapeutic	(88)
miR-373-3p	Suppression	Inhibits TFAP4/PI3K/AKT pathway	*in vitro/in vivo*	prognostic/Therapeutic	(65)
miR-300	Suppression	Targets the FAK/PI3K/AKT signaling pathway	*in vitro*	Therapeutic	(89)
miR-141-3p	Suppression	E-cadherin, occludin, and cytokeratin 18 (CK18)	*in vitro/in vivo*	Therapeutic	(90)
miR-21	Induction	Suppresses PTEN and hSulf-1 expression through AKT/ERK pathways	*in vitro/in vivo*	Therapeutic	(91)
miR-26b	Suppression	Targets USP9X	*in vitro*	Therapeutic	(92)
miR-1296	Suppression	Targets SRPK1/AKT pathway	*in vitro/in vivo*	Prognostic	(85)
miR-501	Induction	Targets JDP2	*In vitro*	Diagnostic	(93)
miR-26a	Suppression	Down-regulates EZH2 expression	*in vitro/in vivo*	Therapeutic	(94)
miR-612	Suppression	Targets HADHA with the inhibition of Wnt/β-catenin	*in-vitro/in-vivo*	Diagnostic/Therapeutic	(95)
miR-214-5p	Suppression	via gankyrin/AKT signaling	*in vitro/in vivo*	Therapeutic	(96)
miR-296-5p	Suppression	Suppresses NRG1/ERBB2/ERBB3/RAS/MAPK/Fra-2 signaling	*in-vitro/in-vivo*	Prognostic/Therapeutic	(97)
miR-6875-3p	Suppression	via the BTG2/FAK/Akt pathway	*in-vitro/in-vivo*	Prognosis/Diagnosis/ Therapeutic	(98)
miR-532-3p	Induction	Activates the gankyrin/AKT/TWIST1 signaling pathway	*in-vitro/in-vivo*	Prognosis/Diagnosis	(99)
miR-199b-5p	Suppression	Targets TGF-b1	*in-vitro/in-vivo*	Therapeutic	(100)
miR-216a/ 217	Induction	Activates the PI3K/Akt and TGF-β pathways by targeting PTEN and SMAD7	*in-vitro/in-vivo*	Prognostic/Therapeutic	(101)
miR-4319	Suppression	by targeting FOXQ1	*in-vitro/in-vivo*	Therapeutic	(102)
miR-9	Suppression	Targets EIF5A2	*in-vitro/in-vivo*	Therapeutic	(103)
miR-542-3p	Suppression	UBE3C	*in-vitro/in-vivo*	Prognostic/Therapeutic	(104)
miR-876-5p	Suppression	BCL6 corepressor like 1	*in vitro*	Therapeutic	(105)
miR-379-5p	Suppression	FAK/AKT signaling	*in-vitro/in-vivo*	Prognostic/Therapeutic	(106)
miR-187-3p	Suppression	S100A4	*in-vitro/in-vivo*	Prognostic/Therapeutic	(107)
miR-221	Induction	AdipoR1	*in vitro*	Prognostic/Therapeutic	(108)
miR-30e	Suppression	ErbB2-dependent pathway	*in vitro/in vivo*	Diagnostic	(109)
miR-139-5p	Suppression	Targeting ZEB1 and ZEB2	*Invitro*	Diagnostic/Therapeutic	(110)

tumor size, tumor-node metastasis (TNM) stage, lymph node metastasis, albumin-bilirubin grade, and poor 5-year survival and novel targets for prognosis and therapeutics of HCC [144]. Therefore, targeting microRNAs that regulate glycolysis-associated pathways presents a novel avenue for HCC therapy.

9. Angiogenic mechanisms and pathways in HCC

Angiogenesis is a highly regulated process for forming new blood vessels. It is fundamental in many biological processes including development, reproduction, and wound repair. Numerous inducers of angiogenesis have been identified, including the members of the vascular endothelial growth factor (VEGF) family, angiopoietins, TGFs, PDGF, tumor necrosis factor-α (TNF-α), interleukins (IL-s), and members of the FGF family [145,146]. Angiogenesis is a process that helps HCC grow and spread by providing nutrients to tumor cells and increasing tumor growth and metastasis [147].

Angiogenesis is the introduction of invasion of lymph and blood vessels followed by metastasis of primary tumor cells to distant organs. The process of angiogenesis is led by different pathways and these pathways are regulated by miRNAs, and any disruption of them will finalize the process of transformation in normal cells. The upregulation of miR-433 and silencing of KDM5A down-regulates the FXYD3-PI3K-AKT axis which inhibits the angiogenesis process in HCC and subsequently hinders cancer growth [148]. The metastatic and pro-angiogenic ability of HCC cells could be suppressed by miR-497. Subsequent studies revealed that miR-497 suppressed the 3′-untranslated regions of VEGF-A and astrocyte elevated gene-1 [149].

The role of microRNAs in the angiogenesis process of cancer cells has been extensively studied for therapeutic and diagnostic purposes. For example, miR-26a targets hepatocyte growth factor (HGF). miR-26a exerts its anti-angiogenesis function, at least in part,

Table 4
Glycolysis -regulating miRNAs in Hepatocellular carcinoma.

MicroRNA	suppression or induction	Mechanism of action	Type of study	Potent application	Ref.
miR-374b	Suppression	Antagonizing PKM2-mediated glycolysis pathway	in vitro/in vivo	Therapeutics	(124)
miR-30a-5p	Suppression	Directly targets CLCF1	in vitro/in vivo	Therapeutics	(125)
miR-139-5p	Suppression	Via a reciprocal regulatory interaction with ETS1	in vitro/in vivo	prognostic and Therapeutics	(126)
miR-3662	Suppression	Inhibits of HIF-1α-mediated Warburg effect	in vitro/in vivo	Diagnosis/prognosis/Therapeutic	(121)
miR-125a	Suppression	Targets hexokinase HK2	in vitro/in vivo	Therapeutics	(127)
miR-183-5p	Induction	Targets PTEN and activates Akt/mTOR signaling	In vitro	Therapeutics	(122)
miR-873	Induction	Through the AKT/mTOR-mediated Warburg effect	in vitro/in vivo	Prognosis/therapeutics	(128)
miR-126	Induction	Targets IRS1 and interferes with the mitochondrial function	in vitro/in vivo	Therapeutics	(129)
miR-181a-5p	Induction	Regulates the electron transport chai	in vitro/in vivo	Therapeutics	(130)
miR-199a	Suppression	Targets hexokinase-2 (Hk2)	in vitro/in vivo	Therapeutics	(131)
miR-34c-3p	Induction	Targets MAGI3 and contributes to enhanced Warburg effect	in vitro and in vivo	Therapeutics	(132)
miR-23a	Induction	Targets PGC-1a and G6PC	in vitro and in vivo	Therapeutics	(133)
miR-199a-5p	Suppression	Targets Hexokinase 2	in vitro and in vivo	Prognostic	(134)
miR-383	Suppression	Targets LDHA	in vitro	Therapeutic	(135)
miR-103a	Induction	via ATP11A and EIF5	in vitro and in vivo	Prognostic	(136)
miR-192-5p	Suppression	Upregulates GLUT1 and PFKFB3 and c-Myc	in vitro	Prognostic	(137)
miR-34a	Suppression	Inhibits LDHA-dependent glucose	in vitro	Prognostic and Therapeutics	(138)
miR-129-5p	Suppression	Targets the pyruvate dehydrogenase kinase 4 (PDK4)	in vitro and in vivo	Therapeutic	(139)
miR-593-3p	Suppression	Targets Slc38a1 and CLIP3	in vitro	Diagnostic	(140)
mIR-455-5p	Suppression	via IGF-1R/AKT/GLUT1 pathway by targeting IGF-1R	in vitro	Therapeutic	(141)
miR-142-3p	Suppression	Targets LDHA	in vitro	Therapeutic	(142)

by inhibiting the HGF-hepatocyte growth factor receptor (c-Met) and its downstream signaling pathway, which in turn suppresses VEGFA production in HCC cells and impairs VEGFR2 signaling in endothelial cells. In HCC patients with high miR-26a, low VEGFA, low HGF, or small vessel density in tumor tissues had a better prognosis in terms of time to recurrence and overall survival (OS). Therefore, the study of miR-26a can suppress HCC tumor angiogenesis through HGF-cMet signaling [150]. Therefore, miR-26a is a promising therapeutic target and an independent prognostic indicator for overall survival (OS) and time to recurrence (TTR) of HCC patients. Considering the role of miR-199a-3p in the regulation of TME, miR-199a-3p reduced several intercellular interactions in HCC by abrogating several TME components such as VEGFA, VEGFR1, VEGFR2, ANGPT1, MMP2, HGF, cMET, CD44, and PDGFRα. In addition, miR-199a-3p limits metastasis, invasion, and angiogenesis in HCC by inhibiting HGF signaling and regulating MMP2, and thus can be considered one of the most powerful effective treatments for HCC patients [151]. Furthermore, BRCA1 can repress PDGFRA promoter activity and miR-146a reduces BRCA1 gene expression to impair the BRCA1-PDGFRA signaling pathway. Therefore, this microRNA increases tumor angiogenesis, and miR-146a and PDGFRA may appear as potential anti-angiogenic targets for HCC treatment based on ECs function (Table 5) [152] (Fig. 7).

10. Exploring regulatory miRNA dynamics in HCC: A bioinformatics approach

In this section, we detail our bioinformatics analysis aimed at uncovering regulatory miRNA dynamics in hepatocellular carcinoma (HCC), offering insights into potential diagnostic and therapeutic targets. The methodology employed in our analysis draws from an autonomous and extensive collection of Liver Hepatocellular Carcinoma (LIHC) data (raw gene count) obtained via RNA-sequencing from The Cancer Genome Atlas (TCGA) available from https://cancergenome.nih.gov.

We conducted differential expression analysis of miRNAs (DEmiRNAs) using the DESeq2 R package in Bioconductor, leveraging the mean expression levels of genes across samples for filtering. Significant DEmiRNAs were identified based on expression differences between 372 tumor and 50 corresponding paracancerous tissue samples, using a log fold change of |1| and an adjusted p-value threshold of 0.05 [153].

To explore miRNA expression patterns in different HCC patient populations, we utilized an interactive microRNA target viewer (miR-TV) from TCGA version 18.0 (http://mirtv.ibms.sinica.edu.tw). Additionally, we employed the miRPathDB database (https://mpd.bioinf.uni-sb.de/) to gain insights into miRNA-regulated pathways, expanding our understanding of target pathways accessible through bioinformatics analysis. The interaction between DEmiRNAs and targets was visualized using alluvial diagrams generated with the gg alluvial tool.

To explore miRNA expression patterns in different HCC patient populations, for this purpose, microRNAs obtained from TCGA were first collected, and then the mature, immature forms of microRNAs, and up-regulation or down-regulation were extracted from the
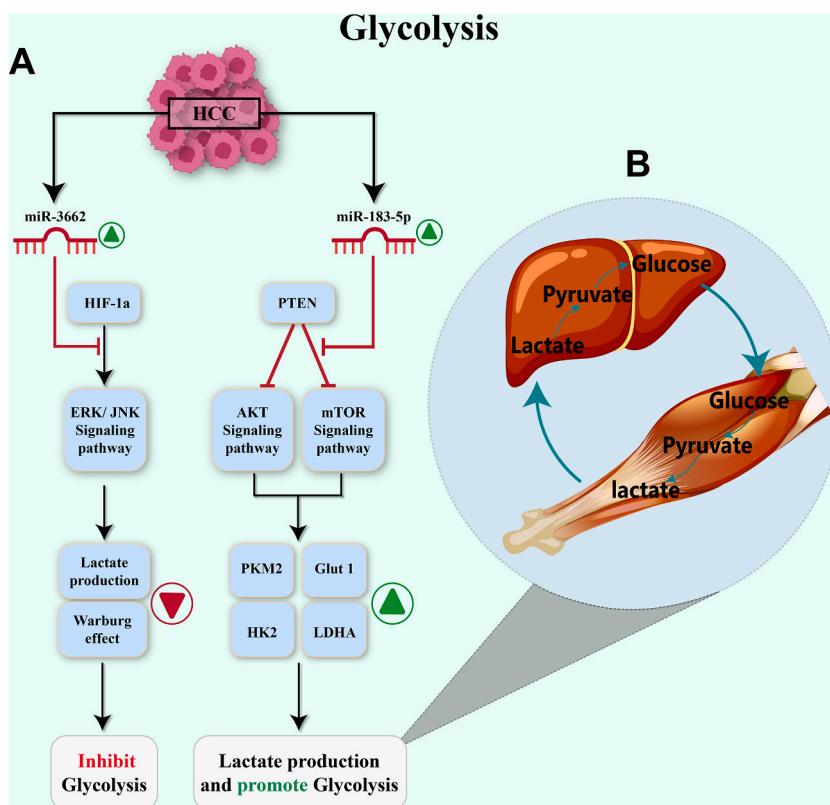
Fig. 6. Role of miRNAs regulating glycolysis in HCC. A. Tumor cells use glucose as their metabolic source to meet their biosynthetic and bioenergetic needs. By inactivating pyruvate dehydrogenase and increasing the expression of lactate dehydrogenase A, pyruvate is converted to lactate, and lactate increases to a large level. Increased levels of lactate become the main energy fuel in tumors.
B. The increase of miR-3662 levels by directly targeting the hypoxia-inducible factor 1α (HIF-1α) by inhibiting the activation of ERK and JNK signaling pathways and by reducing lactate production and reducing the Warburg effect inhibits coagulation in cancer cells. Increasing the level of miR-183-5p by reducing the expression of the PTEN gene in the Akt, p-Akt, and mTOR pathway and increasing the expression of PKM2, HK2, LDHA, GLUT1 genes with lactate production leads to an increase in glycolysis in HCC cells.

Mir-TV database (**Supplementary files 1**).

Additionally, we employed the miRPathDB database to gain insights into miRNA-regulated pathways, expanding our understanding of target pathways accessible through bioinformatics analysis. For this purpose, we entered the obtained data into miRPathDB (https://mpd.bioinf.uni-sb.de/), and the pathways and target genes for the up-regulated and down-regulated microRNAs were obtained separately (**Supplementary files 2 and 3**). The data obtained from the miR PathDB database were from the experimental strong data set. In the next step, we compared the pathways affected by the microRNAs extracted from the bioinformatics databases with the pathways obtained from the original articles specific to HCC, showing the role of these specific pathways in the six features of EMT, glycolysis, angiogenesis, autophagy, apoptosis and metastasis (Table 7).

Based on these comprehensive analyses, we extracted microRNAs specific to each cancer characteristic in the respective tables. These microRNAs were plotted using R software (**Supplementary files 1 to 3**). Simultaneously with the extraction of bioinformatics data, in addition to the extraction of HCC-specific pathways in the examined cancer characteristics, specific microRNAs of each characteristic were also extracted from exclusive HCC research articles from Google Scholar and PubMed databases. The data for each characteristic were extracted into separate tables. The microRNAs extracted from the research articles were compared and analyzed with the microRNAs predicted by the bioinformatics analysis. Finally, the data that have the greatest role in all these features were determined (Figs. 8 and 9).

Our bioinformatics analyses unveiled 26 microRNAs that can simultaneously play a role in all 6 processes including glycolysis, EMT, angiogenesis, metastasis, autophagy, and apoptosis (Table 8). These miRNAs, specific to HCC in TCGA, were predominantly expressed in an upregulated form. Conversely, miRNAs exhibiting low expression levels included miR-130a-3p, miR-139-5p, miR-195-5p, miR-214-5p, miR-214-3p, and miR-424-5p.

Furthermore, we compared the microRNAs obtained from the bioinformatics data with the microRNAs obtained from the research articles. The data of research articles were also analyzed in six characteristics glycolysis, EMT, apoptosis, autophagy, angiogenesis, and metastasis. As shown in Table 9, the results of HCC-specific research articles play a role in the characteristics of glycolysis, angiogenesis, metastasis, autophagy, apoptosis, and EMT. According to the data of research articles and the distribution of these data in the

Table 5
Angiogenesis-regulating miRNAs in Hepatocellular carcinoma.

MicroRNA	suppression or induction	Mechanism of action	Study type	Potent application	Ref
miR-126-3p	Suppression	Targets LRP6 and PIK3R2	*in vitro/in vivo*	Therapeutic	(151)
miR-182	Induction	Targets RASA1	*in vitro*	Therapeutic	(152)
miR-375	Suppression	Inhibits platelet-derived growth factor C (PDGFC)	*in vivo/in vitro*	Therapeutic	(153)
miR-203a	Suppression	through VEGFR by targeting HOXD3	*in vitro*	Therapeutic	(154)
miR-200b-3p	Suppression	Enhances endothelial ERG expression	*in vitro*	Therapeutic	(155)
miR-146a	Induction	Promotes the expression of platelet-derived growth factor receptor α (PDGFRA)	*in vitro*	Therapeutic	(150)
miR-199a-3p	Suppression	Targets VEGFA, VEGFR1, VEGFR2, HGF and MMP2	*in vivo/in vitro*	Therapeutic	(149)
miR-1301	Suppression	Decreases Wnt/β-catenin signaling through targeting BCL9	*in vitro/in vivo*	Therapeutic	(156)
miR-142	Suppression	Targets and inhibits transforming growth factor β (TGF-β)	*in vitro*	Therapeutic	(157)
miR-3064-5p	Suppression	Targets the FOXA1/CD24/Src pathway	*in vitro*	Prognostic/ Therapeutic	(158)
miR-29b	Suppression	Regulates Matrix Metalloproteinase 2 expression	*in vitro/in vivo*	Therapeutic	(159)
miR-195	Suppression	Inhibits the expression of VEGF, VAV2, and CDC42	*in vitro/in vivo*	Therapeutic	(160)
miR-26a	Suppression	by Targeting Hepatocyte Growth Factor-cMet Pathway	*in vitro/in vivo*	Prognostic/ Therapeutic	(148)
miR-584-5p	Induction	Through PCK1-mediated nuclear factor E2-related factor 2 signaling pathway	*in vitro/in vivo*	Therapeutic	(161)
miR-503	Suppression	Targets FGF2 and VEGFA	*in vitro/in vivo*	Therapeutic	(162)
miR-338-3p	Induction	Targets MACC1, b-catenin and VEGF	*in vitro*	Prognostic/ diagnosis	(163)
miR-214	Suppression	Activates the HDGF paracrine pathway	*in vitro/in vivo*	Prognostic/ Therapeutic	(164)
miR-20b	Suppression	Negatively regulated VEGF expression by directly targeting STAT3	*in vitro*	Therapeutic	(165)
miR-433	Suppression	by KDM5A silencing and suppresses the FXYD3-PI3K/AKT axis	*in vitro/in vivo*	Therapeutic	(146)
miR-302c	Suppression	Inhibits expression of metadherin (MTDH)	*in vivo/in vitro*	Therapeutic	(166)
miR-130b-3p	Induction	Down-regulation and dysregulation in the Sp1/miR-130b-3p/ HOXA5 axis	*in vitro/in vivo*	Prognostic/ Therapeutic	(167)
miR-139-3p	Suppression	Represses ANXA2R	*in vitro/in vivo*	Therapeutic	(168)

desired cancer characteristics, it seems that these data can be a suitable candidate for therapeutic purposes.

Finally, bioinformatics data microRNAs were compared with microRNAs examined in HCC research articles and common microRNAs, the most important of these microRNAs include; miR-34a, miR-139-5p, miR-21-5p, miR-373-3p, miR-195-5p, and miR-139-5p. Joint data obtained from bioinformatics and research specific to HCC may be suitable candidates for diagnostic and therapeutic studies. Our bioinformatics approach and data obtained in this section offer a systematic framework for exploring regulatory miRNA dynamics in HCC, providing a foundation for future research aimed at advancing diagnostic and therapeutic strategies for this complex disease.

11. Combinatorial approaches

HCC cancer cells are highly complex, and due to continuous genetic changes caused by heterogeneity, they can evade chemotherapy and radiotherapy drugs over time and show resistance to treatment [154]. HCC cancer cells do not respond to treatment due to changes in the expression pattern of genes that confer resistance to chemotherapy drugs [154,155]. The presence of genetic variations in HCC leads to inconsistencies in the activity of signaling pathways, drug metabolism, transport, and dominance of DNA repair protein expression, all of which affect resistance.

A widely used approach is combination therapy, which uses chemotherapy drugs or radiation therapy together with gene therapy such as RNAi, to eliminate the expression of drug-resistance genes [156,157]. Artificial miRNAs, which mimic the function of natural miRNAs in the cell, regulate the expression of many genes simultaneously. To enhance the delivery of miRNAs into the cells, suitable viral vectors and nanoparticle-scale carriers are recommended and tested in various studies [158,159]. The combined treatment of sorafenib and miR-486-3p has been studied *in vitro* and *in vivo* for HCC. Overexpression of miR-486-3p was observed by lentivirus injection in cell and animal models. miR-486-3p, by targeting FGFR4 and EGFR, was shown to reduce resistance to sorafenib and suppress tumor growth [160]. Paclitaxel (PTX) is an intravenous anticancer drug that can lead to treatment resistance. Shi et al.

Table 6
Metastasis-regulating miRNAs in hepatocellular carcinoma.

microRNAs type	suppression or induction	Mechanism	Study type	Potent application	Ref.
miR-30d	Induction	Targets Galphai2	*in vitro/in vivo*	Therapeutic	(169)
miR-146a	Suppression	Downregulates VEGF	*in vitro/in vivo*	Therapeutic	(170)
miR-188-5p	Suppression	Targets FGF-5	*in vitro/in vivo*	Prognostic/Therapeutic	(171)
miR-135a	Induction	by forkhead box M1 (FOXM1)	*in vitro/in vivo*	Prognostic	(172)
miR-7	Suppression	Targets the Phosphoinositide 3-Kinase/Akt Pathway	*in vitro/in vivo*	Diagnostic/Prognostic/ Therapeutic	(33)
miR-612	Suppression	Targets AKT2	*in vitro/in vivo*	Diagnostic	(95)
miR-1296	Suppression	Targets SRPK1-mediated PI3K/AKT pathway	*in vitro/in vivo*	Prognostic/Therapeutic	(85)
miR-195	Suppression	Inhibits the expression of VEGF, VAV2, and CDC42	*in vitro/in vivo*	Prognostic/Therapeutic	(160)
miR-140-5p	Suppression	by targeting TGFBR1 and FGF9	*in vitro/in vivo*	Prognostic	(173)
miR-137	Suppression	Targets AKT2	*in vitro/in vivo*	Prognostic	(174)
miR-187-3p	Suppression	Targets S100A4	*in vitro/in vivo*	Prognostic/Therapeutic	(107)
miR-23b	Induction	Targets ST7L	*in vitro/in vivo*	Diagnostic/Therapeutic	(175)
miR-10b	Induction	Targets CADM2	*in vitro/in vivo*	Therapeutic	(176)
miR-532-3p	Suppression	Activatesgankyrin/AKT/TWIST1 signaling pathway	*in vitro/in vivo*	Prognostic	(99)
miR-501-3p	Suppression	TargetsLIN7A	*in vitro/in vivo*	Prognostic/Therapeutic	(177)
miR-196b	Induction	Targets FOXP2	*in vitro/in vivo*	Prognostic	(178)
miR-1269b	Induction	Through the PI3K/Akt pathway	*in vitro/in vivo*	Prognostic/Therapeutic	(85)
miR-142	Suppression	Directly targets and inhibitsTGF-β	*in vitro*	Therapeutic	(157)
miR-17-5p miR-20a-5p	Suppression	Blocks HGF/ERBB3-NF-κB	*in vitro/in vivo*	Prognostic/Therapeutic	(179)
miR-487a	Induction	by PIK3R1 and SPRED2	*in vitro/in vivo*	Prognostic/Therapeutic	(180)
miR-197	Induction	Activates Wnt/β-Catenin Signaling	*in vitro/in vivo*	Prognostic/Therapeutic	(181)
miR-182-5p	Induction	Represses FOXO3a	*in vitro/in vivo*	Prognostic	(182)
miR-3650	Suppression	Directly targets NFASC	*in vitro*	Prognostic/Therapeutic	(183)
miR-561-5p	Induction	by CX3CL1 Signaling	*in vitro/in vivo*	Prognostic/Therapeutic	(184)
miR-873	Induction	Directly Targets TSLC1	*in vitro*	Therapeutic	(185)
miR-296-5p	Suppression	Attenuates NRG1/ERBB2/ERBB3 signaling	*in vitro/in vivo*	prognostic/Therapeutic	(97)
miR-6875-3p	Induction	via BTG2/FAK/Akt pathway	*in vitro/in vivo*	prognostic/Therapeutic	(98)
miR-612	Suppression	By HADHA mediated lipid reprogramming	*in vitro/in vivo*	Therapeutic	(95)
miR-140-5p	Suppression	Targets TGFBR1 and FGF-9	*in vitro*	prognostic/Therapeutic	(173)
miR-126-3p	Suppression	Targets LRP6 and PIK3R2	*in vitro/in vivo*	Therapeutic	(151)
miR-373-3p	Suppression	Inhibits TFAP4/PI3K/AKT pathway	*in vitro/in vivo*	prognostic/Therapeutic	(65)
ssmiR-506	Suppression	TargetsIL-8	*in vitro/in vivo*	Therapeutic	(186)
miR-1251-5p	Induction	Targets AKAP12	*in vitro/in vivo*	Therapeutic	(187)
miR-517a	Induction	Targets rapamycin/Akt/phosphatidylinositol (PI)3K pathway	*in vitro/in vivo*	Therapeutic	(188)
miR-509-3p	Suppression	Inhibits Twist expression	*in vitro/in vivo*	Therapeutic	(189)

Table 6 (*continued*)

microRNAs type	suppression or induction	Mechanism	Study type	Potent application	Ref.
miR-374b-5p	Suppression	Through the ERK/ZEB1 pathway	*in vitro/in vivo*	prognostic/Therapeutic	(190)
miR-3677-3p	Induction	Suppresses SIRT5	*in vitro/in vivo*	prognostic/Therapeutic	(191)
miR-211-5p	Suppression	Targets ACSL4	*in vitro/in vivo*	prognostic/Therapeutic	(192)
miR-93-5p	Induction	via a microRNA-93-5p/MAP3K2/c-Jun	*in vitro/in vivo*	Therapeutic	(193)
miR-21-3p	Induction	Upregulates YAP1 expression via direct inhibition of SMAD7	*in vitro/in vivo*	Prognostic	(194)
miR-650	Induction	Directly Inhibits LATS2 Expression	*in vitro*	prognostic/Therapeutic	(195)
miR-766-3p	suppression	Targets Wnt3a	*in vitro/in vivo*	prognostic/Therapeutic	(196)
miR-142-3p	suppression	by TUG1/miR-142-3p/ZEB1 axis	*in vitro/in vivo*	prognostic/Therapeutic	(197)
miR-429	suppression	via inhibiting Raf/MEK/ERK pathway by targeting CRKL	*in vitro*	Diagnostic/Therapeutic	(198)
miR-330-5p	Induction	via MAPK/ERK signaling by targeting SPRY2	*in vitro/in vivo*	prognostic/Therapeutic	(199)
miR-655-3p	suppression	Regulates ADAM10 and β-catenin pathway	*in vitro*	Therapeutic	(200)
miR-876	suppression	via POSTN	*in vitro/in vivo*	prognostic/Therapeutic	(201)
miR-379-5p	suppression	Target FAK/AKT signaling	*in vitro/in vivo*	Prognostic/Therapeutic	(106)
miR-21-5p	Induction	Regulates miR-21-5p/KLF6 Axis	*in vitro/in vivo*	Prognostic	(202)
miR-139-5p	suppression	Target ZEB1 and ZEB2	*In vitro*	Diagnostic/Therapeutic	(110)

investigated the combined effect of miR34a and paclitaxel after loading them into cationic solid lipid nanoparticles (miS-LNs-34a/PTX). The nanoparticle carrying miR34a and paclitaxel was injected into the mouse model. Combined treatment increased the sensitivity of cancer cells and inhibited tumor growth [161]. Another study observed combined treatment of doxorubicin (DOX) and miR-34a in prostate cancer increased programmed cell death and decreased drug resistance to doxorubicin through suppression of CD44 [162]. Combined treatment of miR-375 and DOX via liposomes has been studied in HCC cells and mouse models. The results showed that miR-375 inhibited HCC tumor growth by significantly reducing the expression of YAP1, AEG-1, and ATG7. Aditionally, combined miR-375/DOX treatment showed synergistic effects on HCC tumor inhibition and increased HCC sensitivity to DOX [163]. Combined treatment of chemotherapy drug and gene therapy using Sorafenib (So) as a kinase inhibitor and antimiRNA21 was investigated in lipoprotein modified with pentapeptide RGD in liver cancer. In vitro and *in vivo* findings showed the synergistic effect of antimiRNA21 and Sorafenib (So) on inhibiting tumor growth and increasing sensitivity to Sorafenib| [164]. Combined treatments of chemotherapeutic agents and miRNA are promising approaches to overcome chemotherapy drug resistance. However, further clarification of the pharmacodynamic and pharmacokinetic data regarding the combination of chemotherapy agents and miRNAs is necessary. Moreover, studies on the safety of miRNA in terms of immune response, and toxicity of delivery systems of combined therapeutic agents should be conducted. However, there are still challenges in this regard. RNAi or RNA interference such as siRNA has the potential to control gene expression. In addition to miRNA, it is one of the most promising gene therapy strategies at the laboratory, *in vivo* and clinical levels. The potential advantage of siRNA is that it specifically inhibits the expression of a carcinogenic protein and has no effect on the wild type [165,166]. Development of miRNA/antimiR and siRNA/miRNA-based combinations alongside chemotherapeutic drugs is another strategy of combination therapies that is of interest. Liver cancer shows resistance to doxorubicin after some time of treatment. In one study, (lactic-co-glycolic acid) nanoparticles (PLGA-NP) were designed loaded with two complementary miRNA fusion agents (sense miRNA-122 and antisense antimiR 21). Then, it was transfected and examined by ultrasound method in cells and mouse model. The results showed that miRNA-loaded PLGA-NP simultaneously with doxorubicin increased apoptosis in doxorubicin-resistant HCC cells [167]. In a study in a HCC mouse model, the synergistic effect of microRNA-122 and anti-microRNA-21 was investigated. microRNA-122 and anti-microRNA-21 in nanoparticles PLGA-b-PEG were loaded and delivered by ultrasound-targeted microbubbles (UTMD) method. The findings showed that the combined treatment of microRNA-122 and anti-microRNA-21 prevents HCC tumor growth by creating transient cytokine storms in the HCC microtumor environment and by reducing GM-CSF levels [168].

One of the factors of drug resistance in cancer is the repair of DNA damage. DNA repair pathways can increase the vulnerability of cancer cells to treatment. Identifying the precise mechanisms of DNA damage repair has led to new therapeutic strategies against cancer. New therapeutic strategies based on the combination of miR-146a-5p and radiation therapy have been studied in HCC cells through the DNA damage repair pathway. Replication Protein A (RPA) is a single-stranded protein (ssDNA) that makes HCC cancer cells resistant to radiotherapy. The findings showed that miR-146a-5p as a tumor suppressor by binding to the repeat protein A3 (RPA3), activates the DNA damage repair pathway. By inhibiting the cell cycle and growth of HCC cells and inducing apoptosis, it increases the sensitivity to radiation therapy [169].
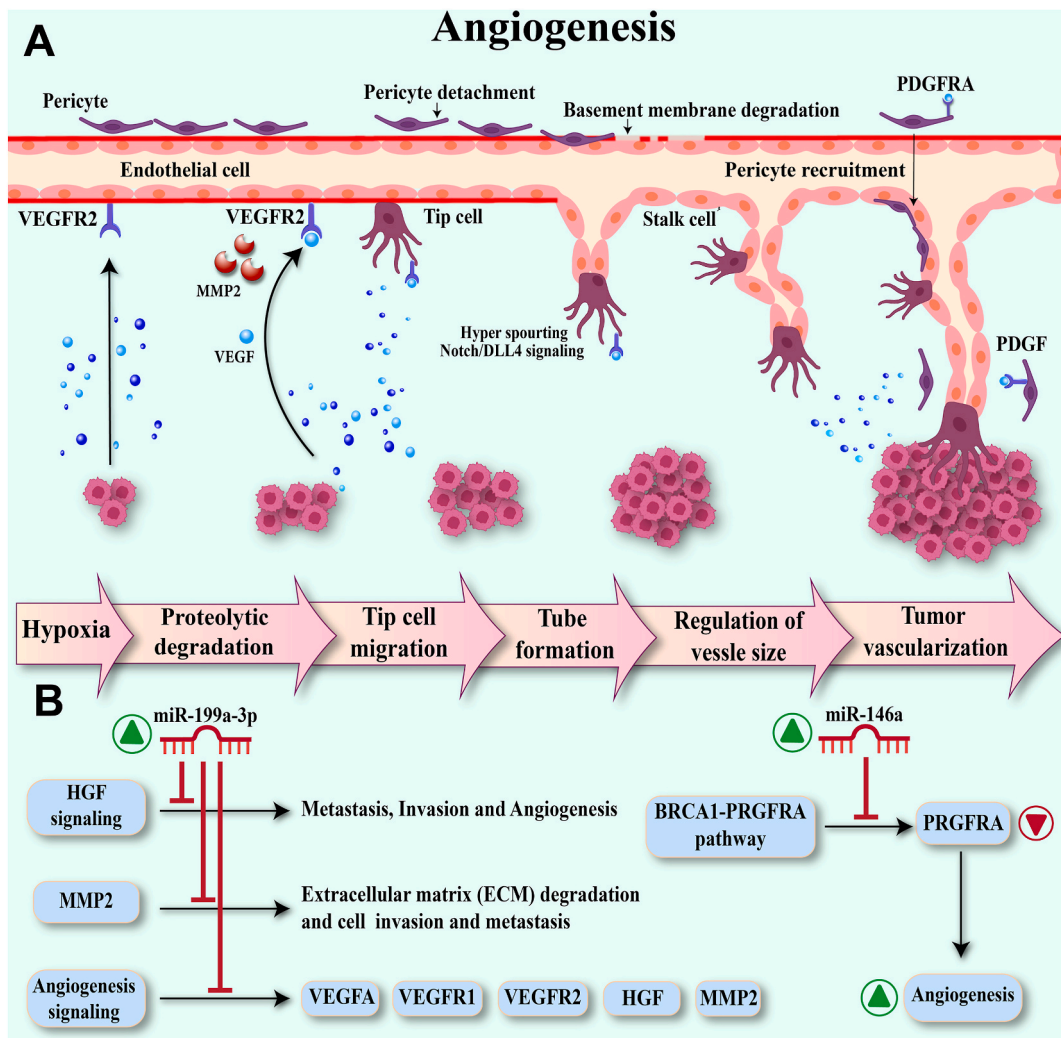
Fig. 7. Role of miRNAs regulating angiogenesis in HCC. A. Pericytes line the vascular endothelium throughout the body. Pericytes cover vascular endothelium throughout the body, platelet-derived growth factor receptors, and vascular endothelial growth factor receptors are located on these vascular surfaces. tip/stalk cells are a group of endothelial cells. Tumor cells widely secrete pro-angiogenic factors and create tumor tissue with poor perfusion. After that, a hypoxic microenvironment is created, which is one of the main drivers of angiogenesis in tumor tissue. PDGF by tip cells is secreted, and the high level of VEGF secretion by tumor cells blocks PDGFRb signaling through the receptor complex consisting of PDGFRb and VEGFR2. VEGF-stimulated tip/stalk cells tend to migrate and proteolytic enzymes such as MMP, elastase or trypsin are secreted by cancer cells. These enzymes destroy cell junctions. The released cells gradually mimic the behavior of blood vessel cells and form channel-like structures similar to vessels.

B. By increasing the expression of miR-199a-3p, the signaling pathways of HGF, and MMP2 are blocked, and at the same time, the expression of VEGFR1 and VEGFR2, HGF, MMP2, VEGFA genes is inhibited, and then angiogenesis is inhibited. Increased miR-146 increases angiogenesis by inhibiting PDGFRA activation in the BRCA1–PDGFRA pathway.

Another combination strategy is the treatment of small molecules, which are used as modulators and enhancers of miRNAs. In a study, CMD-stabilized PEI-PCL nanoparticles were designed. 2′-hydroxy 2,4,4′,5,6′-pentamethoxychalcone named Rubone is an anti-cancer agent that is loaded with miR-34a into PEI-PCL nanoparticle stabilized with CMD and *in vitro* and *in vivo* HC There were thirty studies. The findings showed that Rubon regulates the expression of miR-34a, which leads to inhibiting tumor growth and increasing apoptosis of HCC cells [170]. Also, in another study, sustained release nanoparticles based on chitosan were designed to load miR-128-3p agomir (NA-miR-128-3p) and the natural product Oroxin B and were transferred to HCC cells and mouse model. The combined effect of miR-128-3p agomir (NA-miR-128-3p) and the natural product Oroxin B interferes with the VEGF and PI3K-AKT pathways and enhances tumor growth inhibition [171].

Table 7

Biological pathways specific to six cancer characteristics were extracted from HCC related articles.

| Types of HCC pathways	Types of cancer characteristics	Ref.
PI3K/Akt/mTOR pathways	Autophagy-apoptosis-metastasis-angiogenesis-glycolysis	(203–206)
AMPK-mTOR signaling pathway	Autophagy- glycolysis	(207, 208)
Wnt/β-catenin pathway	autophagy-apoptosis-EMT-Metastasis-angiogenesis-glycolysis	(156, 209–212)
Jak-STAT signaling pathway	Autophagy-apoptosis-EMT -glycolysis	(213–216)
The RAS/RAF/MEK/ERK pathway AMPK pathway, Hippo pathway, EGFR pathway, p53 signaling pathway	Autophagy	(217, 218)
Hedgehog signaling pathway	Autophagy-apoptosis-EMT-Metastasis-angiogenesis-glycolysis	(219–224)
Jagged1-Notch signaling, AMPK/p53/FOXA1 pathways, Ras/Raf/MEK/ERK signaling pathway	Apoptosis	(225–228)
Hippo Signaling	Apoptosis-metastasis	(223, 229)
Signaling by Interleukins	Apoptosis-EMT-Metastasis-angiogenesis	(61, 230, 231)
TGF beta-SMADs signal pathway	Metastasis- angiogenesis	(84, 232)
Notch signaling	Metastasis- EMT- glycolysis	(233–235)
TGF-β1/H-Ras signaling pathway, DOR/EGFR/ERK pathway, TNF signaling pathway	metastasis	(236–238)
FoxO signaling pathway, TGF beta-SMADs Signal Pathway, PI3K/AKT and WNT/β-catenin signaling, PTEN/PI3K/AKT/β-catenin	EMT	(211, 239, 240)
VEGFA-VEGFR2 Signaling Pathway, TGF-β/SMAD3/NF-κB signaling, PIK3C2α/Akt/HIF-1α Pathway, BMP Signaling, FGF pathway signaling	Angiogenesis	(149, 232, 241–244)
HIF-1 signaling pathway, mTOR signaling pathway, PI3K/Akt/FoxO pathway, AMPK-mTOR signaling pathway,	Glycolysis	(208, 245–247)
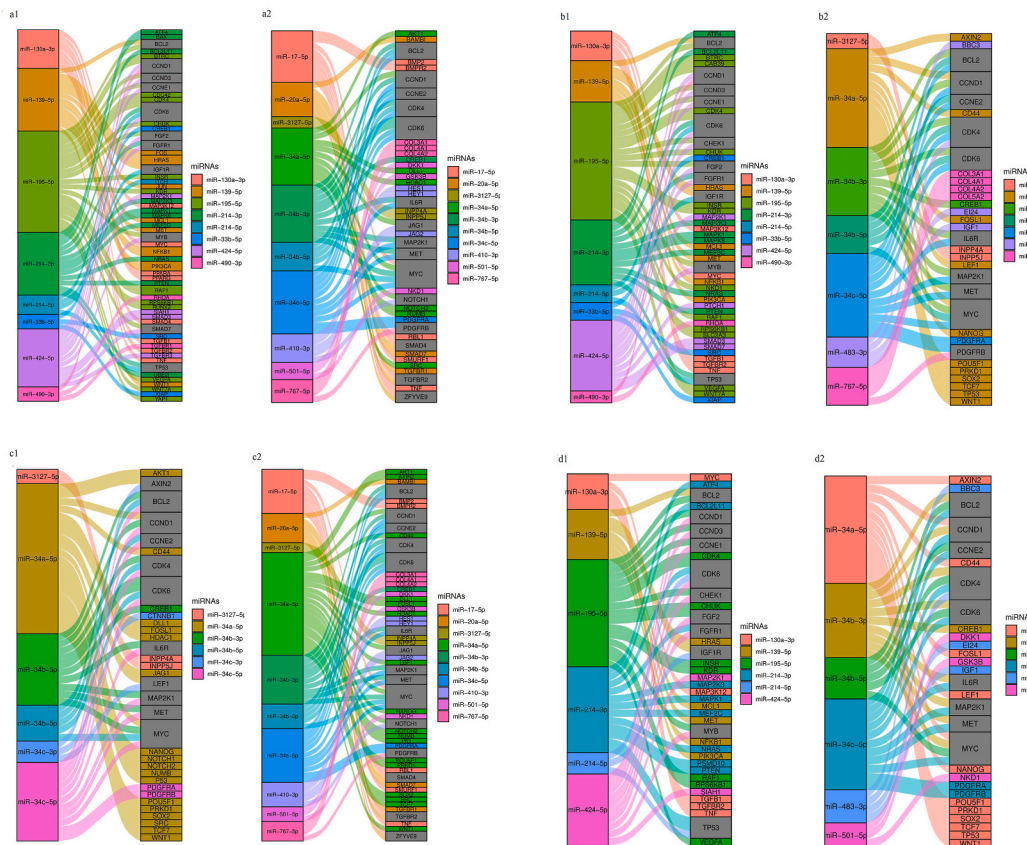
Fig. 8. Alluvial diagrams show the interaction between miRNAs- Targets in Apoptosis (a1 down. a2 up), autophagy (b1 down, b2 up), angiogenesis (c1 down, c2 up), and glycolysis (d1 down, d2 up).
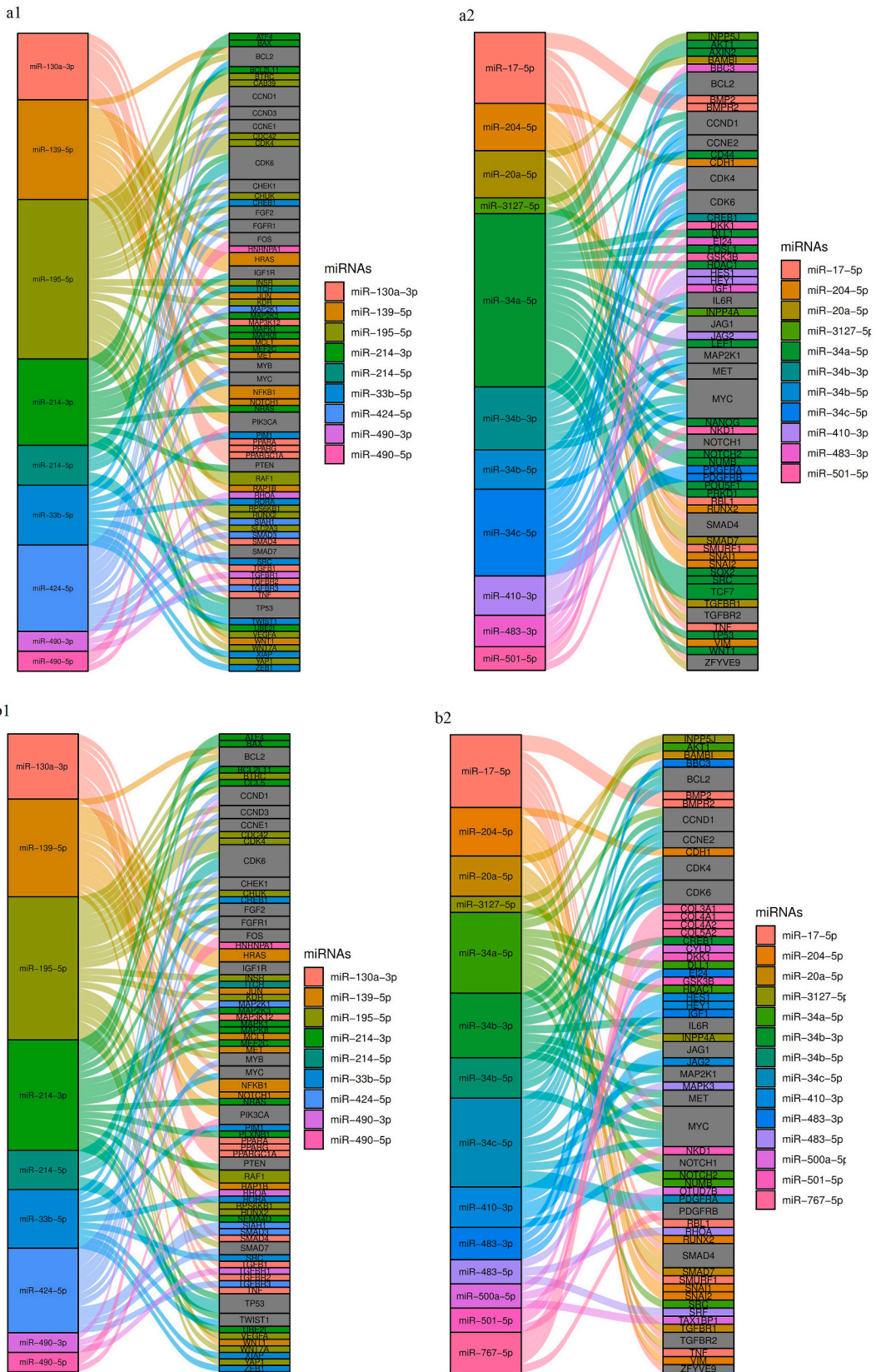
Fig. 9. Alluvial diagrams show the interaction between miRNAs-Targets in EMT (a1 down, a2 up) and metastasis (b1 down and b2 up).

12. Potential strategies to overcome challenges associated with miRNA stability and delivery

RNA-based oligonucleotides, including miRNA gene therapies, are used with many challenges and problems with gene therapy and efficiency. The miRNAs can easily be degraded by internal enzymes after adding them to biological systems, which reduces their therapeutic effect [172]. One of the biggest miRNA issues is having difficulty in delivering them to specific body textures. Delivering miRNAs should be designed should be designed to ensure that miRNAs reach the desired tissue with maximum efficiency. The miRNAs may be recognized by the host's intrinsic immune system. Potentially causing unpredictable side effects. Therefore, it is very important to check their safety before clinical administration [173,174]. The poor affinity of miRNAs for complementary sequences is another challenge of oligonucleotides [175]. They have difficulty crossing the cell membrane, and even if they do, they may trapped by endosomes. Despite these challenges and problems, technological advancement and extensive and ongoing research can provide useful ways to overcome these problems and improve the potential of miRNA as a strong tool for disease treatment [176]. To translate these discoveries into viable therapies, resistant and stable molecules must be brought about, and chemically modified poly (nucleic acids) are planned to be used for miRNA therapy. Chemical modification of miRNAs aims to create suitable and stable molecules with a very short half-life in the blood [177] and to produce specific points to enhance miRNA binding to a set of miRISC complexes [177,178]. Chemical modifications include 2′-O-methyl (2′-O-Me) or 2′-O-methoxyethyl-oligonucleotides (2′-O-MOE), peptide nucleic acids (PNA), locked nucleic acid oligonucleotides (LNA), phosphorothioate-containing oligonucleotides, and fluorine derivatives (FANA and other chemical changes) [179–183]. However, miRNAs generally do not cross the cell membrane efficiently. Therefore, extensive research is underway to develop delivery systems for miRNA oligonucleotide mimics. Systemic delivery of therapeutic agents of miRNAs can overcome defects caused by the miRNA processing machinery in some tumors [184,185]. Chemical modifications of miRNAs may alter their function and change their target gene profile. Guide-strand modifications can also affect the function of RISC interaction with other endogenous miRNAs in the cell. Therefore, most researchers use miRNA products lacking chemical modifications to overcome these problems.

Strategies including *in vivo* miRNA delivery of unmodified miRNA-based oligonucleotides and viral vectors into the target tissue have been pursued. However, these methods are limited due to the degradation of miRNAs and the side effects of viruses. Nanoscale

Table 8

The data obtained from bioinformatics data that are expected to play a role in six characteristics of glycolysis, angiogenesis, metastasis, autophagy, apoptosis, and EMT in HCC.

Upregulated miRNAs	Downregulated miRNAs
miR-3127-5p, miR-34a-5p, miR-34b-3p, miR-483-3p, miR-501-5p, miR-34c-5p	miR-130a-3p
miR-34b-5p, miR-373-3p, miR-409-3p miR-425-5p, miR-410-3p, miR-92b-3p	miR-139-5p
miR-10b-5p, miR-135b-5p, miR-182-5p	miR-195-5p
miR-183-5p, miR-18a-3p, miR-196b-5p, miR-200c-3p, miR-20b-5p, miR-21-5p	miR-214-5p
miR-221-3p, miR-222-3p, miR-224-5p	miR-214-3p
miR-301b-3p, miR-30d-5p	miR-424-5p

Table 9

Shared microRNAs that play a role intumor biology of HCC.

MicroRNA type	A variety of features	Ref.
miR-142	Autophagy-glycolysis-angiogenesis –metastasis	(142, 157, 197, 248)
miR-30a	Autophagy-glycolysis	(35, 249)
miR-26b	Autophagy- apoptosis- EMT	(39, 54, 92)
miR-26a	Autophagy- angiogenesis- EMT	(39, 94, 148)
miR-34a	Autophagy- apoptosis- glycolysis	(34, 138, 250)
miR-7	Autophagy- metastasis	(33, 44)
miR-21	Autophagy- EMT- metastasis	(45, 91, 202)
miR-196b, miR-429, miR-211-5p, miR-135a	Apoptosis- metastasis	(55, 178)(57, 198)(58, 251)(172, 252)
miR-221	Apoptosis- EMT	(56, 108)
miR-199a, miR-383, miR-125a	Apoptosis- glycolysis	(131, 253)(61, 135)(250, 254)
miR-373-3p	Apoptosis- EMT-metastasis	(65)
miR-375	Apoptosis- angiogenesis	(66, 255)
miR-199b	Apoptosis- EMT	(100, 253)
miR-183	Apoptosis- glycolysis	(122, 256)
miR-23b, miR-612, miR-1296, miR-501, miR-296, miR-6875-3p, miR-532-3p, miR-876	EMT- Metastasis	(88, 175)(95)(85)(257, 258)(259)(98) (99)(105, 201)
miR-374b	Glycolysis and Metastasis	(124, 260)
miR-214-5p	EMT and Angiogenesis	(96, 164)
miR-195	Apoptosis-angiogenesis-metastasis	(73, 160)
miR-139	Metastasis, apoptosis, glycolysis, angiogenesis, and EMT	(74, 110, 126, 168)

particles are very important for loading therapeutic agents including gene therapy. Nanoparticles are made from various synthetic and natural materials and can load miRNA-based oligonucleotides. The loading inside the nanoparticles protects the miRNAs from degradation and transfers them to the cytoplasm of the cell with a high volume of miRNAs with high effectiveness to maintain cellular function according to their role [186]. Another approach is to use the administration of miRNA expression vectors, which transfer DNA encoding the target miRNA and provide high levels of miRNA required by the cell. miRNA expression vectors are designed to be locally introduced into the target tissues [187]. Liposomes are one of the most common means of transferring oligonucleotides to the cell cytoplasm. However, these carriers are not effective in oligonucleotide transfer due to toxicity, immune response, and non-specific absorption. The toxicity is caused by the positive charge of the surface of liposomes [188,189]. Polyethyleneimine (PEI) is one of the most widely used gene transfer tools. PEI has a positive charge due to having amine groups and thus binds to the negative charge of oligonucleotides. The combination of PEI with nucleic acids has a more positive charge, the greater positive charge leads to interaction with the negative charge of the cell membrane, and in this way, PEI easily passes through the cell membrane [190]. Besides, dendrimers are of interest in gene transfer due to their high surface-to-volume ratio. These carriers are involved in the transfer of genes including miRNAs [191]. Poly(lactide-co-glycolide) (PLGA) polymers are synthesized at the nanoparticle scale and miRNA loaded and successfully delivered to cancer cells [192]. In addition to synthetic polymer materials, systems based on natural materials have been made that are compatible with the body's biological system. These materials include protamine, atelocollagen, chitosan, exosomes, and natural carbohydrate materials. They are made/secreted on a tiny scale that can load miRNA and other gene factors and successfully enter the cell cytoplasm [193–195]. Finally, miRNAs are stable for intracellular localization at high levels, and the development of efficient delivery systems is crucial.

13. Bridging bench side discoveries to bedside applications: the significance of integrating clinical relevance in MicroRNA research for HCC

Integrating clinical relevance or clinical application into miRNA research is essential for bridging the gap between benchside discoveries and bedside applications in HCC. By elucidating the diagnostic, prognostic, and therapeutic implications of identified miRNAs, researchers can pave the way for precision medicine approaches tailored to the individual needs of patients with HCC [196].

Here, we aim to discuss the significance of integrating clinical relevance in miRNA research for HCC, for either diagnostic, prognostic, and therapeutic applications.

Early detection is a crucial step that is closely linked with improving patient outcomes. By introducing different capabilities, miRNA biomarkers can help clinicians for this purpose. However, further research, validation studies, and regulatory approvals are essential steps towards translating miRNA-based diagnostics into routine clinical use. One of the most preliminary steps toward this aim is to integrate the expression profiles of these miRNAs into computational models or algorithms designed to distinguish between HCC and non-cancerous liver conditions [197].

By incorporating multiple miRNA signatures into diagnostic algorithms, clinicians can enhance the accuracy and reliability of HCC detection, especially in cases where conventional diagnostic methods may be inconclusive. These algorithms can offer biomarker clinical scores (numerical values that show the significance of biomarkers in diagnosing a disease). After development, these scores must be validated by rigorous research and then could be used as valuable tools for healthcare providers in making informed decisions about patient care. Validation studies should assess the sensitivity, specificity, reproducibility, and clinical utility of miRNA-based diagnostic tests in diverse patient populations. Regulatory approval ensures that miRNA-based diagnostic assays meet quality standards and safety requirements, thereby instilling confidence among healthcare providers and patients regarding their reliability and effectiveness [197].

Researches performed by Baldirà et al. [198], Wang et al. [199], and Xue et al. [200] are some examples of these preliminary steps toward translating miRNA-based diagnostics into routine clinical use.

Studies have been conducted in the preclinical and clinical stages. The increase in data and the development of advanced genome, transcriptome (RNA), and proteome (protein) data will improve the conditions for the discovery of miRNA biomarkers in patient care and treatment management for diagnosis and medical intervention decisions, which miRNA biomarkers can be used for valuable medicinal purposes [201]. Phase 4 clinical studies focused on miRNA biomarkers are registered in the Clinicaltrials.gov database. Studies using candidate miRNAs to assess disease progression in patients receiving FDA-licensed drugs have been conducted [202,203] The extraction of miRNAs from the blood sources can be done through various routes, including extracellular vesicles (exosomes) [204]. The levels of miRNAs extracted from biological fluids in preclinical studies in laboratory-research environments of cell and animal models and at the clinical level in industrial environments and hospitals are evaluated and measured. Microarray, next-generation sequencing, and real-time PCR platforms are used to normalize data to small RNA controls.

Early diagnosis and timely prognosis are essential for successful treatment and monitoring of treatment in HCC patients. In a study, Shigoka et al. took blood samples from patients with HCC and evaluated the expression of miR-92a in the plasma of these patients compared to healthy individuals. Results showed that the expression of miR-92a in the plasma samples of HCC patients was significantly reduced compared to the expression in healthy individuals [205]. Another study showed that miR-21, miR-122, and miR223 are highly expressed in the serum of HCC patients compared to healthy individuals. miR-21 and miR-122 have higher expression levels in people with chronic hepatitis compared to healthy people [206]. The expression of miR-500 in the serum of the patients showed a decrease in the expression level observed in three HCC patients after surgery. The findings suggested that miR-500 as an isophthalic miRNA could serve in the diagnosis of HCC [207]. miR-16، miR-195 و miR-199a expression patterns in the serum of three groups of patients with chronic liver disease and HCC and healthy individuals were evaluated. Compared to the chronic liver disease and healthy control group, miR-16 accurately predicted HCC disease in 69.2 % of cases [208]. In a study, a 20-miRNA expression profile was

studied in tumor tissue samples of HCC patients. An evaluation was done in a clinical group consisting of 131 patients. The results showed that the metastasis signature of 20 miRNAs in primary HCC with venous metastases had a tenfold increase compared to single tumors without metastases. However, non-cancerous liver tissues did not show significant expression of these 20 miRNAs. The results of this study identified 20 miRNAs as an important predictive biomarker of HCC survival, which is likely to have recurrence and metastasis in HCC patients [209]. miRNAs are also used as critical prognostic markers. In 116 patients, HCC (miR-128, miR-139-5p, miR-382-5p and miR-410, miR-424-5p, miR-101-3p) were evaluated in patient serum. A regression model was used to estimate univariate Cox proportional hazards. Four miRNAs (miR-128, miR-139-5p, miR-382-5p, and miR-410) had the highest expression associated with larger and more aggressive tumors. While two other miRNAs (miR-424-5p and miR-101-3p) showed a lower expression pattern, these two biomarkers are significantly associated with worse survival outcomes and tumor invasion [210]. In the new age, MiRNAs as biological markers have paved the way for clinically relevant outcomes. In the future, these biomarkers may be recognized as the "Golden Age" in diagnosis, prognosis, and treatment.

Beyond diagnosis, miRNAs hold promise as prognostic biomarkers for HCC. By correlating miRNA expression profiles with clinical outcomes such as overall survival, disease-free survival, and treatment response, researchers can identify miRNAs that predict disease progression and patient prognosis. Understanding the prognostic value of specific miRNAs can guide clinical decision-making, risk stratification, and personalized treatment strategies for patients with HCC. Thus, discussing the prognostic implications of identified miRNAs is crucial for elucidating their clinical relevance [213]. Just as to the diagnostic relevance of miRNAs, here, again, incorporating multiple miRNA signatures into prognostic algorithms and then validation of the results are among the first steps towards translating miRNA-based diagnostics into routine clinical application. Researches held by Zhang et al. [214], Xue et al. [200], and Zhan et al. [215] are some examples of the preliminary steps toward translating miRNA-based prognostic into routine clinical use.

By targeting dysregulated miRNAs implicated in HCC pathogenesis, researchers can develop novel therapeutic interventions aimed at modulating miRNA expression levels and restoring cellular homeostasis. These miRNA-based therapeutics could complement existing treatment modalities such as chemotherapy, targeted therapy, and immunotherapy, providing new avenues for improving patient outcomes. Therefore, it is essential to explore the therapeutic implications of identified miRNAs and their potential role in HCC management [216–219].

Following the identification of dysregulated miRNAs that may be of therapeutic value (via integrating data from experimental studies, bioinformatics analyses, and clinical cohorts), some of the preliminary measures toward translating miRNA-based therapeutics into routine clinical application may include the following steps: i). Target validation and functional studies can be done via *in vitro* and *in vivo* assessments and understanding their molecular mechanisms by elucidating the downstream targets and signaling pathways. ii). Development of miRNA-based therapeutics interventions using various strategies, including miRNA mimics, antagomiRs, miRNA sponges, and viral vectors for miRNA delivery. iii). Preclinical testing. iv). Clinical trials v). Regulatory Approval [220,221].

Therapeutic small RNA drugs (less than 200 nucleotides in length) based on miRNAs are in development in phase 1 and phase 2 clinical trials [27]. Per cancer treatment, miRNA drugs are injected directly into the tumor. In this way, the effectiveness, specific local release of cancer tissue, and side effects are minimized (Mercatelli et al., 2008; Chen et al., 2015). According to studies conducted in clinical phases, patients showed different reactions to miRNA drugs. miR-34 is a tumor suppressor whose function is lost in HCC cells. The miR-34 was evaluated in the form of the drug MRX34 in a phase 1 trial in patients with primary liver cancer [211]. In a study conducted in 2016 by biotech company Synlogic, MRX34, was a liposomal miR-34A that is not expressed in cancer cells. Using the standard dose MRX34 drugs were investigated by venous injection into HCC and melanoma. The results showed that the MRX34 had a controlled poison but showed severe side effects in five HCC patients in Phase 2 in the form of severe immune reactions [212].

Moving forward, further research is needed to elucidate the clinical relevance of miRNAs in HCC fully. This includes conducting prospective clinical studies to validate the diagnostic and prognostic utility of identified miRNAs, exploring their mechanistic roles in HCC pathogenesis, and developing innovative miRNA-based therapeutic approaches. Additionally, leveraging multi-omics approaches and advanced bioinformatics tools can enhance our understanding of the complex interplay between miRNAs and other molecular pathways in HCC.

14. Concluding remarks and future perspectives

Many published works to date has shown that miRNAs are actively involved in cellular processes in HCC initiation, progression, and metastasis. Indeed, gene profiling studies revealed several regulatory miRNAs as "oncomiRs" and inversely some as "tumor suppressors" in HCC. The microRNAs are responsible for regulating the diverse biological processes of HCC cancer cells, including EMT, glycolysis, angiogenesis, apoptosis, autophagy, and metastasis. It has been shown that the dysregulation of miRNAs that control these processes through different pathways and target genes, can push each of these characteristics in a direction that causes cancer to become uncontrollable and eventually metastasize. The discovery of miRNAs associated with different molecular pathways and genes may help to find key targets with multiple anti-cancer functions, which facilitates the efficient treatment, timely diagnosis, and favorable prognosis of HCC.

The High throughput technology, screening, and analysis methods could be developed to identify microRNAs based on laboratory studies along with bioinformatics predictions that can simultaneously regulate the most common cancer characteristics, and introduce to researchers' perfect high-performance biomarkers. In this review, we focused on the recent acknowledgment related to miRNAs involved in HCC and their ability to meet the real needs for the diagnosis, prognosis, and treatment of patients with HCC considering the six features of tumor biological hallmarks. An important question to be answered is what are the potential common target miRNAs shared among EMT, glycolysis, angiogenesis, autophagy, apoptosis, and potential metastasis, and thus could be excellent diagnostic, prognostic, and therapeutic targets for physicians dealing with HCC. Among the data extracted from the research and bioinformatics

data, the miRNAs that played the most significant role in the properties we examined include (i) miR-34a with a therapeutic role, prognosis in the properties of autophagy, apoptosis, and glycolysis, (ii) miR-373-3p with a therapeutic role, prognosis in EMT characteristics, metastasis, and apoptosis,(iii) miR-21-5p with therapeutic goals, prognosis in EMT, metastasis and apoptosis and (iv) miR-214-5p with therapeutic goals, prognosis in EMT, angiogenesis, (v) miR-195-5p with therapeutic goals, prognosis in angiogenesis, apoptosis and metastasis and finally (vi) miR-139-5p have a diagnostic, therapeutic and prognostic role in the characteristics of EMT, metastasis and apoptosis, angiogenesis and glycolysis. These microRNAs are selected as ideal high-performance monitors that may be used as appropriate options for controlling HCC.

However, more miRNAs should be extensively explored and the relationship of miRNAs with the gene network and biological pathways effective in cancer characteristics should be studied on a wider level and selecting very high-performance microRNAs from sensitivity and specificity points. To guarantee the miRNAs' discovery results become as reliable as possible, a set of practical approaches both miRNA technology platforms and extraction procedures should be paralleled with optimized or each other for diverse types of cancer patients with HCC. However, both practical and technical, miRNA stability and its toxic effects are shortcomings for proper delivery of miRNA-therapeutics to the HCC location. Despite the encouraging results *in vitro*, preclinical and clinical studies are rare, and we still face many difficulties such as off-target effects in the long journey of transferring miRNAs as therapeutic targets from the cellular level to the clinic.

CRediT authorship contribution statement

Fereshteh Rahdan: Writing – original draft, Methodology. **Alihossein Saberi:** Formal analysis, Data curation. **Neda Saraygord-Afshari:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Morteza Hadizadeh:** Writing – original draft, Software, Resources, Formal analysis, Data curation. **Tahura Fayeghi:** Visualization, Software, Resources, Methodology, Formal analysis, Data curation. **Elham Ghanbari:** Software, Formal analysis, Data curation. **Hassan Dianat-Moghadam:** Writing – original draft, Visualization, Formal analysis, Data curation. **Effat Alizadeh:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Availability of data and materials

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Ethics approval and consent to participate

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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