Perspective

Future perspectives for body fluid exosomes and cancer

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Abstract: Exosomes are extracellular vesicles which contain DNA, RNAs, proteins, carbohydrates and lipids derived from normal and tumor cells that are secreted into body fluids. Exosomes can influence other cells at distance once in body fluids. There are ongoing studies being conducted to determine how exosomes can be used for both the early detection and treatment of cancer. In this article, we discuss some of these diagnostic and therapeutic investigations, some of which may make their way to clinical applicability in the future.

Keywords: Epithelial mesenchymal transition (EMT); transforming growth factor beta; breast milk; exosomes

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Extracellular vesicles (EVs) (microvesicles, apoptotic bodies and exosomes) appear to form in a variety of cell types. The relative importance of different EV types in cancer remains uncertain, in part due to ongoing difficulty in differentiating these entities. Exosomes, the smallest and most thoroughly characterized of the EVs, have been identified in many body fluids, including urine, semen, saliva, amniotic fluid, cerebrospinal fluid, bile, ascites, tears, breast milk and blood (1). These vesicles are secreted in increased numbers in cancer cells compared to normal cells (2). They carry out processes which may help or harm the organism. Exosomal contents vary based on cell of origin and whether the cell is normal or abnormal (3). Their role in cancer development and progression is becoming increasingly clear, as has been outlined in the manuscripts described in this issue of *TCR*.

Exosomes derived from cancer cells assist the cells in altering the microenvironment to suit the needs of the tumor. This may include promoting tumor growth or progression, evasion of immune surveillance, or drug resistance (3). Exosomes can deliver their contents, which may include proteins, messenger (m)RNA, micro(mi)RNA, lipids and carbohydrates, to a recipient cell (4). In addition, exosomes may interact with receptors on the recipient cell surface (4). For example, it has been shown that exosomal TGF-β derived from cancer cells can induce fibroblast differentiation to myofibroblasts, which may promote tumor growth (4). High (but not low) levels of exosomal TGF-β in breast milk cells have been shown to induce epithelial mesenchymal transition (EMT) in both benign and malignant mammary epithelial cells (5). EMT promotes tumor aggressiveness and increases metastatic potential (6). Tumor derived exosomes contain angiogenesis related genes which promote new blood vessel formation and thereby tumor growth (7). Tumor derived exosomes which contain adrenomedullin mediate the onset of diabetes in patients with pancreatic cancer (8).

Exosomes as cancer biomarkers

It has been proposed that exosomes may be useful for the diagnosis of cancer. Cancer cells generally generate more exosomes than normal cells derived from a similar tissue. Whether this phenomenon is universal, or if the difference is sufficient that it would prove useful, is unclear.

Specific exosomal biomarkers in body fluids, including serum, plasma, cervicovaginal lavage, and urine have been proposed as potential indicators of cancer (3). In some cases, the biomarker was in greater abundance than in controls, whereas in other cases, it was only found in the body fluid of the cancer patient. Generally lacking were validation studies of adequate statistical power to confirm the usefulness of the biomarker(s) in the early detection of cancer. One exception is the protein glypican-1. It has recently been reported (9)

that glypican-1 is highly sensitive and specific in the early detection of pancreatic cancer. A second study (10) confirmed this association.

Targeting tumor derived exosomes to treat cancer

Exosomes from tumors are different from normal cells both in number produced and in the specific components of each exosome. Studies are underway to confirm this observation in other cancer types. Omega-3 fatty acids (ω3-FA) are present in a variety of dietary compounds, most notably fish oil. They have been proposed to have multiple health benefits, including anticancer activity (11). Of many ω3-FA, one of the best studied is docosahexaenoic acid (DHA), which has demonstrated anticancer activity (12). Treatment of both estrogen receptor positive MCF-7 and estrogen receptor negative MDA-MB-231 cells with DHA increased the expression of tumor suppressive miRNAs (let-7a, miR-23b, miR-27a/b, miR-21, let-7, and miR-320b) (12). When DHA-treated MCF-7 cells were added to endothelial cells, expression of these miRNAs increased in the endothelial cells, while the expression of angiogenic target genes decreased.

Genetic manipulation of exosomes may be useful to treat pancreatic cancer (13). Exosomes are protected from phagocytosis by monocytes and macrophages and thereby retained for a longer period than liposomes in the blood. KRAS mutations often drive pancreatic cancer, but they have been difficult to target. Exosomes from normal fibroblasts engineered to contain short interfering or short hairpin RNA targeting the oncogenic KRAS mutation G12D, which is commonly found in pancreatic cancer, suppressed murine models of pancreatic cancer and improved overall survival.

Exosomes derived from dendritic cells, so called "dexosomes", have been developed to increase the immune response to cancer. Dexosomes have abundant cell surface proteins which allow binding to receptor cells and subsequent uptake of dexosomal cargo (14). Moreover, dexosomes contain molecules for antigen presentation leading to MHC class II-T cell responses (15). Dexosomes also increase NK activation and proliferation through IL-15Rα and NKG2D (14). Dexosomes decrease the growth of murine tumors (16). Clinical trials have been initiated in colorectal cancer, melanoma, and non-small cell lung cancer (17). While results thus far have been modest, strategies to improve immunostimulation are underway, including the use of exosomes derived from interferon γ-matured dexosomes and engineering dexosomes (Dex) which stimulate B cell (in addition to T cell) responses (14). To increase efficacy, Dex have been combined with chemotherapy (chemoimmunotherapy). This combination strategy has demonstrated, in some cases, increased antitumor efficacy, even when chemotherapy has suppressed immune stimulation by Dex (18).

In vitro studies have demonstrated the ability of exosomes to deliver the chemotherapeutic agent paclitaxel (19). Short interfering (si)RNA can be loaded into exosomes. Murine dendritic cells engineered to express the exosomal membrane protein Lamp2b fused to an α_v integrin-specific iRGD peptide targeted α_v positive breast cancer cells *in vitro* and α_v positive breast tumors in nude mice (20).

Exosome depletion to treat cancer

In an effort to decrease the tumor promotional effect of cancer derived exosomes, exosome removal strategies have been studied. In cell culture, fetal bovine serum is known to contain exosomes (21), and exosome removal leads to reduced cell growth (22). In mice, depletion of exosomes using dimethyl amiloride restored the efficacy of cyclophosphamide (23). Aethlon Medical, Inc. San Diego, CA, has patented a hemopurifier system to capture tumor derived exosomes for cancers of the breast, ovary, and melanoma.

Confounders to consider in exosome studies

As with most biomarkers, controlling for confounders is critical in order for results to be meaningful. I will highlight a few examples of variables to consider when studying exosomes. A report evaluated exosomal expression in breast milk in transitional-T (3–8 days after delivery) and mature (2 months postpartum) milk. A higher content of exosomes was observed in T than in mature milk. The exosomes in T milk were enriched in HLA-DR (a major histocompatibility complex-MHC class II antigen) and had a lower level of HLA-ABC (a MHC class I antigen) compared to mature milk (24). A second report evaluated whether the mother's diet influences the contents of milk exosomes. Indeed, multiple plant miRNAs are present in both human and porcine breast milk exosomes (25). Thus both diet and timing influenced breast milk exosome expression. The influence of exosome size is yet another variable that has

not been well evaluated in most reports.

Summary

The importance of exosomes in cancer early detection and therapy is unclear, but their potential is very promising. As with all biomarkers and delivery vehicles, confounders must be considered. Since exosomes are made by normal as well as cancer cells, specific markers or a marker panel that are expressed exclusively or at high levels only in cancer cells would be especially useful as early detection biomarkers. Preliminary evidence suggests that glypican-1 may be one such marker. Therapeutic strategies require tumor specific targeting to minimize off target effects. Most needed are validation studies to confirm promising preliminary findings.

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