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WORD COUNT 5419
TIME SUBMITTED 21-SEP-2023 04:04PM
PAPER ID 102906458

Name of Journal: *World Journal of Gastroenterology*
Manuscript NO: 86000
Manuscript Type: REVIEW

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

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Abstract

Small extracellular vesicles (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

Wang HC, Yin WX, Jiang M, Han JY, Kuai XW, Sun R, Sun YF, Ji JL. Function and biomedical implications of exosomal microRNAs delivered by parenchymal and nonparenchymal cells in hepatocellular carcinoma. *World J Gastroenterol* 2023; In press

Core Tip: Hepatocellular carcinoma (HCC) is one of the most serious cancers in adults and microRNAs (miRNAs) in small extracellular vesicles (exosomes) play a vital role in the pathophysiological processes of HCC. Recent studies on exosomal 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

In 2020, liver cancer was ranked the sixth most frequent malignant solid cancer globally. It was also the third reason which led to tumor death in the world[1]. Hepatocellular carcinoma (HCC) stands as the primary histological type of liver cancer, comprising 80% of primary liver cancer cases[2]. It is characterized by the high degree of malignancy and poor prognosis. It is a threat to the health of humans. The symptoms of incipient-stage HCC are strong concealment, and it's challenging to diagnose HCC early. In addition, approximately 70% of patients undergo recurrence and the spreading of cancer cells to other parts of the body within 5 years after surgical resection[3].

The tumor microenvironment (TME) is important in the development of HCC[3], which primarily comprises host cells, both resident and recruited, along with the secreted molecules and extracellular matrix (ECM) proteins[4]. Nonparenchymal cells in the liver, such as sinusoidal endothelial cells, hepatic stellate cells (HSCs), and macrophages, have a critical role in establishing the TME and mediating tumorigenesis by paracrine communication via cytokines and/or angiocrine factors[5]. Accumulating investigations on the TME have revealed novel perceptions of tumor growth as well as metastasis therein exosomes play a crucial function[6-8].

Small extracellular vesicles (sEVs), also known as exosomes, refer to a specific type of extracellular vesicles with a size of 40-160 nm that originate from multivesicular bodies (MVBs), which act as carriers for biological information exchange to shape 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 DNA, lipids, proteins and RNA such as microRNAs (miRNAs), circular RNAs (circRNAs), long noncoding RNAs (lncRNAs) and messenger RNAs (mRNAs), which are involved in intercellular

communication[10,11]. More and more molecules of different classes carried by exosomes have been reported. Based on data retrieved from the ExoCarta database (http://www.exocarta.org), the identified components within exosomes consist of 9769 unique proteins, 3408 distinct mRNAs, 2838 different miRNAs, and 1116 lipids. Initially, exosomes were considered as carriers of cellular waste, and their functions were also underestimated[12]. Over the past few decades, the crucial functions of exosomes in facilitating intercellular communication in both physiological and pathological processes have been extensively studied and validated[13]. In 1996, exosomes derived from murine and human B lymphocytes were proven to execute a crucial function in transporting MHC molecules and eliciting MHC-II restricted T-cell responses[14]. Later, cancer cells and non-tumor cells in the TME were also found to be able to deliver exosomes and thereby participate in the malignant progression of tumors through molecular exchanges mediated by them[15,16]. Exosomes, hence, are recognized as important contributors to cancer initiation and progression[17-19].

MiRNAs represent an extensive collection of post-transcriptional gene expression regulators in eukaryotes. These regulatory molecules typically consist of 20-24 nucleotides and exert their function over various developmental and cellular processes[20]. Due to their essential role in gene expression, exosomal miRNAs have also been widely studied. In 2007, Valadi *et al*[21] reported that exosomes contained miRNAs, which could be delivered to other cells and exert their functions. Researches have demonstrated that exosomes are loaded with a high abundance of miRNAs, which play a crucial role in immune modulation, resistance to chemotherapy, and metastasis in diverse malignancies[22]. These miRNAs can promote tumor development in a paracrine manner in the surrounding microenvironment[23-25]. Furthering the comprehension of cancer mechanisms needs the identification of exosomal miRNAs, which are abnormally expressed in pathological states.

Numerous scientific researches have demonstrated that exosomes play a critical role in the genesis and malignant progression 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 to better understand the role of exosomal miRNAs from stromal cells and immune cells in HCC and develop innovative strategies for HCC prevention and treatment.

FORMATION, COMPOSITION AND FUNCTIONS OF EXOSOMES

Unlike other types of vesicles, exosomes have a different formation mechanism. First, the plasma membrane germinates inwards to form early endosomes (membrane-bound vacuoles)[27,28]. By further inwards budding of early endosomes encompassing several miRNAs, proteins, and other selected substances, late endosomes called MVBs are formed[29]. Following this, the MVBs undergo fusion with the cell membrane, and the intraluminal endosomal vesicles are released into the extracellular area. These vesicles subsequently form exosomes[30] or fuse with the lysosome to decompose the biological information[31].

Studies revealed that the essential system involved in the biogenesis of exosomes is the endosomal sorting complex required for transport (ESCRT)[32]. The ESCRT complex identifies the ubiquitin-labeled "cargo" protein, guides it to MVBs, and subsequently separates the MVB from the peripheral membrane in a highly conserved process similar 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, hereby executing their designated tasks[34,35]. Exosomes transport multiple biologically active substances, such as proteins, RNA, DNA, and cholesterol[36-38]. The sucrose gradient density range in which exosomes float is 1.13 to 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.

EXOSOMAL MIRNAS AND LIVER CANCER

In the past few years, exosomes have been evidenced as crucial mediators of intercellular material and information exchange that can modulate the TME by transmitting nucleic acids and proteins between cells; hence, they are involved in tumor cell proliferation and migration, immune regulation, and drug resistance[44,45]. As an essential component of exosomes, exosomal miRNAs exert crucial functions in HCC tumorigenesis and progression.

Here, we first review the function of exosomal miRNAs derived from HCC cells. MiR-122, which proved to be the most enriched miRNA in the human liver, is found to be decreased in the liver of HCC patients[46-48]. It is expressed and delivered by Huh7 cells (human HCC cell line) and can be transferred into HepG2 cells (human HCC cell line, of which the basal expression of miR-122 is low) in the form of exosomes, reducing the growth and proliferation of recipient HepG2 cells. The restoration of miR-122 inhibits HCC growth and enhances HCC sensitivity to chemotherapeutic drugs[49]. In addition, exosomes delivered by liver cancer cells can affect nonparenchymal cells in the microenvironment, promoting the malignant progression of tumors, which will be discussed in subsequent sections.

On the other hand, exosomal miRNAs secreted by tumor cells other than liver cancer cells can also promote the formation of premetastatic niches in the liver. Colon cancer cell-derived exosomes are able to deliver miR-21, miR-192, and miR-221 to hepatoma cells[50]. Exosomal miR-25-3p delivered by colon cancer cells promotes 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 types of cancers, even remodeling the microenvironment to boost liver metastasis[53].

Exosomal miRNAs might also be linked to different etiology of liver disease related to HCC. The connection between miRNAs and different liver diseases covering hepatitis B virus (HBV) infection, 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 in-depth introduced in previous high-quality reviews[54-56]. In the liver of ALD, NASH, and HCC patient, the level of hepatocyte-specific miR-122 exhibit a remarkable decrease. This specific miRNA directly targets distinct regions at the 5′-UTR of the HCV RNA genome, thereby facilitating the replication of HCV RNA[57]. When it comes to HBV replication, miR-122 functions oppositely. It acts as an inhibitor by downregulating the cyclin G1-p53 complex and preventing the specific interaction between p53 and HBV enhancers[58]. In simple steatosis, the liver shows an increase in the expression of miR-192, which is enriched in hepatocytes. However, this elevation is not observed in NASH[59]. On the other hand, the expression of miR-192 is decreased in HCC[60]. It is the most significantly downregulated miRNA in hepatic cancer stem cells (CSCs) and plays a role in the activation of CSCs. Due to the anti-tumor property of miR-192, administering miR-192 to individuals with HCC can be a potential strategy for HCC therapy[60]. The expression of miR-155, a highly abundant miRNA in immune cells, including macrophages, is elevated in the liver tissues of patients with ALD, AIH, and HCC. It is an oncogenic miRNA that links inflammation with tumorigenesis[61,62]. The activation of NF-κB signaling was reported to induce an upregulation in miR-155 levels in hepatocytes and liver cancer when mice were fed a choline-deficient and amino acid-defined diet[61] or HCV infection in patients[62]. However, few studies have focused on the etiology of HCC and miRNAs delivered by exosomes. According to a recent investigation, extracellular vesicles derived from neutrophils have the capability to transfer miR-223 to macrophages, stimulating the resolution of liver fibrosis[63]. Neutrophil/myeloid-specific miR-223 has been extensively studied for its anti-inflammatory properties. Its function involves the suppression of IL-

6 expression, effectively reducing the activation of the IL-6-p47phox-ROS pathway within neutrophils[64]. The upregulation of miR-223 is observed in the serum and/or liver of patients or mouse models experiencing ALD or NASH, both diseases characterized by significant hepatic neutrophil infiltration. Consequently, the compensatory increase in miR-223 expression is a protective mechanism against ALD[64] and NASH[65]. At the same time, the reduction of miR-223 in HCC might be a causal factor in promoting the HCC progression[66]. Therefore, the administration of miR-223 is thought to be a potent treatment in murine 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.

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 identified as an evolutionary and ecological process characterized by constant, dynamic and reciprocal action upon each other. Nonparenchymal cells in the liver cancer TME, such as HSCs, cancer-associated fibroblasts (CAFs), immune cells (T lymphocytes, B lymphocytes, natural killer cells or NK cells, natural killer T cells, and tumor-associated macrophages or TAMs), and endothelial cells (ECs), are pivotal in mediating tumor-stromal communications, thus regulating the biological processes of HCC[68]. Noncellular components are composed of growth factors like transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), proteolytic enzymes, ECM, and inflammatory cytokines. These components create a beneficial environment for the formation and proliferation of HCCs. Exosomal miRNAs, a crucial element of the TME, play a significant role in transmitting signals between cells and contribute to the development and advancement of tumors. In the next section, the role of the exosomal

miRNAs from different nonparenchymal cells in HCC formation and metastasis is thoroughly discussed. The related investigations are paving the way for novel strategies in clinical diagnosis and treatments aimed at HCC (Figure 1).

Exosome-mediated cell-cell communication between activated HSCs and HCC cells

HSCs can be observed in the space of Disse, located between liver sinusoidal endothelial cells and hepatocytes. These cells are responsible for storing lipid droplets containing vitamin A[69,70]. When there is damage to the liver, quiescent HSCs transform to activated HSCs, which resemble myofibroblasts and produce excessive fibrotic ECM[70]. The migration and accumulation of myofibroblasts are thought to be the key events that initiate liver fibrosis. Although many cell types, such as HSCs[71-73], portal fibroblasts[71,72], mesenchymal stem cell (MSC)-like cells[74], mesothelial cells[75] and bone marrow-derived cells[76], have been reported to contribute to the myofibroblast pool, recently, researchers have evidence that 82%-96% of myofibroblasts in models with toxic, cholestatic and fatty liver diseases are generated from activated HSCs[73].

The initiation and promotion of liver cancer are significantly correlated to the existence of liver fibrosis[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 *et al*[78] reported that reducing activated HSC-delivered exosomal miR-148a-3p inhibited HCC initiation through the ITGA5/PI3K/Akt pathway. Another group found that HSC-HCC cell coculture reduced intracellular miR-335-5p expression in both types of cells. Additionally, *in vitro* and *in vivo* experiments showed that miR-335-5p-loaded HSC exosomes inhibited cancer growth and invasion[79]. In summary, activated HSCs can promote the development of HCC *via* various miRNAs delivered by exosomes, and targeting activated HSC-exosome miRNAs represents an innovative therapeutic strategy in HCC. At the same time, exosomes derived from HCC cells also promote the activation of HSCs. 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]. The progression of cancer was

further accelerated by the activation of CAFs, which release angiogenic cytokines such as VEGF, bFGF, TGF-β, matrix metalloproteinase-2 (MMP2), and MMP9[80]. Another study suggested that a high level of serum exosomal miRNA-21 is associated with increased activation of CAFs and a higher vessel density in patients with HCC[80].

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

CAFs are an important component of the TME[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]. In the latest study, Zhu *et al*[85] successfully identified five CAF subtypes within HCC tumors through single-cell RNA sequencing data obtained from both mouse and human HCC tumors. The subtypes include vascular CAFs (vCAFs), matrix CAFs (mCAFs), lipid processing-mCAFs (lpmCAFs, also known as $CD36^+$ CAFs), lipid-processing CAFs (lpCAFs) and antigen-presenting CAFs (apCAFs). In these cells, CD36+ CAFs are derived from HSCs[85]. Another group also showed that Tcf21 was explicitly expressed in HSCs 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 crucial contributors to the alterations of the ECM that contribute to the development of HCC, CAFs have the potential to stimulate the progression of HCC through communication mediated by exosomes. A recent study found that miR-320a level was remarkably decreased in CAF-derived exosomes compared with corresponding para-neoplastic fibroblast (PAF)-derived exosomes in HCC patients. *In vitro* and *in vivo* experiments evidenced the anti-tumor effects of miR-320a when it was delivered to malignant cells through exosomes. The anti-tumor effect of miR-320a might be achieved by effectively targeting PBX3, thereby impeding the activation of the MAPK pathway[87]. Another study confirmed that miR-150-3p was lost in exosomes released by CAFs. CAF-delivered exosomes potently accelerate the malignant progression of HCC due to the absence of anti-tumoral miR-150-3p. Restoring the expression level of miR-150-3p by

delivering miR-150-3p-loaded exosomes to HCC cells can effectively suppress their migration and invasiveness. Therefore, exosomal miR-150-3p can serve as a prognostic biomarker for HCC, and a supplement with exosomal miR-150-3p might be a potential treatment option[88].

Apart from those under-expressed anti-tumor miRNAs found in CAF-derived exosomes, the oncogenic miR-20a-5p was enriched in CAFs compared to HCC cells. MiR-20a-5p can be transferred from CAFs to HCC cells through exosomes and thereby suppress the expression of the tumor suppressor LIM domain and actin binding 1 (LIMA1), which in turn inhibits the Wnt/β-catenin signaling pathway in HCC[89]. Thus, the distinct expression of exosomal miRNAs in CAFs plays a crucial part in the malignant progression of HCC, so potential therapeutic implications can be expected from anti-CAF medications that aim at certain exosomal miRNAs.

However, exosomal noncoding RNAs other than miRNAs also participate in the CAF-tumor cell communication. Chemoresistance in HCC can be influenced by CAF-exosomal circRNAs. Circular RNA ZFR (circZFR) is highly expressed in CAFs and CAF exosomes. CAF-exosomes transfer circZFR to tumor cells, suppress the STAT3/NF-κB signaling pathway, and consequently enhance the growth of HCC cells, as well as stimulate chemoresistance to cisplatin (DDP)[90]. In addition, the migration, invasion, and glycolytic abilities of HCC cells were enhanced by lncRNA TUG1 loaded in CAF-exosomes by targeting the miR-524-5p/SIX1 axis[91].

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

The involvement of adipose tissue in tumor progression has long been recognized[92]. Adipocytes play a crucial role in the hepatic microenvironment of NAFLD, which is also a proven risk factor for HCC[44]. There is a close association 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*[94] found that exosomes from obese donors' visceral adipose tissue caused dysregulation of genes involved in the TGF-β pathway in HepG2 cells. Recently, Liu *et al*[95] found that the levels

of miR-23a/b in serum exosomes and tumor tissues were significantly elevated in high-body fat ratio (BFR) HCC patients compared to their low-BFR counterparts. In tumor tissues, it is highly probable that miR-23a/b can be transported from adipocytes into cancer cells *via* exosomes, thus promoting the malignant progression of HCC[95]. Moreover, exosomal miR-23a/b affects the von Hippel-Lindau/hypoxia-inducible factor pathway, thus promoting chemoresistance[95]. Exosomal circRNAs also played a role. Adipocyte exosomal circDB 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 valuable therapeutic targets for HCC.

On the other hand, HCC cell-derived exosomes can educate adjacent adipocytes and generate a microenvironment that promotes tumor formation and 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]. Adipocytes treated by tumor-derived exosomes enhance tumor development, angiogenesis, and macrophage recruitment in a mouse xenograft model[44]. The specific exosomal miRNAs that played a role in the process remain to be revealed.

Besides, it is observed in experimental models and human studies that the exposure to adipocyte exosome also increased the expression of various pro-fibrotic molecules in HSCs, including tissue inhibitor of metal protease 1 (TIMP-1), TIMP-4, Smad-3, integrins αvβ-5 and αvβ-8, and MMP-9[94].

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

It is widely acknowledged 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*[99] discovered that the decrease of miR-214 in HCC cells contributed to the upregulation of

hepatoma-derived growth factor, stimulating vascular endothelial cells to promote angiogenesis and tumor growth. Therefore, miR-214 is a potent suppressor of angiogenesis. It is also evidenced that exosomes derived from HCC cells are able to induce the formation of lumens of human umbilical vein endothelial cells[98]. Recently, several HCC cell-derived exosomal miRNAs were found to be vital to angiogenesis. Fang *et al*[100] reported that hepatoma cell-derived exosomal miR-103 can be internalized by endothelial cells and damage the integrity of endothelial junctions and a subsequent elevation in vascular permeability that facilitates tumor metastasis. The underlying mechanism involves the specific targeting of crucial endothelial junction proteins, such as vascular endothelial-cadherin(VE-cadherin) and p120-catenin, by exosomal miR-103[100]. Exosomal miR-210, derived from HCC cells, can be delivered to endothelial cells and lead to the promotion of tumor angiogenesis. This effect is mediated by the specific targeting of SMAD4 and STAT6, key regulators involved in modulating angiogenic processes[101]. Exosomal miRNAs (miR-638, miR-663a, miR-3648, and miR-4258) from HuH-7M (which is established from luciferase-expressing human hepatoma Huh-7 and deemed as a new, highly intrahepatic metastatic cell line) are able to attenuate the integrity of endothelial junctions, thus enhancing permeability by reducing VE-cadherin and zonula occludens-1 (ZO-1) expression[102]. These findings revealed that HCC-exosomal miRNAs could be delivered to endothelial cells to promote HCC progression.

On the other hand, the exosomes released by endothelial cells might also affect tumor cells. A recent study showed that engineered human cerebral endothelial cell-derived exosomes containing increased 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.

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

The tumor immune microenvironment (TIME) is an important part of the TME[104]. The complicated interactions between cancer cells and host immune cells significantly

influence TIME[105]. In HCC, the poor overall survival outcome arises as a result of immune surveillance disruption, which is strongly associated with the suppression of host immune reactions[105-107]. The growing evidence shows that the intricate interplay of exosome exchange-based cancer immunity shapes the tumor microenvironment, causing immune suppression and immune tolerance.

TAM presents the major leukocyte component infiltrating 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-infiltrating macrophages play a more critical 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, metastasis, and evasion of immune attack[110,111]. The role of TAM-derived exosomes is now attracting more and more attention. Liu *et al*[112] found a role of exosomal miR-92a-2-5p derived from M2 macrophages in promoting HCC cell invasion. This process is mediated through the regulation of the AR/PHLPP/p-AKT/β-catenin signaling pathway by miR-92a-2-5p. Increased expression of miR-27a-3p and miR-660-5p in M2 macrophage-derived exosomes facilitates HCC development by downregulating thioredoxin-interacting protein and KLF Transcription Factor 3 (KLF3)[113,114]. Exosomes derived from TAMs exhibit a reduction of miR-125a and miR-125b expression, which have been proven to promote HCC cell proliferation, sphere cell formation, and metastasis. While miR-125a/b exerts inhibitory effects on the HCC proliferation and attenuates their stem cell-like characteristics by specifically targeting CD90, a recognized stem cell marker in HCC[115].

Modulating TAM exosomal miRNAs provides a new way to suppress HCC. A tumor suppressor miRNA - miR-375, enriched in exosomes from IL-2 modulated TAMs, can ameliorate HCC development[116]. Moreover, propofol can stimulate TAMs to secrete exosomes overexpressing miR-142-3p. When miR-142-3p exosomes are transferred to HCC cells, they can inhibit HCC cell invasion[117].

Conversely, M1 macrophages perform proinflammatory and anti-tumor effects. M1 macrophage-derived exosomal miR-628-5p suppresses HCC development by restraining the m6A modification of circFUT8[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 are also involved in HCC. In mice, NK-exosomes rich in miR-223 inhibited carbon tetrachloride (CCL4)-induced liver fibrosis by inhibiting TGF-β1 induced HSC activation by directly targeting ATG7. So, the overexpression of ATG7 in HSCs abolished the HSC activation-suppressive effect of NK cell exosomes[120]. Hepatitis C virus E2 envelope glycoprotein can stimulate mast cells, which in turn secrete a considerable amount of miR-490 enriched exosomes. When these exosomes are transferred into HCC cells, they inhibit 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, characterized by elevated IL-10 production and decreased IL-6 production upon LPS stimulation[122].

On the other hand, tumor-derived exosomal miRNAs also affect the distribution and function of immune cells. Tregs constitute the most prominent subset of suppressor cells in the TME and release immunosuppressive productions, including IL-10 and TGF-β, contributing to tumor progression. Tregs also present various chemokine receptors and surface molecules like CTLA4 and PD-1, which make them susceptible to immune checkpoint inhibitor immunotherapy. The development of immune-related adverse events may partly be attributed to Treg destabilization[123]. Tumor cell-delivered miR-214 has the potential to augment the population of CD4+CD25highFoxp3+ Treg by reducing the expression of PTEN in CD4+ T cells, which results in the suppression of the host immune response and accelerates tumor development[124]. Indeed, the expansion of Treg populations through tumor-secreted miR-214 is believed to be a shared mechanism employed by various cancer cells to establish an immune-tolerant environment. This miRNA is crucial in modulating immune responses and promoting immune tolerance within the tumor microenvironment. Consequently, the inhibition of tumor-secreted

miR-214 transportation to immune cells shows potential as an innovative approach to counteract 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.

CLINICAL APPLICATIONS OF EXOSOME-DELIVERED MIRNAS IN HCC

Radical resection and trans-arterial chemoembolization remain the most efficacious therapeutic approaches 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 exosomes exhibit distinct characteristics as a vehicle for drug transport, encompassing diminished immunogenicity, enhanced biocompatibility, reduced toxicity, and the capacity to traverse the blood-brain barrier, exosomes have garnered considerable attention as an innate delivery vector for conveying miRNA molecules[128]. Among the various cell types recognized for their ability to produce exosomes, MSC is a promising choice for the large-scale production of exosomes for drug delivery. It has been proved in regenerative medicine and tumor treatment studies that MSC-derived exosomes can serve as effective vehicles for drug delivery[129,130]. Based on the above findings, engineered MSC-derived exosomes loaded with specific miRNAs present a novel therapeutic approach for HCC treatment.

Exosomal miRNAs have been utilized to enhance the chemosensitivity of tumor cells[131,132]. Recent research demonstrated that miR-122 overexpression could sensitize the response of HCC cells to chemotherapy drugs by suppressing multidrug resistance-

associated genes, the anti-apoptotic gene Bcl-w, and the cell cycle-related gene cyclin B1[47]. The miR-122 overexpression amniotic membrane MSCs (AMSCs) can effectively encapsulate miR-122 to secreted exosomes, which are in turn delivered to HCC cells and further increase the sensitivity of HCC cells to sorafenib[133]. The miR-199a loaded AMSC exosomes produced through miR-199a overexpression lentivirus infection and subsequent puromycin selection are able to potently transport miR-199a and enhance the sensitivity of HCC cells towards doxorubicin by specifically targeting the mTOR pathway. Furthermore, tumor tissue can be effectively targeted by AMSC exosomal miRNA-199a through intravenous injection, thereby enhancing the therapeutic effect of Dox on HCC *in vivo*[134].

Liver fibrosis is the precursor stage of cirrhosis and liver cancer. MSC-derived exosomes alleviated CCL4-induced liver fibrosis in mice through the expression of miR-148a. MiR-148a directly targets KLF transcription factor 6 and successfully converts the M1 macrophages to the M2 macrophages in vitro and liver fibrosis models[135]. *In vitro* studies have shown that transplanted human chorionic plate-derived MSCs (CP-MSCs) reduce lung and liver fibrosis in murine models[136,137]. One study supported that CP-MSCs released exosomes containing miRNA-125b into hedgehog-responsive HSCs and restrained the activation of hedgehog signaling by blocking the expression of smoothened receptors, consequently reducing the severity of hepatic fibrosis[138]. As a new candidate therapeutic strategy, MSC exosomes have excellent application prospects for HCC.

In addition, human liver stem cells derived exosomes are loaded with multiple antitumor miRNAs (miR451, miR223, miR24, miR31, miR214, and miR122), which can downregulate multi-drug resistance 1, migration inhibitory factor, ras-associated protein 14 and E2F transcription factor 1. These exosomes have been proven to be able to inhibit the growth of hepatoma cells both *in vitro* and *in vivo*[139].

CONCLUSION

Despite significant advances in diagnosis and therapeutics, HCC remains exceedingly fatal. 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 emphasized the function and mechanism of exosomal miRNAs from nonparenchymal cells for the initiation and malignant 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 significant progress has been achieved in elucidating the functions of exosomes and their miRNA cargoes in HCC, some challenges remain. Sometimes, 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 techniques can lead to the isolation of varied subtypes of extracellular vesicles, each exhibiting unique miRNA profiles, protein compositions, and biological functions[140-142]. In clinical applications, problems include low targeting efficiency and easily phagocytosed by the immune system. The exosome separation and purification method also have limitations and could be time-consuming and laborious. Therefore, further research must be done to address these problems and come up with more feasible and effective clinical translational

applications of exosomes. The integration of nanoengineering and molecular biology allows for the utilization of exosome-mediated miRNAs in precision nanomedicine, presenting novel approaches for the diagnosis and treatment of HCC.

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