Review Article

Exosomes in blood and cancer

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Abstract: Exosomes are the smallest of the extracellular vesicles (EVs). They have been detected in a variety of body fluids, including serum and plasma. Exosomes from tumor cells that are found in the circulation (and elsewhere) are enriched in certain intracellular components, including miRNAs. While many small studies document the association of miRNAs, DNA, protein or lipid alterations within exosomes with the presence of a given type of cancer, few if any of these findings have been independently validated. Indeed, there are many gaps in our knowledge of the role of extracellular RNA, DNA, proteins or lipids in cancer detection, prognosis or therapy. Some of the important questions which need to be addressed for a given cancer type include assessing which: (I) body fluid(s) to study; (II) molecular components within a given body fluid to study (RNA, DNA, proteins or lipids contained within and/or outside of EVs); and (III) specific markers within the body fluid fraction provide the most information regarding diagnosis, prognosis, and/or treatment of a given cancer. The most common body fluid analyzed to evaluate the role of exosomes in cancer is blood. Herein we review published findings related to studies that have analyzed exosomes obtained from blood or its components serum or plasma. We also review resources that are available to assist the clinician and scientist wishing to learn more about exosomes. Vesiculopedia and Exocarta are websites dedicated to gathering molecular information on EVs (all sizes) and exosomes, respectively. They contain information on EV content by molecular type (DNA, RNA, protein, lipid) and species from which the EV study originated. The International Society of Extracellular Vesicles and the *Journal of Extracellular Vesicles* are dedicated to advancing knowledge of EVs. The National Institutes of Health has funding 30 research grants which seek to increase our understanding of extracellular RNA, including that contained within EVs.

Keywords: Exosomes; blood; cancer

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Introduction

Of the three types of extracellular vesicles (EVs) (exosomes, microvesicles and apoptotic bodies), exosomes are the smallest (1). They are formed by inward budding, and released into body fluids after fusion with the cell membrane. Exosomes contain proteins, DNA, mRNA, miRNA, and lipids (1). There are a variety of proteins common to all exosomes, such as CD9, CD63, CD81 and CD82, as well as proteins specific to exosomal cell of origin, and whether the cell of origin is diseased or healthy (1).

A variety of studies document that tumor derived exosomes promote tumor development and progression. Exosomes from colorectal cancer (CRC) cells (2), gastric cancer cells (3), and liver cancer cells (4) have increased levels of proteins and/or mRNAs that promote endothelial cell proliferation. In response to hypoxia, tumor cells increase the release of exosomes to facilitate tumor angiogenesis (5). Tumor derived exosomes facilitate immunosuppression (6), and increase the resistance of cancer cells to treatment (7).

It has been estimated that exosomes can carry up to approximately 500 miRNAs. The estimated carrying capacity is about 10,000 nucleotides per exosome (8), enough for about 500 miRNAs. The concentration of exosomes in serum or plasma has reported to range from 0.88×10^8 to 13.38×10^8 exosomes/mL (9).

Tracking exosomes in body fluids

It is known that exosomes released from a cell into a body fluid can be taken up by another cell at distance from the cell of origin. The authors wished to trace the fate of exosomes and other EVs in body fluids (10). To do this, they tagged CD63, a protein highly expressed on EVs, with green fluorescent protein (GFP). CD63-GFP was found on EVs in rat serum, milk and amniotic fluid. CD63-GFP EVs in the serum were taken up by rat embryonic fibroblasts in culture. The authors suggest the model could be used to better understand intercellular transfer of exosomes and other EVs, as well as mother-child EV transfer.

Exosomal pan-cancer marker?

Telomerase is known to be active in over 90% of malignancies, but repressed in normal human cells (11). The authors measured serum levels of telomerase mRNA (hTERT) in serum and found the levels to be very low. Notably, mRNA levels in exosomes have been reported to be more stable than when not within exosomes, as the mRNA is protected from RNases (12). The authors measured hTERT levels in exosomes derived from patients with 15 different types of cancer. Overall, hTERT was detected in 67.5% of patients, with the highest rate of detection (80%) in patients with "other" types of cancer, including squamous cell carcinoma and adenocarcinoma, and the lowest level (0%) in patients with prostate and kidney cancer. For cancers with at least 10 patient samples analyzed (breast, CRC, acute leukemia, and lymphoma), detection rates were 63%, 71%, 42%, and 64%, respectively. Exosomal hTERT levels decreased in patients after clinical evidence of effective treatment of their cancer.

Exosomal lipids and cancer

Exosomes contain a variety of lipids. Compared to the parent cells, exosomes are either enriched or depleted for many different lipids (13). Cholesterol makes up over 40% of the lipids isolated from exosomes derived from a variety of cell types. Certain lipid metabolizing enzymes and lipids such as ceramide, neutral sphingomyelinase, and phospholipase D2 appear to influence the release of exosomes (14). The lipids and enzymes involved appear to be cell type specific.

Exosomal studies of blood and cancer: miRNAs

CRC

The authors wished to identify miRNAs that were differentially expressed in exosomes derived from the serum of patients with CRC compared to controls (15). After an initial screen by microarray and confirmation by quantitative real-time PCR, 5 exosomal miRNAs (miR638, miR5787, miR8075, miR6869-5p and miR548c-5p) were downregulated and 2 (miR486-5p and miR3180-5p) upregulated in serum. Lower levels of miR638 in serum exosomes were associated with advanced disease stage. The authors conclude that specific exosomal miRNAs in the serum of CRC patients may serve as biomarkers of and as therapeutic targets for CRC.

Urothelial malignancies

miR141 and miR375 are the miRNAs most often associated with aggressive prostate cancer (those with high Gleason score and/or lymph node spread), both in the serum (16) and in serum derived exosomes (17). Levels of both miRNAs in the blood have been reported to discriminate advanced from localized prostate cancer (18). miR141 levels are also increased in the urine of patients with prostate cancer (17). While many studies of bladder cancer have evaluated miRNAs in urine, there is evidence that plasma miRNAs may also aid in diagnosis. miR148b, miR200b, miR487, miR541, and miR566 were increased while miR25, miR33b, miR92a/b, and miR302 were decreased in patients with bladder cancer compared to healthy controls (19). Expression levels of these miRNAs discriminated patients with bladder cancer from controls with 89% accuracy (19). von Brandenstein *et al.* reported significant up-regulation of miR15a in the urine of renal cell carcinoma (RCC) patients compared to patients with other medical conditions (20), suggesting urinary miR15a as a potential biomarker for RCC. Serum levels of both miR378 and miR451 have been reported to distinguish patients with RCC from healthy controls (21). Other serum miRNAs associated with RCC include miR1233 (22) and miR210 (23).

Pancreatic cancer

miR17-5p and miR21 are highly expressed in exosomes from patients with pancreatic cancer (24) compared to normal controls. Increased miR17-5p correlates with

advanced disease. While levels of miR21 were elevated in pancreatic cancer compared to controls, levels do not appear to correlate with disease stage.

Non-small cell lung cancer (NSCLC)

EGFR, KRAS, claudins and RAB-family proteins are found in exosomes from NSCLC patients (25). EGFR expression was identified in 80% of the exosomes from NSCLC samples compared to 2% of the exosomes in chronic inflammatory lung tissue (26). A variety of exosomal miRNAs are overexpressed in NSCLC (27). One of these, miR146a, targets EGFR, an important driver of some NSCLCs. The addition of miR146a to NSCLC cells decreased their growth and induced apoptosis (27). Moreover, miR146a enhanced the antiproliferative effect of agents that target EGFR.

Biliary tract cancers (BTC)

Levels of miR21, miR187 and miR202 were elevated in the blood of patients with gallbladder carcinoma compared to controls (28). miR21 has been associated with BTC in a number of studies (29). In a separate study, miR150 was increased in the plasma of patients with intrahepatic cholangiocarcinoma and downregulated in matched tissue (30).

Ovarian cancer

As with most other malignancies, survival is high in early stage ovarian cancer, but poor in patients with advanced disease (31). While the initial response to chemotherapy is high, patients with ovarian cancer generally eventually relapse and response to additional therapy after relapse is less favorable (31). CA125 remains the best biomarker for the early detection of ovarian cancer, but it has false positives and false negatives making it a suboptimal biomarker. There have been many studies evaluating the usefulness of measuring miRNAs in the plasma or serum to detect ovarian cancer. Some of the studies isolated miRNAs from EVs, others from serum or plasma without isolating EVs (31). miRNAs elevated in multiple studies included miR21, miR200a, and miR200c. Separate studies have identified miRNAs associated with a poor prognosis among women with ovarian cancer (31). A miRNA commonly associated with a poor prognosis was miR21.

Exosomal studies in blood and cancer: circular RNAs (cRNAs)

cRNAs are stable noncoding RNAs that may regulate gene expression (32). Over 1,000 cRNAs have been found in human serum exosomes. cRNAs are enriched at least two-fold in exosomes compared to the cells of origin (32), suggesting some regulation of how the cRNAs are incorporated into exosomes. There appears to be an association between cRNA levels in exosomes and miRNA levels in the cells of origin, suggesting that the miRNAs in the producer cells may regulate the cRNA levels in the exosomes (32). Exosomal cRNA from xenograft colon cancer tumors entered the serum of mice, with cRNA content correlated with tumor mass (32). cRNAs in sera from CRC patients was also found to be different from that of normal controls (32).

Exosomal studies in blood and cancer: proteins

Pancreatic cancer

Mass spectrometry was used to identify a proteoglycan, glypican-1, on the surface of exosomes in the serum of patients and mice with pancreatic cancer (33). Exosomes expressing glypican-1 were found in all patients with pancreatic cancer studied, but not in patients with benign pancreatic disease. Moreover, levels of glypican-1 correlated with disease burden and survival (33).

Exosomal studies in blood and cancer: genomic DNA

Pancreatic cancer

Exosomes containing >10 kb fragments of double stranded genomic DNA, mutant KRAS and p53 can be found in the serum of patients with pancreatic cancer (34). KRAS and p53 DNA were only found in exosomes, not in exosome depleted DNA, with mutations only in the serum from patients with cancer (34).

Discussion

There has been an outpouring of research to evaluate the potential usefulness of exosomal contents in body fluids for disease diagnosis, prognosis, and therapy. The most common body fluid analyzed is blood plasma or serum.

Most of the studies have evaluated miRNAs. As with many early studies, application of the findings requires validation studies to confirm the preliminary results. To date few validation studies have been performed to demonstrate the usefulness of exosomal contents in cancer.

In 2013, the NIH awarded 5 years of funding to 30 research projects focusing on biomarkers or therapeutics involving extracellular RNAs, including miRNAs (https://ncats.nih.gov/exrna/projects/therapeutics2013). Phase I (preliminary studies/choosing markers to target for validation) was from 2013–2015, and phase II (validation/therapeutic application) from 2015–2018. The funded projects included diseases of the central nervous system-CNS (Huntington's Disease, Alzheimer's disease and CNS demyelination), kidney disease, and cancer, focusing on miRNA delivery strategies as well as targeting tumor derived EVs. Among the topics of the funded cancer projects was HER2 overexpressing breast cancer, ovarian cancer, hepatocellular carcinoma, and castration resistant prostate cancer. Body fluids analyzed include circulating miRNAs for multiple sclerosis, urinary miRNAs for kidney disease, cerebrospinal fluid-CSF miRNAs for Alzheimer's disease, salivary miRNAs for gastric cancer, plasma and CSF miRNAs for gliomas, and blood miRNAs for placental dysfunction.

Vesiculopedia (http://www.microvesicles.org/) is a website dedicated to gathering molecular data on EVs. As of July 11, 2017, data from 538 independent studies involving 33 species had been cataloged on the site. Exocarta (http://www.exocarta.org/) is a database focused specifically on exosomes. Additional links of interest include the *Journal of Extracellular Vesicles* (http://www.tandfonline.com/toc/zjev20/current), the International Society of Extracellular Vesicles (http://www.isev.org/), and the extracellular RNA portal (exrna.org).

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