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Review

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Exosome-based liquid biopsy in the management of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) commonly presents at an advanced stage due to the lack of efficient early screening tools. Early, non-invasive biomarkers useful in the diagnosis and prognosis of HCC would be of significant benefit for HCC management. Development of exosome-based liquid biopsy as a non-invasive method for the management of HCC has gained much traction. Exosomes are small membranous vesicles secreted by most cell types including HCC cells. Exosomes serve as couriers for the intercellular transfer of important biomolecules, including, protein, nucleic acids and lipids to nearby and distant cells in the body. The molecular cargos carried by exosome have been described to play significant roles in cancer progression. Herein, we will dissect how HCC-derived exosomes confer aggressive traits such as tumour growth, invasion, immune remodelling and drug resistance to HCC cells. We review the current literature concerning exosomes as biomarkers in a diagnostic setting, evaluating their prognostic, predictive and monitoring capabilities. This review will highlight and discuss emerging research in the utility of exosome-based liquid biopsies therapeutic tools in HCC management. Here we will also focus on advances in exosome biology in preclinical studies.

Keywords: Hepatocellular carcinoma, liquid biopsy, exosomes, microRNA, epithelial-to-mesenchymal transition, cancer stem cell

INTRODUCTION

Hepatocellular carcinoma (HCC), a fatal primary malignancy of hepatocytes remains a global challenge due to its high mortality rates and high frequency of recurrence[1-3]. Surgical resection, chemotherapeutic or

radiotherapeutic interventions are intensively used in the clinic; however, the survival benefit is limited[4]. The poor prognosis of advanced HCC is, in part, related to the lack of reliable biomarkers for early diagnosis and the lack of effective therapeutic agents for unresectable tumours. Currently, the clinical diagnosis of HCC relies on serum alpha-fetoprotein (AFP) levels and imaging examination, including ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and invasive tumour biopsy[5]. However, it is widely known that these screening methods have low sensitivity, high false negative rates and cannot predict post-therapy recurrence and also fail to monitor real time disease and therapy[6,7]. Furthermore, conventional tumour biopsies provide a small sample size and may fail to reflect the entire tumour heterogeneity that is essential in treatment procedures and prescribing a targeting therapy based on the genotype. Therefore, to improve the prognosis and survival of HCC patients, there is an urgent need for more sensitive and effective tools for early detection, screening, real time monitoring of the disease and prognosticating risk of relapse.

EXOSOME-BASED LIQUID BIOPSY

Liquid biopsy, as a minimally invasive and cost effective method for sampling of genetic, proteomic and metabolic material from different types of cancers, has drawn much attention in recent years[8]. Recently, the discovery that exosome-based liquid biopsy may have diagnostic and therapeutic applications has garnered considerable interest[9]. Exosomes are small membranous cell-derived extracellular vesicles of endocytic origin with 50-100 nm in diameter[10]. These nano-vesicles are secreted by most type of cells and can be detected and isolated from various body fluids such as serum, urine, plasma, saliva, milk and malignant ascites[9].

The exact function of exosomes remains largely unknown. Initially, exosomes were considered to function as cellular garbage bags for the disposal of excess or non-functional cellular constituents[9,11]. Emerging studies have revealed that exosomes serve as an intercellular courier of important functional biomolecules including protein, lipid, DNA, messenger RNA, and microRNA[12]. Exosomes have a unique function in modulating intercellular communication among both nearby and distant cells in the body and thereby influencing both pathological and physiological processes. Exosomes interact with their target cells by fusion of membranes and transfer their content to regulate cellular activities in target cells[13]. Additionally, proteins on the surface of exosomes have been known to interact with cell surface receptors on target cells to mediate intracellular signalling[13].

In cancers, the production and composition of exosomes are markedly altered. For instance, it is estimated that approximately 2000 trillion exosomes are contained in normal human blood and the number of exosomes increase to approximately 4000 trillion in blood of cancer patients[9,14]. The underlying cause for enhanced levels of exosome production remains unclear. Cancer cell-derived exosomes function in an autocrine or paracrine manner to modulate the tumour microenvironment[15]. Moreover, the cargo shuttled by tumour-derived exosomes determines their effect on target cells, and the exosomes play important roles in their ability to influence tumour growth and progression. The role of exosomes in the areas of diagnosis, prognosis and treatment of tumours have been intensively investigated in many cancers, including HCC[13,16]. Tumour cell-derived exosomes were shown to carry and transfer oncogenes, pathogens and microRNAs[17]. Understanding the role of exosomes and their relevance to HCC offers the potential for new biomarkers for diagnosis and new druggable targets for treatment.

BENEFITS OF EXOSOME-BASED LIQUID BIOPSIES

Exosome-based liquid biopsies have several advantages over traditional biopsies. First, due to its minimally invasive nature, multiple samples of exosomes can be collected at different time points during treatment. Whereas, the deeply located tumours are often not accessible to be monitored during treatment and obtaining multiple tumour biopsies is difficult in clinical settings[18]. Second, similar to cells, the cargo of exosomes reflect the metabolic state of cells they originate from, in real time. Exosomes also express specific markers seen in their cells of origin, making it easier to track the origin of exosomes[9]. Third, they

are distinguishable by their size and morphology (cup-shaped appearance) through electron microscopy. Moreover, exosome surface profiling through flow cytometry and ELISAs allow classification of these subcellular vesicles to an extent[19]. Fourth, many detection and isolation techniques have been developed for exosomes in research and therapy. Many commercial kits are available for high efficiency exosomes isolation from small amounts of body fluids[10]. Fifth, the lipo-proteinous architecture of exosomes also protects the exosomal constituents from degradation. For example, microRNAs (miRs) within the exosomes are resistant to RNases and are stable in the circulation and may be promising candidates as novel biomarkers of cancers[20]. Sixth, the routinely used serum HCC markers such as AFP and des-gamma-carboxyprothrombin (DCP) are not accurate for the early detection of HCC as they lack adequate sensitivity and specificity for effective HCC surveillance[21]. Furthermore, several factors unrelated to HCC such as obstructive jaundice, vitamin K deficiency, alcohol intake, or warfarin treatment may elevate the serum DCP levels[22]. A recent study has highlighted that exosomal serum miRs are promising biomarkers to improve sensitivity, specificity, early detection and prognostic prediction of HCC[20]. Thus, exosome-based diagnostics may improve the detection of early HCC and prove to be more superior to the frequently used HCC biomarkers such as AFP and DCP.

Finally, exosome-based liquid biopsy is preferred as a robust standalone diagnostic and prognostic method compared with other liquid biopsy-based biomarkers such as circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs). Both ctDNA and CTCs have limitations biologically and technically and appear unsuited for clinical practice at the present moment. ctDNA is a single stranded or double stranded DNA, shed by either living or dying tumor into the blood[23]. Clinical use of ctDNA levels alone as cancer biomarker is currently not recommended as it is not a cancer-specific biomarker and elevated ctDNA levels have been detected in healthy controls with infections. Moreover, increased ctDNA levels are associated with pathological conditions unrelated to cancer such as chronic inflammation and autoimmune disease[24]. ctDNA are less stable as they have a short half-life[25].

CTCs are cancer cells that have detached from tumor tissue and are present in the bloodstream. They have the potential to seed the cancer to other sites[26]. CTC application is confronted with many challenges. A major challenge with CTCs is to obtain tumor cells in adequate numbers for evaluation, as CTCs are rare in blood (1-10 CTCs per 10 mL). CTCs also lack cancer-specific surface markers, making detection and isolation difficult[25]. In summary, the many benefits of exosome-based liquid biopsy application render it a useful method for both diagnosis and prognosis of cancers including HCC and it also appears promising in providing a new dimension to personalized cancer care.

ISOLATION AND IDENTIFICATION METHODS OF HCC-DERIVED EXOSOMES

Differential ultracentrifugation is considered the gold standard method for purification of exosomes and most of the studies have applied this technique for isolating HCC-derived exosomes[27,28]. However, depending on the starting material and downstream applications, other methods for the purification and enrichment of HCC-derived exosomes have also been used such as ultrafiltration, size exclusion chromatography (SEC) or the ExoQuick TC method[19,29].

Majority of HCC-derived exosomes have been identified by a combination of different methods including, round or cup shaped morphology by transmission electron microscopy, size of 50-100 nm in diameter by nanoparticle tracking analysis and exosomal surface profiling for markers such the tetraspanins (CD9, CD63, and CD81), heat shock proteins (HSP90 and HSP60), Alix and Tsg101 by immunoblot and flow cytometry[19,20,29-32]. Studies have demonstrated high expression of glypican-3 (GPC-3) and AFP, traditional markers of HCC, within the HCC-derived exosomes, thereby confirming the hepatoma-based origin of these exosomes[31].

Table 1. HCC exosome-derived liquid biopsy-based biomarkers

Exosome-derived biomarker	Region	Patient cohort	Samples	References
Exosomal RNA biomarkers				
miR-21	China	HCC (n = 30) and chronic hepatitis B (n = 30)	Serum exosome	[27]
miR-178	Japan	HCC patients before surgery (n = 6) and HCC patients who underwent LDLT (n = 59)	Serum exosome	[20]
miRs-221, 191, 181a, 26a, let-7a	China	HCC (n = 50), Hepatitis B patients (n = 50) and healthy subjects (n = 50)	Serum exosome	[42]
miRs-18a, 221, 222 and 224	Korea	HCC (n = 20), cirrhosis (n = 20) and Hepatitis B (n = 20)	Serum exosome	[43]
miRs-21, 519d and 494	Italy	HCC patients (n = 118)	Serum and tissue exosome	[17]
miR-320a	China	HCC patients (n = 6)	CAFs and PAFs	[44]
miR-125b	China	HCC (n = 30 and n = 128), CHB (n = 30), cirrhosis (n = 30)	Serum exosome	[39]
miR-665	China	HCC (n = 30), healthy (n = 10)	Serum exosome	[40]
miR-638	China	HCC (n = 126), healthy (n = 21)	Serum exosome	[41]
miR-1247-3p	China	HCC without lung metastasis (n = 90), HCC with lung metastasis (n = 20), healthy (n = 25)	Serum exosome	[19]
Xist	China	206 females including HVs, CHB, cirrhosis and HCC	Peripheral blood exosomes	[45]
Exosomal protein biomarkers				
LG3BP and PIGR	Spain and Poland	HCC (n = 29), healthy individuals (n = 32), CCA (n = 43), PSC (n = 30)	Serum extracellular vesicles	[30]

CLINICAL UTILITY OF EXOSOMES IN THE MANAGEMENT OF HCC

Exosomes have been extensively studied for diagnostic purposes and as drug delivery vehicles[33]. Notably, it was demonstrated that engineered cells can produce exosomes capable of preferentially binding to tumour cells[34]. In this section, we will highlight the studies that address the utility of exosomes as biomarkers and therapeutic tools in the management of HCC.

Exosomes as biomarkers of HCC

The contents of exosomes may serve as novel specific diagnostic biomarkers for detection of early stage and advanced HCC, summarised in Table 1. Exosomes may help discriminate HCC patients with high risk of recurrence and poor prognosis and guide timely comprehensive therapy for these patients.

Exosomal RNA biomarkers

Serum exosomal miRNAs have received considerable attention as potential non-invasive biomarkers for diagnosing cancers. miRNAs are non-coding RNAs that are 22 nucleotides long and target mRNAs for cleavage or translational repression, thus modulating a variety of biological processes[35,36]. Wang *et al.*[27] found enriched miR-21 in serum exosomes from 30 patients with HCC and negligible amounts in chronic hepatitis B patients or healthy volunteers. These authors also reported that miR-21 enrichment in serum exosomes provided increased sensitivity of detection than whole serum. Conversely, another study described that miR-21 expression was much lower in patients with HCC[37]. In line with this study, Qi *et al.*[38] confirmed low expression of miR-21 in HCC patients. Reasons for this conflicting data may be due to differences in detection techniques, as well as, differences in patient cohorts.

The content of serum exosomes has been associated with aggressiveness, prognosis and survival of HCC patients. For instance, downregulation of miR-718 in serum exosomes was associated with the recurrence of HCC after liver transplantation in 59 HCC patients. *HOXB8* was identified as a potential target gene of miR-718, such that the downregulation of miR-718 resulted in the overexpression of HOXB8 in HCC patients. High expression of HOXB8 plays an important role in the progression and recurrence of HCC[20]. Exosomes extracted from serum samples collected from two cohorts of HCC patients showed high levels of miR-125b which was an independent predictive factor for postoperative recurrence and overall survival of HCC patients[39]. Serum exosomal miR-665 levels were significantly higher in HCC patients than those in healthy subjects. Additionally, exosomal miR-665 levels were elevated in larger tumours with local invasion

and at an advanced clinical stage (stage III/IV) of HCC[40]. Another study found decreased expression of serum exosomal miR-638 in HCC patients[41]. High miR-1247-3p in serum exosomal levels correlated with lung metastasis, poor overall survival and poor disease-free survival in HCC patients[19].

A study identified a panel of miRs including miR-221, miR-191, let-7a, miR-181a, and miR-26a to be an optimal gene reference set for normalising the expression of liver-specific miRNAs[42]. The serum levels of a panel of exosomal miRs including miR-18a, miR-221, miR-222 and miR-224 were significantly higher in patients with HCC than those with Hepatitis B and cirrhosis[43]. The serum levels of exosomal miR-101, miR-106b, miR-122 and miR-195 were lower in patients with HCC than in patients with hepatitis B[43]. Circulating miRNAs, miR-939, miR-595, miR-519d and miR-494 could identify cirrhotic patients with HCC. Upon comparison of serum and tissue miR levels in 14 patients surgically treated for HCC, a correlation between circulating and tissue levels of miR-519d, miR-494 and miR-21 was found in HCC patients[17]. A whole micro-RNAome microarray analysis was applied to explore dysregulated expression of miRNAs in patients with cirrhosis, early, intermediate and advanced HCC. This study identified exosome-mediated dysregulation of circulatory miRNAs, miR-519d, miR-21, miR-221 and miR- 1228[17]. A significant reduction in miR-320a level was detected by miRNA sequencing of exosomes derived from cancer-associated stromal fibroblasts (CAFs) when compared to corresponding paracancer fibroblasts (PAFs) of 6 HCC patients[44]. By using nanoparticle tracking analysis, the serum exosome concentration in HCC patients was found to be higher than in cholangiocarcinoma (CCA) and primary sclerosing cholangitis (PSC) patients[30]. Long noncoding ribonucleic acid (lncRNA) X-inactive-specific transcript (Xist) was upregulated in peripheral blood of HCC patients[45].

Exosomal protein biomarkers

Furthermore, when the protein content of serum exosomes was characterised in 29 HCC patients and 32 healthy individuals, Galectin-3-binding protein (LG3BP) and polymeric immunoglobulin receptor (PIGR) was found to be abundant in HCC patients. In particular LG3BP could distinguish patients with HCC from CCA and PSC patients[30]. Together these studies suggest exosomal miRNAs, lncRNA and proteins may serve as novel diagnostic and prognostic biomarkers of HCC.

Exosomes as delivery vehicles for HCC therapeutics

Emerging studies demonstrate the importance of exosomes as potential targets for therapeutic intervention. Exosomes can be used as biological delivery vehicles for incorporating specific cargo into target cells. One study used exosomes to horizontally transfer therapeutic miRNAs into HCC cells[46]. A recent study demonstrated the inhibitory effects of mesenchymal stem cells on HCC. In this study, rats models of HCC treated with adipose-derived mesenchymal stem cell (ADMSC) exosomes harboured significantly smaller tumours and more intratumoural invariant (CD8α+) natural killer T (NKT)-cells and low-grade HCC than the controls[47]. As ADMSCs produce large amounts of exosomes, these cells are well suited for the mass production of exosomes[48]. Another study utilised ADMSCs derived exosomes for miR-122 delivery into HCC xenograft models[49]. This study also demonstrated that miR-122 promoted cheomsensitivity of HCC cells[49]. Furthermore, exosomes isolated from human hepatic stellate (LX2) cells were loaded with miR-335-5p and these exosomes were taken up by HCC cells *in vitro* and *in vivo*. This preclinical study showed an inhibition of HCC cell proliferation and invasion *in vitro* and also demonstrated HCC tumour shrinkage *in vivo* upon uptake of these engineered exosomes[50]. There are several advantages of using exosome-based therapy, as exosomes show low immunogenicity, toxicity and are stable in tissue and in circulation. Together, this information suggests that exosomes have great translational potential as therapeutics or delivery vehicles for targeted therapy. Therefore, further studies must identify the optimal delivery method of exosomes to HCC patients.

HCC-derived exosome functions in preclinical studies

HCC-derived exosomes have pleiotropic biological functions, including roles in tumour growth, metastasis, immune response, intercellular communication, and drug resistance. In this section, we will dissect

Table 2. Preclinical studies demonstrating function of exosomes in HCC

Process	HCC cell lines	Effect	References
mRNA surveillance	HepG2, Hep3B	Nup98 prevents p21 mRNA degradation by the exosome	[52]
Intercellular communication, microRNA-based communication	Hep3B, HepG2 and PLC/PRF/5	Modulate the constitutive expression and downstream signalling of TAK1	[54]
Long noncoding RNA-based communication	Hep3B and PLC/PRF/5	Transfer of TUC339 to regulate HCC growth	[55]
Tumour growth and metastasis	SMMC-7721	Self-derived exosomes promote growth and motility	[15]
	HKCI-C3, HKCI-8, MHCC97L and MIHA	Motile cell-derived exosomes induced motility in non-motile cells	[56]
	MHCC97-H and SMMC-7721	miR-320a suppresses HCC cell migration	[44]
	Hep3B cell, 97H and LM3	Motile HCC cells secret more sugar metabolism regulatory proteins	[28]
	HepG2 and Hep3B	miR-490 rich mast cell-derived exosomes blocked motility of HCC cells	[29]
	MHCC97-H and MHCC97-L	Enriched adenylyl cyclase associated protein 1 in motile HCC cells	[57]
	CSQT-2, HCC-LM3, HepG2 and MHCC-97L	miR-1247-3p promotes lung metastasis	[19]
Immune modulation	PBTC, MHCC97H, SMCC-7721	14-3-3ζ promotes anti-tumour immune response	[32]
	DC2.4, Hepa1-6	Induces immune response to suppress tumour growth	[4]
	Hepa1-6	Induces anti-tumour response by decreasing T regulatory cells	[31]
Chemoresistance	HepG2, Hep3B, PLC/PRF-5 and Huh-7, MzChA1 cells	Exposure of HCC cells to diverse anti-cancer agents increased exosomal linc-VLDLR expression	[59]
	HepG2 and Hep3B	miR-122 delivered via exosomes sensitised cells to doxorubicin and sorafenib	[49]
	MHCC-97 L, MHCC-97H and LO2	Larger tumours formed in mice treated with sorafenib and invasive cell-derived exosomes	[60]
	MHCC97H, MHCC97L, HepG2, Huh7, LX2	Conditioned media from activated fibroblast with high miR-1247-3p conferred sorafenib resistance	[19]
Cancer stem cells	HepG2 and PLC/PRF/5	linc-RoR and TGF-β modulated stemness	[59]
	SMMC-7721	miR-1247-3p enhanced stemness	[19]
EMT	MHCC97-H	Overexpression of miR-320a induces an EMT	[44]

the diverse functions of exosomes derived from HCC cells. These functions are summarised in Table 2. Collectively, these data may provide the foundation for further studies into the regulatory roles of exosomes in the development and progression of HCC.

mRNA surveillance

Exosomes have been known to participate in control mechanisms that remove aberrant RNAs in the nucleus and the cytoplasm[51]. In HCC cell lines, HepG2 and Hep3B, the exosomes recognise and degrade p21mRNA upon Nup98 depletion as a process of mRNA surveillance related either to impaired export or defects in RNA protein complex formation in the 3'UTR region[52].

Intercellular communication

Exosomes have emerged as important mediators of intercellular communication that can shuttle protein and RNA to recipient cells and can elicit a potent overall effect on transformed cell tumours[13,53]. For example, Hep3B, HepG2 and PLC/PRF/5 cell-derived exosomes can modulate the expression of transforming growth factor-β activated kinase-1(TAK1) and associated downstream signalling and enhance transformed cell growth in recipient cells[54]. Furthermore, vacuolar protein sortin 4 homolog A (VPS4A) regulates exosome-mediated aberrant miRNA expression in HCC cells[15]. The potential of exosomes to transfer lncRNA is increasingly recognised. Kogure *et al.*[55] first demonstrated that lncRNA with highly conserved sequences ultraconserved RNAs (ucRNAs) influences intercellular signalling. In HCC cell lines PLC/PRF/5 and Hep3B, the intercellular transfer of ucRNA TUC339 by exosomes represents a unique signalling mechanism by

which tumour cells can promote HCC growth and spread[55]. Thus, the use of exosomes as biological delivery vehicles is of considerable interest.

Modulation of HCC tumour growth and metastasis

Exosomes are considered to serve essential roles in tumour growth and metastasis by regulating complex interactions between tumour cells and their microenvironment. Several studies addressed whether HCC cell-derived exosomes can influence the biological behaviour of the parental HCC cells. A study revealed that incubation of SMMC-7721 cells with self-derived exosomes caused a notable increase in cell growth, migration, and invasion[15]. Another study described a comprehensive RNA and protein profiling of exosomes derived from motile and non-motile HCC cell lines. Exosomes derived from metastatic HCC cell lines HKCI-C3, HKCI-8 and MHCC97L were enriched in protumorigenic RNAs and proteins, such as MET protooncogene, S100 family members and the caveolins. Of interest, exosomes from motile HCC cell lines could significantly enhance the migratory and invasive abilities of non-motile immortalised hepatocyte line MIHA. Motile behaviour in MIHA cells was triggered by activation of PI3K/AKT and MAPK signalling pathways which in turn increased secretion of active matrix metalloproteinase, MMP-2 and MMP-9[56]. A comparative proteome analysis of exosomes from the non-motile Hep3B cell, and the motile 97H and LM3 cells found the motile HCC cells to secrete more sugar metabolism regulatory proteins via exosomes in the tumour microenvironment[28]. The ability of exosomes to modulate the motile ability of tumour cells was tested by comparing protein profiles of cell lines with distinct metastatic potential. Among these, adenylyl cyclase associated protein 1, a protein implicated in HCC metastasis, was significantly enriched in exosomes from cells with high motile ability. Moreover, incubating low motile MHCC97 L cells with highly motile MHCC97 H cell-derived exosomes, enhanced the motile ability of MHCC97-L cells[57]. Thus, it is conceivable that highly motile HCC cell-derived exosomes could modify normal hepatocytes and less motile HCC cells in their microenvironment to facilitate tumour growth, invasion and metastases.

Alteration in exosomal miRs also influences tumour behaviour. For instance, HCC cell-derived exosomes have been shown to activate the MAPK/ERK pathway through miR-665 and further promote the proliferation of tumour cells[40]. Whereas, the expression of miR-320a was significantly downregulated in HCC cell lines. miR-320a binds to its direct downstream target and suppresses HCC cell proliferation, migration and metastasis[44]. Previous studies have shown that the increase of mast cells (MCs) usually indicates a poor prognosis of HCC patients[58]. MC-derived exosomes showed increased expression of miR-490 and the transfection of HepG2 and Hep3B cells with these exosomes inhibited migration and invasion in both the HCC cell lines[29]. Exosomes derived from high-metastatic cancer cells contribute to fibroblast activation to foster lung metastasis of liver cancer via transfer of miR-1247-3p[19].

Immune modulation

Emerging evidence suggests that HCC-derived exosomes can mediate dialogue between cancer cells and immune cells to promote antitumor immune responses for tumour growth. For instance, Wang *et al.*[32] demonstrated that 14-3-3ζ, also called 14-3-3 protein zeta was transmitted from HCC cells to T cells via exosomes and resulted in the inhibition of anti-tumour functions of tumour-infiltrating T cells in the HCC microenvironment. HCC–derived exosomes have been shown to be enriched in HCC antigens, which in turn can prime cytotoxic T lymphocytes and elicit a stronger immune response *in vitro* and *in vivo* compared with cell lysates[4]. Exosomes from HCC cells can also present tumour antigens to versatile mediators of the immune system, the dendritic cells to induce a strong immune response and to suppress tumour growth[4]. Similarly, another study combined tumour-derived exosome-pulsed dendritic cells and PD-1 antibody with sorafenib and observed the effects on tumours in mice with orthotopic HCC. This treatment combination induced antitumor responses and changed the tumour microenvironment by decreasing T regulatory cell accumulation in tumour tissue after sorafenib treatment[31].

Chemoresistance

Cell behaviour can be modulated by the content within exosomes during chemotherapeutic treatment in HCC cells. For example, exposure of HCC cells to diverse anti-cancer agents such as sorafenib, camptothecin, and doxorubicin increased the lncRNA, linc-VLDRL within the exosomes released from these treated cells and promoted chemotherapeutic resistance[59]. Another study explored how transforming growth factor (TGF)-β selectively enriched lncRNA linc-RoR within exosomes and thereby facilitated chemoresistance[59]. Treating HepG2 and Hep3B cells with miR-122 loaded exosomes derived from adipose tissue derived mesenchymal stem cells (ADMCs) sensitised these cells to doxorubicin and sorafenib[49]. Furthermore, a study found that exosomes derived from HCC cells can block the therapeutic effects of sorafenib and promote tumor growth[60]. This study demonstrated that exosomes derived from highly invasive hepatoma cells, MHCC-97 L and MHCC-97H had greater efficacy than that of exosomes derived from less invasive cells, LO2. Notably, combined treatment with sorafenib and exosomes derived from highly invasive hepatoma cells resulted in the formation of larger tumours in mice than those in mice treated with sorafenib alone or sorafenib plus exosomes derived from less invasive cells[60]. After treatment with conditioned media collected from fibroblasts pre-treated by exosomes derived from high-metastatic cancer, tumour cells showed increased spheroid formation ability, motility, and resistance ability to sorafenib[19]. Thus, exosomes can modulate chemoresistance in recipient cells that incorporate these exosomes. Understanding how exosomes confer resistance to cellular stress will enable us to develop more effective treatments for HCC.

Cancer stem cells

Accumulating evidence implicates cancer stem cells (CSCs) in the growth and spread of HCC[61]. CSCs have the capacity for self-renewal and ability to differentiate, and have been identified to confer resistance to chemotherapy. Exosomes have been implicated in promoting stemness in HCC. For example, TGF-β treatment enhanced the growth of CD133+ CSCs in HepG2 cells. Both stemness and chemoresistant phenotype of CSCs were modulated by lncRNA linc-RoR within the exosomes derived from TGF-β treated cells[59]. SMMC-7721 cells treated with miR-12473p revealed increased sphere formation with elevated expression in CSC marker genes such as CD133, lrg5, Oct4, nanog and CD90[19].

Epithelial-to-mesenchymal transition

Epithelial-to-mesenchymal transition (EMT) is a cellular process during which epithelial cells undergo a phenotypic switch to become more aggressive and motile mesenchymal cells[62]. The process of EMT is also a major contributor for metastasis and drug resistance[61]. More recently, exosomes have been described to mediate EMT in many cancers[63]. In HCC cell lines, the expression of miR-320a was significantly downregulated and induced EMT as evidenced by changes in EMT marker expression. miR-320a simultaneously enhanced the expression of mesenchymal marker, N-cadherin and suppressed the expression of epithelial marker E-cadherin, thereby eliciting EMT[44].

DISCUSSION

Numerous studies suggest clinical usefulness of exosomes as minimally invasive biomarkers for the detection, diagnosis and prognosis of different cancers[9,10]. In HCC, there is poor consensus regarding the use of exosome-derived miRs as diagnostics. This could be ascribed to diversity of study designs, analytical conditions, choice of internal control genes, choice of body fluid used such as serum or plasma, choice of control and patient populations and sample size[17]. Furthermore, most of the data reported in HCC literature have been obtained on eastern patients, whose tumour biology might not match that of western patients. Indeed, obtaining exosome-based liquid biopsies might prove to be beneficial in cases where obtaining tumour biopsies is difficult in clinical settings. However, it is still necessary to standardise methods for exosome isolation and characterisation by using guidelines proposed by the EV-TRACK Consortium[64]. Furthermore, the potential use of exosomes as delivery vector needs more critical evaluation.

Tumour-derived exosomes have been described as regulators of metabolic reprogramming in various tumour microenvironments[65]. Metabolic reprogramming is a process whereby tumours increase their glucose availability by suppressing uptake of glucose by non-tumour cells[66]. However, their role in HCC remains to be elucidated. Although a few studies have explored the role of exosomes in EMT and cancer stem cells, further studies are warranted in these areas. Another relevant aspect is angiogenesis, a major process which regulates nutrient availability of fast growing solid tumours[10]. The role of exosomes in facilitating angiogenesis and its consequence on HCC metastasis remain unexplored. Collectively, these phenomena impose major challenges on cancer treatment and both *in vitro* and *in vivo* studies in these areas will lay the foundation for future clinical trials.

CONCLUSION

Exosomes are biologically active nanovesicles that can transfer information to recipient cells to mediate local as well as distant cell-cell communication. In summary, increasing number of studies has shown that HCC-derived exosomes are potent mediators of tumor growth, proliferation and motility. They also play a pivotal role in moulding the host immune response. Other relevant aspects influenced by HCC-derived exosomes are chemoresistance, EMT and CSCs. The ease of isolating exosomes and their content from different body fluids may provide a new source of biomarkers with application in diagnosis, prognosis and in monitoring disease progression during and after treatment. Moreover, exosomes have shown great potential as drug delivery systems for the treatment of HCC. Overall, exosomes show a tremendous potential for better cancer care and effective treatment outcomes for HCC.

DECLARATIONS

Authors' contributions

Conception and manuscript writing, provision of study materials, collection and assembly of data, and final approval of manuscripts: Jayachandran A, Manda SV, Shrestha R, Bridle KR, Prithviraj P, Crawford DHG

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