An emerging role of radiation-induced exosomes in hepatocellular carcinoma progression and radioresistance (Review)

QING FAN[1], YUE YU[2], YUELING ZHOU[2], SHENG ZHANG[1] and CHUNLI WU[2]

Departments of [1]General Surgery and [2]Radiation Oncology, The Fourth Affiliated Hospital of China Medical University, Shenyang, Liaoning 110032, P.R. China

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Abstract. The incidence rates of hepatocellular carcinoma (HCC) worldwide are increasing, and the role of radiotherapy is currently under discussion. Radioresistance is one of the most important challenges in the therapy of HCC compared with other local advanced, recurrent and metastatic cancers. The mechanisms of radioresistance are complex and remain to be fully understood; however, extracellular vesicles have been investigated in recent studies. Exosomes, which are 40- to 150-nm extracellular vesicles released by cancer cells, contain multiple pathogenic components, including proteins, nucleic acids and lipids, and play critical functions in cancer progression. Emerging data indicate a diagnosis potential for exosomes in HCC, since radiation-derived exosomes promote radioresistance. Radiation-based therapy alters the contents and components of exosomes, suggesting that exosomes and their components may serve as prognostic and predictive biomarkers to monitor radiation response. Therefore, understanding the roles and mechanisms of exosomes in HCC progression and radiation response during HCC therapy may increase our knowledge concerning the roles of exosomes in radioresistance, and may lead to novel approaches for HCC prognosis and treatment. The current review summarizes recent studies on exosome involvement in HCC and the molecular changes in exosome components during HCC progression. It also discusses the functions of exosomes in HCC therapy, and highlights the importance of exosomes in HCC progression and resistance for the development of novel therapies.

Correspondence to: Dr Chunli Wu, Department of Radiation Oncology, The Fourth Affiliated Hospital of China Medical University, 4 Chongshan East Road, Huanggu, Shenyang, Liaoning 110032, P.R. China
E-mail: clwu@cmu.edu.cn

Abbreviations: HCC, hepatocellular carcinoma; mRNA, messenger RNA; miRNA, micro-RNA; lncRNA, long non-coding RNA; circRNA, circular RNA; ncRNA, non-coding RNA; HEV, hepatitis E virus; ctDNA, circulating tumor DNA; EMT, epithelial-mesenchymal transition; TNM, tumor, node, metastasis; ceRNA, competitive endogenous RNA

Key words: hepatocellular carcinoma, radioresistance, exosomes, radiotherapy, ncRNA

Contents

1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer (1,2) and the second leading cause of cancer-associated mortality worldwide (3). HCC occurs most frequently in patients with chronic liver diseases, including cirrhosis caused by hepatitis B or C infection, which accounts for ~50% of cases (4,5). The risk of HCC, which is the most common type of liver cancer, is higher in patients with long-term liver diseases as well as in patients infected with hepatitis B or C (6,7). HCC is also more common in individuals who consume large quantities of alcohol and in those with accumulated fat in the liver (8). Other factors, such as tobacco smoke inhalation and intake of aflatoxin B1, are also well-described contributors to HCC (8).

As HCC is one of the most common malignant tumors in clinical practice, its diagnosis, treatment and prognosis attract significantly considerable attention. Surgical interventions, including resection and transplantation, are the most common methods for early HCC therapy (9). However, since HCC is difficult to diagnose at an early stage, and the majority of patients tend to be diagnosed at advanced stages and are not ideal for resection, conservative treatment strategies are generally useful for advanced HCC (2). However, radiotherapy, including stereotactic body radiation therapy and radiofrequency ablation, is affected by increased radioresistance, indicating that HCC therapy should be improved due to the

high morbidity, mortality and recurrence of this disease (10,11). Radioresistance is a complex biological process associated with multiple factors, such as abnormal DNA damage response, gene mutations, cell autophagy, apoptosis, cell cycle checkpoint and other dysregulated signaling pathways (12). These mechanisms lead to poor prognosis in patients and cause major clinical obstacles for radiotherapy, ultimately leading to tumor metastasis and relapse (13). Radioresistance is also highly associated with the tumor microenvironment (14,15), which is an important factor associated with tumor progression and therapeutic response (16,17) and is actively achieved by exosome-regulated cell-cell communication.

Exosomes are single-membrane secreted organelles 40-150 nm in diameter. Exosomes have similar topologies with cells, and are enriched in nucleic acids, selected proteins, lipids and glycoconjugates. The biogenesis of exosomes relies on a mechanism of protein quality control, and exosomes display activities in remodeling the extracellular matrix and transmitting signals and molecules from the contributor cells to other cells. This pathway plays important functions in numerous aspects of human homeostasis and disease, such as development, tissue homeostasis, immunity, neurodegenerative diseases and cancer (18,19). Increasing evidence suggests that cancer-derived exosomes play multiple and critical functions in cancer. Exosomes, as well as their components, may serve as cancer prognostic markers, therapeutic targets or anticancer drug carriers (20,21). Regarding the advantages of exosomes in cancer diagnosis and their newly suggested application, previous studies have indicated that radiation-derived extracellular vesicles, particularly exosomes, decrease survival, increase tumor burden, cause radiation-induced bystander effects and promote radioresistance. The present study reviews recent reports on radiation-induced changes in extracellular vesicles (particularly exosomes) in HCC, and discusses the molecular mechanisms of exosome-regulated HCC progression and radioresistance for the development of novel therapeutic strategies.

2. Biological components of exosomes in HCC

The majority of commonly studied cells are characterized by the production of a set of lipid membrane-coated vesicles that can be secreted to the extracellular space. They are named extracellular vesicles, and the most well-known ones are exosomes (22). Exosomes, which were first identified in the late 1990s, are a set of membranous vesicles released into the extracellular space by contributor cells after multiple intracellular vesicles fuse into the cell membrane (23). The majority of known cell types, including normal and pathogenic cells, are capable of secreting exosomes, and numerous studies suggest that, compared with that of normal epithelial cells, the release of exosomes by tumor cells is more active (24). Exosomes are present in almost all types of body fluids, including urine, saliva, blood, breast milk, bile, synovial fluid, seminal fluid and amniotic liquid, indicating their critical functions in intercellular communication by transducing both genomic and proteomic materials among cells and subsequently modulating physiological responses (25). However, the mechanisms of the biological formation of exosomes remain to be clarified. The functions and characteristics of exosomes are mostly based on their sizes, expression of surface markers and composition, including DNA, RNA, lipids, metabolites and surface proteins (18,26). This variety of characteristic protein components on the outer membrane of exosomes, which serve as surface markers, includes CD9, CD81, CD63, CD53, CD82, CD37, TSG101, Hsp70 and Alix (26,27). These proteins are commonly used as markers to confirm the presence of exosomes, since they are detectable by western blotting. In addition, exosomes contain numerous lipid molecules, which participate in multiple biological processes and play critical roles in the morphological stability of exosomes in the extracellular fluid (28).

In the tumor microenvironment, cancer cells secret numerous exosomes, which are transferred from cancer cells to other cell types to participate in signaling transduction, and in the processes of tumor formation and progression (29). In addition, exosomes derived from both tumor and stromal cells have been reported to be implicated in all stages of cancer progression and to exhibit crucial functions in tumor therapy resistance. Due to their characteristics as mediators of cell-cell communication, exosomes are integral to tumor microenvironment-dependent therapy resistance (30). Furthermore, exosomes are also able to deliver oncogenes to normal epithelial cells under pathological conditions, which has been reported to be one of the mechanisms involved in tumor invasion and metastasis (31). Numerous previous studies have demonstrated that exosomes can deliver various proteins and ribonucleic acids to recipient cells (18). These studies are validated by the activation and expansion of exosome-producing immune cells in murine models of acute or chronic inflammation (32).

Exosomes are of different phenotypes in different body fluids and contain a variety of biologically active molecules, which leads to diverse and heterogenous exosome types (26). These molecules are divided into lipids (including sphingomyelin, phosphatidylserine and cholesterol) (33), proteins (including tumor-suppressor proteins, oncoproteins and transcription regulators) (34), DNA (including genomic DNA, single-strand DNA, and retrotransposon elements) (35,36) and RNAs [including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), circular RNAs (circRNAs), messenger RNAs (mRNAs) and other non-coding RNAs (ncRNAs)] (37). Multiple studies have confirmed that these exosomal active components exhibit critical functions in the resistance of a variety of tumors, particularly in HCC radioresistance (18). Therapeutic interventions such as chemotherapy and radiotherapy, could affect exosome uptake and subsequent tumor biological responses, which in return could influence the secretion and components of exosomes (13) (Fig. 1).

3. Protein transduction by HCC-derived exosomes

Exosomes contain abundant proteins, which include not only self-proteins, but also proteins derived from contributor cells.

Exosomal proteins as HCC biomarkers. Exosomal proteins derived from cancer cells are becoming novel biomarkers for cancer monitoring and efficacy evaluation, and are also becoming a popular research topic in studies on cancer radioresistance (38), particularly in HCC. Mass spectrometry (MS) analysis demonstrated that 213 proteins could be

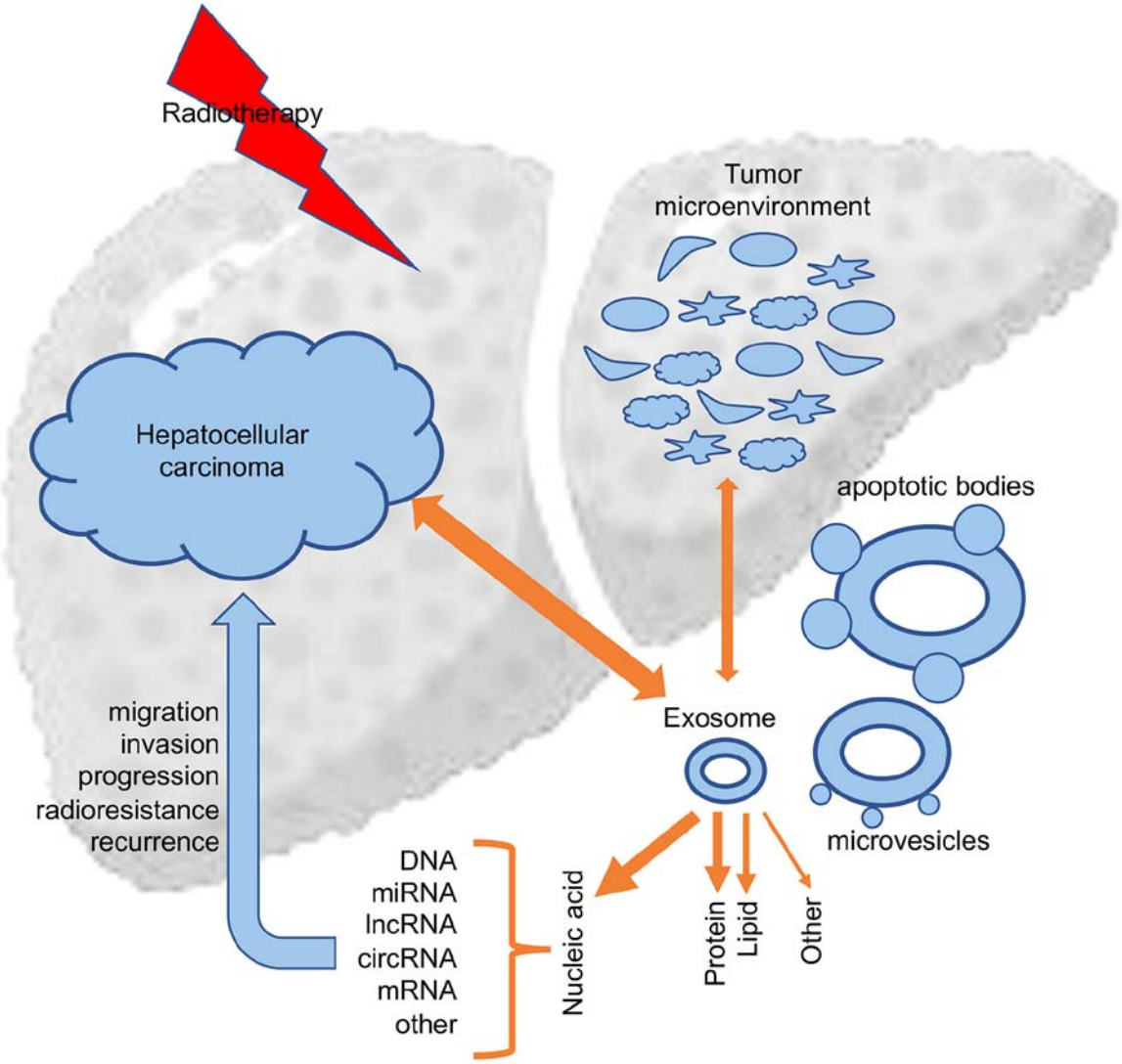

Figure 1. The involvement of exosomes and their contents in the development, progression and recurrence of hepatocellular carcinoma. mRNA, messenger RNA; miRNA, microRNA; lncRNA, long non-coding RNA; circRNA, circular RNA.

detected in exosomes secreted by an HCC cell line, and, among them, 158 proteins were specifically expressed in exosomes secreted by highly malignant HCC cells compared with the exosomal proteome of each cell line (39). Exosomes secreted by metastatic HCC cell lines were found to carry several protumorigenic RNAs and proteins, including MET proto-oncogene, caveolins and S100 family members. Notably, exosomes from motile HCC cell lines significantly enhanced the migratory and invasive abilities of non-motile MIHA cells. Uptake of exosomal molecules may trigger signaling pathways, including the PI3K/AKT and MAPK signaling pathways, in MIHA cells with increased secretion of active matrix metalloproteinases MMP-2 and MMP-9. These findings demonstrated that HCC-derived exosomes had the potential of mobilizing normal hepatocytes, which may have implications in promoting the protrusive activities of HCC cells through the liver parenchyma during the process of metastasis (39). Another study using MS investigated exosomes isolated from the HCC cell line HepG2 and identified a total of 1,428 proteins in HepG2 cell exosomes. These proteins were found to facilitate the activation of several kinases and the NF-κB signaling pathway in HCC cells (39). Eukaryotic translation initiation factor 3 subunit C (EIF3C), a protein associated with poor patient survival that is upregulated during HCC tumor progression, was found to significantly increase extracellular exosome secretion, exosome size and exosome marker expression diversity. EIF3C-overexpressing exosomes were oncogenic, and enhanced tumor angiogenesis and blood vessel growth. Subcutaneous inoculation of EIF3C-enriched exosomes together with Huh7 HCC cells increased the growth of vessels and upregulated the expression of EIF3C in tumors. Furthermore, EIF3C activated the expression of S100A11, which is involved in EIF3C-enriched exosome-mediated increased tube formation in angiogenesis (40). A previous study performed protein profiling of exosomes derived from different HCC cell types, and identified 129 proteins, among which, adenylyl cyclase-associated protein 1 (CAP1), a protein implicated in HCC metastasis, was significantly enriched in exosomes secreted by HCC cells with high motile abilities (41). Similarly, super-stable isotope labeling by/with amino acids in cell culture-based MS analysis on exosomes secreted by three human HCC cell lines, non-motile Hep3B cells, and motile 97H

and LM3 cells quantified >1,400 exosomal proteins in each MS analysis with highly biological reproducibility. In total, 469 and 443 exosomal proteins, respectively, represented differentially expressed proteins in LM3/Hep3B vs. 97H/Hep3B cells. These proteins were involved in sugar metabolism-centric canonical pathways according to signaling pathway analysis. Specifically, these pathways included the glycolysis I, gluconeogenesis I and pentose phosphate pathways, and the proteins enriched in these signaling pathways could form a tightly connected network. The above findings suggest that motile HCC cells have the potential to preferentially export more sugar metabolism-associated proteins by exosomes that facilitate the differentiation of motile HCC from non-motile HCC cells (42).

Exosomal proteins in HCC metastasis and therapy resistance. Another study investigated the proteome of macrovesicles present in urine samples acquired from liver injury experimental models as a method to identify potential biomarkers of hepatic diseases. The biochemical and proteomic characterization of highly purified exosomes identified 28 previously unreported proteins, a number of which were closely associated with liver diseases. This analysis suggested that urinary exosomes could potentially be a sample source for identifying biomarkers of liver injury, and certain proteins such as CD26, CD81, solute carrier family 3 member 1 (SLC3A1) and CD10 were differentially expressed in urinary exosomes derived from experimental models, thus serving as potential biomarkers for liver injury (43). HCC cells were reported to promote cancer cell proliferation and lung metastasis formation in a paracrine/endocrine manner, which was achieved by primary HCC-derived exosomes. In the presence of these malignant exosomes, enhanced cell adhesion was observed. Notably, attached HCC cells released exosomes containing both mothers against decapentaplegic homolog 3 (SMAD3) protein and mRNA, and delivered them to detached HCC cells to subsequently facilitate their adhesion. These exosomes contained activated SMAD3 signaling in the recipient HCC cells and improved their adhesive capacities. Clinically, SMAD3-containing exosomes were detected in the peripheral blood of patients with HCC, and their levels were closely associated with disease stage and SMAD3 expression in primary tumors. That study suggested a potential mechanism by which exosome SMAD3 mediated communication between primary and circulating HCC cells (44). Tumor-derived exosomes were found to activate B cells, which expressed high levels of T-cell immunoglobulin and mucin domain 1 (TIM-1) protein and suppressed the activities of $CD8^+$ T cells in a similar manner to TIM-1^+ regulatory B (Breg) cells isolated from HCC tissues. Enrichment of TIM-1^+ Breg cells in HCC tissues was involved in advanced disease stage, and predicted early recurrence in HCC as well as reduced patient survival in HCC. High mobility group box protein 1 (HMGB1) derived from exosomes activated B cells and promoted the expansion of TIM-1^+ Breg cells through the Toll-like receptor 2/4 (TLR2/4) and MAPK signaling pathways (45). T cells could uptake exosomes containing 14-3-3ζ secreted by HCC cells, which suggested that 14-3-3ζ is delivered from HCC cells to tumor-infiltrating T lymphocytes by exosomes. In tumor-infiltrating T cells of the HCC microenvironment, 14-3-3ζ was upregulated and inhibited their antitumor functions. Specifically, 14-3-3ζ is potentially transferred from HCC cells to T cells partially through exosomes (46). Exosomes with increased C-X-C motif chemokine receptor 4 (CXCR4) levels secreted by high metastatic mouse hepatocarcinoma Hca-F cells were confirmed to increase the migration and invasion of paired syngeneic Hca-P cells with low metastatic capacities. The increase in cell migratory and invasive capacities of Hca-P cells was facilitated by the internalization of exosomes isolated from Hca-F cells and subsequent transferring of CXCR4 via exosomes. Hca-F cell migration and invasion were increased by lymphatic endothelial cells via upregulation of stromal cell-derived factor-1α (SDF-1α) binding to C-X-C motif chemokine receptor 4 (CXCR4) in Hca-F cells and subsequently upregulating the secretion of vascular endothelial growth factor (VEGF)-C, MMP-9 and MMP-2. In addition, CXCR4 derived from Hca-F exosomes significantly increased the abilities of lymphatic endothelial cell proliferation and lymphatic tube formation (47). C-type lectin domain family 3 member B (CLEC3B) downregulation in HCC was found to suggest a poor prognosis. Exosomes secreted by HCC with downregulated CLEC3B significantly increased the epithelial-mesenchymal transition (EMT), migration and invasion of tumor cells. CLEC3B downregulation in HCC exosomes decreased VEGF secretion in HCC cells and inhibited angiogenesis. Mechanistically, VEGF expression mediated by CLEC3B in tumor cells relied on the activation of the AMP-activated protein kinase signaling pathway (48). Both *in vivo* and *in vitro*, overexpression of chitinase 3 like 1 (CHI3L1), a cancer poor prognosis-associated secreted glycoprotein, significantly facilitated the growth, migration and invasion of liver cancer cells. Overexpression of CHI3L1 influenced cell-cell adhesion, extracellular exosomes and adherent junction-related genes via TGF-β signaling pathway activation (49). The expression of golgi membrane protein 1 (GOLM1) was inhibited by miR-145 via targeting a coding sequence of the *GOLM1* gene. The expression of GOLM1 and miR-145 showed an inverse correlation in human HCC tissues. Mechanistically, exosomes with GOLM1 enrichment activated the GSK-3β/MMP signaling axis of recipient cells, and accelerated HCC migration and proliferation (50).

4. Lipid transduction by HCC-derived exosomes

Exosome structures and contents consist of proteins, nucleic acids and lipids, which are mostly derived from contributor cells. Similar to structures such as cell membranes, exosomes contain a lipid bilayer membrane that protects the encapsulated material (nucleic acids, proteins, metabolites and lipids) from the extracellular environment (33,51). Lipids not only act as essential components of exosomal membranes, which protect exosome contents from various stimuli in the circulating body fluids, but also are necessary players in exosome formation and release to the extracellular environment (33). Increasing evidence suggests that specific lipid components are enriched in exosome particles compared with their parental cells.

In contrast to those of proteins and nucleic acids, the functions of exosomal lipids in HCC development, progression, radioresistance and chemoresistance are less established. Increasing data suggest that lipid molecules in exosomes may

serve as potential cancer diagnostic biomarkers, as confirmed by data obtained in urine (52) and cancer cell lines (53). Exosomes derived from the oligodendroglial precursor cell line Oli-neu were enriched in cholesterol, and contained higher levels of sphingolipids (sphingomyelin and hexosylceramide) and lower levels of phosphatidylcholine than those found in the cell membrane (54). In previous studies on hepatitis and HCC, exosomes were found to play key roles in hepatitis E virus (HEV) egress; however, HEV infection did not modify the characteristics of exosomes produced by infected cells. Enrichment in phosphatidylserines and cholesterol in these exosomes could be critical for HEV entry into cells. Meanwhile, HEV particles in exosomes are protected from the attack of the immune system, which leads to wide spreading of HEV in the circulatory system of the host (55). A previous high-resolution lipidomic and proteomic study reported the analysis of exosomes and extracellular vesicles obtained by differential ultracentrifugation from the glioblastoma cell line U87, the HCC cell line Huh7 and human bone marrow-derived mesenchymal stem cells (MSCs). Exosomes enriched in glycolipids and characterized by the presence of free fatty acids, as well as exosomes from Huh7 cells and MSCs were specifically enriched in cardiolipins (56). Unlike circulating tumor cells, exosomes are highly abundant in biofluids and cells, and are diverse in vesicle type and lipid composition. Different from other single-molecule circulating biomarkers, exosomes protect their molecular cargo from degradation and may carry molecular signatures involved in specific phenotypes (57). Increased understanding of the role of exosomal lipids in HCC progression and radioresistance suggests that exosome-derived lipids have a considerable potential as HCC biomarkers and a tool to monitor radiotherapy in the future (26).

5. Nucleic acids derived from HCC exosomes

Exosome-derived nucleic acids are diverse, but the majority of the currently identified exosome-derived nucleic acids are RNAs, and few studies have focused on exosomal DNA in HCC.

DNA. Analyses of circulating cell-free tumor DNA and circulating tumor cells have paved the way for novel diagnostic approaches, and are the basis of diagnostic techniques based on liquid biopsies. The application of circulating cell-free tumor DNA on early cancer detection is of high public interest (58). Exosomal DNA has attracted broad interest in cancer research; however, few studies on HCC have been conducted to date (36,59). The majority of DNA associated with tumor exosomes has been confirmed to be double-stranded in three different cancer models: human chronic myeloid leukemia cell model K-562; human colorectal carcinoma cell model HCT116; and murine melanoma cell model B16-F10. This double-stranded DNA in exosomes represents the whole genomic DNA. Exosomal DNA has been confirmed to be used to identify mutations in parental tumor cells, which suggests its translational potential as a circulating biomarker of cancer in the clinic (36). A recent study on liquid biopsy samples of 194 patients, who were undergoing treatment for localized or metastatic pancreatic adenocarcinoma, showed a marked increase in exosomal DNA levels after neoadjuvant therapy, which suggested a close association with disease progression. Significantly shorter times of progression-free and overall survival were found in patients with metastases and detectable circulating tumor DNA (ctDNA) at baseline status compared with those of patients without detectable ctDNA (60). Plasma cell-free DNA (cfDNA) levels in patients with HCC and hepatitis B virus-related liver fibrosis were closely associated with the degree of liver inflammation, body mass index and α-fetoprotein level, but showed no association with liver fibrosis stage. Significantly elevated plasma cfDNA levels were detected in patients with HCC compared with those found in patients without HCC. The HCC index, which is generated by a combination model including age, and cfDNA and α-fetoprotein levels, had an area of 0.98 under the receiver operating characteristics curve for the diagnosis of HCC, suggesting that the diagnostic power of the HCC index was more promising than that of cfDNA or α-fetoprotein levels alone (61).

miRNA. Exosomal RNAs include miRNAs, lncRNAs, mRNAs, circRNAs and other non-coding ncRNAs (37). ncRNA families, including miRNAs, lncRNAs, circRNAs and other ncRNAs, are not functional to encode proteins, but function as specific RNA inhibitors by degradation or transformation to regulate post-transcriptional gene expression, which is associated with the control of nuclear architecture, transcription in the nucleus, and modulation of mRNA stability, translation and post-translational modifications in the cell cytoplasm (62,63).

miRNAs are small ncRNAs of 17-24 nt, which mediate post-transcriptional gene silencing by binding to the 3'untranslated or open reading frame region of target mRNAs (64). miRNAs have been detected in exosomes, which have been found to be taken up by neighbor or distant cells, and then modulate the activities of recipient cells. Accumulating evidence has shown that miRNAs can be stable in body fluids, including saliva, urine, breast milk and blood. Besides being packed into exosomes or other microvesicles, extracellular miRNAs can also be loaded into high-density lipoprotein (65) or be bound by argonaute-2 protein outside these microvesicles (66). All these three modes of action protect miRNAs from degradation and ensure their stability (67). Changes in the expression levels of miRNAs contribute to the pathogenesis of numerous human malignancies. These changes are potentially caused by multiple different mechanisms, such as gene mutations, amplifications or deletions involving miRNA loci, epigenetic silencing or dysregulation of transcription factors targeting specific miRNAs. Malignant cells depend on the dysregulated expression of multiple miRNAs, and in turn control or are controlled by dysregulation of multiple protein-coding oncogenes or tumor-suppressor genes, which suggests that these small RNAs may provide opportunities for the development of novel miRNA-based therapies (68). Circulating exosomal miRNA-21 has been associated with TNM stages and other prognostic factors, including T stage and portal vein thrombosis, in HCC. High miRNA-21 level was reported to be a promising predictor of increased mortality and disease progression, as well as larger tumor size and higher C-reactive protein levels. Patients with higher circulating levels of exosomal miRNA-21 exhibited significantly lower overall

and progression-free survival (69). Another study found that the migration, invasion and proliferation of recipient HCC cells were significantly increased after treatment with exosomes secreted by HCC cells cultured in acidic medium. miR-21 and miR-10b were the most critically functional miRNAs in acidic HCC cell-derived exosomes. Activation of hypoxia-inducible factor (HIF)-1α and HIF-2α was facilitated by the acidic microenvironment, which also stimulated the expression and secretion of exosomal miR-21 and miR-10b, substantially promoting HCC cell proliferation, invasion, migration and proliferation both *in vivo* and *in vitro*. Advanced tumor stage, and HIF-1α and HIF-2α expression in patients with early HCC were closely associated with serum exosomal miR-21 and miR-10b levels, suggesting that exosomal miR-21 and miR-10b were independent prognostic factors for disease-free survival of patients with early HCC (70). A previous study demonstrated that the levels of 19 miRNAs were markedly upregulated in the sera of patients with HCC (71). A following study demonstrated that HCC cells secreted miR-210 via exosomes. The *in vitro* tubulogenesis of endothelial cells and the *in vivo* angiogenesis of HCC were significantly promoted by exosomal miR-210 by inhibiting the expression levels of SMAD4 and STAT6 in the recipient endothelial cells (72). Another study focused on the functions of exosomal miR-224 in the development of HCC, and assessed its diagnostic and prognostic value in HCC. Patients with HCC who had high expression levels of serum exosomal miR-224 exhibited a lower overall survival, suggesting that exosomal miR-224 is potentially a tumor-promoting gene, and may serve as a biomarker for diagnosis and prognosis in patients with HCC (73). In addition to these studies, a number of other exosome-derived miRNAs have been found to be closely associated with the pathogenesis and progression of HCC. Their potential roles include being biomarkers for HCC diagnosis and serve as therapeutic targets for HCC treatment.

lncRNAs. lncRNAs, another type of ncRNAs with a size >200 nt that lack protein-coding functions, have been reported to be critical modulators of intercellular communications (74). Cancer cell-derived exosomes have been confirmed to contain various lncRNAs, usually defined exosomal lncRNAs, which regulate the cancer microenvironment by modulating multiple cellular functions, including regulation of the transcription of certain critical genes that determine cancer growth and progression, thus defining the biological functions of cancer exosomes (75,76). Furthermore, deregulation of lncRNA expression has been identified in multiple human tumors, and lncRNAs may serve as attractive diagnostic biomarkers to predict different cancer types (77).

Similar to miRNAs, exosomal lncRNAs are also important contributors to the occurrence, development and transferring of HCC. A stress-responsive lncRNA, long intergenic non-protein coding RNA, regulator of reprogramming (linc-ROR), was significantly upregulated in HCC cells and was also found to be enriched in tumor cell-derived extracellular vesicles. Incubation with HCC-derived extracellular vesicles significantly increased the expression of linc-ROR and blunt chemotherapy-induced cell death in recipient cells (78). Incubation with linc-very low density lipoprotein receptor (VLDLR)-enriched exosomes reduced chemotherapy-induced cell death in HCC cells, and increased the expression of linc-VLDLR in recipient cells (79). The expression levels of lncRNA-focally amplified lncRNA on chromosome 1 (lnc-FAL1) were significantly upregulated in HCC tissues, and lnc-FAL1 was found to function as an oncogene in HCC (80). In serum exosomes of patients with HCC, the levels of lnc-FAL1 were upregulated, and transferring lnc-FAL1 to HCC cells increased their cell migration and proliferation abilities (81).

Numerous studies have reported HCC-derived exosomes as cargo of novel lncRNA biomarkers. Exosomes derived from non-HCC serum contained a large number of lncRNAs with a high level of alternative splicing compared with those found in patients with other hepatic diseases. Exosomal lncRNAs, including lnc-G protein-coupled receptor 89B-15 (lnc-GPR89B-15), lnc-family with sequence similarity 72 member D-3 (lnc-FAM72D-3), lnc-enhancer of polycomb homolog1-4 (lnc-EPC1-4) and lnc-zinc finger E-box binding homeobox 2-19 (lncZEB2-19), showed differential expression in the serum of patients with HCC (82). Another study revealed that five lncRNAs, namely CTD-2116N20.1, AC012074.2, RP11-538D16.2, long intergenic non-protein coding RNA (LINC) 00501 and RP11-136I14.5, showed significantly different expression. Co-expression and competing endogenous (ce)RNA network analyses revealed possible mechanisms of CTD-2116N20.1 and RP11-538D16.2 as exosome-related lncRNAs, and CTD-2116N20.1 and RP11-538D16.2 were correlated with poor prognosis in patients with HCC (83). The expression of lncRNA-HEIH (lnc-HEIH) was increased in the serum and exosomes of patients with hepatitis C virus-related HCC compared with the findings in patients with chronic hepatitis C (84). High levels of ENSG00000258332.1 in HCC were associated with overall survival, portal vein tumor emboli, TNM stage and lymph node metastasis, and high levels of LINC00635 were closely associated with overall survival, TNM stage and lymph node metastasis (85). Another study evaluated the relative expression levels of 8 selected serum lncRNAs in the training and validation sets of patients with HCC and matched healthy controls. The expression of LINC00161 was markedly upregulated, and exhibited high stability and specificity in serum samples of patients with HCC, indicating the biomarker potential of LINC00161 in HCC diagnosis and prognosis (86). The expression level of X-inactive-specific transcript (Xist), another lncRNA, was significantly increased in peripheral blood mononuclear cells and granulocytes of patients with HCC. Another lncRNA, Jpx, also showed increased upregulation in mononuclear cells, granulocytes and exosomes of female patients with HCC. Delivery of Jpx from HCC cells to blood cells via exosomes activated Xist expression in blood cells, possibly by inhibiting the *trans*-regulatory effects of CCCTC-binding factor (87). A previous study also identified Xist as a mediator of miRNA-92b, which promoted HCC progression by targeting SMAD7 (88). Another commonly reported HCC-associated lncRNA is TUC339. The most highly significantly expressed lncRNA in extracellular vesicles derived from HCC cells was identified as TUC339 in a previous study. Functionally, TUC339 was found to be involved in regulating tumor cell adhesion and growth (89). Compared with those from healthy controls, exosomes derived from HCC cells contained upregulated levels of TUC339, and HCC-secreted exosomes could be taken up by THP-1 cells. These THP-1 cells also

showed a significant increase in pro-inflammatory cytokine production, increased co-stimulatory molecule expression and enhanced phagocytosis upon inhibition of TUC339 (90). LncRNA acetylserotonin O-methyltransferase like antisense RNA 1 (ASMTL-AS1) was found to be upregulated in HCC tissues, and exhibited increased expression in tumors after radiofrequency ablation (RFA) insufficiency. The expression levels of ASMTL-AS1 were found to be closely associated with disease stage, metastasis and prognosis of HCC, and were found to contribute to the malignancy of HCC cells. ASMTL-AS1 could be packaged by exosomes and then contribute to the malignancy of HCC cells via the nemo-like kinase (NLK)/yes-associated protein (YAP) regulatory axis between cells even in residual HCC after RFA insufficiency (91). High expression of exosomal H19, an exosomal lncRNA, could enhance the motility and proliferation of HCC cells while reducing their apoptosis through binding to and sponging miR-520a-3p (92).

lncRNAs may play a role in HCC recurrence. A set of deregulated lncRNAs showed potential to differentiate HCC from cirrhotic tissue. Among these lncRNAs, cancer susceptibility 9 (CASC9) and lung cancer associated transcript 1 (LUCAT1) showed upregulation in a subset of HCC-derived cell lines and in certain HCC tissues, whose donors exhibited decreased recurrence after surgery. LUCAT1 was found to directly sponge the onco-miR-181d-5p, a novel regulatory element of liver cancer stem/progenitor cells. Both lncRNAs, LUCAT1 and CASC9, were detected in secreted exosomes, and upregulated circulating CASC9 levels were closely associated with tumor size and HCC recurrence after surgery in human patients (93).

circRNAs. circRNAs, first identified in RNA viruses in 1976 by electron microscopy (94), are another type of ncRNAs that form a covalently circled continuous loop via the process of back-splicing, during which the downstream splice donor site is joined with the upstream splice acceptor site (95). Different to common linear RNAs, circRNAs are characterized by featuring a covalently circled continuous loops without 5' or 3'polarities (96). Previous studies have demonstrated that circRNAs are capable of absorbing miRNAs by stably competitively binding and play a role as sponges of miRNAs to regulate gene expression (97). Numerous studies have demonstrated that circRNAs are abundant, stable and one of the major components of exosomes (98). Increasing evidence has indicated that dysregulation of exosomal circRNAs contributes to tumorigenesis and progression in human liver cancer (99).

circRNAs secreted by adipose tissue were found to regulate deubiquitination in HCC cells to facilitate cell growth. Exosome circ-de-ubiquitination (circ-DB) levels were significantly upregulated in patients with HCC who had higher body fat ratios. circ-DB has been demonstrated to promote HCC cell growth and inhibit DNA damage by suppressing miR-34a expression and upregulating the de-ubiquitination of ubiquitin specific protease 7 (USP7). The effects of adipose-secreted exosomes on HCC cells could be reversed by downregulation of circ-DB. These findings indicated that exosome circRNAs secreted from adipocytes promoted tumor growth and reduced DNA damage via miR-34a suppression and activation of the USP7/cyclin A2 signaling pathway axis in HCC cells (100). CircRNA circ-0051443 was significantly downregulated in the tissues and plasma exosomes of patients with HCC compared with the levels found in those of healthy controls, and was mainly packaged into exosomes in HCC cells. HCC cells could be distinguished from healthy control cells by the presence of circ-0051443 in exosomes. Transmitting circ-0051443 from healthy normal cells to HCC cells via exosomes significantly suppressed the malignancy of HCC cells via cell apoptosis increase and cell cycle arrest (101).

Numerous studies have reported critical roles of exosomal circRNAs in HCC progression. The level of another circRNA, circ-tripartite motif containing 33-12 (TRIM33-12), was also decreased significantly in HCC tissues and cell lines. circ-TRIM33-12 downregulation in HCC was closely associated with HCC malignant behavior, and served as a promising risk factor of recurrence-free and overall survival in patients with HCC after surgery (102). A recent study characterized the facilitation of MET expression by exosome circ-prostaglandin reductase 1 (PTGR1) competing with the seed sequence of miR-449a, a miRNA with roles in the inhibition of tumor growth and metastasis in HCC (103). Further mechanistic analyses confirmed that three different isoforms of exosomal circPTGR1 promoted HCC metastasis through the miR-449a/MET pathway (104). The level of circTMEM45A was markedly reduced in HCC. circTMEM45A levels were closely associated with clinicopathological features as well as decreased prognosis of patients with HCC. Clinically, circTMEM45A expression levels were markedly increased in exosomes from patients with HCC (105). Several studies have demonstrated that exosomal circRNAs, particularly those derived from pathogenic exosomes, may have an important influence on the pathophysiological stage and processes of HCC; however, only a limited number of circRNAs have been reported to establish critical functions or clinical applications (26). Exosomal circRNAs are likely to attract more interest in HCC research.

mRNAs. mRNAs are mediators of genetic information transferred from the cell nucleus to ribosomes in the cytoplasm, in which mRNAs serve as templates for protein coding and synthesis (106). Although relatively few studies have focused on exosomal mRNAs, recent reports have been published on the promising and increasing functions of exosomal mRNAs in cancer development and prognosis (18,107), particularly in liver disease and cancer (108). An increasing number of exosomal mRNAs have been reported to be potential biomarkers in HCC diagnosis. By using bioinformatics, a recent study identified an HCC-exosomal RNA-based biomarker selection panel, which was mostly associated with RAB11A gene expression and its competing endogenous network, including lncRNA-RP11-513I15.6 and miR-1262. The expression levels of this network were validated in the sera of 60 patients with HCC, and were compared with those found in 42 patients with chronic hepatitis C virus infection and 18 healthy donors. Panels of three novel exosomal RNA-based genes, including RAB11A, miR-1262 and lncRNA-RP11-513I15.6, displayed good sensitivity and specificity in predicting and differentiating patients with HCC from patients with chronic hepatitis C virus infection and healthy donors. Of these three RNAs, the

RAB11A mRNA levels in serum were the most independent and promising prognosis factor (109). Another cohort study on patients with HCC, liver cirrhosis, chronic hepatitis B and healthy controls quantified serum exosomal heterogeneous nuclear ribonucleoprotein H1 (hnRNPH1) mRNA, and found that the hnRNPH1 mRNA levels in serum exosomes from patients with HCC were markedly upregulated compared with those in the other groups. The mRNA levels of hnRNPH1 discriminated patients with HCC from patients with chronic hepatitis B. Furthermore, the hnRNPH1 mRNA levels in serum exosomes from patients with HCC were closely associated with portal vein tumor emboli, lymph node metastasis, Child-Pugh classification, overall survival and TNM stage. These data indicated that hnRNPH1 mRNA levels in serum exosomes were potentially a promising biomarker for HCC diagnosis in areas with a high prevalence of patients with hepatitis B virus infection (110). A recent study enrolled a cohort consisting of healthy donors (n=159), as well as patients with benign hepatic tumors (n=24), hepatitis (n=11), hepatic cirrhosis (n=8), HCC (n=104), and breast (n=10), gastric (n=9), kidney (n=15) and colorectal cancer (n=12). The authors detected >10,000 extracellular vesicle-derived long RNAs, including mRNAs, circRNAs and lncRNAs, in plasma. Among these RNAs, a majority of the total mapped reads were mRNAs. Blood extracellular vesicles contained a substantial fraction of intact mRNAs, and 8 extracellular vesicle-derived long RNAs were suggested to serve as potential biomarkers for HCC diagnosis in areas with high diagnostic efficiency (108).

6. Exosomes in the tumor microenvironment of HCC

Tumor progression depends on the complexity and heterogeneity of the tumor microenvironment, which consists of a network of cellular and acellular constituents. Increasing evidence reveals that this process is also impacted by exosome-mediated cross-talks or communications within the tumor microenvironment (111). Communications between HCC cells and their microenvironmental cell components are necessary mechanisms that modulate tumor development and progression. As one of the major components of the ncRNA exosomal cargo, miRNAs play multiple critical roles in modulating cellular pathways in targeted cells and microenvironments, and in regulating multiple processes associated with tumor progression, invasion and metastasis, including EMT, invasion, angiogenesis, multi-drug resistance and immune escape. Increasing evidence suggests that exosomal miRNAs function as important players in the dynamic cross-talk among cancerous, stromal and immune cells to establish the microenvironment of tumorigenesis (112). In the HCC microenvironment, exosomes participate in multiple steps during tumor progression and radioresistance, and numerous cell types (particularly stromal and inflammatory cells) within the microenvironment are involved in these processes (113). Exosomes have been reported to be critical in mediating the regulation of the inflammatory microenvironment to promote cancer progression and metastasis (114). HCC cells exposed to arsenite secreted miR-155-rich exosomes that enhanced inflammation and were positively correlated with IL-6 or IL-8 levels (115). The bidirectional communication between HCC and its exosome-mediated inflammatory microenvironment was also confirmed by a previous study, which reported that the inflammatory microenvironment promoted tumorigenesis while the tumor also created an inflammatory environment that promoted its own development. The β1-integrin/NF-κB signaling pathway in cancer-associated fibroblasts (CAFs) was activated by exosomes with miR-1247-3p secreted by high-metastatic HCC cells via directly targeting β-1,4-galactosyltransferase 3. These activated CAFs further promoted pleiotropic actions, including the secretion of proinflammatory cytokines, such as IL-6 and IL-8, that governed tumor progression (116). Exosomes with miR-320a overexpression derived from CAFs showed potential to inhibit tumorigenesis, thus suggesting a possible cause of HCC progression mediated by CAFs by deficiency in antitumor miR-320a in CAF-derived exosomes (117). CAFs could transfer miR-320a to HCC cells via exosomes and then inhibit EMT, while loss of antitumor miR-320a in CAF-derived exosomes could induce EMT and promote tumor progression. In solid HCC tumors, a number of the main components of the extracellular matrix, such as collagens, laminins, fibronectin, glycosaminoglycans and proteoglycans, play crucial roles in changing the phenotypic and functional characteristics of HCC and stroma cells via various mechanisms, including exosome-involved interaction (118). Beside these studies, additional evidence in recent years has implied the roles of exosomes in establishing and modifying the HCC microenvironment in a manner that enhances tumor progression, metastasis and tumor resistance.

7. Other extracellular vesicle subtypes in HCC progression and radioresistance

Besides exosomes, which are well studied, the other two major extracellular vesicle subtypes, microvesicles and apoptotic bodies (which are differentiated based upon their biogenesis, release pathways, size, content and function) (119), are less studied in the context of the development, progression and radioresistance of HCC. Microvesicles derived from human liver stem cells induced the *in vitro* proliferation and apoptosis resistance of hepatocytes by transporting mRNA into hepatocytes (120). Microvesicles derived from human adult liver stem cells may reprogram *in vitro* HepG2 liver cancer and primary HCC cells by inhibiting their growth and survival. *In vivo* intratumor administration of microvesicles induced the regression of ectopic tumors developed in SCID mice by a mechanism involving the delivery of miRNAs from human adult liver stem cell-derived microvesicles to tumor cells according to various studies. The antitumor effect of adult liver stem cell-derived microvesicles was also observed in tumors other than liver, such as lymphoblastoma and glioblastoma (121). These studies suggest that the delivery of selected miRNAs by microvesicles derived from stem cells may inhibit tumor growth and stimulate apoptosis. Apoptotic bodies, another major class of extracellular vesicles released as a product of apoptotic cell disassembly as well as insufficient apoptosis, have been associated with the development and progression of tumors of the liver and biliary tree (122). Accumulating evidence suggests a defective apoptotic process in human HCC, and numerous potential therapeutic strategies that

induce the apoptotic process in various ways have the potential to aid the management of HCC (123).

8. Conclusions and perspectives

Exosomes are emerging as novel modes of intercellular communications in human homeostasis and diseases. Exosomes act as bioactive components that transfer proteins, lipids, DNA, mRNAs, ncRNAs and recently identified molecules from contributor to recipient cells, which leads to the exchange of genetic information and the transcriptional re-programming of recipient cells (22,124). Circulating exosomes, as well as their main components, may potentially be used as liquid biopsies, and are promising biomarkers for early detection, diagnosis and multi-therapy in patients with cancer (125), such as HCC (59,126). Clinically, HCC is considered a radioresistant tumor, and increasing evidence suggests a critical role of HCC-derived exosomes in radioresistance (13,127), due to their influence on tumor initiation, growth, progression, metastasis, drug radioresistance and recurrence. Despite these adverse conditions in HCC therapy, as increasing novel technologies are developed and to monitor and modify exosomes in cancer progression and radiotherapy, exosome-associated HCC diagnosis, therapies and post-surgery prediction are becoming more promising, and exosome-containing molecules are valuable in HCC research as novel biomarkers.

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Authors' contributions

QF, YY and CW designed and wrote the manuscript. YZ and SZ collected and summarized the related papers and references. CW revised and conceived the final approval of the version to be submitted and obtaining of the funding.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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