Review1

Q1. In Figure 1, please add M1 and M2 associated MiRs in the Figure.
Answer: Thanks for your suggestion. We have added M1 and M2 associated miRNAs in the Figure.

Q2. In Figure 1, please label or express the anti-tumor/tumor inhibition function or pro-tumor function of each MiR to make the functions more clearly.
Answer: Thanks for your suggestion. In the revised Figure to make the functions clearer, the anti-tumor/tumor inhibition-miRNAs are highlighted in green, and pro-tumor-miRNAs are highlighted in red.

Q3. miR-125a/b in Figure and Table is not described in the text of the manuscript. Please add the description in the text.
Answer: Thanks for your careful checks. Based on your comments, we added the description in the revised paper as: "TAM-derived exosomes with low levels of miR-125a and miR-125b have been proven to promote HCC cell proliferation, sphere cell formation, and metastasis. The miR-125a/b suppressed HCC cell proliferation and stem cell properties by targeting CD90, a stem cell marker of HCC stem cells". (Line 322-326)

Q4. Please put the full names of the abbreviations in the Figure legend and under the Table 1.
Answer: Thanks for your suggestion. In the revised paper, we have put the full names of the abbreviations in the Figure legend and under Table 1.

Attachment

Revised manuscript with changes highlighted

Review2

Q1. The title does not reflect the conclusion of this study. Hence, it would be better to revise the title so that it aligns with the conclusion of the study.
Answer: Thanks for your comment. In the revised paper, the Title has been revised as "Function and biomedical implications of exosomal microRNAs delivered by parenchymal and nonparenchymal cells in hepatocellular carcinoma."

Q2. This is highly narrative and lacking in critical review of the literature.
Answer: Thanks for your comment. In the revised paper, besides the briefly described findings, we have added statements to discuss and interpret the finding, hoping to help readers better understand the significance of the relevant findings. Please refer to the highlighted statements in the context. We believe these revisions have improved the quality of this review and hope that they meet your requirements.

Q3. The conclusion does not summarize the role of particular miRNAs in the pathogenesis and reversion of the HCC. Hence, the conclusion requires major revision.
Answer: Thanks for your comment. Sorry for missing the statement about the role of miRNAs in the pathogenesis and reversion of the HCC in the section of the Conclusion of the early version. According to your

suggestion, in the section of the Conclusion of the revised paper, though we did not discuss the role of particular miRNAs, we added the statement that exosomes and exosomal miRNAs play an important role in HCC pathogenesis and treatment. (Line 408-416)

Q4. There are several typos and grammatical errors throughout this manuscript. This manuscript requires major English corrections.
Answer: Thanks for your suggestion. The revised manuscript was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English-speaking editors at Spring Nature Author Service (SNAS). And we hope the revised manuscript could be acceptable to you.

Attachment

Revised manuscript with changes highlighted

Review3

Q1. Many facts in the introduction are mentioned without their references.
Answer: Thanks for your comment. In the revised paper, we added and updated the references in the part of the Introduction, which are highlighted in the paper. Please refer to Ref [1-13], [17-20], [23-26].

Q2. The conclusion is very long.
Answer: Thanks for your comment. The section of the Conclusion was condensed in the revised version. The total word count was reduced from 419 in the original to 349 in the revised version.

Q3. Some references are old (2011, 2005, 2012, ….etc).
Answer: Thanks for your comment. We added and updated the references within five years throughout the revised paper. Please refer to the highlighted references in the text. Sometimes, the text was updated as needed.

Q4. Other comments are in the attached manuscript word file.
Answer: Thanks for your comment. In the revised paper, we added the necessary references in the places you indicated, which are highlighted in the paper. The specific document number is reference [1] in line 30 and reference [3] in line 35.

Attachment

Revised manuscript with changes highlighted

Review4

Q1. The manuscript would benefit of a section discussing available data on the role of etiology of HCC and relationship with miRNAs, in particular whether there are differences according to etiology of underlying liver

disease in patients with HCC.

Answer: Thanks for your comment. In the revised paper, we added a paragraph discussing available data on the role of the etiology of HCC and its relationship with miRNAs, in particular, whether there are differences according to the etiology of underlying liver disease in patients with HCC in the section “Exosomal miRNAs and liver cancer.” (Line 129-160)

Q2. The authors recall the major role of CD4+ CD25+ regulatory T cells as they mentioned that tumor-derived exosomal miR-214 efficiently enhanced IL-10 expression by promoting CD4(+)CD25(high)Foxp3(+) regulatory T cell (Treg) expansion, thereby accelerating tumor growth. Since Tregs are now recognized as the most prominent immunosuppressive immune cell population in tumor microenvironment of HCC, they should further expand such a relevant issue also quoting their pathogenic role in HCC as well as in the development of immune-related adverse events under immune checkpoint inhibitors as well described and summarized in a comprehensive review (Hepatocellular carcinoma in viral and autoimmune liver diseases: Role of CD4+ CD25+ Foxp3+ regulatory T cells in the immune microenvironment. World J Gastroenterol. 2021 Jun 14;27(22):2994-3009. doi: 10.3748/wjg.v27.i22.2994).

Answer: Thanks for your thoughtful comments. In the revised paper, we quoted the pathogenic role of Tregs in HCC. Briefly, we discussed their involvement in developing immune-related adverse events under immune checkpoint inhibitors: “Tregs that produce inhibitory factors such as IL-10 and TGF-β are among the most prevalent suppressor cells in TME and have been related to tumor progression. Tregs also express a panel of chemokine receptors and surface molecules such as CTLA4 and PD-1, thus making them a direct target of immune checkpoint inhibitor immunotherapy. The development of immune-related adverse events may partly be attributed to Treg destabilization[123]. Tumor cell-secreted miR-214 could expand the CD4+CD25highFoxp3+ Treg population by decreasing the levels of PTEN in CD4+ T cells, leading to host immune suppression and rapid tumor growth[124]. The expansion of the Treg population by tumor-secreted miR-214 likely serves as a common mechanism for various cancer cells to create a tolerant immune environment. Inhibiting the transport of tumor-secreted miR-214 to immune cells may be a novel strategy to reverse tumor-induced immune tolerance[124].” (Line347-357 in revised paper) The review mentioned above is cited as Ref 123.

Please note that this description of the effects of HCC-derived exosomal miRNAs on Tregs was moved from the section “2. Exosomal miRNAs and liver cancer” to the section “3.5 Exosome-mediated cell-cell communication between immune cells and HCC cells” in the revised paper.

Attachment

Revised manuscript with changes highlighted

Name of Journal: World Journal of Gastroenterology

Manuscript Type: REVIEW

Function and biomedical implications of exosomal microRNAs delivered by parenchymal and nonparenchymal cells in hepatocellular carcinoma

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Ling Ji drafted the paper; Hai-Chen Wang prepared the figure; Wen-Xuan Yin, Meng Jiang, Jia-Yi Han, and Yu-Feng Sun researched on the background of the study; and Ju-Ling Ji, Xing-Wang Kuai, and Rui Sun reviewed and revised the paper. All authors read and approved the final manuscript.

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Abstract

Small extracellular vesicles (sEVs, exosomes) are important components of the tumor microenvironment (TME). They are small membrane-bound vesicles derived from almost all cell types and play an important role in intercellular communication. Exosomes transmit biological molecules obtained from parent cells, such as proteins, lipids and nucleic acids, and are involved in cancer development. MicroRNAs (miRNAs), the most abundant contents in exosomes, are selectively packaged into exosomes to carry out their biological functions. Recent studies have revealed that exosome-delivered miRNAs play crucial roles in the tumorigenesis, progression, and drug resistance of hepatocellular carcinoma (HCC). In addition, exosomes have great industrial prospects in the diagnosis, treatment, and prognosis of patients with HCC.

This review summarized the composition and function of exosomal miRNAs of different cell origins in HCC and highlighted the association between exosomal miRNAs from stromal cells and immune cells in the TME and the progression of HCC. Finally, we described the potential applicability of exosomal miRNAs derived from mesenchymal stem cells in the treatment of HCC.

Key words: Hepatocellular carcinoma; MicroRNA; Exosomes; Extracellular vesicles; Nonparenchymal cells

Core tip: Hepatocellular carcinoma (HCC) is one of the most serious cancers in adults and microRNAs (miRNAs) in small extracellular vesicles (sEVs, exosomes) play a vital role in the pathophysiological processes of HCC. Recent studies on exosomal microRNAs (miRNAs) in HCC mainly focus on miRNA profiling but place little emphasis on where miRNAs come from and what target cells they act on. This review focuses on the origin of exosomal miRNAs according to their parent cells in the tumor microenvironment (TME) and their role in HCC pathogenesis, contributing to a better understanding of exosomal miRNAs in TME.

INTRODUCTION

Liver cancer was the sixth most common malignant solid tumor and the third leading cause of cancer death worldwide in 2020[1]. Hepatocellular carcinoma (HCC) is the main histological subtype of liver cancer, accounting for 80% of primary liver cancer[2]. It is characterized by a high degree of malignancy and poor prognosis and is a serious threat to human health. Due to the strong concealment of incipient symptoms, it's difficult to diagnose HCC early. In addition, approximately 70% of patients undergo recurrence and metastasis within 5 years after surgical resection[3].

The tumor microenvironment (TME) plays a critical role in the tumorigenesis and progression of HCC[3]. The TME mainly consists of a variety of resident and infiltrating host cells, secreted factors and extracellular matrix proteins [4]. Nonparenchymal hepatic cells, such as liver sinusoidal endothelial cells, hepatic stellate cells, and hepatic macrophages, play an important role in establishing the TME and stimulating tumorigenesis by paracrine communication through cytokines and/or angiocrine factors[5]. Recent studies on the TME have provided novel insight into tumor growth and metastasis, in which exosomes play an important role[6-8].

Small extracellular vesicles (sEVs), also known as exosomes, refer to a subpopulation of extracellular vesicles with a 40-160-nm diameter derived from multivesicular bodies (MVBs), which act as substance transport carriers for biological information exchange to regulate the cellular microenvironment[9]. To maintain consistency in nomenclature across studies published at different stages, we use the name exosome for the rest of this review. Studies have shown that exosomes contain various cargoes including proteins, DNA, lipids, messenger RNAs (mRNAs), microRNAs (miRNAs), long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs), which are involved in intercellular communication[10, 11]. An increasing number of molecules within exosomes have been identified. According to data from the ExoCarta database (http://www. exocarta. org), the contents inside exosomes that have been identified include 9769 proteins, 3408 mRNAs, 2838 miRNAs and 1116 lipids. Initially, exosomes were considered as carriers of cellular waste, and their functions were also underestimated[12]. Research during the past decades has confirmed the important role of exosomes in mediating intercellular communication under physiological and pathological conditions[13]. In 1996, exosomes derived from

murine and human B lymphocytes were proven to play an essential role in delivering MHC molecules and induced antigen-specific MHC class II-restricted T-cell responses[14]. Later, cancer cells and stromal cells in the TME were also found to deliver exosomes and modulate tumor progression through exosome-mediated molecular exchanges[15, 16]. Exosomes have thus become important contributors to cancer initiation and progression[17-19].

MicroRNAs (miRNAs) are a large family of posttranscriptional regulators of gene expression with a length of approximately 20-24 nucleotides and control developmental and cellular processes in eukaryotic organisms[20]. Due to their important role in gene expression, miRNAs in exosomes have also been widely studied. In 2007, Valadi et al. reported that exosomes contained miRNAs, which could be delivered to other cells and exert their functions[21]. Studies have shown that exosomes contain high levels of miRNAs, which contribute to immune regulation, chemoresistance, and metastasis in a variety of tumors[22]. These miRNAs can promote tumor development in a paracrine manner in the surrounding microenvironment[23-25]. The identification of abnormally expressed miRNAs in pathological states might further the understanding of the mechanisms of cancers.

Accumulating studies have shown that exosomes are involved in the genesis and development of tumors by transmitting signals between cells and regulating the TME[26]. This paper summarizes the studies of exosomal miRNAs released from nonparenchymal cells in the TME of HCC and discusses the association between these exosomal miRNAs and HCC. This study will help researchers in the field in better understanding the role of exosomal miRNAs from stromal cells and immune cells in HCC and in developing innovative strategies for HCC prevention and treatment.

1. Formation, composition and functions of exosomes

Exosomes are a subtype of extracellular vesicles with a diameter of 40-160 nm[27]. Unlike other types of vesicles, exosomes have a different formation mechanism. First, the plasma membrane germinates inwards to form early endosomes (membrane-bound vacuoles)[28]. By further inwards budding of early endosomes encompassing several miRNAs, proteins and other selected substances, late endosomes called multivesicular bodies (MVBs) are

formed[29]. Subsequently, the MVBs fuse with the cell membrane and release intraluminal endosomal vesicles into the extracellular space, which then become exosomes[30] or fuse with the lysosome to degrade the biological information contained inside[31].

The endosomal sorting complex required for transport (ESCRT) mainly guides special molecules into the exosomes of MVBs and is regarded as an important mechanism of synthesis[32]. The ESCRT complex selects the "cargo" protein that is labeled by ubiquitin, directs it to MVBs, and then separates the MVB from the peripheral membrane in a highly conserved process that is homologous to the process of cytokinesis and virus budding[33].

Exosomes can be produced by any cell under normal or pathological conditions and might be taken up by other cells to carry out their function[34, 35]. Exosomes carry multiple biologically active substances, including proteins, RNA, DNA, and cholesterol[36-38]. The density at which exosomes float in a sucrose gradient is between 1.13 and 1.19 g/mL[39]. Of note, the composition of exosomes varies depending on their cellular origin[40], and different cell-derived exosomes or even the same cell-derived exosomes contain different components in different physiological or pathological states[41]. The amount of exosomal miRNAs secreted by hepatoma cells could also vary under different stimuli[42]. Research has shown that 55 miRNAs in Heb3B cell-derived exosomes were expressed at levels that were four times higher than those in donor cells, while 30 miRNAs were expressed at lower levels, and 11 miRNAs were expressed only in exosomes[43]. These changes may be a potential mechanism for disease progression.

2. Exosomal miRNAs and liver cancer

In recent years, exosomes have been shown to be important mediators of intercellular material and information exchange, that can modulate the TME by transmitting nucleic acids and proteins between cells, thus playing a role in tumor cell growth, metastasis, drug resistance, and immune regulation[44, 45]. As an essential component of exosomes, exosomal miRNAs exert crucial functions in HCC tumorigenesis and progression.

Here we first review the role of exosomal miRNAs derived from liver cancer cells. Specifically, miR-122, the most abundant miRNA in the human liver is decreased in the liver of HCC patients[46-48]. It can be expressed and released by Huh7 cells and transferred

into miR-122-deficient HepG2 cells in the form of exosomes, reducing the growth and proliferation of recipient HepG2 cells. The restoration of miR-122 inhibits HCC growth and sensitizes HCC to chemotherapeutic drugs[49]. In addition, exosomes delivered by liver cancer cells can affect nonparenchymal cells in the microenvironment, promoting the progression and recurrence of tumors, which will be discussed in subsequent sections.

On the other hand, exosomal miRNAs secreted by tumor cells outside the liver can also promote the formation of premetastatic niches in the liver. Colon cancer cell-derived exosomes can deliver miR-21, miR-192, and miR-221 to hepatoma cells[50]. Colon cancer cell-derived exosomal miR-25-3p induced premetastatic niche formation in the liver by improving vascular permeability and angiogenesis[51]. Exosomes from colorectal cancer highly expressed miR-135a-5p, which could be transmitted to hepatic Kupffer cells to regulate the LATS2-YAP1/TEAD1-MMP7 pathway and promote cell adhesion, forming premetastatic niches[52]. These results showed that exosomes could communicate between different kinds of cancers, even changing the microenvironment to boost liver metastasis[53].

Exosomal miRNAs might also be associated with different etiology of underlying liver disease in patients with HCC. The relationship between miRNAs and different liver diseases including, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alcohol-associated liver disease (ALD), nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), autoimmune hepatitis (AIH), and drug-induced liver injury (DILI) has been discussed extensively in previous high-quality reviews[54-56]. Hepatocyte-specific miR-122 is decreased in the livers of ALD, NASH, and HCC patients. This microRNA directly pairs with distinct regions at the 5′-UTR of the HCV RNA genome and promotes the replication of HCV RNA[57]. Diverging from its role in HCV infection, miR-122 suppresses HBV replication by downregulating the cyclin G1-p53 complex and blocking the specific binding of p53 to HBV enhancers[58]. The liver expression of hepatocyte-enriched miR-192 is elevated in simple steatosis but not in NASH [59], and is decreased in HCC[60]. It is the most significantly downregulated miRNA in hepatic cancer stem cells (CSCs) and contributes to CSC activation. Owing to the anti-tumorigenic effects of miR-192, delivering miR-192 to HCC may be a potent strategy for HCC therapy[60]. The

expression of miR-155, highly expressed in immune cells, including macrophages, is increased in the livers of ALD, AIH, and HCC patients. It is an oncogenic miRNA that links inflammation with tumorigenesis[61, 62]. Activation of NF-κB signaling seems to upregulate miR-155 expression in hepatocytes and liver cancer associated with choline-deficient and amino acid-defined die feeding in mice[61], or HCV infection in patients[62]. However, few studies have focused on the etiology of HCC and miRNAs delivered by exosomes in HCC. A recent study reported that neutrophils can transmit miR-223 via extracellular vesicles to macrophages, promoting liver fibrosis resolution[63]. Neutrophil/myeloid-specific miR-223 is a well-documented anti-inflammatory miRNA. It inhibits IL-6 expression and subsequently attenuates the IL-6-p47phox-ROS pathway in neutrophils[64]. The expression of miR-223 is elevated in serum and/or liver in patients or mouse models with ALD or NASH, of which hepatic neutrophil infiltration is a hallmark. Thus, elevation of miR-223 compensatively protects against ALD[64] and NASH[65], while downregulation of miR-223 in HCC likely acts as a causal factor to accelerate HCC progression[66]. Injection of miR-223 is an effective therapy in mouse models of acute hepatitis and NASH[67]. Future studies of the above-reported miRNAs associated with different etiology of liver diseases underlying HCC could be extended to the area of exosomes.

3. The interactions between TME and tumor cells via exosomal miRNAs in HCC

Since Stephen Paget proposed the "seed-soil" theory of tumor metastasis in 1889 to explain the organ specificity of tumor metastasis, there has been increasing evidence that tumor metastasis requires coordination between tumor cells and the TME, which has been recognized as an evolutionary and ecological process, including constant, dynamic and reciprocal interactions. Nonparenchymal cells in the liver cancer TME, such as hepatic stellate cells, fibroblasts (cancer-associated fibroblasts or CAFs), immune cells (T lymphocytes, B lymphocytes, NK cells, natural killer T cells, and tumor-associated macrophages or TAMs), and endothelial cells (ECs), play a pivotal role in tumor-stromal interactions, thus regulating the biological activity of HCC[68]. Noncellular components include growth factors such as transforming growth factor-β (TGF-β), insulin-like growth

factor (IGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF), as well as proteolytic enzymes, ECMs, and inflammatory cytokines. These factors can provide a flexible environment for the further growth and proliferation of HCCs. As an essential component of the TME, exosomal miRNAs are involved in cell-to-cell signal transduction and the processes of tumor formation and progression. In the next section, the role of the exosomal miRNAs from different nonparenchymal cells in HCC formation and metastasis is thoroughly discussed, which may provide new insights for the clinical diagnosis and treatment of HCC (Figure 1).

3.1 Exosome-mediated cell-cell communication between activated hepatic stellate cells (HSCs) and HCC cells

Hepatic stellate cells (HSCs) are situated in the space of Disse between hepatocytes and liver sinusoidal endothelial cells (LSECs), which store vitamin A in lipid droplets[69, 70]. When the liver is damaged, quiescent hepatic stellate cells (qHSCs) transform into activated hepatic stellate cells (aHSCs) to secrete proteins such as elastin that promote cross-linking, maturation and insolubility of the fibrotic ECM[70]. Liver fibrosis occurs as a result of chronic liver disease, and the migration of fibroblasts is thought to play an important role in fibrosis. Many cell types, such as HSCs[71-73], portal fibroblasts (PFs)[71, 72], mesenchymal stem cell-like cells[74], mesothelial cells[75] and bone marrow-derived cells[76], have been reported to contribute to the myofibroblast pool. Researchers have shown that 82-96% of myofibroblasts in models of toxic, cholestatic and fatty liver disease are derived from activated HSCs[73].

Liver fibrosis is a substantial risk factor for the development and progression of liver cancer[70]. Activated HSC is a major factor mediating liver fibrosis and promotes liver cancer progression. Activated HSCs cocultured with HCC cells promoted tumor growth and invasiveness in nude mice[77]. In 2022, Zhang X et al. reported that reducing activated HSC-derived exosomal miR-148a-3p suppressed HCC tumorigenesis through the ITGA5/PI3K/Akt pathway[78]. Another group found that HSC-HCC cell coculture reduced intracellular miR-335-5p expression in both types of cells. HSC-exosomes loaded

with miR-335-5p decreased cancer growth and invasion *in vitro* and *in vivo*[79]. In summary, activated HSCs can promote the development of HCC via various miRNAs delivered by exosomes, and targeting activated HSC-exosome miRNAs could be a novel therapeutic strategy in HCC.

At the same time, HCC cells also promote the activation of HSCs through exosomes. The HCC cell derived exosome-miRNA-21, which targets the PETN gene in HSCs, activates the PDK1/AKT pathway and converts HSCs to CAFs[80]. Activated CAFs further promoted cancer progression by secreting angiogenic cytokines, including VEGF, MMP2, MMP9, bFGF and TGF-β[80]. A high level of serum exosomal miRNA-21 was correlated with greater activation of CAFs and higher vessel density in HCC patients[80].

3.2 Exosome-mediated cell-cell communication between CAFs and HCC cells

Cancer-associated fibroblasts (CAFs) are important in the tumor microenvironment[81]. However, the concepts of HSCs and CAFs in early literature sometimes needed to be clarified. Researchers used to believe that in the HCC microenvironment, HSCs frequently differentiate into CAFs, which have been extensively reported to influence HCC progression[81-84]. Recently, Zhu et al. identified five CAF subtypes in HCC tumors, namely, vascular CAFs (vCAFs), matrix CAFs (mCAFs), lipid processing-mCAFs (lpmCAFs, $CD36^+$ CAFs), lipid-processing CAFs (lpCAFs) and antigen-presenting CAFs (apCAFs), from single-cell RNA sequencing data of mouse and human HCC tumors. In these cells, $CD36^+$ CAFs are derived from hepatic stellate cells[85]. Another group also showed that Tcf21 was explicitly expressed in hepatic stellate cells in mouse and human livers. Tcf21-positive HSCs, representing approximately 10% of all HSCs, can transdifferentiate into the majority of myofibroblasts in fibrotic liver and CAFs in HCC[86].

As key players in the multicellular matrix-dependent alterations leading to the pathogenesis of HCC, CAFs can accelerate HCC progression by exosomal-mediated communication. A recent study found that miR-320a level was significantly reduced in CAF-derived exosomes compared with corresponding paraneoplastic fibroblast (PAF)-derived exosomes from HCC patients. In vitro and in vivo studies revealed that transferring miR-320a to tumor cells via exosomes could function as an antitumor miRNA

by targeting PBX3 and subsequently inhibiting the activation of the MAPK pathway[87]. Another study confirmed that miR-150-3p was significantly reduced in CAF-derived exosomes. The loss of antitumoral miR-150-3p in CAFs-derived exosomes greatly promotes HCC progression. Exosomal miR-150-3p is a potential prognostic biomarker, and transferring miR-150-3p-loaded exosomes to HCC cells could abrogate the migration and invasiveness of HCC and might become a novel therapeutic option[88].

Apart from those under-expressed antitumor miRNAs in CAF-derived exosomes, the expression of oncogenic miR-20a-5p was much higher in CAFs than in HCC cells. MiR-20a-5p can be loaded to CAF-derived exosomes and transferred from CAFs to HCC cells and resulting in inhibited expression of the tumor suppressor LIM domain and actin binding 1 (LIMA1), which inhibits the Wnt/β-catenin signaling pathway in HCC[89]. Thus, differential expression of exosomal miRNAs in CAFs plays a vital role in the developing and progressing of HCC, so anti-CAF drugs targeting specific exosomal miRNAs may yield a potential therapeutic strategy.

However, other exosomal noncoding RNAs other than miRNAs also participate in the CAF-tumor cell communication. Chemoresistance in HCC can be influenced by CAF-exosomal circRNA. CircZFR is highly expressed in CAFs and CAF exosomes. CAF-derived exosomes delivered circZFR to HCC cells, which inhibited the STAT3/NF-κB pathway and thereby promoted tumor growth and enhanced cisplatin (DDP) drug resistance[90]. In addition, CAF-derived exosomes promoted migration, invasion, and glycolysis in HepG2 cells by releasing lncRNA TUG1, which suppressed miR-524-5p/SIX1 axis[91].

3.3 Exosome-mediated cell-cell communication between adipocyte and HCC cell

Adipose tissue has long been considered to be involved in tumor progression[92]. Adipocytes are an important component of the hepatic microenvironment in nonalcoholic fatty liver disease (NAFLD), a significant risk factor for HCC[44]. There is a strong correlation between the adipocyte-HCC cell interaction and the risk of HCC development and progression[93]. Adipocyte-derived exosomes can affect the gene expression of liver cancer cells. In 2014, Koeck et al. reported that exosomes from obese donors' visceral adipose tissue caused dysregulation of genes involved in the TGF-β pathway in HepG2

cells[94]. Recently, Liu et al. found that miR-23a/b was significantly higher in serum exosomes and tumor tissues of high-body fat ratio (BFR) HCC patients than in low-BFR HCC patients. In tumor tissues, miR-23a/b was most likely to be derived from adipocytes and transported into cancer cells via exosomes, thus promoting the growth and migration of HCC cells[95]. Moreover, exosomal miR-23a/b confers chemoresistance by targeting the von Hippel-Lindau/hypoxia-inducible factor axis[95]. Exosomal circRNAs also played a role. Adipocyte exosomal circ-DB can suppress miR-34a expression in HCC cells and subsequently activate the deubiquitination-related USP7/Cyclin A2 signaling pathway and promote tumor growth of HCC[96]. These studies provided evidence that high BFR-related exosomal miRNA could be a promising target for future treatment of HCC.

On the other hand, exosomes derived from HCC cells can educate surrounding adipocytes to create a favorable microenvironment for tumor progression. HepG2 exosomes induced an inflammatory phenotype in adipocytes by activating several phosphorylated kinases (p-AKT, p-Erk1/2, p-GSKb, p-stat5a, and p-p38) and NF-kB signaling pathway[44]. Tumor exosome-treated adipocytes promoted tumor growth, enhanced angiogenesis, and recruited more macrophages in a mouse xenograft model[44]. The specific exosomal miRNAs that played a role in the process remain to be revealed.

Besides, the exposure to adipocyte exosome also increased the expression of TIMP-1, TIMP-4, Smad-3, integrins anb-5 and anb-8, and matrix metalloproteinase-9 in HSCs, all of which are intimately involved in the development of fibrosis in liver disease and showed increased expression in human studies and experimental models[94].

3.4 Exosome-mediated cell-cell communication between vascular endothelial cells and HCC cells

It is well known that angiogenic factors from tumor cells activate vascular endothelial cells, promote their proliferation and migration, and contribute to aberrant tumor angiogenesis[97]. HCC is a typical hyper-vascular tumor, so understanding the mechanisms of angiogenesis in HCC is very important[98]. In an early study, Shih et al. reported that the downregulation of miR-214 in HCC cells induced hepatoma-derived growth factor (HDGF) expression and secretion so as to stimulate vascular endothelial cells for angiogenesis and

promote tumor growth[99]. Therefore, miR-214 is a potent suppressor of angiogenesis. It was also evidenced that HCC cell-derived exosomes could induce lumen formation of human umbilical vein endothelial cells[98]. Recently, several HCC cell-derived exosomal miRNAs were found to play an important role in angiogenesis. Fang et al. reported that hepatoma cell-derived exosomal miR-103 could be delivered into endothelial cells, then impair endothelial junction integrity and increase vascular permeability and promote tumor metastasis by targeting multiple endothelial junction proteins, including VE-cadherin and p120-catenin[100]. Exosomal miR-210 secreted by HCC cells can also be transferred to endothelial cells, thereby promoting tumor angiogenesis by targeting SMAD4 and STAT6[101]. Exosomal miRNAs (miR-638, miR-663a, miR-3648, and miR-4258) from HuH-7M can attenuate the integrity of endothelial junctions and increase permeability by inhibiting VE-cadherin and ZO-1 expression[102]. These findings revealed that HCC-exosomal miRNAs could be delivered to endothelial cells to promote HCC progression.

At the same time, the exosomes released by endothelial cells might also affect tumor cells. A recent study showed that engineered human cerebral endothelial cell-derived exosomes carrying elevated miR-214 (hCEC-Exo-214) could enhance HCC cells' sensitivity to anticancer drugs, such as oxaliplatin and sorafenib[103]. However, how endothelial cell-derived exosomes and exosomal miRNAs act on HCC cells is poorly studied. It is worth paying attention to in the follow-up studies.

3.5 Exosome-mediated cell-cell communication between immune cells and HCC cells

The tumor immune microenvironment (TIME) is an important part of the TME[104]. It is influenced by intricate interactions between tumor cells and host immune cells[105]. In HCC, the poor overall survival outcome results from the collapse of immune surveillance, which is closely associated with the suppression of host immune responses[105-107]. Mounting evidence has indicated that the interplay of exosome exchange-based cancer immunity is involved in the modulation of the microenvironment, imparting immune-suppressive and immune-tolerogenic characteristics.

TAM presents the major leukocyte component that infiltrates in the HCC TIME[107].

Hepatic macrophages, also known as Kupffer cells, are the most abundant immune cells in the liver[108]. During the early stages of carcinogenesis, pro-inflammatory activation of Kupffer cells is important in tumor development. Once the primary tumor is established, the liver-infiltrated macrophages play a more prominent role than Kupffer cells in HCC progression[109]. M2-polarized TAMs promote HCC progression by preventing T cells from recognizing and killing cancer cells, promoting tumor growth, angiogenesis, invasion, and metastasis, and resisting immune damage[110, 111]. The role of TAM derived exosomes is now getting more and more attention. It has been reported that M2 macrophage-derived exosomal miR-92a-2-5p can increase the invasion of HCC cells by regulating the AR/PHLPP/p-AKT/β-catenin signaling pathway[112]. M2 macrophage-derived exosomal miR-27a-3p and miR-660-5p augmented HCC development by downregulating TXNIP and KLF3[113, 114]. TAM-derived exosomes with low levels of miR-125a and miR-125b have been proven to promote HCC cell proliferation, sphere cell formation, and metastasis by downregulating CD90, a stem cell marker of HCC. The miR-125a/b suppressed HCC cell proliferation and stem cell properties by targeting CD90, a stem cell marker of HCC stem cells[115].

Modulating TAM exosomal miRNAs provide a new way to suppress HCC. A tumor suppressor miRNA - miR-375 was found to be upregulated in exosomes from IL-2 modulated TAMs and ameliorated HCC development[116]. Moreover, propofol can stimulate TAMs to secrete exosomes overexpressing miR-142-3p. MiR-142-3p exosomes were transferred to HCC cells, inhibiting HCC cell invasion[117].

Conversely, M1 macrophages perform proinflammatory and antitumor effects. M1 macrophage-derived exosomal miR-628-5p inhibited the m6A modification of circFUT8, thereby inhibiting HCC development[118]. Peripheral blood monocyte-derived exosomal miR-142 and miR-223 can directly inhibit the proliferation of HCC[119].

The exosomes from other immune cells also play a role in HCC. In mice, NK-exosomes rich in miR-223 inhibited CCL4-induced liver fibrosis by inhibiting TGF-β1-induced HSC activation. ATG7 was confirmed as a direct target of miR-223, so the overexpression of ATG7 in HSCs abolished the HSC activation-suppressive effect of NK cell exosomes[120].

Mast cells can be stimulated by hepatitis C virus E2 envelope glycoprotein and secrete

large amounts of miR-490-rich exosomes, which can be transferred into HCC cells and inhibited tumor cell metastasis through the ERK1/2 pathway[121]. Besides, miR-150-5p and miR-142-3p can be transferred from regulatory T cells (Tregs) to dendritic cells DCs via exosomes, resulting in the induction of a tolerant phenotype in these cells, with increased IL-10 and decreased IL-6 production after LPS stimulation[122].

On the other hand, tumor-derived exosomal miRNAs also affect the distribution and function of immune cells. Tregs that produce inhibitory factors such as IL-10 and TGF-β are among the most prevalent suppressor cells in TME and have been related to tumor progression. Tregs also express a panel of chemokine receptors and surface molecules such as CTLA4 and PD-1, thus making them a direct target of immune checkpoint inhibitor immunotherapy. The development of immune-related adverse events may partly be attributed to Treg destabilization[123]. Tumor cell-secreted miR-214 could expand the $CD4^{+}CD25^{high}Foxp^{3+}$ Treg population by decreasing the levels of PTEN in CD4+ T cells, leading to host immune suppression and rapid tumor growth[124]. The expansion of the Treg population by tumor-secreted miR-214 likely serves as a common mechanism for various cancer cells to create a tolerant immune environment. Inhibiting the transport of tumor-secreted miR-214 to immune cells may be a novel strategy to reverse tumor-induced immune tolerance[124].

In summary, exosome-delivered miRNAs from immune cells were intensely involved in the biological processes of HCC, and HCC-derived exosomal miRNAs also affect the distribution and function of immune cells.

4. Clinical applications of exosome-delivered miRNAs in hepatocellular carcinoma

Radical resection and trans-arterial chemoembolization (TACE) are still the most effective curative methods for patients with early-stage liver cancer. Still, the treatment efficacy remains unsatisfactory due to the compensatory effect of vascular proliferation after hypoxia[125, 126]. For patients with advanced liver cancer, targeted therapy, and traditional chemotherapy can only prolong the survival of these patients to a certain extent. Innovative and alternative therapies are continuously needed to improve the prognosis of HCC patients.

Studies have recently confirmed that specific miRNAs can be transported through exosomes, thereby controlling tumor growth and achieving therapeutic effects[127]. Since exosome has unique features as a drug delivery system, such as low immunogenicity, high biocompatibility, low toxicity, and the ability to cross the blood-brain barrier, exosome is gaining traction as a natural delivery vector for miRNA[128]. Among the cell types known to produce exosomes, mesenchymal stem cell (MSC) is an ideal candidate for the large-scale production of exosomes for drug delivery. MSC-derived exosome has been used as a drug delivery vehicle in some studies for tumor treatment and regenerative medicine[129, 130]. Based on the above findings, engineered MSC-derived exosomes loaded with specific miRNAs provide a new therapeutic strategy for HCC treatment.

Exosomal miRNAs have been used to improve the chemosensitivity of tumor cells[131, 132]. The research showed that overexpression of miR-122 could regulate the sensitivity of HCC cells to chemotherapy drugs by downregulating multidrug resistance-associated genes, the anti-apoptotic gene Bcl-w and the cell cycle-related gene cyclin B1[47]. The miR-122-modified amniotic membrane mesenchymal stem cells (AMSCs) can effectively package miR-122 into secreted exosomes, which mediate miR-122 communication between AMSCs and HCC cells and further increase the sensitivity of HCC cells to sorafenib[133]. AMSC exosomal miR-199a (AMSC-Exo-199a), constructed by miR-199a lentivirus infection and puromycin selection, acts as an effective carrier for miR-199a delivery to sensitize HCC cells to doxorubicin by targeting the mTOR pathway. In addition, intravenous injection of AMSC-Exo-199a can be delivered to tumor tissue, significantly increasing the effect of Dox on HCC in vivo[134].

Liver fibrosis is the precursor stage of cirrhosis and liver cancer. MSC-derived exosomes alleviated carbon tetrachloride (CCL4)-induced liver fibrosis in mice through the expression of miR-148a. MiR-148a directly targeted KLF6 to effectively convert the polarization state of macrophages from the M1 to the M2 phenotype in vitro, and liver fibrosis models[135]. In vitro studies have shown that transplanted human chorionic plate-derived mesenchymal stem cells (CP-MSCs) reduce lung and liver fibrosis in murine models[136, 137]. One study supported that CP-MSCs released exosomes containing miRNA-125b into target cells, such as hedgehog-responsive HSCs, and hindered hedgehog

signaling activation by inhibiting smoothened receptor expression, eventually alleviating hepatic fibrosis[138]. As a new candidate therapeutic strategy, MSC exosomes have excellent application prospects for HCC.

In addition, MVs released from human liver stem cells (HLSCs) inhibited the growth of hepatoma cells both in vitro and in vivo by delivering antitumor miRNAs (miR451, miR223, miR24, miR31, miR214, and miR122) that downregulated MDR1, MIF, ras-associated protein 14 (RAB14) and E2F-2[139].

CONCLUSION

Despite significant advances in diagnosis and therapeutics, HCC remains a highly lethal disease. In most cases, HCC develops from chronic liver inflammation, which provides a tumor-promoting microenvironment composed of immune and stromal cells. As a novel cellular communicator in TME, exosomes mediate the intricate interaction of nonparenchymal cells (including immune and stromal cells) with cancer cells. They are involved in the etiology of HCC and multiple processes related to tumor initiation, development, metastasis, and drug resistance. Exosome cargoes, especially miRNAs, are key communication factors in the complicated cross-talk, indicating that they are promising prognostic markers and therapeutic targets for HCC. In this review, we focused on the role and mechanism of exosomal miRNAs from nonparenchymal cells for the development and progression of HCC. Also, we introduced the influences of exosomal miRNAs delivered by tumor cells on nonparenchymal cells. The functions of the exosomal miRNAs in HCC were also summarized (Table 1). Finally, the therapeutic potential of exosomes for HCC was discussed. With the development of nanoengineering technology, exosomes can be modified to carry specific miRNAs and target specific cells, thus enabling precision and individualized treatment of HCC.

Although remarkable advances have been made in understanding the role of exosomes and their miRNA cargoes in HCC, some challenges remain. Different investigators reported different experimental observations for the same exosomal miRNAs. The inconsistency of experimental subjects and study designs might cause these discrepancies. Therefore, factors such as the environment, age and sex of the subjects, cause of HCC

occurrence, and data collection from multiple centers should be considered to produce more accurate results. Moreover, different isolation methods may result in different subpopulations of extracellular vesicles with different miRNAs, proteins, diameters, and functions[140-142]. In clinical applications, problems include low targeting efficiency and easy phagocytosis by the immune system. The exosome separation and purification method also have limitations and could be time-consuming and laborious. Therefore, more research must be done to solve these problems and develop more effective clinical applications of exosomes. With the combination of nanoengineering and molecular biology, exosome-mediated miRNAs for precision nanomedicine will provide new HCC diagnosis and treatment approaches.

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Footnotes

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Figure Legends

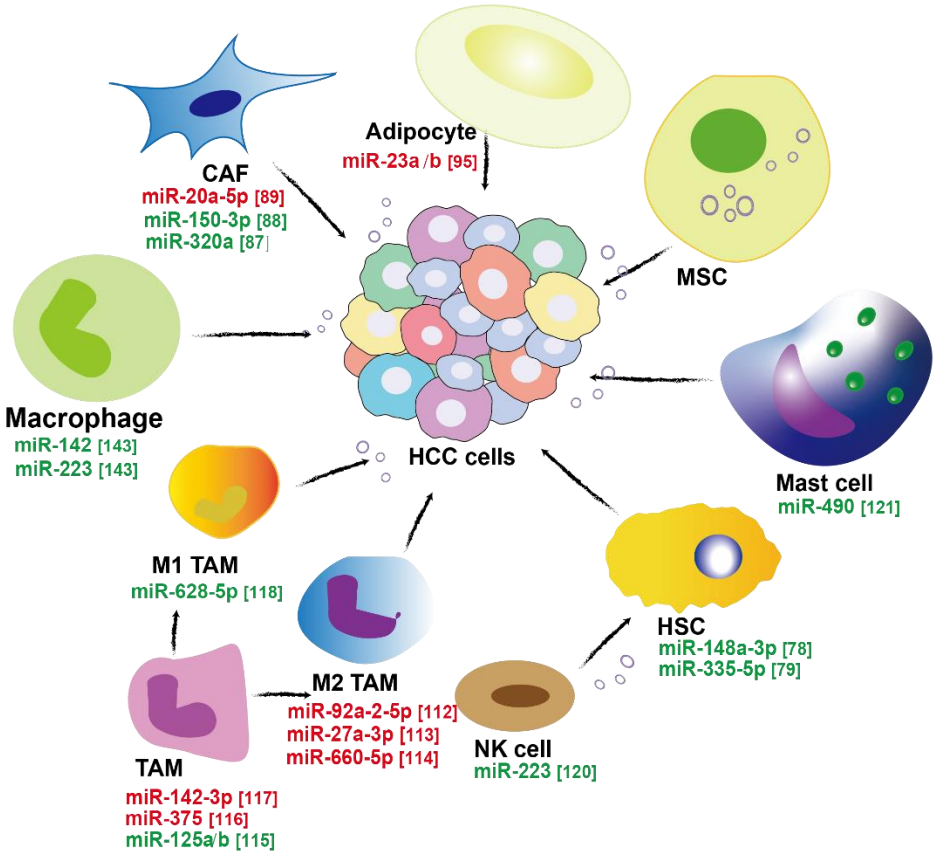

Figure 1 A schematic of exosomal microRNAs in the tumor microenvironment of hepatocellular carcinoma. **HCC,** hepatocellular carcinoma**; CAFs,** cancer-associated fibroblasts; **TAMs,** tumor-associated macrophages; **NK cells,** Natural Killer cells**; HSCs,** hepatic stellate cells; **MSC,** mesenchymal stem cell. Red represents the promoting effect of miRNA on HCC proliferation, and green represents the inhibitory effect of miRNA on HCC proliferation.

Table 1 The function of exosomal microRNAs from interstitial cells in the liver

miR Species in Exosomes	Exosome-Secreting Cells	Exosome Isolation Methods	Target Cells	miRNA Expression of Exosome	Downstream Targets	Functions of miRNA	Additional Information	Reference	Year
miR-148a-3p	Primary fibroblasts（The HSC cell line LX-2）	The ExoQuick-TC kit	Human HCC cell lines PLC, HCCLM3 and SMMC-7721	Reduced in the exosomes of HSCs after cocultivation with primary liver cancer-associated fibroblasts	ITGA5/PI3K/Akt Axis	Inhibits HCC cell malignancy	Primary fibroblasts were isolated from primary HCC tumor and paired peritumor tissues in 17 primary HCC patient samples	[78]	2022
miR-335-5p	The HSC cell line LX-2	Ultracentrifugation	Human HCC cell lines MHCC97H, MHCC97L, HepG2 and Huh7	Reduced in the exosomes of fibroblasts, as well as in HCC cells after cocultivation	CDC42？CDK2？	Inhibits neighboring cancer cell proliferation, invasion and motility		[79]	2019
miR-320a	CAFs	Life Technology exosome precipitation solution	Human HCC cell lines MHCC97-H, SMMC-7721, Huh7, and the human normal liver cell line 7702	Reduced in the exosomes of CAFs derived from human HCC patients	PBX3	Inhibits HCC cell proliferation and metastasis ability	PAFs and CAFs derived from 6 pairs of matched primary hepatocarcinoma and adjacent tumor-free tissues (5 cm from the cut edge of the tumor edge)	[87]	2017
miR-150-3p	CAFs	0.22-μm PVDF filter and Total Exosome Isolation Reagent	Human HCC cell lines Huh7 and Hep3B	Decreased in CAF-derived exosomes		Inhibits HCC proliferation and metastasis	Stromal fibroblasts isolated from tumor tissue and adjacent (>5 cm from the tumor edge) tissues from 6 HCC patients	[88]	2021
miR-20a-5p	CAFs	Centrifuged and filtered	Human HCC cell lines SMMC7721,	Higher in exosomes from cancer tissues	LIMA1	Contributes to HCC cell	CAFs were from the HCC tissues and NFs in paired	[89]	2022

		through a 0.22-μm PVDF membrane	Huh7, YY8103, Hep3B, Focus, HepG2 and HCCLM3 and a normal liver cell line MIHA, WRL68	than in matched adjacent para-tumoral tissues		proliferation, metastasis and EMT	adjacent normal tissues from 92 HCC patients		
miR-214	hCECs	Centrifuged and filtered through a 0.22-μm PVDF membrane and ultracentrifugation	Human HCC cell lines HepG2, Hep3B, the human liver epithelial cell line THLE-2	Lower levels in HCC cells than in normal human liver epithelial cells	P-gp/SF3B3	Reduced cancer cell viability and invasion compared with monotherapy with oxaliplatin or sorafenib		[103]	2021
miR-23a/b	Adipose cell mouse preadipocyte 3T3-L1 cells	Differential centrifugation	The human HCC cell lines BEL-7402 and BEL-7402/5-Fu Mouse hepatoma cell line Hepa1–6	High in exosomes from HCC patients with high BFR	VHL/HIF-1α	Promoted HCC cell growth and migration	Adipose cell were isolated from human tumor tissues from obese and nonobese patients	[95]	2019
miR-142 miR-223	Monocyte-derived Macrophages Human acute monocytic leukemia THP-1, B-lymphoblast	Microfiltration and ultracentrifugation	The human HCC cell lines Huh7 and HepG2	High when cocultured with HCC cells	STMN-1	Inhibited HCC proliferation	PBMCs were isolated from lymphocyte cones or fresh blood by density gradient centrifugation and were incubated for 2 h in plastic plates before the flask was washed intensively to remove any nonadherent cells. After 4 days of incubation in serum-free medium supplemented with 1% autologous serum, adherent	[143]	2014

	oid 721.221 and mouse lymphoblast-like mastocytoma P815 cell lines						cells were washed with PBS and cultured in standard DMEM-based medium for 3 to 6 extra days to generate monocyte-derived macrophages, phenotyped to be $CD14^+$, $CD11a^+$, $CD3^-$, $CD56^-$, and $CD19^-$.		
miR-490	Human MC line HMC-1 (treated with HCV-E2)	Total exosome separation reagent from Invitrogen	The human HCC cell lines HepG2 and HepG3b	High when HCV-E2-stimulated MC-derived exosomes were incubated with the two types of HCC cells for 24 h compared with the incubation with normal MC-derived exosomes	ERK1/2	Inhibited HCC proliferation		[121]	2017
miR-223	Human NK cell line NK92-MI	Differential centrifugation	The human HSC line LX-2	Higher in Exosomes derived from NK cells than in parental NK-92MI cells	AGT7	Attenuated TGF-β1-induced HSC activation and inhibited liver fibrosis	LX-2 cells were treated with TGF-β1 (5 ng/mL) for 24 h to stimulate HSC activation. LX-2 cells in the Exosomes derived from NK cells-treated groups were pretreated with Exosomes derived from NK cells (10 μg/mL) before TGF-β1 treatment. LX-2 cells in the rapamycin-treated groups were pretreated with the autophagy activator rapamycin (2 mM) in DMSO for 12 h	[120]	2020

							before TGF-β1 treatment.		
miR-125a/b	TAMs	ExoQuick exosome precipitation solution	The human HCC cell lines Huh7, HepG2 and BEL‐7404	Downregulated in exosomes from HCC-associated macrophages	CD90	Suppressed HCC cell growth and sphere formation	TAMs and nontumor macrophages were isolated from primary human HCC, adjacent nontumor liver tissues from 6 patients with HCC	[115]	2019
miR-628-5p	M1 macrophage		The human HCC cell lines Huh7, HCCLM3, Hep3B, and MHCC97H , Immortalized human liver epithelial THLE-3 cell line	High in M1-Exos	METTL14/circFUT4/CHMP14B	Inhibited HCC cell progression	THP-1 cells were differentiated into M0 macrophages by a 24h incubation with 150 nM phorbol 12-myristate 13-acetate followed by a 24h incubation in RPMI medium. M0 macrophages were polarized to M1 macrophages by incubation with 20 ng/ml IFN-γ and 10 pg/ml lipopolysaccharide	[118]	2022
miR-92a-2-5p	M2 macrophage（monocytic leukemia cell line THP-1）	Centrifuged and filtered through a 0.22-μm PVDF membrane and ultracentrifugation	Human liver cancer SK-HEP-1 and HepG2 cell lines, HA22T cell line and mouse HCC Hepa 1-6 cell line	Increased after coculture with liver cancer cells	AR/PHLPP/p-AKT/β-catenin signaling	Promoted HCC growth and invasiveness	To induce differentiation into macrophages, THP-1 cells were cultured with 100 ng/ml PMA (Sigma) for 48 h. and the macrophage was cultured with the addition of DMSO to promote M2 polarization	[112]	2020
miR-660-5p	M2 macrophage（monocyti	Differential centrifugation	Human HCC cell lines HepG2 and Bel-7402	High	KLF3	Augmented the tumorigenic ability of HCC	THP-1 monocytes were stimulated by 100 ng of phorbol 12-myristate 13-acetate (Sigma −	[114]	2021

	c leukemia cell line THP-1）					cells	Aldrich, MO, USA) for 48h, thus differentiating into M0 macrophages. Then, M0 macrophages were treated with 20 ng/mL interleukin 4 (AF-200–04-5, PeproTech, NJ, USA) for 72 h to polarize into M2 macrophages		
miR-27a-3p	M2 macrophage（monocytic leukemia cell line THP-1）	SBI ExoQuick-TC Kit	Human HCC cell lines Huh7, 97H, HepG2, LM3 and SMMC-7721	——	TXNIP	Induced the cancer stemness of HCC	Differentiation of THP-1 cells to macrophages was performed using 200 ng/mL phorbol myristic acetate, and the cells were then cultured with 20 ng/mL interleukin-4 for 72h to induce M2-type polarization	[113]	2021
miR-142-3p	TAMs treated by propofol（The mouse macrophage cell line Raw 264.7 cells）	Differential centrifugation	The mouse HCC cell line Hepa1-6	Dose-dependent increase when treated with propofol	RAC1	Enhanced the antitumor activity of propofol	Raw 264.7 cells were cultured in complete RPMI 1640 with 10% FBS and treated with propofol (dissolved in RPMI 1640) in complete medium. TAMs were isolated from tumor-bearing mice treated with 0 mg/kg, 20 mg/kg and 50 mg/kg propofol by i.p. injection.	[117]	2014
miR-375	TAMs (IL-2 induced)	Total Exosome Isolation Reagent	The human HCC cell lines HepG2 and QJY–7703 cells	High		Ameliorated HCC development and progressio	Primary human HCC specimens were collected from patients who suffered from	[116]	2022

	n	hepatectomy. The macrophages were isolated and cultured by Percoll (GE Healthcare) density gradient centrifugation. TAMs were treated with IL-2 for 24 h before the supernatants were collected. The treatment concentration was 20 ng/ml.

Notes: HCC: Hepatocellular carcinoma; CAFs: Cancer-associated fibroblasts; TAMs: Tumor-associated macrophages; NK cells: Natural Killer cells; HSCs: Hepatic stellate cells; MSC: Mesenchymal stem cells; ITGA5: Integrin α5; PI3K: Phosphoinositide 3 - kinase; CDC42: Cell Division Cycle 42; CDK2: Cyclin dependent kinase 2; PBX3: Pre-B-cell leukemia homeobox 3; PAFs: Para-cancer fibroblasts ; LIMA1: LIM domain and actin binding 1; P-gp: P-glycoprotein; SF3B3: Splicing factor 3b subunit 3; hCECs: Human cerebral endothelial cells; BFR: Body fat ratio; VHL: Von Hippel-Lindau; HIF-1α: Hypoxia-inducible factor 1α; STMN1: Stathmin-1; PBMCs: Peripheral blood mononuclear cells; MCs: Mast cells; HCV-E2: Hepatitis C virus E2 envelope glycoprotein; ERK1/2: Extracellular regulated protein kinases 1/2; AGT7: Autophagy-related 7; Exos: Exosomes; METTL14: Methyltransferase-like 14; AR: Androgen receptor; KLF3: Kruppel-like factor 3; TXNIP: thioredoxin-interacting protein; RAC1: Rac family small GTPase 1