Recent progress in the emerging role of exosome in hepatocellular carcinoma

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Abstract
Exosomes are small membrane vesicles 50-150 nm in diameter released by a variety of cells, which contain miRNAs, mRNAs and proteins with the potential to regulate signalling pathways in recipient cells. Exosomes deliver nucleic acids and proteins to participate in orchestrating cell-cell communication and microenvironment modulation. In this review, we summarize recent progress in our understanding of the role of exosomes in hepatocellular carcinoma (HCC). This review focuses on recent studies on HCC exosomes, considering biogenesis, cargo and their effects on the development and progression of HCC, including chemoresistance, epithelial-mesenchymal transition, angiogenesis, metastasis and immune response. Finally, we discuss the clinical application of exosomes as a therapeutic agent for HCC.

1 | INTRODUCTION

Since the first observation of exosomes as "trash cans" that simply allows cells to dispose of unwanted proteins,1 the further functions of exosomes have recently been explored. It has been proven that exosomes could be secreted by most cell types.2 With regard to the liver, exosomes mainly released from three types of cells: hepatocytes, non-parenchymal immune cells (such as Kupffer cells, natural killer cells, T cells and B cells) and non-parenchymal liver cells (e.g., liver stellate cells).3 As for a subtype of the extracellular vesicle, they implicated in many normal and pathological processes.4 Especially in tumours, they play a vital role in tumour chemoresistance, angiogenesis, epithelial-mesenchymal transition (EMT) and metastasis by modulating extracellular communication. On the one hand, tumour cells impact adjacent cells through exosomes and establish tumorigenic microenvironment. On the other hand, the stroma cells (such as stellate cells and MSCs) and immune cells could influence tumour cells to promote or prevent tumorigenesis through exosomes.5

Importantly, the versatile roles of exosomes are mostly determined by their donor cells and their contents including lipids, nucleic acids and proteins6,7 (Figure 1). The information has been deposited in ExoCarta (www.exocarta.org).

Furthermore, exosomes have the potential to be utilized in therapeutic tools due to their numerous characteristics, which we will discuss as follow.

2 | EXOSOMES BIOGENESIS

Recently, there has been a great interest in the study of exosomes as the major regulator in tumorigenesis. Based on recent studies, endosomal sorting complexes required for transport (ESCRT) is considered as the main mechanism of exosomes production8 (Figure 2).
which was first defined as a ubiquitin-dependent protein sorting pathway in yeast.\(^9\)

Vps4, one of the compositions of ESCRT complex, is known as a multimeric mechanoenzyme with an ATP-binding domain which binds to ESCRT-III subunits then provides energy through dehydrating ATP to disassociate them from the cell membrane.\(^{10-12}\)

Surprisingly, Wei et al.\(^{13}\) found that the downregulation of Vps4 is an independent risk factor for recurrence-free survival of hepatocellular carcinoma (HCC) patients. Their study showed that Vps4A is associated with inhibition of biological activity of HCC cell-derived exosomes and the recipient cells’ response to exosomes. PI3K/Akt signalling pathway might be a candidate mechanism due to its inactivation occurrence while Vps4 overexpressed in HCC cells.\(^{13}\) This study extends our knowledge that the exosomes production is associated with tumour progression, metastasis and worse prognosis.

Numerous studies demonstrated that exosomal cargo sorting is an active process.\(^{9,14}\) The content of exosomes is determined by their donor cells. Up to now, a bunch of molecules have been found in exosomes such as heat shock proteins (eg, Hsp90 and Hsp70),\(^{15,16}\) cytoskeletal proteins (actin, tubulin, coflin, etc), lipids and enzymes, along with RNAs, including microRNAs, mRNAs, and other non-coding RNAs (ncRNAs), and mitochondrial DNA (mtDNA) and single strand DNA (ssDNA).\(^{17}\)

3 | EXOSOME CONTENTS

It has been reported that cancer cells produce and secrete an increased amount of exosomes as tumour-inducing agents compared to non-cancer cells.\(^{18}\) Exosomes play a critical role in manipulating the microenvironment that favours cancer cells by transferring oncogene,\(^{20}\) inducing angiogenesis,\(^{21}\) establishing pre-metastasis niche\(^{22}\) and inducing EMT in recipient cells.\(^{23}\) Importantly, it has been demonstrated that the functions of exosomes are mainly determined by their cargoes which are different in various situations.
These results indicated that there is a possibility to reveal the mechanisms that altered by exosomes uptake. Thus, we summarize the molecules found in exosomes in patients with HCC including proteins and RNAs (miRNA, IncRNA), and the purpose is to clarify the mechanism by which exosomes promote HCC progression.

3.1 Proteins

According to Vesiclepedia database, the number of proteins in exosomes is at ~1800 levels, and in HCC cell line-derived exosomes, 213 unique proteins were found by mass spectrometry analysis. Exosomal proteins include cargo proteins and membrane proteins, depending on location in exosomes. Membrane proteins are associated with exosomal internalization by recipient cells and target organ selection. Cargo proteins composition is different in exosomes during tumour progression in different cells.

3.2 Nucleic acids

Considering that liver biopsy, a gold-standard method for monitoring and evaluating liver disease, has the risk of bleeding and infection, noninvasive diagnostic tools are urgently needed. Thus, a “Liquid biopsy” which implements early diagnosis and prognostic prediction of HCC through serum exosomes becomes more attractive. However, “Liquid biopsy” is based on markers for HCC development and progression. In addition to protein, it has been demonstrated that nucleic acids, particularly miRNAs, are also one of the compositions of exosomes (Table 1). Kogure et al have documented 134 miRNAs expressed in Hep3B-derived exosomes and 11 of miRNAs exclusively expressed in exosomes compared to their donor cells.

Li et al reported that miR-429 the significant prognosis factor for HCC is secreted into exosomes and taken up by recipient cell. Sohn et al compared the serum level of exosomal miRNAs in HCC, CHB and LC patients. Their study showed that the expression level of miR-18a, miR-221 and miR-222 is significantly higher and that of the miR-101, miR-106b, miR-122 and miR-195 is lower in HCC patient comparing with CHB or LC. These raised the possibility of exosomal cargoes, particularly miRNAs, serving as biomarkers for HCC formation and progression.

Sugimachi et al have shown that miR-718 can serve as a preoperative biomarker for the prediction of HCC recurrence after surgery. Their study showed that the expression level of miR-718 in exosomes collected in patients with HCC recurrence after liver transplantation was significantly lower than those without HCC recurrence. Furthermore, a validated cohort study showed that decreased expression of miR-718 and overexpression of the potential target gene HOXB8 were associated with tumour aggressiveness and poor prognosis. These results show the potent value of selecting patients who need liver transplantation, and therefore use donor organs properly. In addition, Liu et al have reported that exosomal miR-125b could serve as a prognostic marker due to miR-125b level in exosomes was an independent factor for time to recurrence and overall survival of HCC patients.

Although exosomal miRNAs might be useful tools to reflect their donor cells feature that can be used as biomarkers for tumour cell, the extent to which exosomal miRNAs play a role in HCC remains poorly understood. Furthermore, there are controversial results of miRNA expression level and functions under specific conditions, and some cohort studies did not include healthy participants, due to the conveniences of collecting serum sample from patients with liver disease compared with healthy people.

Recently, increased studies have focused on a role of long non-coding RNA in exosome in addition to miRNA. Long non-coding RNAs (lncRNAs) are defined as non-coding RNAs more than 200 nucleotides in length. Lnc-ROR and Lnc-LVDR which expressed in HCC-derived exosome had widely explored. It has recently been found that the ultraconserved lncRNA (ucRNA) expression is dramatically altered within extracellular vesicles as compared to donor cells. For instance, the ucRNA named TUC339 is mostly enriched in HCC cell-derived exosomes and promotes HCC growth and spread. Above all, these studies explored the nucleic acids that transferred within cells via exosome that modulate tumour cells and function as an intracellular signalling mediators.

4 MECHANISMS OF INTERACTION BETWEEN EXOSOMES AND RECIPIENT CELLS

Recently, dynamic regulation of exosomes uptake by recipient cells extensively explored. There are several models considered as a possible mechanism of exosomes internalization by recipient cells, the receptor-mediated endocytosis, and classic fluid-phase endocytosis. The latter one is considered to be a common approach for microvesicle internalization that lacks the specificity. However, Schneider et al documented that the mechanism of exosomes uptake by alveolar epithelial cells is similar, but not same, to classic macropinocytosis depending on dynamin function and actin polymerization.

In contrast, receptor-mediated endocytosis attracted more interest for its cell-specific feature that allows further modifications of exosomes for therapeutic use. Integrins are one of the receptors commonly expressed on exosomes membrane. It has been found that exosomal integrins have the ability to predict metastatic organ. For instance, exosomes expressing ITGa1β1 specifically bind to Kupffer cells, mediating liver tropism whereas exosomal ITGa1β2 and ITGa4β1 bind lung-resident fibroblasts and epithelial cells governing lung tropism. Thus, targeting exosomal integrins has a potential to prevent tumour metastasis.

Furthermore, the blockade of Scavenger Receptor Class A family (SR-A), a novel monocyte/macrophage uptake receptor for exosomes, with dextran sulphate in vivo enhances tumour accumulation by reducing exosomes clearance in mice liver. These findings have advanced the development of exosomes therapeutic method.

Intriguingly, the process of taking up exosomes is not always necessary for modulating recipient cells function; even it is a basis of...
transporting exosomal cargo. Muller et al.\textsuperscript{56} showed that the tumour-derived exosomes (TEX) mediate Treg suppressor functions dependent on cell surface signalling and do not require TEX internalization by recipient cells.

Furthermore, an oncogenic transformation of the recipient cells was observed following exposure of exosomes isolated from serum of cancer patients.\textsuperscript{57} This phenomenon has a synergy when combined with mutations in tumour suppression gene in recipient cells.\textsuperscript{57,58} Collectively, these results indicate a hypothesis that the migration of cancer cells might not be necessary for metastasis and that this can be achieved by exosomal transport.

### TABLE 1

| miRNA  | Source of exosome       | Source of compared      | Expression level | Function                                                                 | Reference |
|--------|-------------------------|-------------------------|------------------|--------------------------------------------------------------------------|-----------|
| miR-584| HEP3B-exo               | HEP3B cell              | Exclusively       | Target TAK1, enhance transformed cell growth in recipient cells          | 27        |
| miR-517c|                         |                         |                  |                                                                          |           |
| miR-378|                         |                         |                  |                                                                          |           |
| miR-520f|                        |                         |                  |                                                                          |           |
| miR-142-5p|                      |                         |                  |                                                                          |           |
| miR-451|                         |                         |                  |                                                                          |           |
| miR-518d|                        |                         |                  |                                                                          |           |
| miR-215|                         |                         |                  |                                                                          |           |
| miR-376a|                        |                         |                  |                                                                          |           |
| miR-133b|                        |                         |                  |                                                                          |           |
| miR-367|                         |                         |                  |                                                                          |           |
| miR-18a| Serum of HCC patients   | LC and CHB patients     | Upregulated      | Novel serological biomarkers for HCC                                     | 96        |
| miR-221|                         |                         |                  |                                                                          |           |
| miR-222|                         |                         |                  |                                                                          |           |
| miR-224|                         |                         |                  |                                                                          |           |
| miR-106b|                      | CHB patients            | Downregulated    |                                                                          |           |
| miR-122|                         |                         |                  |                                                                          |           |
| miR-195|                         |                         |                  |                                                                          |           |
| miR-101|                         |                         |                  |                                                                          |           |
| miR-21 | Serum of HCC patients   | CHB patients and healthy volunteers | Upregulated | Potential biomarker for HCC diagnosis                                     | 98        |
| miR-10b|                         |                         |                  |                                                                          |           |
| miR-21 |                         |                         |                  |                                                                          |           |
| miR-122|                        |                         |                  |                                                                          |           |
| miR-200a|                       |                         |                  |                                                                          |           |
| miR-125b|                      | CHB patients and LC patients | Downregulated | Prognostic marker for HCC; An independent predictive factor for TTR and OS | 106       |
| miR-665| Serum of HCC patients   | Healthy volunteers      | Upregulated      | Prognostic and diagnostic marker for HCC                                | 95        |
| miR-718| Serum from patients with no recurrence | Serum from patients who suffer HCC recurrence after | Downregulated | Target HOXB8, suppress cell proliferation                                | 105       |

#### 5 | THE ROLES OF EXOSOMES IN HCC PROGRESSION

Intercellular communication is essential in liver physiology and pathology including tumorigenesis since liver is a multicellular organ. Exosomes provide new form of intercellular communication, besides autocrine, paracrine and cell-cell contact. Moreover, this process could be affected by many factors, such as microenvironment pH, oncogenic transformation and stress response.\textsuperscript{59-61} The role of exosomes in HCC progression has been extensively studied. Exosomal miRNAs derived from HCC cell activate transforming growth
factor-β activated kinase-1 (TAK1) and the downstream signalling molecules, resulting in further growth of recipient cells, indicating that exosomes have an ability to modulate receptor cell signalling and biological effects. In this part, we summarize the recent studies on the progress of HCC involving exosomes.

5.1 Exosomes participate in HCC chemoresistance

Sorafenib is the first-line molecular targeted drug for advanced HCC approved by US Food and Drug Administration. However, after long-term treatment of sorafenib, HCC cells exhibit resistance to sorafenib. Accumulating evidence has shown that exosomes are involved in HCC chemoresistance as well. Here, we summarize several possible mechanisms related to exosomes.

First, exosomes promote drug efflux to develop chemoresistance. Tumour cells can excrete anti-cancer drugs and the metabolites by encapsulation in exosomes. Takahashi et al showed that the expression of lincRNA-LVVDL increased in HCC cells in the presence of diverse anti-tumour agents including sorafenib. Altered expression of lincRNA-LVVDL in cells is related to increased expression of ABCG2, a member of ATP-binding cassette (ABC) transporter superfamily involved in drug elimination of cancer cells. Furthermore, overexpression of lincRNA-LVVDL was also found in HCC cell-derived exosomes, indicating that cancer cells maintain chemoresistance not only by eliminating chemodrug via exosomes but also by inducing molecular transfer.

Second, exosomes participate in chemoresistance by enhancing the viability of tumour cells in the presence of chemo drugs. Qu et al for the first time showed that exosomes derived from HCC cells induce sorafenib resistance in hepatoma cells by inhibiting sorafenib-induced apoptosis. The underlying mechanism is that HCC-derived exosomes result in overexpression of hepatocyte growth factor (HGF) in hepatoma cells and lead to subsequent c-Met phosphorylation and downstream signalling pathways such as PI3K/Akt and MAPK/Erk activation. Takahashi et al also found that sorafenib increases the expression of linc-ROR, a stress response long non-coding RNA, in HCC cells. Intriguingly, linc-ROR selectively enriched in exosomes in response to TGFβ that modulates chemotherapy-induced apoptosis and allows cell survival under chemotherapeutic stress through p53 dependent manner.

These results indicate that exosomal cargoes participate in chemical therapeutic response modulation and provide therapeutic targets that enhance the chemosensitivity of HCC cells.

5.2 Exosomes modulate epithelial-mesenchymal transition of HCC cells

Epithelial-mesenchymal transition (EMT) is an initial step in cancer distance metastasis. EMT defined as a process by which cell lose epithelial markers like E-cadherin and acquire mesenchymal cell hallmarks like N-cadherin. EMT and the reverse process MET are the basis of the complex three-dimensional structure of the internal organs. However, tumour cells achieve mobility and invasiveness through the EMT process, leading to cancer metastasis. For example, it has been demonstrated that Hakai an E-cadherin ubiquitination protein that mediates E-cadherin ubiquitination and finally degradation plays a crucial role in EMT. It is considered to be a better therapeutic target than proteasome in the tumour subtypes. Exosomes provided a new research perspective for studying EMT. For example, it has been found that EMT reprogramming occurs in cancer cells after receiving miR-223 from polymorphonuclear leucocyte-derived exosomes. However, this impact of miR-223 is transient because it is rapidly inactivated by the exonuclease XRN1, indicating that ectopic miRNAs and endogenous miRNAs act in different ways. In addition, MSC-derived exosomes have been found to induce EMT in adjacent epithelial cells in many different cancers types.

Taken together, these results support the notion that exosomes participate in EMT that associated with aggressive, invasive and metastatic potential in cancer cells. However, more research is needed to better understand the exact mechanism by which exosomes modulate EMT in HCC.

5.3 Exosomes promote angiogenesis in HCC tissue

It has been demonstrated that cancer cells undergoing EMT capable of efficiently transferring angiogenetic proteins to the recipient endothelial cell via exosomes. In addition, secretion of exosomes increased in HCC tissue under stringent conditions, such as deficiency of oxygen or nutrition, chemodrug stimulation and ethanol exposure. Among them, oxygen and nutrition deficiency are the main causes of angiogenesis. These results lead us to hypothesize that under stringent conditions, cancer cells transmit angiogenic molecules through exosomes to establish a tumour-promoting microenvironment. In the study conducted by Gonzalez-King showed that hypoxic MSCs-derived exosomes induce angiogenesis by horizontally transferring Jagged-1 and activating the downstream Notch pathway in endothelial cells. In another study, Sruthi found that HepG2 cells express a higher level of miR23a both in the cytoplasm.
and secreted exosomes under hypoxic conditions and the exosomal miR23a downregulates SIRT1 in recipient cells, thereby inducing angiogenesis.70

Interestingly, the increasing evidence suggested that a relationship between cancer stem cells (CSCs) and angiogenesis exists in tumour microenvironment, called “crosstalk” which synergistically promotes tumour growth.3,4,91,92 For example, Conigliaro et al99 demonstrated that CD90+ CSC like liver cells could influence epithelial cells by transferring exosomes. The increased level of vascular endothelial growth factor (VEGF) production and tube formation was observed in epithelial cells after exosomes internalization. By identifying lncRNA profiling, they found that lncRNA H19 is enriched in CD90+ CSC like liver cell-derived exosomes, and could be a major mediator of angiogenesis and the therapeutic target for HCC.49

In addition to the intracellular environment, exogenous stimuli such as ethanol exposure induce angiogenic endothelial phenotypes in multiple pathways.93-96 Lamichhane et al97 reported that ethanol increases the vascularized bioactivity of endothelial cell-derived EVs through downregulating anti-angiogenic miRNA cargo (miR-106b) and upregulating pro-angiogenic long non-coding RNA (lncRNA) cargo (MALAT1 and HOTAIR). Importantly, this might be one of the molecular mechanisms by which alcohol causes liver cancer.

5.4 | Exosomes promote HCC metastasis

Long-term survival rate is low in patients with HCC due to the high metastases and/or high post-surgical recurrence rate.98 Tumour metastasis is a multistep process that includes invasion, intra-vasation and colonization of distal sites through the circulatory system.99 EMT, the initial step of metastasis, has been described above.

It has been found that exosomes facilitate the pre-metastatic niche formation and metastasis, whether derived from cancer cells or adjacent stromal cells.26,34,100-105 The characteristic of promoting metastasis is based on the variation of exosomal cargo during tumour progression.3,76,106 Various oncogenic RNAs and proteins, such as MET protooncogene, caveolins, and S100 family members, have been found in motile HCC cell line-derived exosomes by the full characterization of exosomal transcriptome and proteome.26 Internalization of these exosomes by hepatocytes activates PI3K/AKT and MAPK signalling pathway and increases matrix degrading proteases, MMP-2 and MMP-9 that are favourable for cell invasion.26 Furthermore, Zhang et al105 demonstrated that loss of miR-320a in cancer associated fibroblast (CAF)-derived exosomes in HCC leads to PBX dysregulation in recipient cells (hepatocyte) leading to lung metastasis. These results suggested that exosomes could mobilize normal hepatocyte to construct tumorigenic microenvironment, and consequently lead to metastasis.

5.5 | Exosomes trigger immune responses

Immune tolerance, the unique immune microenvironment of the liver, is the main obstacle to immunotherapy for treating HCC.4 It is paradoxical that exosomes trigger immune response. On the one hand, exosomes are found in a variety of known immunosuppressive mechanisms, such as activation of immune suppressor cells, antigen presentation defects and induction of T-cell apoptosis.107,108 On the other hand, exosomes are a key source of tumour antigens exposed by tumour cells and immune cells.109

For example, Lv et al15 demonstrated that anti-cancer drugs stimulate HCC-derived exosomes secretion and generate more exosome-carried HSPs, which known as “stress response” proteins. According to their study, HSP-bearing exosomes stimulate potent anti-tumour immune response through several mechanisms, including stimulation of NK cell cytotoxicity, anti-Hepatoocytes, up-regulation of the expression of inhibitory receptor CD94 and downregulation of the expression of activating receptors CD69, NKG2D and NKP44.25

Rao et al compared the level of immune responses elicited by dendritic cells pulsed by HCC tumour cell-derived exosomes (TEX) or cell lysates. Their study showed that increased numbers of T lymphocytes, increased expression of interferon-γ, and decreased levels of interleukin-10 and tumour growth factor-β were observed in HCC mice treated with HCC TEX-pulsed DCs, rather than treated with cell lysates-pulse DCs.109 These results indicated that TEX-carrying tumour associated antigens (TAAs) can be presented to DCs to initiate DC-mediated immune responses.107,109

Furthermore, Lu et al110 demonstrated that potent T-cell activation was observed in HCC mice treated with HCC antigen-modified DC (a-fetoprotein [AFP]-expressing DC) derived exosomes (DEXs). These findings demonstrated that exosomes not only present TAA from tumour cells to APCs but also are capable of presenting them to T lymphocytes that elicit an antigen-mediated anti-tumour immune response. This greatly promotes the development of HCC immunotherapy by providing cell-free vaccines.

5.6 | Exosomes are a promising agent for anti-cancer therapy

Cell membrane-derived nanoparticles have many properties, such as protecting their cargo, low immunogenicity and proper size through the endothelium,88 which can be used as drug delivery agents.111-114 For example, Lou et al112 reported that adipose-derived MSCs have full ability to transfer miR-122 via exosomes, thereby sensitizing HCC cells to chemotherapeutic agents. It has been demonstrated that the miR-122 negatively regulates the expression of the disintegrin and metalloproteinases family member 17 (ADAM17), ADAM10, IGF1R and MADS-box transcription factor SRF113,114 and is correlated with poor prognosis and metastasis in human HCC patient.113,115

MSCs are widely used due to they are the most prolific producer of exosomes among the cell types.116 In addition to adipose-derived MSCs,117 the bone marrow-derived exosomes are commonly used in stem cell-based therapies.86 Furthermore it has been reported that the DC-derived exosomes are used as cancer vaccines.110,118

Tian et al111 suggested that it may have a potential value for clinic application that modifying exosomes by targeting ligands
which used for a drug delivery vesicle. For instance, modification of exosomes membrane with Arg-Gly-Asp (RGD) peptide elicits blood vessel targeting effect, which may be a new strategy for therapeutic angiogenesis.119

Meanwhile, exosomes have been reported to be involved in chemodrug resistance, and several studies indicated that inhibition of exosomes secretion has been shown to be effective in sensitizing cancer cells to therapeutic drugs.8,120

Overall, exosomes are promising agents for HCC treatment therapy.

6 | CONCLUSION

Exosomes in cancer include HCC is a research hot spot over the past few years. In this review, the aim was to better understand the exosomes in HCC development. To the best of our knowledge, exosomes promote HCC progression by regulating multiple tumorigenic processes, including chemoresistance, EMT, angiogenesis, metastasis and immune response. An implication of this is the possibility that exosomes may be promising candidates for the treatment of HCC. It had been found that exosomes have several advantages as a drug delivery agent in the treatment of HCC. These findings had offered a framework for the exploration of new therapeutic tools for HCC. However, research is limited by the lack of information on the clinical safety and efficacy of exosomes. Therefore, further studies are still required to better understand the relationship between exosomes and HCC development.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Morelli AE, Larregaia AT, Shufesky WJ, et al. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. Blood. 2004;104(10):3257-3266.
2. Saleem SN, Abdel-Mageed AB. Tumor-derived exosomes in oncogenic reprogramming and cancer progression. Cell Mol Life Sci. 2015;72(1):1-10.
3. Wu Z, Zeng Q, Cao K, Sun Y. Exosomes: small vesicles with big roles in hepatocellular carcinoma. Oncotarget. 2016;7(37):60687-60697.
4. Moris D, Beal EW, Chakedis J, et al. Role of exosomes in treatment of hepatocellular carcinoma. Surg Oncol. 2017;26(3):219-228.
5. Zhou S, Abdou M, Arena V, Arena M, Arena GO. Reprogramming malignant cancer cells toward a benign phenotype following exposure to human embryonic stem cell microenvironment. Plos One. 2017;12(1):e0169899.
6. Williams C, Rodriguez-Barrueco R, Silva JM, et al. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res. 2014;24(6):766-769.
7. Farooqi AA, Desai NN, Qureshi MZ, et al. Exosome biogenesis, bioactivities and functions as new delivery systems of natural compounds. Biotechnol Adv. 2018;36(1):328-334.
8. Zhou L, Lv T, Zhang Q, et al. The biology, function and clinical implications of exosomes in lung cancer. Cancer Lett. 2017;407:84-92.
9. Henne WM, Buchkovich NJ, Emr SD. The ESCRT pathway. Dev Cell. 2011;21(1):77-91.
10. Raiborg C, Stenmark H. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. Nature. 2009:458(7237):445-452.
11. Babst M, Sato TK, Banta LM, Emr SD. Endosomal transport function in yeast requires a novel AAA-type ATPase, Vps4p. Embo J. 1997;16(8):1820-1831.
12. Han H, Monroe N, Votteler J, et al. Binding of substrates to the central pore of the Vps4 ATPase is autoinhibited by the microtubule interacting and trafficking (MIT) domain and activated by MIT interacting motifs (MIMs). J Biol Chem. 2015;290(21):13490-13499.
13. Wei JX, Lv LH, Wan YL, et al. Vps4A functions as a tumor suppressor by regulating the secretion and uptake of exosomal microRNAs in human hepatoma cells. Hepatolog. 2015;61(4):1284-1294.
14. Juan T, Furthauer M. Biogenesis and function of ESCRT-dependant extracellular vesicles. Semin Cell Dev Biol. 2018;74:66-77.
15. Lv LH, Wan YL, Lin Y, et al. Anticancer drugs cause release of exosomes with heat shock proteins from human hepatocellular carcinoma cells that elicit effective natural killer cell antitumor responses in vitro. J Biol Chem. 2012;287(19):15874-15885.
16. Saha B, Momen-Heravi F, Furi I, et al. Extracellular vesicles from mice with alcoholic liver disease carry a distinct protein cargo and induce macrophage activation via Hsp90. Hepatology. 2018;67(5):1986-2000.
17. Guesscini M, Genedani S, Stocchi V, Agnati LF. Astrocytes and glioblastoma cells release exosomes carrying mtDNA. J Neural Transm (Vienna). 2010;117(1):1-4.
18. Szabo G, Momen-Heravi F. Extracellular vesicles in liver disease and potential as biomarkers and therapeutic targets. Nat Rev Gastro Hepat. 2017;14(8):455-466.
19. Kahler C, Kalluri R. Exosomes in tumor microenvironment influence cancer progression and metastasis. J Mol Med. 2013;91(4):431-437.
20. Al-Nedawi K, Meehan B, Micallef J, et al. Intracellular transfer of the oncogenic receptor EGFRII/V by microvesicles derived from tumour cells. Nat Cell Biol. 2008;10(5):619-624.
21. Cho JA, Park H, Lim EH, Lee KW. Exosomes from breast cancer cells can convert adipose tissue-derived mesenchymal stem cells into myofibroblast-like cells. Int J Oncol. 2012;40(1):130-138.
22. Jung T, Castellana D, Klingbeil P, et al. CD44v6 dependence of premetastatic niche preparation by exosomes. Neoplasia. 2009;11(10):1093-1105.
23. Tauro BJ, Mathias RA, Greening DW, et al. Oncogenic H-ras reprograms Madin-Darby canine kidney (MDCK) cell-derived exosomal
proteins following epithelial-mesenchymal transition. Mol Cell Proteomics. 2013;12(8):2148-2159.

24. Lu J, Li J, Liu S, et al. Exosomal tetraspanins mediate cancer metastasis by altering host microenvironment. Oncotarget. 2017;8(37):62803-62815.

25. Zhang J, Lu S, Zhou Y, et al. Motile hepatocellular carcinoma cells preferentially secret sugar metabolism regulatory proteins via exosomes. Proteomics. 2017;17(13-14). doi:10.1002/pmic.201700103

26. He M, Qin H, Poon TC, et al. Hepatocellular carcinoma-devised exosomes promote motility of immortalized hepatocyte through transfer of oncogenic proteins and RNAs. Carcinogenesis. 2015;36(9):1008-1018.

27. Dalla Pozza E, Forciniti S, Palmieri M, Dando I. Secreted molecules inducing epithelial-to-mesenchymal transition in cancer development. Semin Cell Dev Biol. 2017;78:62-72.

28. Liu C, Yang Y, Wu Y. Recent advances in exosomal protein detection via liquid biopsy biosensors for cancer screening, diagnosis, and prognosis. Aaps J. 2018;20(2):41.

29. Li L, Tang J, Zhang B, et al. Epigenetic modification of MiR-429 promotes liver tumour-initiating cell properties by targeting Rb binding protein 4. Gut. 2014;64(1):156-167.

30. Kogure T, Lin W, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology. 2011;54(4):1237-1248.

31. Sohn W, Kim J, Kang SH, et al. Serum exosomal microRNAs as novel biomarkers for hepatocellular carcinoma. Exp Mol Med. 2015;47:e184.

32. Sugimachi K, Matsumura T, Hirata H, et al. Identification of a bona fide microRNA biomarker in serum exosomes that predicts hepatocellular carcinoma recurrence after liver transplantation. Br J Cancer. 2015;112(3):532-538.

33. Liu W, Hu J, Zhou K, et al. Serum exosomal miR-125b is a novel prognostic marker for hepatocellular carcinoma. Onco Targets Ther. 2017;10:3843-3851.

34. Liu WH, Ren LN, Wang X, et al. Combination of exosomes and circulating microRNAs may serve as a promising tumor marker complementary to alpha-fetoprotein for early-stage hepatocellular carcinoma diagnosis in rats. J Cancer Res Clin Oncol. 2015;141(10):1767-1778.

35. Hung CS, Liu HH, Liu JJ, et al. MicroRNA-200a and -200b mediated hepatocellular carcinoma cell migration through the epithelial to mesenchymal transition markers. Ann Surg Oncol. 2013;20(Suppl 3):S360-S368.

36. Petrelli A, Perr A, Cora D, et al. MicroRNA/gene profiling unveils early molecular changes and nuclear factor erythroid related factor 2 (NRF2) activation in a rat model recapitulating human hepatocellular carcinoma (HCC). Hepatology. 2014;59(1):228-241.

37. Yeh TS, Wang F, Chen TC, et al. Expression profile of microRNA-200 family in hepatocellular carcinoma with bile duct tumor thrombus. Ann Surg. 2014;259(2):346-354.

38. Yuan JH, Yang F, Chen BF, et al. The histone deacetylase 4/SP1/microRNA-200a regulatory network contributes to aberrant histone acetylation in hepatocellular carcinoma. Hepatology. 2011;54(6):2025-2035.

39. Qi P, Cheng SQ, Wang H, et al. Serum microRNAs as biomarkers for hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. Plos One. 2011;6(12):e28486.

40. Wang H, Hou L, Li A, et al. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. Biomed Res Int. 2014;2014:1-5.

41. Xu J, Wu C, Che X, et al. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. Mol Carcinog. 2011;50(2):136-142.
inhibition of mesenchymal cancer stem cells characterized by the expression of CD90. Sci Rep. 2017;7(1):11292.

62. Dong J, Zhai B, Sun W, et al. Activation of phosphatidylinositol 3-kinase/AKT/snail signaling pathway contributes to epithelial-mesenchymal transition-induced multi-drug resistance to sorafenib in hepatocellular carcinoma cells. Plos One. 2017;12(9):e0185088.

63. Shedden K, Xie XT, Chandaroy P, Chang YT, Rosania GR. Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles. Cancer Res. 2003;63(15):4331-4337.

64. Safaei R, Larson BJ, Cheng TC, et al. Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. Mol Cancer Ther. 2005;4(10):1595-1604.

65. Dean M, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. J Lipid Res. 2001;42(7):1007-1017.

66. Sukowati CH, Rosso N, Pascut D, et al. Gene and functional up-regulation of the BCRP/ABCG2 transporter in hepatocellular carcinoma. Bmc Gastroenterol. 2012;12:160.

67. Qu Z, Wu J, Wu J, et al. Exosomes derived from HCC cells induce sorafenib resistance in hepatocarcinoma both in vivo and in vitro. J Exp Clin Cancer Res. 2016;35(1):159.

68. You H, Ding W, Dang H, Jiang Y, Rountree CB. c-Met represents a potential therapeutic target for personalized treatment in hepatocellular carcinoma. Hepatology. 2011;54(3):879-889.

69. Ma PC, Moulig K, Christensen J, Salgia R. c-Met: structure, function and potential for therapeutic inhibition. Cancer Metastasis Rev. 2003;22(4):309-325.

70. Graziani A, Gramaglia D, Cantley LC, Comoglio PM. The tyrosine-phosphorylated hepatocyte growth factor/scatter factor receptor associates with phosphatidylinositol 3-kinase. J Biol Chem. 1991;266(33):22807-22809.

71. Ponsetto C, Bardelli A, Zhen Z, et al. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. Cell. 1994;77(2):261-271.

72. Fixman ED, Naujokas MA, Rodrigues GA, Moran MF, Park M. Efficient cell transformation by the Trp-Met oncoprotein is dependent upon tyrosine 489 in the carboxy-terminus. Oncogene. 1995;10(2):237-249.

73. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2(6):442-454.

74. Gopal SK, Greening DW, Rai A, et al. Extracellular vesicles: their role in cancer biology and epithelial-mesenchymal transition. Biochem J. 2017;474(1):21-45.

75. Markopoulos GS, Roupaiki E, Tokamani M, et al. A step-by-step microRNA guide to cancer development and metastasis. Cell Oncol. 2017;40(4):303-339.

76. Greening DW, Gopal SK, Mathias RA, et al. Emerging roles of exosomes during epithelial-mesenchymal transition and cancer progression. Semin Cell Dev Biol. 2015;40:60-71.

77. Blackwell R, Foreman K, Gupta G. The role of cancer-derived exosomes in tumorigenesis & epithelial-to-mesenchymal transition. Cancers. 2017;9(8):105.

78. dos Anjos Pultz B, Andrés Cordero da Luz F, Socorro Faria S, et al. The multifaceted role of extracellular vesicles in metastasis: priming the soil for seeding. Int J Cancer. 2017;140(11):2397-2407.

79. Diaz-Diaz A, Casas-Pais A, Calama V, et al. Proteomic analysis of the E3 ubiquitin-ligase hakai highlights a role in plasticity of the cytoskeleton dynamics and in the proteasome system. J Proteome Res. 2017;16(8):2773-2788.

80. Zangari J, Ille M, Rouaud F, et al. Rapid decay of engulfed extracellular miRNA by XRN1 exonuclease promotes transient epithelial-mesenchymal transition. Nucleic Acids Res. 2017;45(7):4131-4141.

81. Aga M, Bentz GL, Raffa S, et al. Exosomal HIF1alpha supports invasive potential of nasopharyngeal carcinoma-associated LMP1-positive exosomes. Oncogene. 2014;33(37):4613-4622.

82. Greening DW, Xu R, Ji H, Tauro BJ, Simpson RJ. A protocol for exosome isolation and characterization: evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods. Methods Mol Biol. 2015;1295:179-209.

83. Qin W, Tsukasaki Y, Dasgupta S, et al. Exosomes in human breast milk promote EMT. Clin Cancer Res. 2016;22(17):4517-4524.

84. Xiao D, Barry S, Kmetz D, et al. Melanoma cell-derived exosomes promote epithelial-mesenchymal transition in primary melanocytes through paracrine/autocrine signaling in the tumor microenvironment. Cancer Lett. 2016;376(2):318-327.

85. Vallabhaneni KC, Formonis P, Dhule S, et al. Extracellular vesicles from bone marrow mesenchymal stem/stromal cells transport tumor regulatory microRNA, proteins, and metabolites. Oncotarget. 2015;6(7):4953-4967.

86. Bruno S, Collino F, Dereugibus MC, et al. Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth. Stem Cells Dev. 2013;22(5):758-771.

87. Gopal SK, Greening DW, Hanssen EG, et al. Oncogenic epithelial cell-derived exosomes containing Rac1 and PAK2 induce angiogenesis in recipient endothelial cells. Oncotarget. 2016;7(15):19709-19722.

88. Zhang X, Ng H, Lu A, et al. Drug delivery system targeting advanced hepatocellular carcinoma: current and future. Nanomed Nanotechnol Biol Med. 2016;12(4):853-869.

89. Gonzalez-King H, Garcia NA, Ontoria-Oviedo I, et al. Hypoxia-inducible factor-1alpha potentiates 1-mediated angiogenesis by mesenchymal stem cell-derived exosomes. Stem Cells. 2017;35(7):1747-1759.

90. Sruhti TV, Edatt L, Raji GR, et al. Horizontal transfer of miR-23a from hypoxic tumor cell colonies can induce angiogenesis. J Cell Physiol. 2018;233(4):3498-3514.

91. Yao H, Liu N, Lin MC, Zheng J. Positive feedback loop between cancer stem cells and angiogenesis in hepatocellular carcinoma. Cancer Lett. 2016;379(2):213-219.

92. Yang XR, Xu Y, Yu B, et al. High expression levels of putative hepatic stem/progenitor cell biomarkers related to tumour angiogenesis and poor prognosis of hepatocellular carcinoma. Gut. 2010;59(7):953-962.

93. Morrow D, Cullen JP, Cahill PA, Redmond EM. Ethanol stimulates endothelial cell angiogenic activity via a Notch- and angiopoietin-1-dependent pathway. Cardiovasc Res. 2008;79(2):313-321.

94. Wang L, Song YO, Ding S, et al. Ethanol enhances tumor angiogenesis in vitro induced by low-dose arsenic in colon cancer cells through hypoxia-inducible factor 1 alpha pathway. Toxicol Sci. 2012;130(2):269-280.

95. Wang S, Xu M, Li F, et al. Ethanol promotes mammary tumor growth and angiogenesis: the involvement of chemoattractant factor MCP-1. Breast Cancer Res Treat. 2012;133(3):1037-1048.

96. Lu Y, Ni F, Xu M, et al. Alcohol promotes mammary tumor growth through activation of VEGF-dependent tumor angiogenesis. Oncol Lett. 2014;8(2):673-678.

97. Lamichhane TN, Leung CA, Douti LY, Jay SM. Ethanol induces the expression of CD90. Sci Rep. 2017;7(1):13794.

98. Sruthi TV, Edatt L, Raji GR, et al. Ethanol-induced exosomes target epithelial-mesenchymal transition and endothelial transmigration. Mutat Res. 2016;736(1-2):179-209.

99. van Zijl F, Krupitza G, Mikulits W. Initial steps of metastasis: cell invasion and endothelial transmigration. Mutat Res. 2011;728(1-2):23-34.

100. Zhang H, Deng T, Liu R, et al. Exosome-delivered EGFR regulates liver microenvironment to promote gastric cancer liver metastasis. Nat Commun. 2017;8:15016.

101. Yu Z, Zhao S, Ren L, et al. Pancreatic cancer-derived exosomes promote tumor metastasis and liver pre-metastatic niche formation. Oncotarget. 2017;8(38):63461-63483.
102. Plebanek MP, Angeloni NL, Vinokour E, et al. Pre-metastatic cancer exosomes induce immune surveillance by patrolling monocytes at the metastatic niche. *Nat Commun*. 2017;8(1):1319.

103. Li L, Li C, Wang S, et al. Exosomes derived from hypoxic oral squamous cell carcinoma cells deliver miR-21 to normoxic cells to elicit a prometastatic phenotype. *Cancer Res*. 2016;76(7):1770-1780.

104. Sharma A. Role of stem cell derived exosomes in tumor biology. *Int J Cancer*. 2018;142(6):1086-1092.

105. Zhang Z, Li X, Sun W, et al. Loss of exosomal miR-320a from cancer-associated fibroblasts contributes to HCC proliferation and metastasis. *Cancer Lett*. 2017;397:33-42.

106. Park JE, Tan HS, Datta A, et al. Hypoxic tumor cell modulates its microenvironment to enhance angiogenic and metastatic potential by secretion of proteins and exosomes. *Mol Cell Proteomics*. 2010;9(6):1085-1099.

107. Czernek L, Düchler M. Functions of cancer-derived extracellular vesicles in immunosuppression. *Arch Immunol Ther Ex*. 2017;65(4):311-323.

108. Sullivan R, Maresh G, Zhang X, et al. The emerging roles of extracellular vesicles as communication vehicles within the tumor microenvironment and beyond. *Front Endocrinol*. 2017;8:194.

109. Rao Q, Zuo B, Lu Z, et al. Tumor-derived exosomes elicit tumour suppression in murine hepatocellular carcinoma models and humans in vitro. *Hepatology*. 2016;64(2):456-472.

110. Lu Z, Zuo B, Jing R, et al. Dendritic cell-derived exosomes elicit tumor regression in autochthonous hepatocellular carcinoma mouse models. *J Hepatol*. 2017;67(4):739-748.

111. Tian Y, Li S, Song J, et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. *Biomaterials*. 2014;35(7):2383-2390.

112. Lou G, Song X, Yang F, et al. Exosomes derived from miR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma. *J Hematol Oncol*. 2015;8:122.

113. Tsai WC, Hsu PW, Lai TC, et al. MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology*. 2009;49(5):1571-1582.

114. Bai S, Nasser MW, Wang B, et al. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J Biol Chem*. 2009;284(46):32015-32027.

115. Couloaur C, Factor VM, Andersen JB, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene*. 2009;28(40):3526-3536.

116. Yeo RW, Lai RC, Zhang B, et al. Mesenchymal stem cell; an efficient mass producer of exosomes for drug delivery, *Adv Drug Deliv Rev*. 2013;65(3):336-341.

117. Ko SF, Yip HK, Zhen YY, et al. Adipose-derived mesenchymal stem cell exosomes suppress hepatocellular carcinoma growth in a rat model: apparent diffusion coefficient, natural killer T-cell responses, and histopathological features. *Stem Cells Int*. 2015;2015:853506.

118. Yang N, Li S, Li G, et al. The role of extracellular vesicles in mediating progression, metastasis and potential treatment of hepatocellular carcinoma. *Oncotarget*. 2017;8(2):3683-3695.

119. Wang J, Li W, Lu Z, et al. The use of RGD-engineered exosomes for enhanced targeting ability and synergistic therapy toward angiogenesis. *Nanoscale*. 2017;9(40):15998-16005.

120. Li XQ, Liu JT, Fan LL, et al. Exosomes derived from gefitinib-treated EGFR-mutant lung cancer cells alter cisplatin sensitivity via up-regulating autophagy. *Oncotarget*. 2016;7(17):24585-24595.

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