MiniReview

Progress of Exosome in Hepatocellular Carcinoma

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Abstract

An exosome is a type of tiny vesicle characterized by a lipid bilayer membrane structure, with a diameter ranging from 30 to 100nm. Exosomes can be secreted by nearly all cells, containing cell-specific proteins, lipids, nucleic acids, and other components. They play a crucial role in transmitting signal molecules to target cells, thereby influencing their biological functions. The investigation of exosomes’ role in hepatocellular carcinoma (HCC) has garnered significant attention. As exosome content accurately reflects the characteristics of their cell of origin, enabling the differentiation between normal and diseased tissues, and considering their widespread presence in the human body and ease of accessibility, exosomes hold promise as potential biomarkers for the detection, diagnosis, and treatment of HCC. This paper provides a comprehensive review of the potential biological effects of exosomal miRNAs in the development of HCC and explores the associated roles of exosomes in this context.

Keywords: exosome, miRNA, hepatocellular carcinoma, biomarker

1 INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, jeopardizing public health worldwide. Primary liver cancer can be divided into HCC, intrahepatic cholangiocarcinoma, and mixed HCC according to its cellular origin. HCC is the most common type of liver cancer, accounting for about 80%-90% of all primary liver cancers[1]. The insidious onset of HCC, in which most patients are detected in the middle to late stages of liver cancer, makes clinical treatment difficult and passive, and its prognosis is often unsatisfactory. Currently, the gold standard for the diagnosis of HCC is pathologic biopsy, which, despite its high sensitivity and specificity, is usually not accepted by patients and clinicians because it is an invasive test. In addition alpha-fetoprotein (AFP) is currently considered an ideal serum protein marker for the diagnosis of HCC. Some studies have shown that the sensitivity of the AFP test in the diagnosis of HCC is about 60% and the specificity is only 80%[2,3]. Therefore, it is particularly important to find a specific, sensitive and non-invasive biomarker.

Exosomes are tiny vesicles located extracellularly with a lipid bilayer structure first identified by Johnstone et al.[4] in 1989 in studies of reticulocytes. Exosomes are endosomal in origin, with a diameter of about 30 to 100nm and an average diameter of about 100nm[5], which exist in a variety of body fluids such as blood, urine, and amniotic fluid.
Many studies have shown that exosomes are novel non-invasive biomarkers for cancer detection. Compared to other indicators, exosomes are stable in blood and other body fluids and have the advantage of being minimally invasive and easy to sample. The liver is one of the most essential organs of the human body, and many hepatocytes can either secrete exosomes or act as target cells for exosomes, such as biliary epithelial cells, hepatic stellate cells, monocyte macrophages, lymphocytes, etc.[6,7]. Exosomes can be involved in cellular communication between the liver and other organs, contributing to processes such as inflammation, immunomodulation, fibrosis, and angiogenesis.[8] It has been confirmed that exosomes are involved in the pathological progression of precancerous lesions in the liver, making them a potential crucial target for the treatment of such lesions. Exosome formation can be divided into two stages: the first stage is the first invagination of the plasma membrane to form a cup-shaped structure that includes cell surface proteins and soluble proteins associated with the extracellular environment and leads to the formation of early-sorting endosomes (ESEs); the second stage is that the ESEs can mature into late-sorting endosomes, which are formed into multivesicular vesicles (MVBs) (which contain several intraluminal vesicles) by double invagination of the plasma membrane.[9,10]. The fusion of MVBs with the plasma membrane releases the contained luminal vesicles to act as exosomes. MVBs can also fuse with lysosomes or autophagosomes to digest and degrade the contained substances, thus cycling these substances. Exosomes play a crucial role in cellular communication as carriers. They deliver bioactive substances to receptor cells, altering their biological responses.[11]. A growing number of laboratory and clinical studies have shown that abnormalities in exosome secretion and function play an integral role in malignant tumorigenesis, progression, and treatment. Specific enrichment proteins (e.g., CD9, CD63, CD81, and CD82) are widely used as exosome markers. The current method for extracting exosomes primarily involves ultracentrifugation. In addition, there are also commercial kits available for the isolation of exosomes.

2 EXOMES REGULATE THE DEVELOPMENT OF HCC

Tumorigenesis is the transformation of normal cells into tumor cells. The transfer of various genes into tumors via exosomes is one of the main causes of oncogenic transformation and the spread of malignant biological behavior. Recently, increasing attention has been paid to circRNAs stored in HCC cell exosomes. Exosomal circUHRF1[12], exosomal circPTGR1[13], exosomal circRNA-100,338[14] and exosomal circ MMP2[15] play important roles in HCC cell communication and in assisting cancer metastasis. Circ_002136 was first identified in gliomas, where it has a predominantly oncogenic function, activating glioma cell growth and migration.[16]. Yuan et al.[17] found that circ_002136 had a similar distribution pattern in both HCC cell lines and was enriched in both intracellular and exosomes. Circ_002136 ablation slightly inhibited cell growth and metastasis in vitro and increased apoptotic capacity. However, the addition of exosomes offset this effect, possibly by incorporating circ_002136 into exosomes, reintroducing it into tumor cells and disrupting its biological activity.

A number of circRNAs have been identified that can bind and associate with important miRNA molecules involved in oncogenic transformation to alter cancer progression. The inhibitory effect of circ_002136 on miR-19a-3p activity has been identified, and the coexistence of circ_002136 and miR-19a-3p partially restores cell function and partially prevents the effect of circ_002136 on HCC progression. Extensive research has also long shown that miR-19a-3p is associated with various cancer phenotypes, which usually include cancer metastasis and drug resistance[18-20].

More importantly, high levels of miR-19a-3p may also be an independent prognostic factor in cancer patients. MiRNAs function as active regulatory elements that destabilize or manipulate the translation of target miRNAs. MiR-19a-3p is often the target of different genes in different cancers, suggesting a complex network regulation pattern. RAB1A has been identified as a gene encoded by miR-RAB1A. RAB1A belongs to the Rab1 protein isoform family and is an important protein for vesicle trafficking between the endoplasmic reticulum and the Golgi apparatus.[21]. In cancer development, aberrant expression of RAB1A has been associated with the development of various cancers, such as colon cancer[22], lung cancer[23] and human gliomas[24].

Exosomes can initiate and promote the progression of hepatic fibrosis-cirrhosis-HCC[25-27]. MiR-222, an exosome derived from hepatitis B virus-infected hepatocytes, promotes hepatic fibrosis by inhibiting the transferrin receptor-induced iron death. In contrast, in a rat model of cirrhosis, 400μg of rat bone marrow mesenchymal stem cell-derived exosomes inhibited hepatocyte pyroptosis, alleviated cirrhosis, and enhanced hepatic function. Exosomes may also contribute to developing HCCs by increasing secretion or participating in intercellular communication. Exosomes RAB11A mRNA and IncRNA-RP11-583F2.2 were increased in liver tissue and serum of patients with hepatocarcinogenic precancerous lesions. After inducing HCC in rats, the cytoplasm of hepatocytes formed multiple tiny nano-vesicles, which then aggregated into multivesicular bodies and detached from the hepatocytes. Hesperidin inhibited HCC by decreasing the exosomal RAB11A mRNA and IncRNA-RP11-583F in liver tissue and serum[28].

Therefore, exosomes may be an essential target for treating HCC. Exosomes in HCC cells are produced by...
HCC cells, and the RNA and proteins in the exosomes are different from those in normal cells\(^{[29,30]}\). These exosomes can be ingested and internalized by other cells, delivering genes with a specific function. Similar to the serum tumor markers that can be used in screening HCC, exosomal miRNAs can be used as a valuable non-invasive tool for treating HCC\(^{[31]}\). As a valuable non-invasive biomarker to differentiate the type and grade of liver inflammation and to assist in the early diagnosis of HCC, studies have shown that circulating miRNAs could be a biomarker for the diagnosis of HCC due to the large number of miRNA variants in HCC cells.

3 EXOSOMES PROMOTE THE METASTASIS OF HCC

Several studies have shown that exosomes have a significant impact on the metastasis and progression of HCC. Exosomes induce physiological changes in cells by facilitating the transport of molecules, thereby affecting cell differentiation and migration. Exosomes exert their effects through a variety of mechanisms, including directly stimulating tumor growth, conferring migratory and invasive potential on cells with limited or no metastatic potential, inducing epithelial-mesenchymal transition (EMT), restoring the pre-metastatic microenvironment, and promoting angiogenesis to create an environment favorable for metastatic potential. The effects are exerted by a variety of mechanisms. Previous studies have shown that MET proto-oncogenes, S100 family members, and cabolin mobilize normal hepatocytes and participate in the migration and progression of HCC. In addition, interleukin-6 and Golgi 1 have been shown to play an important role in the invasion of HCC through exosome release. Several studies have reported that S100A4 and CircPTGR1 have been identified as major components of exosomes derived from highly metastatic HCC cells. These components have been observed to affect neighboring cells and contribute to the metastatic potential of less metastatic HCC cells.

EMT is a biological process of cell transformation characterized by the loss of epithelial properties and the acquisition of mesenchymal properties, which allows cells to acquire metastatic and invasive capabilities, promoting tumor progression and metastasis. Exosomal cyclic RNA hsa_circ_003288 affects miR-145, which regulates PD-L1 expression and may promote EMT, migration, and invasion of HCCs. The exosome circ_MMP2 (also known as has_circ_0039411), which acts as a sponge for miR-136-5p, resulted in overexpression of metalloproteinase 2 from the host genetic matrix. This interaction has been shown to lead to HCC progression and is associated with worse overall survival in HCC patients. Overexpression of miR-4669 leads to increased migration capacity of HCC cells, conferring resistance to sorafenib treatment, which is accompanied by upregulation of sirtuin 1.

4 EXOSOMES AND DRUG RESISTANCE

Drug resistance in tumor cells is a major cause of chemotherapy failure in patients with HCC, especially in patients with advanced HCC. Sorafenib, adriamycin and platinum are the traditional systemic or local chemotherapeutic agents for HCC, although there is a high rate of resistance to them. As an important component of cell-to-cell communication, exosomal circRNAs can interact with cells in different tumor microenvironments and act as miRNA sponges or protein scaffolds, e.g., to regulate antitumor drug resistance and affect tumor progression\(^{[33,34]}\). Recently, it has been reported that exosomes can transfer circRNAs from drug-resistant cells to susceptible cells and participate in the regulation of cancer drug resistance through multiple mechanisms. In HCC cells resistant to sorafenib, circSORE promotes the resistance of HCC cells to sorafenib via exosomes\(^{[35]}\). Mechanistically, sorafenib-resistant circRNA (circSORE) in HCC cells binds to the large oncogenic protein Y box binding protein 1 (YBX1) found in the cytoplasm and blocks the nuclear interaction between YBX1 and the E3 ubiquitin ligase upstream pre-RNA processing factor 19 (PRP19), thereby preventing PRP19-mediated degradation of YBX1. In addition, sorafenib resistance was significantly overcome in various HCC mouse models by circSORE silencing with small interfering RNA injection, providing a potential strategy to clinically reverse sorafenib resistance. The above studies showed that exosomal circRNA induces drug resistance in HCC cells. Therefore, these identified exosomal circRNAs may be potential therapeutic targets to overcome drug resistance. Influencing the synthesis and secretion of exosomes and their uptake into target cells can reduce resistance to tumor drugs and increase clinical benefits for patients.

Exosomes may also play a role in tumor resistance, as drugs can stimulate exosome formation in tumors and promote drug leakage through exosomes\(^{[36]}\), increasing the likelihood of new resistance mechanisms. Furthermore, drug resistance may also be related to cellular autophagy\(^{[37,38]}\). As shown by Liang et al.\(^{[39]}\), the cellular stress transcription factor FOXO3a plays a role in the regulation of cell proliferation, apoptosis, stress response and metabolic
pathways, and FOXO3a-dependent activation of autophagy-related gene transcription and autophagy flux is a key mechanism mediating hypoxia-induced resistance of HCC cells to sorafenib. Inhibition of FOXO3a suppresses sorafenib-induced hypoxia and significantly improves the efficacy of sorafenib. However, these studies have not been thoroughly described and require further investigation. In conclusion, the identification of exosomal circRNA markers that can predict the development and changes in drug resistance is crucial for the accurate diagnosis and treatment of tumors, particularly when drug resistance emerges during tumor therapy.

5 IMMUNOTHERAPY OF EXOSOMES

Immunotherapy of exosomes in HCC The liver has a unique immune-tolerant microenvironment, which poses a significant challenge for immunotherapy of HCC. Many studies have shown that exosome-derived cell-cell interactions can modulate the immune microenvironment. For example, exosomes can induce specific cytotoxic T-lymphocytes in vivo and inhibit the growth of established nude mice tumors in a T cell-dependent manner. HCC-associated tumor cell-derived exosomes (TEX) can efficiently carry antigens to dendritic cells (DCs), generating a more robust immune response than cancer cell lysates. TEX-pulsed DCs (DCTEX) significantly inhibited tumor growth in both ectopic and in situ HCC mice compared to DCs pulsed with cell lysates.

In conclusion, in situ HCC mice, DCTEX treatment could further improve the immune and tumor microenvironment by decreasing interferon and T-lymphocytes while decreasing tumor growth factor and interleukin-10 at the tumor site. Not only that, but the exosomes could synergize with antitumor drugs. The application of drugs such as 5-Aza-CdR and the epigenetic drug MS-275 resulted in an increase in tumor-associated antigens and immune-associated molecules in HCC-derived exosomes, enhancing the antitumor effects. These results suggest that HCC-derived exosomes could be used to treat HCC, providing new clues for the immunotherapy of HCC.

6 CONCLUSION AND PROSPECTS

In conclusion, due to the ability of exosomes to reflect the disease state and the role of cellular communication, they can be used as biomarkers for the diagnosis, recurrence, and prognosis of HCC and are expected to be used in the treatment of HCC. The study of exosomes is still in its infancy, moreover, the composition of exosomes from different cells, body fluids and different stages of liver cancer patients are different. In the future, large sample studies are needed to screen exosome components with high specificity and sensitivity as biomarkers. Secondly, exosomes have been identified as other somatic biomarkers in addition to blood. Exosomes have been identified as biomarkers in other body fluids, such as saliva, breast milk, etc. Exosomal PD-L1 mRNA from saliva can distinguish periodontitis from healthy groups, and its level correlates with the severity of periodontitis. Breast milk exosomes containing high levels of TGF2 lead to changes in the epithelial cells of both benign and malignant mammary glands, which contributes to the development and progression of breast cancer. Exosomes in these fluids are more organotypic than those in the blood. Exosomes in these body fluids are more organ-specific and sensitive than those in blood, and detecting exosomes in the peritoneal fluid or bile of patients with HCC is an up-and-coming area for further studies. Although some progress has been made in research on exosomes, there are challenges and difficulties ahead. Firstly, there is no ideal high-purity and high-efficiency exosome isolation strategy, resulting in less reproducible and convincing results. Second, how cancer cells release specific exosomes to maintain HCC plasticity and metastasis. Finally, determining which exosome sources to apply targeted therapies. In conclusion, exosome application is an attractive research area that still needs to be explored in terms of development, diagnosis and therapeutic efficacy in HCC.

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Conflicts of Interest
The author declared no conflict of interest.

Author Contribution
Liu L conceptualized and crafted the manuscript, meticulously reviewing and refining it. Liu L contributed to the manuscript and approved its final version.

Abbreviation List

- AFP: Alpha-fetoprotein
- DCs: Dendritic cells
- DCTEX: TEX-pulsed DCs
- EMT: Epithelial-mesenchymal transition
- ESEs: Early-sorting endosomes
- HCC: Hepatocellular carcinoma
- MVBs: Multivacuolated vesicles
- PRP19: Pre-RNA processing factor 19
- TEX: Tumor cell-derived exosomes
- YBX1: Y box binding protein 1

References


