Cancer exosomes: wanted by many, explored by few, waiting for one

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Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related deaths worldwide and has a five-year survival rate of fifteen percent. It is projected that liver cancer will be one of the top three cancer killers by 2030 (1). HCC is an aggressive tumor type with poor prognosis due to the diverse etiological factors implicated in the development of HCC. In addition, heterogeneity and late diagnosis further complicate survival of HCC patients. Despite many potential therapeutic targets, the overall survival of HCC remains very poor.

Tumor cell-derived exosomes (TEXs), secreted nanovesicles that transfer proteins, DNAs, messenger RNAs, and miRNAs, are emerging as key players in HCC progression. Recent models suggest that exosomes play an important role in cancer progression by mediating immune responses. Exosomes also participate in horizontal intercellular communication (i.e., trafficking among different cell types). A recent review by Wu *et al.* provided insights into the roles of exosomes in HCC and its potential as diagnostic and prognostic tools to improve HCC therapies (2).

Exosomes are extracellular vesicles (EVs) released from cells for meditating intercellular communications. While there are many nomenclatures for exosomes, it should be noted that exosomes are different from ectosomes, which are larger in size, released by the plasma membrane, and are characterized by TyA and C1q markers. Exosomes, however, are smaller in size, released through multivesicular bodies (MVB) at a much slower rate, and are characterized by specific markers such as CD63 and CD61. These subtle differences, while distinct, are difficult to differentiate during purification or isolation. While exosomes and ectosomes presumably function very similarly once released, it remains to be clarified whether they possess different biological effects. The lack of standardized procedures to purify, isolate, or prepare EVs and the inevitable contaminants, such as protein aggregates, lipoproteins, or apoptotic fragments (3), further complicate the exact roles of exosomes in cancer.

As highlighted by Wu *et al.*, the cargoes of TEXs differ greatly and are dependent on the cell types. Hepatocytes, non-parenchymal immune cells, and parenchymal liver cells in liver can release exosomes. Thus far, the majority of exosomal cargoes have been characterized from hepatocytes. These include miR-21 as a biomarker for chronic cirrhosis and HCC from healthy patients (4,5). Members of the *let*-7 miRNA family and the MET oncoprotein are also found (6,7). Further investigations on exosomes released by hepatic stellate cells and Kupffer cells, however, will be equally important to elucidate their roles in tumorigenesis, inflammation, and fibrosis in the liver.

The unusual participation of secreted exosomes in horizontal communication further compounds the sophistication of exosomes in liver pathology. As discussed

by Wu *et al.*, miRNAs, such as miR-21, miR-192, and miR-221, enriched in colorectal cancer cell exosomes could mediate invasion and metastasis of HCC cells. Uptake of pancreatic cancer cell exosomes by Kupffer cells further illustrates previously uncharacterized mechanism of action in establishing pre-metastatic niche required for liver metastasis (6). TEXs have also been demonstrated to transfer its contents to modulate immune functions in T and B lymphocytes, promote angiogenesis *via* endothelial cells, and enhance cell migration or invasion by modulating macrophages in microenvironment (6,8,9). Thus, exploring trafficking of exosomes among different cell/tissue types will also be important to investigate HCC progression.

The regulation on exosome stability and its carried cargoes are also emerging as critical parameters in exosome research. TEXs are encapsulated in a lipid bilayer that is stable, allowing protection of its cargos within. Wu *et al.* discussed that modulation and degradation of exosomal RNA species may lie on RNA binding proteins (RBP), such as high-density lipoproteins, AGO2 and ELAVL1. For example, recent evidence suggests that RBP-RNA complex is important in maintaining the stability and loading of cargoes into exosomes (10). Since RBPs regulate gene expression by modulating the maturation, stability, transport, and translation of its RNA targets, the biological function of exosomes could be altered by changes in RBP-RNA complex. This is demonstrated in a recent study which showed that active KRAS signaling suppresses AGO2 interactions with endosomes and secretion of miRNA *via* exosomes (10). Given that RAS activation has been shown to promote HCC and approximately 5% of HCC harbors a KRAS mutation, it is reasonable to surmise that exosomal dysregulation due to aberrant RAS activation also drives HCC progression (11). In addition to RAS activation, other key cancer drivers, such as β-catenin, may promote tumorigenesis through the deregulation of RBP-RNA complexes (12). It has also been suggested that the alterations of the tumor suppressor p53 would affect overall exosome biogenesis, landscape of EVs, and the secretory profile in tumorigenesis as previously proposed (13).

Besides RNA species, TEXs have been described to carry oncoproteins and tumor suppressors such as PTEN (14). Membrane bound ligands and growth factor signaling receptors are also found in exosomes. The vast difference in protein cargoes expands the diversity and subtypes of exosomes. Investigation of exosomes and its cargoes will be key to illuminate diagnosis and prognosis for HCC and other cancers.

Last but not least, exosomes serve as a great means for delivery. Given that many ideal therapeutic targets are expressed intracellularly, and thus "un-targetable" by conventional therapy, a Trojan horse exosome approach may find utility for cancer drug delivery. Previous studies propose a Trojan horse model for customized exosomes (15). In reference to current trends in artificial nanoparticles, selection of cargoes, destination of specific targeting, and functional consequence upon its uptake/delivery will be critical parameters for the potential therapeutic use of customized exosomes.

In conclusion, exosome research is certainly an exciting field with strong clinical implications and vast basic biology for future investigation. Complexity and confusion of current exosome research, however, should strongly be noted. For example, the lack of standardized protocols for isolating and purifying exosomes make it difficult to distinguish different EVs, their sub-types and trafficking in HCC. We suspect that as the field progresses, new and improved isolation methods including biophysical, molecular and microfluidic methods will significantly augment the current limitations. With these improvements, our understanding of exosome stability and secreted cargoes will evolve, giving hints to the underlying mechanism of exosomal targeting and kinetic profile. Another important highlight in exosome research is our current understanding of how components of exosomes such as proteins, miRNAs and lipids may be effectively used for prognostic and diagnostic purposes. While recent studies demonstrated the potential importance of exosomes for clinical diagnostics, the field is still at its early stage. Future studies will broaden the understanding of exosome biology and improve our ability to exploit exosomes for the treatment of HCC and other cancers.

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Footnote

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