

Review

Exosomes in hepatocellular carcinoma microenvironment and their potential clinical application value

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ARTICLE INFO

Keywords:

Hepatocellular carcinoma

Exosome

Microenvironment

ABSTRACT

Hepatocellular carcinoma (HCC) has become a challenging disease in the world today. Due to the limitations on the current diagnosis and treatment as well as its high metastatic ability and high recurrence rate, HCC gradually becomes the second deadliest tumor. Exosomes are one of the types of cell-derived vesicles and can carry intracellular materials such as genetic materials, lipids, and proteins. In recent years, it has been verified that exosomes are linked to numerous physiological and pathological processes, including HCC. However, how exosomes affect HCC progression remains largely unknown. In this review, the exosome-mediated cellular material transfer between cells of different types in the HCC microenvironment and their effects on the behaviors and functions of recipient cells are studied. Furthermore, we also addressed the underlying molecular mechanisms. We believe that new light on the diagnosis of this cancer as well as its treatment strategies will be shed after a collation of literature in this area.

1. Introduction

Primary liver cancer (PLC) includes many types, among them, hepatocellular carcinoma (HCC) occupies about 75–85% of PLC, which makes it the most common type [1]. HCC has become very

commonplace nowadays, recent research shows that about 840,000 people suffering from HCC on the original basis every year, moreover, there are 780,000 people die due to this deadly disease every year [1]. Till now, the major diagnostic methods of HCC include clinical manifestations, imaging such as ultrasonography, molecular markers such as

Abbreviations: ABC-G2, sub-family G member 2; AFP, α -fetoprotein; AKT, protein kinase B; AMPK, AMP-activated protein kinase; ANGPT2, angiotensin II type 2 receptor; AUC, area under the curve; B4GALT3, beta-1,4-galactosyltransferase 3; BAK1, BCL2 antagonist/killer 1; bFGF, basic fibroblast growth factor; Breg, Regulatory B; CA, carbonic anhydrase; CAFs, cancer-associated fibroblasts; CAP1, cyclase-associated protein 1; CAV1, caveolin 1; CAV2, caveolin 2; CDK2, cyclin-dependent kinase 2; CLEC3B, C-type lectin domain family 3 member B; CTLA-4, cytotoxic T-lymphocyte associated protein 4; CXCR, C-X-C chemokine receptor; DLL4, delta-like 4 ligand; DOX, doxorubicin; DVT, deep vein thrombosis; ECM, extracellular matrix; ECs, endothelial cells; EMT, epithelial-mesenchymal transition; ENO1, alpha-enolase; eNOS, endothelial nitric oxide synthase; EPCs, endothelial progenitor cells; ER, endoplasmic reticulum; ERG, ETS-related gene; ETS, erythroblast transformation-specific; EVs, vesicles; exoDOX, exosomal DOX; FAK, focal adhesion kinase; GLUT-1, glucose transporter 1; HCC, hepatocellular carcinoma; HIF, hypoxia inducible factor; HIF-1 α , hypoxia-inducible factor 1 α ; HK2, hexokinase II; HSCs, hepatic stellate cells; HSPGs, heparin sulfate proteoglycans; IL-6, interleukin-6; IL6R, interleukin 6 receptor; IL-8, interleukin-8; IL-10, interleukin-10; ILVs, intraluminal vesicles; IME, immune microenvironment; ING4, inhibitor of growth family member 4; LAG3, lymphocyte-activation gene 3; LHX6, LIM homeobox 6; LOXL4, lysyl oxidase-like 4; MAPK, mitogen-activated protein kinase; MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9; MVBs, multivesicular bodies; NF- κ B, nuclear factor-kappa B; NK, natural killer; p120, p120-catenin; PAX2, paired box 2; PBX3, PBX homeobox 3; PDK1, pyruvate dehydrogenase kinase 1; PD-L1, programmed death-ligand 1; Pgp, P-glycoprotein; PI3K, phosphatidylinositol 3-kinase; PLC, primary liver cancer; PTEN, phosphatase and tensin homolog; ROC, receiver operating characteristic curve; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; SALL4, sal-like protein 4; SMAD3, SMAD family member 3; SMAD4, SMAD family member 4; SOX2, SRY-box 2; STAT6, signal transducer and activator of transcription 6; TAMs, tumor-associated macrophages; TGF- β , transforming growth factor- β ; TIM-1, T cell immunoglobulin and mucin domain 1; TIM-3, T cell immunoglobulin and mucin domain 3; TILs, tumor-infiltrating lymphocytes; TLR, Toll-like receptor; TME, tumor microenvironment; ucRNA, ultraconserved lncRNA; USP7, ubiquitin-specific protease 7; VASN, vasorin; VE-Cad, VE-Cadherin; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau; ZFP36, zinc finger protein 36 homolog; ZO-1, zonula occludens 1.

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<https://doi.org/10.1016/j.bioph.2021.111529>

Received 17 January 2021; Received in revised form 15 March 2021; Accepted 17 March 2021

Available online 26 March 2021

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serum α -fetoprotein (AFP), and diagnostic biopsy, while the treatment options for HCC mainly include surgical resection, ultrasound-guided radiofrequency ablation, liver transplantation, local radiotherapy or chemotherapy and comprehensive treatment [2,3]. So far, although we have been working on the diagnosis and treatment of HCC, certain drawbacks still possess and we have a quite long distance to go. For example, AFP is a commonly used diagnostic marker for this disease, however, the area under the receiver operating characteristic (ROC) curve (AUC) of AFP is not very satisfactory, it only exhibits AUC values of 0.726, and has a sensitivity of 0.779 and a specificity of 0.823 [4]. With the advancement of medical research, we have discovered some potential diagnostic markers one after another, making the shortcomings of AFP more obvious [4]. Besides, surgical resection is suited for the therapy of patients with a solitary tumor, when the number of tumors is high, limitations and disadvantages have also emerged [3]. According to a recently released cancer statistics report, the survival rate of most common cancers, including HCC, have improved to a certain degree, but the prognosis of HCC is still not very satisfactory, due to its high metastatic ability as well as its high recurrence rate, its 5-year survival rate only reached 18%, which makes HCC the second most lethal tumor after pancreatic cancer [5]. Therefore, understanding the underlying molecular mechanisms of this disease is urgently needed, to shed new light on the diagnosis as well as its treatment strategies.

In the 1960s, Wolf discovered a new substance isolated from human platelets, which he called 'platelet-dust', this is the first detection of extracellular vesicles (EVs) [6]. With the progress in this area, we found that there are two types of EVs, including ectosomes and exosomes, among them, exosomes are smaller in size, they have diameters of 40–160 nm, with an average size of 100 nm [7]. The mechanism of exosome formation is gradually understood, intraluminal vesicles (ILVs) are formed in late endosomes through the endocytic pathway, they can be released outside the cell by the mechanism of multivesicular bodies (MVBs) fuse with the cell plasma membrane, these vesicles that be released outside the cell we call as exosomes [8]. Because of the way it is produced, exosomes can carry a variety of intracellular materials such as genetic materials, lipids, and proteins [7]. However, the physiological functions of exosomes are still not very well known and need further studies. Exosomes were initially thought to be artifacts or fragments of degraded or dead cells and had little functional potential [8]. In recent years, it has been verified that exosomes are linked to numerous physiological and pathological processes, including cancers, this may be related to the transport of genetic materials, proteins, and lipids by exosomes to recipient cells [7,9,10]. For example, Lugini et al. found that exosomes from colorectal cancer cells could transform normal mesenchymal stem cells from human colon into cancer-like cells [9]. In addition, Cossetti et al. found that somatic cell-derived exosomes could transfer genetic material into germ cells, which was different from Mendelian modes of inheritance [10]. Because of these functions, exosomes can be designed to serve as the carrier for multiple therapeutic substances and deliver contents to target cells, which is becoming a research hotspot [7]. In addition, over the past few decades, vesicles with exosomal characteristics were isolated from different body fluids, such as blood [11], urine [12], breast milk [13], ascites fluid [14], and bile [15], prompting the widely distributed of exosome in living organisms. What's more, the levels of exosomes, as well as their contents are substantially different in diverse disease states, and the number of plasmatic exosomes has shown great potential for the screening and follow-up of cancer patients in recent years [16–18]. Osti et al. found that the plasmatic exosome concentration was higher in glioblastoma patients than in healthy people, however, the level of plasmatic exosomes decreased following surgery, intriguingly, they noticed a significant increase in relapsed patients [19]. Additionally, Logozzi et al. discovered that melanoma patients exhibited a high level of plasmatic exosomes compared to healthy individuals, and a reduction in the level of plasmatic exosomes was observed while undergoing chemotherapy [20]. Similar phenomena have also been observed in other cancers, such

as Oral cancer and prostate cancer [21,22]. All these suggest that exosomes may be a potential non-invasive approach for disease diagnosis.

2. The role of exosomes in HCC microenvironment

Cancers develop in an environment which is known as tumor microenvironment (TME), a complicated system, except for tumor cells, it also includes extracellular matrix (ECM) and many other cell species, such as endothelial cells, fibroblasts, and immune cells [23]. Microenvironmental acidity, hypoxia and low nutrient supply are hallmarks of tumors, the abnormal tumor vasculature results in different blood perfusion in TME, hypoxia, and low nutrient supply are generalized phenomena in tumor tissue that with perfusion deficit; hypoxia leads to anaerobic glycolysis in cells, which increases the production of lactic acid, in addition, tumor cells favor glycolysis even there exists abundant oxygen, this phenomenon is termed "Warburg Effect", then tumor cells translocate H⁺ into extracellular milieu via a variety of mechanisms, which finally causes a low pH in TME [24]. Among these mechanisms, carbonic anhydrase (CA) comes in the focus for its potent ability in proton transport. Two studies have found that the expression and activity of carbonic anhydrase in tumor cells were increased due to the low pH which made it possible to allow the survival of tumor cells in this harsh environment [25,26]. Recently, the interaction between TME and exosomes has become a focus of research. Exosome secretion is regulated through various mechanisms in TME, such as the levels of phosphorylated molecules and noncoding RNA-mediated pathway [27–30]. Moreover, microenvironmental acidity and hypoxia have also been found to regulate the secretion of exosomes. Logozzi et al. demonstrated that microenvironmental acidity was involved in exosome secretion in almost all tumor types, in their research, they discovered that exosome secretion of tumor cells was increased in pH 6.5, while pH 7.4 showed the opposite result [31]. Interestingly, in another study, they found that the intraluminal acidity of plasmatic exosomes was increased as well while tumor cells were exposed to the acidic condition [25]. Furthermore, Wang et al. discovered that the production of exosomes was increased when tumor cells were exposed to hypoxia in advanced breast cancers [32]. The simultaneous presence of these factors in TME regulates the evolution and progression of cancers including immune escape, drug resistant, and metastases [33]. For instance, Federici et al. discovered that acidic microenvironment contributed to the resistance of melanoma cells to cisplatin, the mechanism related to decreased uptake or neutralization of weakly basic drugs by the acidic TME, furthermore, they also found another mechanism in this study, exosome-mediated drug transport from cell interior into the external environment, these two mechanisms formed a very efficient framework of tumor resistance to drugs, inasmuch as acidic microenvironment could increase the exosome secretion of tumor cells [34]. After the study of several cancers including HCC, we find that whether tumor cell-derived exosomes or stromal cell-derived exosomes, they all have an impact on TME and regulate the progress of cancer [35–37]. In our review, we observed that the substances in exosomes fall into two main categories, those targeting stromal cells (Table 1) and those that target HCC cells (Table 2). However, we need further researches to explore the role exosomes play in TME and the mechanisms of how exosomes influence TME. Due to this reason, we will emphasize the effect of exosomes on HCC microenvironment and their mechanisms (Fig. 1). In this way, we try to explore their potential clinical application value.

2.1. Exosomes regulate the progression of hepatocellular carcinoma via targeting endothelial cells

Tumor cells can regulate endothelial cells (ECs) behaviors via the release of contents in exosomes, in turn, increased vascular permeability, as well as active angiogenic process, lead to tumor metastasis and growth. Fang et al. observed that miR-103 was obviously increased in HCC cell-derived exosomes and could be delivered into ECs, which

Table 1
The effect of exosomes that target stromal cells and their mechanisms.

Exosomal cargos	Cellular origin	Expression level	Recipient cells	Effects on cell behaviors and functions	Mechanism	Reference
miR-103	HCC cells	High	ECs	Attenuate endothelial junction integrity and enhance vascular permeability	Down-regulate VE-Cad, p120, and ZO-1	[38]
miR-210	HCC cells	High	ECs	Enhance angiogenic capabilities	Down-regulate SMAD4 and STAT6	[39]
ephrin-B2	HCC cells	High	EPCs	Enhance angiogenic capabilities	Not mentioned	[41]
ANGPT2	HCC cells	High	ECs	Enhance angiogenic capabilities	Up-regulate AKT/eNOs and AKT/ β -catenin pathways	[43]
circ-100338	High-metastatic HCC cells	High	ECs	Promote proliferation, angiogenesis, and increase vascular permeability	Not mentioned	[45]
VASN	HCC cells	High	ECs	Promote migration	Not mentioned	[46]
miR-21	HCC cells	High	EPCs	Suppress proliferative, migratory, and invasive capabilities	Down-regulate IL6R	[49]
CLEC3B	HCC cells	Low	ECs	Enhance the expression of VEGF	Down-regulate AMPK signaling pathway	[51]
miR-200b-3p	HCC cells	Low	ECs	Enhance angiogenic capabilities	Upregulate ERG	[52]
miR-1247-3p	High-metastatic HCC cells	High	Normal fibroblasts	Transform into CAFs	Down-regulate B4GALT3 to up-regulate β 1-integrin/NF- κ B signaling pathway	[55]
miR-21	HCC cells	High	HSCs	Transform into CAFs	Down-regulate PTEN to up-regulate PDK1/AKT signaling pathway	[56]
TUC339	HCC cells	High	Macrophages	Suppress phagocytic activity	Down-regulate TLR signaling pathway, Fc γ R-mediated phagocytosis pathway, and the actin cytoskeleton pathway	[59,60]
miR-150	HCC cells	High	Macrophages	Enhance the secretion of VEGF	Down-regulate ING4 to up-regulate HIF	[61]
miR-23a-3p	HCC cells	High	Macrophages	Increase T-cell apoptosis and suppress T-cell functions	Down-regulate OS9	[63]
miR-146a-5p	HCC cells	High	Macrophages	Promote macrophages toward M2-polarized TAMs and suppress T-cell functions	Up-regulate PTEN/AKT signaling pathway to up-regulate PD-L1	[64]
RTKs	HCC cells	High	Monocytes	Block apoptosis	Not mentioned	[65]
circ-UHRF1	HCC cells	High	NK cells	Suppress the anti-tumor functions	Up-regulate MAPK signaling pathway to inhibit caspase cleavage	[67]
miR-92b	HCC cells	High	NK cells	Suppress cytotoxicity	Down-regulate miR-449c-5p to up-regulate TIM-3	[68]
14-3-3 ζ	HCC cells	High	TILs	Suppress the anti-tumor functions	Down-regulate CD69	[71]
HMGB1	HCC cells	High	TIM-1+Breg cells	Promote TIM-1+Breg cells expansion and suppress CD8+ T cells functions	Not mentioned	[72]
MET proto-oncogene, S100A4, CAV1, and CAV2	Metastatic HCC cell	High	Normal hepatocytes	Enhance the migratory and invasive capabilities	Up-regulate TLR2/4-MAPK signaling pathway	[73]
circ-MMP2	High-metastatic HCC cells	High	Low-metastatic HCC cells and normal hepatocytes	Enhance migratory and invasive capabilities	Up-regulate PI3K/AKT and MAPK signaling pathways to up-regulate active MMP-2 and MMP-9	[75]
Cdr1as	HCC cells	High	Normal hepatocytes	Enhance proliferative and migratory capabilities	Down-regulate miR-136-5p to up-regulate MMP2 expression	[76]
linc-ROR	HCC cells	High	Normal hepatocytes	Endow stem cell properties	Not mentioned	[77]

resulted in the destruction of endothelial integrity as well as an increase in vascular permeability, eventually promoting HCC metastasis, the mechanism related to the inhibition of the expression of membrane-associated adhesion molecules such as VE-Cadherin (VE-Cad), p120-catenin (p120) and zonula occludens 1 (ZO-1) by miR-103 [38]. In HCC cell-derived exosomes, six substances were upregulated, including miR-210, ephrin-B2, Delta-like 4 ligand (DLL4), angiopoietin-2 (ANGPT2), circ-100338, and Vasorin (VASN), which had been verified to have links with active endothelium-dependent angiogenesis, however, the mechanisms were not identical [39,41,43,45,46]. MiR-210 could be delivered into ECs through exosomes, then miR-210 directly targeted SMAD family member 4 (SMAD4) and signal transducer and activator of transcription 6 (STAT6) and down-regulated their expressions, which promoted the tubulogenesis of ECs, this finally promoted tumor angiogenesis [39]. Endothelial progenitor cells (EPCs) had a close relationship with ECs, the latter evolved from EPCs [40]. One study observed that ephrin-B2 and DLL4 could be delivered into EPCs through exosomes and promoted tumor angiogenesis, previous studies

uncovered that high expression levels of ephrin-B2 and DLL4 played important roles in arterial differentiation of ECs, in this study they found that DLL4 functions via the upregulation of DLL4/Notch signaling pathway, regrettably, ephrin-B2 mechanism of action remained unknown [41,42]. On the surface of exosomes which were secreted by HCC cells, a high ANGPT2 level was detected and it could be delivered into ECs, which could promote angiogenesis of ECs via the upregulation of protein kinase B (AKT)/endothelial nitric oxide synthase (eNOs) as well as AKT/ β -catenin signaling pathways [43]. This was distinct from the ANGPT2/Tie2 pathway we knew before, the latter was the way free ANGPT2 works [44]. In addition, a high circ-100338 level was detected in exosomes which were secreted by high-metastatic HCC cells and it could be delivered into ECs, unlike the abovementioned substances, circ-100338 not only promoted ECs proliferation and angiogenesis but also increased vascular permeability, however, the mechanisms need to be further understood [45]. In another study, a high VASN level was detected in exosomes which were secreted by HCC cells and it could be delivered into ECs through heparin sulfate proteoglycans (HSPGs)

Table 2

The effect of exosomes that target HCC cells and their mechanisms.

miR-92a-3p	High-metastatic HCC cells	High	Low-metastatic HCC cells	Promote EMT progression	Down-regulate PTEN and up-regulate Akt/Snail signaling pathway	[78]
LOXL4	High-metastatic HCC cells	High	Low-metastatic HCC cells	Enhance the migratory and invasive capabilities	Up-regulate FAK/Src signaling pathway	[79]
CAP1	High-metastatic HCC cells	High	Low-metastatic HCC cells	Enhance the migratory and invasive capabilities	Not mentioned	[80]
circ-PTGR1	High-metastatic HCC cells	High	Low-metastatic HCC cells	Enhance the migratory and invasive capabilities	Down-regulate miR-449a to up-regulate MET	[81]
CXCR4	High-metastatic HCC cells	High	Low-metastatic HCC cells	Enhance the migratory and invasive capabilities	Up-regulate MMP-9, MMP-2, and VEGF-C	[82]
ENO1	High-metastatic HCC cells	High	Low-metastatic HCC cells	Promote proliferation, migration, and invasion	up-regulate integrin $\alpha 6\beta 4$ to up-regulate FAK/Src-p38MAPK signaling pathway	[86]
linc-VLDLR	Resistant HCC cells	High	Non-resistant HCC cells	Enhance chemoresistance	Upregulate ABCG2	[89]
linc-ROR	Resistant HCC cells	High	Non-resistant HCC cells	Enhance chemoresistance	Not mentioned	[91]
miR-32-5p	Resistant HCC cells	High	Non-resistant HCC cells	Enhance multidrug resistance	Down-regulate PTEN to up-regulate the PI3K/Akt signaling pathway	[92]
miR-744	Resistant HCC cells	Low	Non-resistant HCC cells	Promote proliferation and suppress the chemosensitivity	Up-regulate PAX2	[93]
miR-21/ miR-10b	Acidic microenvironment HCC cells	High	Non-acidic microenvironment HCC cells	Promote proliferation, migration, and invasion	Up-regulate Vimentin and Snail while down-regulate PTEN and E-cadherin	[95]
miR-1273f	Hypoxic HCC cells	High	Normoxic HCC cells	Induce proliferation and malignant transformation	Down-regulate LHX6 to up-regulating Wnt/ β -catenin signaling pathway	[96]
SMAD3	Attached HCC cells	High	Detached HCC cells	Enhance cell adhesion	Up-regulate SMAD3 signaling pathway to up-regulate ROS	[97]
circ-CCT3	CAFs	High	HCC cells	Enhance glucose metabolism	Upregulate HK2	[102]
miR-320a	CAFs	Low	HCC cells	promote proliferation and migration	up-regulate MAPK signaling pathway	[103]
miR-150-3p	CAFs	Low	HCC cells	Promote migration and invasion	Not mentioned	[104]
miR-335-5p	HSCs	High	HCC cells	Suppress proliferation and invasion	Not mentioned	[105]
CD11b/ CD18	TAMs	High	HCC cells	Boost migratory	Up-regulate MMP-9 signaling pathway	[106]
miR-125a/b	TAMs	Low	HCC cells	Promote proliferative capabilities and stem cell properties	Up-regulate CD90	[107]
circ-0051443	Normal hepatocytes	High	HCC cells	Promote cell apoptosis and suppress the cell cycle	Down-regulate miR-331-3p to up-regulate BAK1	[108]
SENP3-EIF4A1	Normal hepatocytes	High	HCC cells	Suppress proliferation and migration	Down-regulate miR-9-5p to up-regulate ZFP36	[109]
circ-DB	Adipocytes	High	HCC cells	Promote proliferation and decrease DNA damage	Down-regulate miR-34a to up-regulate the USP7/Cyclin A2 signaling pathway	[110]
miR-23a/b	Adipocytes	High	HCC cells	Promote chemoresistance, growth, and migration	Down-regulate VHL to up-regulate HIF-1 α , GLUT-1, and VEGF	[111]

mediated endocytosis, which promoted migration of ECs, and finally promotes angiogenesis, however, the mechanisms need to be further understood [46].

Deep vein thrombosis (DVT) was very common in cancer patients, those who suffered from DVT were associated with a higher death risk [47,48]. The high miR-21 level was detected in exosomes which were secreted by HCC cells and it could be transported into EPCs, which suppress proliferative, migratory, and invasive capabilities of EPCs via down-regulating interleukin 6 receptor (IL6R), previous study verified that EPCs were closely related to human thrombus resolution, as a result, high-level miR-21 expression increased the mortality of HCC patients [49,50]. Contrary to these up-regulated substances, there are also some substances that are downregulated in exosomes secreted by HCC cells. Dai et al. discovered that C-type lectin domain family 3 member B (CLEC3B) was detected to have a low level in HCC-derived exosomes and could be delivered into ECs, which might result in the down-regulation of AMP-activated protein kinase (AMPK) signaling pathway, this related to an increase of vascular endothelial growth factor (VEGF) level in ECs, high level of VEGF enhanced angiogenesis and this finally promoted the tumor progression [51]. Another study found that there was a low expression of miR-200b-3p in exosomes which were secreted by HCC cells and it could be delivered into ECs, low level of miR-200b-3p enhanced angiogenic capabilities of ECs via upregulating erythroblast transformation-specific (ETS)-related gene (ERG) [52].

2.2. Exosomes regulate the progression of hepatocellular carcinoma via targeting fibroblasts and hepatic stellate cells

Cancer-associated fibroblasts (CAFs) occupy a key position in HCC stroma, which are transformed from activated fibroblasts and are closely related to tumor progression and metastasis [53,54]. A study showed that high miR-1247-3p level was detected in exosomes which were secreted by high-metastatic HCC cells and it possessed the ability to transform normal fibroblasts into CAFs via acting directly on beta-1, 4-galactosyltransferase 3 (B4GALT3) and up-regulating $\beta 1$ -integrin/NF- κ B signaling pathway, this ultimately promoted lung metastasis of tumor cells, further study showed that CAFs could secrete pro-inflammatory cytokines, including interleukin-6 (IL-6) and interleukin-8 (IL-8) and this resulted in stemness, epithelial-mesenchymal transition (EMT), chemoresistance as well as tumorigenicity of HCC cells [55]. Moreover, miR-21 was aberrantly high expressed in HCC-derived exosomes and could be imported into hepatic stellate cells (HSCs), which would transform HSCs into CAFs, further study showed that this might be related to the down-regulation of phosphatase and tensin homolog (PTEN) and the upregulation of pyruvate dehydrogenase kinase 1 (PDK1)/AKT signaling pathway, in the past, PTEN was considered a tumor-suppressor gene, further studies showed that CAFs could secrete angiogenic cytokines including transforming growth factor- β (TGF- β), matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), basic fibroblast growth factor (bFGF) and VEGF, which enhanced angiogenic capabilities of ECs, this finally promoted tumor progression [56,57].

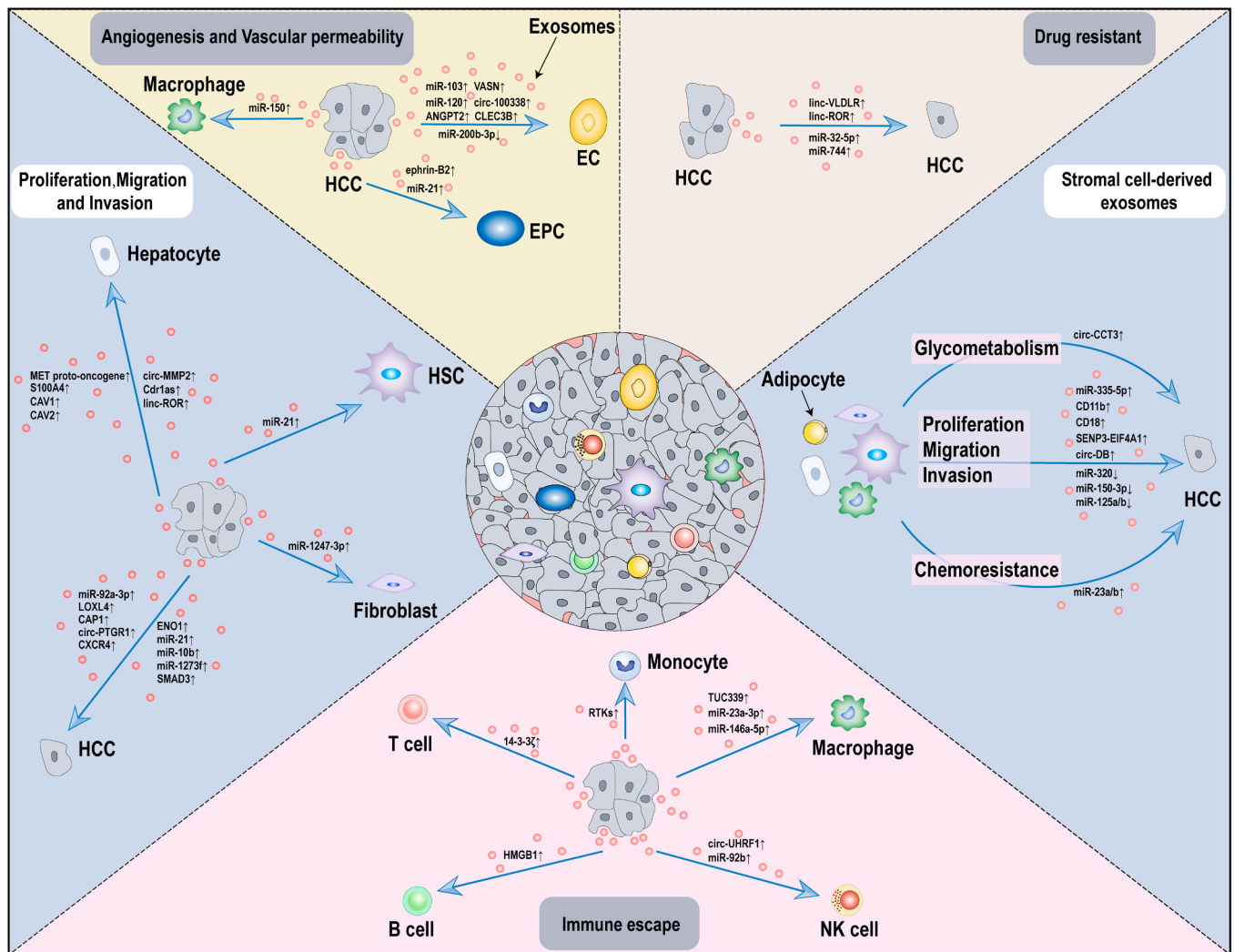


Fig. 1. The effect of exosomes on HCC microenvironment and their mechanisms.

2.3. Exosomes regulate the progression of hepatocellular carcinoma via targeting immune cells

2.3.1. Macrophages and monocytes

Tumor-associated macrophages (TAMs) occupy a prominent position in TME and they play an important role in HCC development. During HCC progression, macrophages can be converted from M1 type to M2 type, the former inhibits tumor progression, in contrast, the latter promotes tumor progression [58]. Ultraconserved lncRNA (ucRNA) TUC339 is overexpressed in the HCC-derived exosomes, which is connected with tumor cell growth and adhesion; two pieces of research discovered that TUC339 was a key mechanism regulating macrophages activation by regulating the M1/M2 polarization, they found the mechanism may be TUC339 down-regulate FcγR-mediated phagocytosis pathway, Toll-like receptor (TLR) signaling pathway and the actin cytoskeleton pathway to decrease the ability of macrophages to phagocytize tumor cells; they also discovered that TUC339 was associated with another two signaling pathways, C-X-C chemokine receptor (CXCR) chemokine receptor binding pathways and cytokine receptor signaling pathways, however, the research on mechanisms remained only at an early stage [59,60]. Liu et al. demonstrated TAMs secreted VEGF to pro-angiogenesis in HCC progression, in this research high miR-150 level was detected in both human HCC plasma and exosomes which were secreted by HCC cells, high miR-150 level were related to high VEGF level and TAMs were the target cells of miR-150, later

intensive studies showed that miR-150 could be targeted to inhibitor of growth family member 4 (ING4), the latter was previously considered a tumor suppressor and could regulate hypoxia inducible factor (HIF) activity, ultimately increasing the level of VEGF; they also predicted another target, OS9, which could be down-regulated by miR-150, the knockdown of OS9 increase the level of VEGF [61,62]. Unlike the behavioral changes mentioned above, two studies had found another novel function of macrophages in HCC microenvironment. Among them, Liu et al. demonstrated that the release of exosomes that contained high-level miR-23a-3p could be promoted by endoplasmic reticulum (ER) stress in HCC cells, and these exosomes are then transported into macrophages, which could increase programmed death-ligand 1 (PD-L1) expression through the up-regulation of PTEN/AKT signaling pathway, this resulted in a negative impact on CD8+ T cells, including a suppression on functions as well as increased apoptosis [63]. In addition, Yin et al. found that the transcription factor Sal-like protein 4 (SALL4) could up-regulate the expression of miR-146a-5p in HCC cells through binding to the promoter, this enhanced miR-146a-5p level in exosomes and could be transferred into macrophages via endocytosis in HCC microenvironment, leading to macrophages toward M2-polarized TAMs and suppression of T-cell functions [64]. The precursors of TAMs are monocytes that undergo apoptosis in about two days, however, the inflammatory tumor microenvironment allows monocytes to survive longer in order to produce more TAMs, regrettably, the mechanisms remain obscure [65]. A recent study identified HepG2 cells-derived

exosomes participates in this process, which transmitted phosphorylated receptor tyrosine kinases (RTKs) to monocytes, this process could up-regulated mitogen-activated protein kinase (MAPK) signaling pathway, Ras-Raf-MEK-ERK, finally led to an inhibition of caspase cleavage as well as a cell cycle block [65].

2.3.2. Natural killer cells

Natural killer (NK) cells belong to a special class of cytotoxic lymphocytes and can mediate anti-tumor response through lysing cells charged with 'non-self' antigens thus it can be seen, they are strongly related to tumor immune microenvironment (IME), their activities are largely related to activating and inhibitory receptor signaling and the reduction of their activities results in increased susceptibility to cancers [66]. The dysfunction of NK cells had been verified in HCC, in one study, a high level of circ-UHRF1 was detected in exosomes which were secreted by HCC cells, it could be transferred into NK cells via endocytosis, which inhibited the anti-tumor functions of receptor cells via sponging miR-331-3p and up-regulating T cell immunoglobulin and mucin domain 3 (TIM-3), it previously showed that TIM-3 could inhibit antitumoral immunity in HCC, they also observed this might be associated with anti-PD1 therapy [67]. Another research discovered that there existed a high miR-92b level in HCC-derived exosomes and could be transferred into NK cells, which down-regulated cytotoxicity of NK cells by suppressing its target mRNAs, in this study, they mainly focused on CD69 which was a key activation marker on NK cells [68].

2.3.3. T cells and B cells

Tumor-infiltrating lymphocytes were another main component of tumor IME, the anti-tumor effects of T cells were largely suppressed in HCC [69,70]. In a study by Wang et al. high 14-3-3 ζ level was detected in HCC cells, further study showed that 14-3-3 ζ could be transferred into tumor-infiltrating T lymphocytes (TILs) through exosomes, in this study TILs with high 14-3-3 ζ level presented a decrease in inflammatory cytokines levels but an increase in anti-inflammatory cytokines levels and there was a high percentage of CD8⁺ T cells which carried exhaustion markers such as TIM-3, lymphocyte-activation gene 3 (LAG3) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), they found that the differentiation of naive T cells deviated at the same time, additionally, they also discovered that the activity of CD3⁺ T cells was inhibited in the high-level 14-3-3 ζ group, all these alterations mentioned above finally inhibited the anti-tumor functions of TILs, however, the mechanisms remained to be investigated [71]. Regulatory B (Breg) cell was a subtype of B cell, it was closely related to tumor progression as a component of humoral immunity, Ye et al. discovered a new type of Breg cell, TIM-1⁺Breg cell, different from traditional peripheral Breg cells, it exhibited a CD5^{high}CD24^{CD27^{+/+}CD38^{+/high}} phenotype, the further study discovered that T cell immunoglobulin and mucin domain 1 (TIM-1)⁺Breg cells were transformed from B cells, the mechanism might relate to the activation TLR2/4-MAPK pathway by high mobility group box 1 (HMGB1) which presented a high level in HCC-derived exosomes and could be transferred into B cells via endocytosis, this mechanism also promoted TIM-1⁺Breg cells expansion, then the receptor cells promoted the progress of HCC by secreting interleukin-10 (IL-10) and suppressing CD8⁺ T cells functions [72].

2.4. Exosomes regulate the progression of hepatocellular carcinoma via targeting normal hepatocytes

As is well known, HCC cells develop from normal hepatocytes and their interactions affect HCC progression, in recent years we found a novel mode of action of HCC cells on normal hepatocytes. He et al. found that metastatic HCC cell could affect the biological behaviors of normal hepatocytes through exosome-based intracellular materials delivery, in this research, they observed two HCC cell lines MHCC97L and HKCI-8 could deliver MET proto-oncogene, S100A4, caveolin 1 (CAV1), and caveolin 2 (CAV2) to immortalized hepatocyte MIHA through exosomes,

then up-regulated phosphatidylinositol 3-kinase (PI3K)/AKT and MAPK signaling pathways to increase secretion of active MMP-2 and MMP-9, previous studies demonstrated that these two proteins were related to metastasis, this finally enhanced the migratory capability of MIHA as well as its invasive ability [73,74]. Moreover, high circ-MMP2 level was detected in exosomes which were secreted by high-metastatic HCC cells and it could be delivered into normal hepatocytes, which promoted HCC metastasis, interestingly, they observed the same result in another type of recipient cells, low-metastatic HCC cells, the mechanism was circ-MMP2 up-regulated MMP2 expression via binding to miR-136-5p [75]. In addition, high circRNA Cdr1as level was detected in exosomes which were secreted by HCC cells and could induce cellular responses after captured by normal hepatocytes, then enhanced proliferative and migratory capabilities of recipient cells, however, the mechanisms were not mentioned [76]. In another study, a high Linc-ROR level was detected in exosomes which were secreted by HCC cells and it could be delivered into normal hepatocytes, which endowed stem cell properties to normal hepatocytes through increasing stem cells related proteins such as CD133, NANOG, SRY-box 2 (SOX2), P53 and OCT4, however, the mechanisms remained to be investigated [77].

2.5. Exosomes regulate the progression of hepatocellular carcinoma via targeting HCC cells

2.5.1. Exosome-mediated delivery of substances between different types of HCC cells

HCC cells can be divided into several types according to their biological characteristics such as migratory capability and drug resistance. The level of substances varies greatly among the exosomes secreted by different types of HCC cells. Importantly, the exosome-mediated communication between these HCC cells changes the biological characteristics of recipient cells to some extent.

2.5.1.1. Between high-metastatic HCC cells and low-metastatic HCC cells.

There was growing evidence that the migratory and invasive capacities of low-metastatic HCC cells could be increased by high-metastatic HCC cells via the exosome-mediated transfer of substances, studies on the underlying mechanisms contribute to a better understanding of HCC progression. The high miR-92a-3p level was detected in exosomes which were secreted by high-metastatic HCC cells and it could be delivered into low-metastatic HCC cells, which promote EMT progression of recipient cells, further studies of the mechanism found that this might be related to the down-regulation of PTEN and the up-regulation of Akt/Snail signaling pathway [78]. In addition to the EMT process, Li et al. found another important process of tumor cell invasion, cell-matrix adhesion, they discovered that lysyl oxidase-like 4 (LOXL4) could be transported from highly metastatic HCC cells into the low metastatic ones through exosomes, following this, the migratory capabilities of recipient cells, as well as their invasive abilities, were enhanced, further studies of the mechanism found that this might be related to the upregulation of focal adhesion kinase (FAK)/Src pathway, which promoted cell-matrix adhesion [79]. Moreover, a high level of adenylyl cyclase-associated protein 1 (CAP1) was detected in exosomes which were secreted by high-metastatic HCC cells, previous studies found that CAP1 had a tight relationship with HCC metastasis, in this study, they discovered that CAP1 could be delivered into low-metastatic HCC cells through exosomes, this finally enhanced the migratory capabilities of recipient cells as well as their invasive abilities, however, the mechanisms remained to be investigated [80]. Similar findings had also been identified in another two studies. Among them, Wang et al. found that circ-PTGR1 could be transported from highly metastatic HCC cells into the low metastatic ones through exosomes, and circ-PTGR1 could enhance the migratory capabilities of low-metastatic HCC cells as well as their invasive abilities by binding to miR-449a and up-regulating MET [81]. Another study obtained the same results, in this study they mainly focused on CXCR4

and the mechanism was CXCR4 up-regulated the expression of MMP-2, MMP-9, and VEGF-C [82]. With the rapid development of this emerging field, many metabolism-related enzymes have been found to regulate exosome release [83,84]. Moreover, exosomes are also found to regulate cell metabolism by delivering metabolism-related enzymes. Alpha-enolase (ENO1) is considered a multifunctional protein that mainly participates in glycolytic reactions and acts as a plasminogen receptor [85]. A recent study by Jiang et al. found that high-metastatic HCC cells could deliver ENO1 into low-metastatic HCC cells, which eventually promoted proliferative, migratory, and invasive capabilities of recipient cells, mechanistically, ENO1 could up-regulate integrin $\alpha 6\beta 4$ which was strongly related to the activation of the FAK/Src-p38MAPK signaling pathway [86].

2.5.1.2. Between resistant HCC cells and non-resistant HCC cells. Sorafenib can delay the progress of tumors as well as angiogenesis via blocking a wide variety of growth factor pathways [87]. For advanced HCC patients, sorafenib remains the only possible cure which is also the only FDA-approved treatment [88]. However, the chemoresistance to drugs contributes to high HCC-related deaths. One research discovered that while HCC cells were exposed to sorafenib, the linc-VLDLR level increased, which promoted chemoresistance via upregulating sub-family G member 2 (ABC-G2), the previous study had shown that ABC-G2 could export active drug out of the cells and this contributed to drug resistance; in addition, this research found that resistant HCC cells could transport linc-VLDLR to non-resistant HCC cells through exosomes and thus promote chemoresistance of non-resistant HCC cells [89,90]. This team had also found a similar result in another lincRNA, linc-ROR [91]. In addition, a marked rise in the expression level of miR-32-5p contributed to promoting multidrug resistance of HCC cells, further study had shown that resistant HCC cell-secreted exosomal miR-210 may be transferred into non-resistant HCC cells, leading to enhanced cancer multidrug resistance through the down-regulation of PTEN and the up-regulation of PI3K/Akt signaling pathway [92]. In contrast to the above up-regulated substances, miR-744 presented a low level in exosomes which were secreted by resistant HCC cells and these exosomes could be endocytosed by non-resistant HCC cells, the low expression of miR-744 up-regulated the expression of paired box 2 (PAX2), this finally promoted proliferation and inhibit the chemosensitivity of non-resistant HCC cells [93].

2.5.1.3. Between other types of HCC cells. It had been previously reported that the pH gradient in and around tumor cells was dysregulated, with intracellular pH (pHi) higher than 7.4 and extracellular pH (pHe) between 6.6 and 7.2, creating a good environment for metastasis [94]. Tian et al. discovered that Low pH conditions would significantly enhance the release of exosomes from HCC cells, these acidic pH-derived exosomes could transfer miR-21 and miR-10b into non-acidic microenvironment HCC cells, these two miRNAs could up-regulate Vimentin and Snail expression while down-regulate PTEN and E-cadherin levels in the recipient cells, which led to enhanced non-acidic microenvironment HCC cells proliferation, migration, and invasion [95]. In addition, Yu et al. discovered that hypoxia played a pivotal role in HCC, which could increase exosomal production in HCC microenvironment, leading to the tumor progress, in their study, they observed that miR-1273f was significantly increased in exosomes which were derived from hypoxic HCC cells and could down-regulate the expression level of tumor suppressor gene LIM homeobox 6 (LHX6) in normoxic HCC cells, which would cause the up-regulation of Wnt/ β -catenin signaling pathway, as a result, promoting malignant progression in normoxic HCC cells [96]. Moreover, Fu et al. found that attached HCC cell-secreted exosomal SMAD family member 3 (SMAD3) might be transferred into detached HCC cells and thereby promoted cell adhesion by up-regulating SMAD3 signal pathway and increased the level of reactive oxygen species (ROS) [97]. In a previous study, ROS was found to play an important role in cell

adhesion, this finally promoted tumor metastases [98].

2.5.2. Exosome-mediated delivery of substances from stromal cells to HCC cells

While stromal cells uptake HCC cell-derived exosomes, they also secrete their own exosomes and can be transferred into HCC cells via endocytosis, in this way, biological behaviors of HCC cells are modified.

2.5.2.1. CAFs and HSCs. Glucose metabolism disorder was a characteristic feature of tumor cells, despite oxygen sufficiency, tumor cells were more likely to perform glycolysis [99]. Previous studies found that hexokinase II (HK2) was the rate-limiting enzyme for glycolysis and the high HK2 level was closely related to a poorer prognosis in HCC patients, however, how HK2 was up-regulated remained largely unknown [100, 101]. One research identified circ-CCT3 as a candidate molecule for this process, they found that circ-CCT3 was highly expressed in CAF-derived exosomes and could be transferred into HCC cells, high levels of circ-CCT3 upregulated HK2 and thus promoted glucose metabolism of HCC cells [102]. In addition, two other studies discovered that the expression levels of two molecules, including miR-320a and miR-150-3p, were significantly decreased in CAF-derived exosomes and could be delivered into HCC cells [103,104]. Among them, miR-320a was an antitumor miRNA and could bind to PBX homeobox 3 (PBX3) in HCC cells, which down-regulated MAPK pathway and then suppressed MMP2 and cyclin-dependent kinase 2 (CDK2) expressions, in contrast, low levels of miR-320a induced the opposite effects and eventually promoted proliferation and migration of HCC cells [103]. Low miR-150-3p expression levels were associated with high incidences of migration and invasiveness of HCC cells and could promote the tumor progress, regrettably, the mechanism had not been mentioned in detail in this study [104]. In addition to CAFs, similar results were found in HSCs. Wang et al. discovered that high levels of miR-335-5p could be transported from HSCs to HCC cells through exosomes, which result in the inhibition of HCC cell proliferation and invasion abilities in vitro and the volume reduction of HCC tumor in vivo, however, the mechanisms remained elusive [105].

2.5.2.2. TAMs. TAM-derived exosomes are also closely related to HCC progress. Wu et al. found that integrin, $\alpha M \beta 2$ (CD11b/CD18), was highly expressed in M2 macrophage-derived exosomes and could be transferred into HCC cells, which could boost migratory of HCC cells by up-regulating MMP-9 signaling pathway [106]. Another study had reported that TAMs potentially promote HCC progress by exosome-mediated transfer of low-expressing miR-125a and miR-125b to HCC cells, the main mechanism was the low miR-125a and miR-125b levels could up-regulate CD90, which promoted cell proliferation and stem cell properties [107].

2.5.2.3. Normal hepatocytes. Two molecules including circ-0051443 and SENP3-EIF4A1 were down-regulated in HCC cells but exhibited high expressions in normal hepatocyte-derived exosomes [108,109]. Low levels of circ-0051443 in HCC cells could block apoptosis and promote cell cycle progression, however, normal hepatocytes could transfer high-level circ-0051443 into HCC cells via exosomes, then it up-regulated BCL2 antagonist/killer 1 (BAK1) by sponging miR-331-3p, this eventually suppressed the malignant biological behaviors [108]. In addition, Wang et al. found that low SENP3-EIF4A1 level in HCC cells promoted tumor progress, however, normal hepatocytes could transfer SENP3-EIF4A1 into HCC cells through exosomes, which could suppress proliferation and migration abilities of HCC cells by binding to miR-9-5p and up-regulating zinc finger protein 36 homolog (ZFP36) [109].

2.5.2.4. Adipocytes. Two recent studies reported new advancements about adipocyte-derived exosomes in HCC microenvironment. Zhang et al. found that circ-DB was highly expressed in adipocytes and could be

transported into HCC cells through exosomes, further research showed that circ-DB could function as a sponge by adsorbing miR-34a to up-regulate the ubiquitin-specific protease 7 (USP7)/Cyclin A2 pathway, this eventually promoted the proliferation in parallel with a decreased DNA damage [110]. Another study found that miR-23a/b was highly expressed in adipocyte-derived exosomes and could be transferred into HCC cells, which promoted chemoresistance, growth, and migration of HCC cells, mechanistically, they found that von Hippel-Lindau (VHL) was down-regulated by miR-23a/b and thereby the expressions of hypoxia-inducible factor 1 α (HIF-1 α), glucose transporter 1 (GLUT-1) and VEGF were up-regulated [111].

3. The potential diagnostic and therapeutic values of exosomes in HCC

With the in-depth study in this emergent field, the potential clinical application value of exosomes is gradually explored. As discussed in the preceding sections, more than 50 substances have been reported to have dysregulated expression in HCC and the number is still increasing. In addition, numerous studies have discovered that the types of molecules inside exosomes, as well as their levels exist differences as the disease progresses [112–114]. For instance, Xue et al. found that miR-93 was highly expressed in HCC cell-derived exosomes and they discovered an intriguing phenomenon, the higher the miR-91 level, the later the tumor stage [112]. Lee et al. also found a similar result on another molecule, lncRNA-ATB [113]. Therefore, a deep analysis of the substances in these exosomes such as components and their levels may help achieve an accurate diagnosis of HCC [114]. Treatment of HCC remains a challenge, particularly in an advanced stage. One study discovered that doxorubicin (DOX) could be loaded into exosomes, the effectiveness of exosomal DOX (exoDOX) was almost the same as doxorubicin treatment alone, however, the cardiotoxicity produced by the former was lower [115]. Moreover, Kim et al. found that exosomes could carry drugs and transport them inside tumor cells, which could avoid the loss of active drug out of the cells via P-glycoprotein (Pgp), a drug efflux transporter [116]. These findings entail the promise of novel therapeutic approaches for HCC patients to an extent.

4. Conclusions and future perspectives

Exosomes as one of the types of cell-derived vesicles play a crucial role in HCC progression via mediating cell-cell communications. As a result, it has become a hot research topic in recent years and considerable research progress has been obtained in this area. The present review first introduced the exosomal cargos secreted by HCC cells in HCC microenvironment and their effects on recipient cells' behaviors and functions through regulating signaling pathways and downstream molecule expression after being delivered into stromal cells. Then we discussed that stromal cells could in turn influence HCC cells by a similar pathway. Moreover, this phenomenon was also present between different types of HCC cells. However, the mechanisms of some of the exosomal cargos mentioned above still remain unknown. Although exosomes have shown their potential clinical application value for HCC, this area of research is still at the preclinical stage and there still exists many challenges, for example, we still lack technologies to efficiently assay exosomes and the artificial synthetic exosomes are still difficult to obtain, additionally, the technology in the delivery of exosomes that contain drugs to the target cells is quite immature. Therefore, more research efforts should be devoted to these problems. We believe that in-depth explorations of this field will shed new light on the diagnosis of this cancer as well as its treatment strategies.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81902431); the Hong Kong Scholars Program (Grant

No. XJ2020012); the Foundation of Key Laboratory of Myocardial Ischemia, Ministry of Education (Grant No. KF201810); and the Chen Xiaoping Foundation for the Development of Science and Technology of Hubei Province (Grant No. CXPJJH1800004-001).

Authors' contributions

All authors: Conception and design. Risheng He: Manuscript writing. Yi Xu, Judy Wai Ping Yam and Yunfu Cui: Manuscript revision. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Research involving human and animal participants

This article does not contain any studies with animals performed by any of the authors.

Consent for publication

Not applicable.

Conflict of interest statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results

Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Acknowledgments

Not applicable.

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