Correspondence

Exosomes: an evolving source of urinary biomarkers and an up-and-coming therapeutic delivery vehicle

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Background

Exosomes are a subset of small, extracellular vesicles secreted by all cell types, and can be isolated for virtually all bodily fluids that have been investigated. The importance of exosomal signaling in normal physiology and cancer is clear, with the transfer of host-cell cytoplasmic RNA, intracellular and membrane-bound proteins being well-described.

Locally, cancer-derived exosomes are able to be internalized and even alter recipient-cell expression and behavior (1). Epithelial-to-mesenchymal transition, a process associated with tumor progression and malignant transformation, has been observed with the application of cancer-derived exosomes on normal cells in several malignancies, with documented stimulation of angiogenesis, as well as enhanced cell migration and invasion (2,3). Distantly, the excretion of cancer-exosomes systemically has been shown to be an organotropic determinant of metastases for specific malignancies via the delivery of specific integrins expressed by tumor-derived exosomes (4).

With the role in signaling exosomes demonstrate in malignancy, they represent a potential, largely-untapped, reservoir for biomarker identification and delivery of therapeutics.

Obstacles of urinary exosome study

We agree with Drs. Lee and Liu on their commentary regarding obstacles to the study of urinary tract exosomes (5). With multiple exosomal sources (including renal, urothelial and prostatic), there is a definite need for characterization of exosomal markers to specifically delineate the source. We have found that by performing a bladder barbotage (a standard procedure in the evaluation for bladder cancer in which at the time of cystoscopy saline is irrigated vigorously in the bladder and the fluid sample taken, typically for cytologic analysis) exosomal yields are higher than in the voided urine, and that barbotage samples had increased expression of mesenchymal markers than in the voided urine (2). In these cases where any residual urine had been emptied from the bladder, the saline barbotage sample should provide a more homogenous sample of urothelial exosomes from the bladder, with less contamination from upper tract (renal) and prostatic sources.

While this is a promising first step in using exosomes for the detection and study of bladder cancer, improvement in specific exosome markers will allow the voided urine sample, an entirely non-invasive test, to become more practical in the evaluation of the mixed population of urinary exosomes.

Use of exosomes in diagnostics

At present there are only two commercially-available exosome-based diagnostic tests on the market, for lung and prostate cancer. Despite the limitation noted above, for prostate cancer a non-invasive test using a patient's voided urine sample to assess exosomes has been developed based

on a proprietary, three-gene signature for the detection of high-grade prostate cancer. In the recently published validation study, McKiernan *et al.* demonstrate that the use of this test, compared to standard of care, demonstrated improved prediction of clinically significant prostate cancer on biopsy, as opposed to less aggressive disease or a benign biopsy (6).

Use of exosomes for delivery of therapeutics

Exosomes hold exquisite promise in the delivery of therapeutics given their low immunogenicity, the environmental protection provided by their lipid bilayer membranes, and potential for targeting to cell types of interest.

Bladder cancer is unique given its precedent for direct delivery of therapeutic agents intravesically in the treatment of cancer (e.g., mitomycin C, Bacillus Calmette-Guérin vaccine). *In vitro*, we have demonstrated that following co-incubation with non-cancer-derived exosomes, bladder cancer cells internalize exosomes at a 50-fold higher rate than normal uroepithelial cells (7). We were then able to load exosomes with siRNA directed toward the gene Polo-like kinase-1 (PLK-1, a key regulator of mitotic progression) via electroporation (confirmed via Amnis ImageStreamX), and subsequently treat bladder cancer cells lines with PLK-1-loaded exosomes. With this technique, we were able to demonstrate not only knockdown of PLK-1 gene expression (via qRT-PCR), but also induction of apoptosis and necrosis of bladder cancer cells compared to those treated with negative control siRNA (7).

A recent study lead by Dr. Kim demonstrated the use of exosomes in the delivery of a chemotherapeutic agent (8). In this study, paclitaxel was loaded into exosomes via sonication and, *in vitro*, loaded-exosomes were shown to have uptake into cancer cells with demonstration of increased cytotoxicity compared to standard chemotherapeutic administration. In an *in vivo* mouse model for pulmonary metastasis, intranasal administration of loaded-exosomes demonstrated not only co-localization of exosomes with cancer cells on confocal microscopy, but also greater inhibition of metastasis growth compared to negative controls or chemotherapeutic administration.

While the above studies use a direct delivery of loaded-exosomes to the tumor cells, promising work in the systemic administration of targeted exosomes has also begun. In the *in vivo* mouse study by Dr. Alvarez-Erviti *et al.*, it was convincingly shown that by producing self-derived exosomes engineered to express a modified exosomal membrane protein (Lamp2b) fused to a cell specific peptide [in this case rabies viral glycoprotein (RVG), a neuron-specific peptide], that exosomes could be injected systemically and not only specifically target neuronal structures, but in doing so also cross the blood-brain barrier (9). When these exosomes were loaded with siRNA to BACE1 (a therapeutic target of Alzheimer's disease), there was significantly decreased mRNA and protein expression in neural tissue compared to controls.

These results should address Dr. Panfoli's concern regarding the ability of exosomes to not only deliver an RNA (or chemotherapeutic) payload, but to also affect target-cell expression (10).

Conclusions

Urinary exosomes represent the forefront of innovation and discovery, and we anticipate great strides to be made in the near future in biomarker discovery and therapeutic advancement. As of this year a urine-based, non-invasive test has become commercially available for highly prevalent prostate cancer to improve discrimination between high- and low-risk disease. The ability to determine exosomal biomarkers to similarly detect urothelial or renal cell carcinoma, or benign but progressive renal conditions, may provide opportunities to spare patients from invasive procedures, or to improve clinical risk stratification of disease. Further, the promise of packaging biologically active molecules or chemotherapeutics for targeted delivery is an exciting prospect in the treatment of benign and malignant disease.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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