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Editorial on "Exosome-mediated transfer of circRNA563 promoting hepatocellular carcinoma by targeting the microRNA148a-3p/metal-regulatory transcription factor-1 pathway"

Papadopoulos N *et al*. Exosome-mediated transfer of circRNA563 promoting HCC

Nikolaos Papadopoulos, Eleni-Myrto Trifylli

Abstract

In this editorial, we comment on the article by Lyu *et al* published in the recent issue of the *World Journal of Gastroenterology* 2023; 2219-2840. Hepatocellular carcinoma (HCC) is a frequently encountered and highly aggressive primary liver cancer, which remains the third-commonest cause of cancer-related death despite the current therapeutic modalities. There is urgency in developing novel therapeutic approaches, such as by manipulating extracellular vesicles, which constitute a highly heterogeneous nanoparticle population that contains various cargoes. These cargoes have a pivotal role in cell-to-cell communication and can modify the functional level of the recipient cells *via* their uptake by other recipient cells. There is a particular evolving significance of exosomal- non-coding RNA in HCC, such as circular RNAs, which have been found differentially expressed in normal hepatic and HCC tissue. The aberrations in their expression levels have a key role in the HCC development and progression and the overall prognosis. In this editorial, we will shed light on the emerging role of exosomal-circular RNA in HCC development and progression, focusing on the oncogenic or potentially tumor suppressive effect of mesenchymal stem cells-derived exosomal non-coding RNA.

Key Words: Exosomes; Hepatocellular carcinoma; Non-coding RNAs; Circular RNA; Tumor microenvironment; Anti-tumor immunity; Mesenchymal-stem cells

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Core Tip: Shedding light on the role of exosomal-circular RNAs s as microRNA sponges and their potential targeting for suppressing hepatocellular carcinoma growth and progression.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a frequently encountered and highly aggressive primary liver cancer, characterized by a high amount of cancer-related deaths worldwide, constituting the third most common cause[1]. Despite the novelties in the diagnostic and therapeutic strategies, including immunochemotherapy, targeted and local treatments, radiotherapy, and surgical approaches, overall survival remains low due to chemoresistance and tumor recurrence[2]. There is a call for the discovery and development of novel therapeutic approaches, with the understanding of the molecular complexity of this malignancy being pivotal. The role of extracellular vesicles (EVs) and non-coding RNA molecules and their implication in HCC development and progression are in the spotlight of their ongoing studies, aiming to discover novel therapeutic targets and strategies[3].

EVs constitute a highly heterogeneous population of nanostructures composed of a lipid membrane (lipid bilayer) with several enclosed cargoes[4]. Their heterogeneity is attributed to their various sizes, biogenetic mechanisms, and the diversity of their cargoes, including coding or non-coding RNA molecules, proteins, lipids, several ligands, DNA molecules, and autophagosomes. A wide variety of cells can produce these vesicles *via* different mechanisms, including the inward or outward budding of the cell membrane or apoptosis, which lead to exosome, microvesicle, and apoptotic bodies generation, respectively[5]. The main classification of EVs is based on their size, including: (1) Exosomes, (2) microvesicles, and (3) apoptotic bodies, being the smallest

(40-150 nm), the medium-sized (150-1000 nm), and the largest (> 1000 nm) subclass, respectively.

However, there is also another classification based on their biogenetic mechanism, including exosomes and ectosomes, with the former being produced by the integration of the multivesicular body (MVB) with the plasma membrane and the exosomes exocytosis, while the latter *via* plasma membrane budding[6].

Focusing on the mechanism of exosome generation, the inward plasma membrane budding is primarily required, accompanied by the internalization of transmembrane proteins and the formation of vesicles. This procedure is followed by several distinct steps for the final generation and release of exosomes from the parental cell, including the formation of early endosomes, the maturation of early endosomes into late endosomes, which either lead to intraluminal vesicles (ILVs) *via* the invagination of the membrane of the latter or they are retransferred to the cell membrane. Afterward, the incorporation of ILVs will lead to MVBs, which are either destructed in lysosomes or fused with the cell membrane to release exosomes[7]. All the aforementioned biogenetic pathways are strictly orchestrated under the influence of endosomal sorting (ESCRT) complex 0-III to modify and remodel the membrane and generate ILVs and, eventually, MVBs. Moreover, soluble NSF attachment protein receptor (SNARE) proteins are required for the MVB fusion with plasma membrane release of exosomes in the extracellular space (Figure 1)[8]. These nanostructures have a pivotal role in cell-to-cell communication, as they can modify the functional and transcriptional level of the recipient cells *via* the uptake of their cargoes. Meanwhile, their high biocompatibility, low immunogenicity, and their role in intercellular communication make them ideal drug delivery vectors or targets[9,10]. There is an increased interest in the role of exosomal non-coding circular RNA (circRNA) in HCC development and progression[11]. Novelties in RNA sequencing have given new opportunities to identify several non-coding molecules, such as circRNA. CircRNA is found in abundance in eukaryote cells under physiological conditions. However, it is also closely related to several diseases, including cancer[12]. These molecules are biologically functional and can regulate gene

expression. This phenomenon is implied by the fact that circRNAs can protect mRNA translation from miRNAs that can silence the mRNA translation or lead to its degradation (microRNA "sponges")[13]. Additionally, they can enhance the expression of several genes in the parental cells *via* interacting with polymerase II, they interact with RNA-binding proteins, leading to significant alterations in the gene expression and translation, as well as they can alter protein locations[14] (Figure2).

Several aberrations are observed in the circRNA expression levels in HCC tissue compared to physiological ones. The mechanisms that circRNA implicates in HCC are still not adequately clear; however, their significant contribution cannot be doubted[15]. Taking advantage of their functions will open new horizons in the development of circRNA-centered therapeutic and diagnostic perspectives.

ROLE OF EXOSOMAL-CIRCRNAS AS A THERAPEUTIC TOOL IN HCC

It is demonstrated that the role of circRNAs is dual in HCC, as they can either promote or suppress tumor progression *via* interacting with oncogenic or tumor-suppressive miRNAs. The aforementioned phenomenon is attributed to their role as a "sponge" for oncogenic miRNAs or tumor suppressive miRNAs, which can lead to tumor inhibition or promotion, respectively[16]. There are several aberrations in the expression levels of circRNAs, which may lead to tumor growth and progression and generally poor prognosis or they can induce suppression of HCC development. The study by Lyu *et al*[17], which was published in the recent issue of *World Journal of Gastroenterology*, demonstrated the role of mesenchymal stem cells (MSCs)-derived exosomal-hsa_circ_0000563 (circ-563) as a sponge for miR-148a-3p leading to tumor progression, whereas the silencing of circ-563 suppressed the HCC growth and development[17].

Exploring earlier studies, in the study by Zhang *et al*[18], tumor-suppressive miR-148a-3p was found to be downregulated in the HCC tissue, compared to physiological hepatic tissue, being associated with aggressive tumor behavior and worrisome prognosis[18]. Additionally, they observed that hepatic stellate cell (HSC)- derived exosomes, in which miR-148a-3p was depleted, led to HCC progression *via*

ITGA5/PI3K/Akt axis, which is involved in cell proliferation, migration, survival, as well as in cancer development and drug resistance. However, the increased expression of exosomal miR-148-3p in HCC tissue leads to tumor suppression[18].

Another study about the role of miR-148a-3p in HCC by Lyu *et al*[19] has demonstrated the interplay between the aforementioned miRNA and Metal-Regulatory transcription factor-1 (MTF-1) in HCC progression[19]. More specifically, MTF-1 is closely implicated in metal homeostasis, while its overexpression leads to hepatocarcinogenesis, tumor proliferation, and metastatic dissemination, as was demonstrated in conditions like copper exposure. MiR-148a-3p enhanced expression successfully suppressed HCC growth and progression that was induced *via* MTF-1. However, it was observed that exosomal-miR-148a-3p was notably downregulated in HCC patients, whereas its enhanced expression led to suppression of MTF-1 and HCC inhibition[19].

Eventually, the development of RNA sequencing, the identification of the circRNAs, and the development of the competitive endogenous RNA (ceRNA) theory expanded the research approaches for HCC pathogenesis and therapeutic targets. The ceRNA theory suggests that several RNA molecules like long non-coding RNA, circRNAs, and mRNAs can competitively share miRNA binding sites. In this recent study of Lyu *et al*[17], they also focused on the significant role of tumor microenvironment (TME) in HCC, focusing on the implication of MSCs *via* releasing exosomes. MSCs are recruited in HCC TME, exerting various effects, including suppression of anti-tumor immunity, and promoting neoangiogenesis that favors tumor growth and progression. Additionally, they differentiate into stromal cells, enhancing the tumor stroma. They can also induce several signaling pathways *via* secreting cytokines and EVs, like exosomes. The manipulation of MSC-derived molecules like exosomes for modifying the functionality of the recipient cells through the delivery of anti-HCC agents or genetic modulatory molecules can potentially widen the therapeutic perspectives. In the aforementioned study, they utilized labeled isolated MSC-derived exosomes co-cultured with HCC. As demonstrated in their previous study by Lyu *et al*[19],

overexpression of exosomal-miR-148a-3p, which targets MTF-1, notably decreased HCC progression, whereas, in the present study, they reported that among the various circRNAs from the databases, circRNA_0000563 (circ563), has_circ563 had the most partially complementary sequence for miR-148a-3p[17]. In addition, they demonstrated a correlation between MTF-1 and circ563 overexpression, as well as a correlation between circ563 upregulation and miR-148-3p decreased expression levels, implying its potential role as an "miRNA sponge" and eventually as an HCC promoter. On the other hand, silencing of circ-563 led to tumor suppression, suggesting its potential use as an anti-HCC therapeutic strategy[17].

CONCLUSION

New opportunities for HCC management could be opened up *via* the deep understanding of ceRNA theory, suggesting the role of circRNAs as "miRNA sponges", accompanied by the utilization of exosomes as delivery vectors.

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Figure Legends

Figure 1 Extracellular vesicles biogenesis. Exosome generation is initiated by the budding of the inward plasma membrane and the internalization of transmembrane proteins, forming early endosomes, which are further maturated into late endosomes. Under the contribution of endosomal sorting complex 0-III, the invagination of late endosomal membrane forms the intraluminal vesicles (ILVs). Eventually, the multivesicular bodies (MVBs), which have several ILVs in their lumen, are formed. Another site of cargo, except of the membrane, is the trans-Golgi complex or cytoplasm. Afterward, MVBs are either fused with cell membrane for the exocytosis of exosomes or degraded in lysosomes. However, they can form amphisomes *via* their fusion with autophagosomes, which are either degraded in lysosomes or fuse with plasma membrane to release the vesicles into the extracellular space. A broad spectrum of cells/tissue, including mesenchymal stem cells can produce extracellular vesicles. ILV: Intraluminal vesicles; MSC: Mesenchymal stem cells (MSCs).
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Figure 2 The role of circular RNA as "microRNA sponge". After the production of the active microRNA (miRNA) strand (leading strand/mature miRNA) is loaded on RISC complex, the so-called miRISC (seed sequence), which is capable of binding on a target mRNA strand, leading in the suppression of mRNA translation, its silencing or even its degradation. Circular RNA (circRNA), are non-coding RNA molecules that have a pivotal contribution in gene expression, as they can protect messenger RNA translation from miRNAs, *via* miRNA sponging, as well as they can interact with several proteins, including RNA-

binding proteins and polymerase II, as well as they can translocate them. circRNA: Circular RNAs; mRNA: Messenger RNA; miRs: MicroRNAs.

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