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Full Length Article

Exosomal protein interactors as emerging therapeutic targets in urothelial bladder cancer

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KEYWORDS

Exosome;
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Abstract *Background:* Exosomes are rich sources of biological material (proteins and nucleic acids) secreted by both tumor and normal cells, and found in urine of urinary bladder cancer patients.
Objective: The objective of the study was to identify interacting exosomal proteins in bladder cancer for future use in targeted therapy.
Methods: The Exocarta database (www.exocarta.org) was mined for urinary bladder cancer specific exosomal proteins. The urinary bladder cancer specific exosomal proteins (n = 248) were analyzed to identify enriched pathways by Onto-tool Pathway Express (http://vortex.cs.wayne.edu/ontoexpress).
Results: Enriched pathways included cellular architecture, motility, cell to cell adhesion, tumorigenesis and metastasis. Proteins in the 9 top-ranked pathways included CTNNA1 (alpha-catenin), CTNNB1 (beta-catenin), VSAP, ITGA4, PAK1, DDR1, CDC42, RHOA, NRAS, RHO, PIK3AR1, MLC1, MMRN1, and CTTNBP2 and network analysis revealed 10 important hub proteins and identified inferred interactor NF2.
Conclusions: The importance of identifying interactors is that that they can be used as targets for therapy, for example, using Bevacizumab (avastin – an angiogenesis inhibitor) against NF2 to inhibit protein–protein interactions will inhibit tumor growth and progression by hindering the exosome biogenesis.

Introduction

Exosomes are microvesicles shed by vesiculation events from various living cells secreted by most cell types, both cancerous and normal and secreted in biological fluids [1] and are thought to play important roles in intercellular communications. They are generated via diverse biological mechanisms triggered by pathways involved in oncogenic transformation, microenvironmental stimulation, cellular activation, stress, or

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death [2]. Biogenesis of the exosome begins with the secretion of signal peptide proteins in the cytoplasm. Intraluminal vesicles (ILVs) are formed through inward budding of endosomes to form multivesicular bodies (MVBs) or multivesicular endosomes (MVE). The MVB fuses with the cellular membrane, and the exosomes are released. MVBs can also be either degraded (by fusion with lysosomes) or recycled [3].

Although exosomes were originally described in 1983, interest in these vesicles has increased dramatically in the last few years, after the finding that they contain RNA, protein, lipid and other biomolecules [4]. It is clear that exosomes have specialized functions and play a key role in intercellular signaling and waste management. It is also well accepted that urinary exosomes (UEs) may be used as a novel biomarker source for early disease detection, classification, prediction severity, outcome and response to treatment [5]. Consequently, there is a growing interest in the clinical application of exosomes as it can potentially be used for prognosis, therapy, and as biomarkers for health and disease.

Cell-derived vesicles are released in many and perhaps all biological fluids, including blood [6], urine [1], breast milk [7], malignant ascites [8,9], amniotic fluid [10], bronchoalveolar lavage fluid [11] and culture medium supernatant in cell cultures [12,13]. The content and secretion of these vesicles are dependent on cellular origin. It may be hypothesized that exosomes present in the urine may be secreted from any of the abdominal and pelvic organs such as the kidney, prostate, ovary, and urinary bladder. Studies were done on identification of exosomal proteins, since the first publication on proteomic profiling of UEs by Pisitkun et al., [1] while Mathivanan et al., [14] created a database, named "Exocarta" specifically for exosomes. Vesiclepedia database and urinary exosome protein database also contain information about exosomes, but these databases include those proteins which are also in the Exocarta database.

Urinary bladder cancer is the sixth most leading cancer in India and the second most common malignancy of the genitourinary tract [15] with a high recurrence rate. Urine cytology and cystoscopy along with biopsy are commonly used diagnostic and monitoring tools, but cytology has a sensitivity of only 29% [16] and cystoscopy is an invasive procedure. Hence, there is a diagnostic need of early detection markers which helps in surveillance of patients. Urinary exosomes may originate from the lining of the urinary tract, including glomerular podocytes, renal tubule cells, and urinary bladder. The number and content of UEs may change over time in association with disorders that affect the urinary system. Most of the membrane and cytoplasmic proteins of tumor cells will be excreted into urine by the process of endo and exocytosis making the microvesicles and exosomes a rich source of cancer-specific proteins [1,2,5]. Exosomes specifically secreted by the bladder lining or urinary bladder cancer cells into the urine may be the result of the interaction of some specific pathways. Identification of the interacting molecules of these pathways will identify targets of therapeutic interest and prevent tumor progression.

Data available on Exocarta database was analyzed using computational tools. Pathway based studies and system biology approach were performed to identify exosomal proteins specific to urinary bladder cancer. The present study identifies urinary bladder cancer-specific urinary exosomal proteins and analysis of their interactions revealed proteins which can be used as therapeutic targets.

Methodology

Fig. 1 provides the study design employed for identifying important proteins in urinary exosomes.

Exosomal proteins present in the urine were identified from the Exocarta database (www.exocarta.org) [1]. A seed list was prepared by selecting exosomal proteins specific only for bladder cancer. The 'seed list' was imported to the Pathway-Express module of Onto-tool (http://vortex.cs.wayne.edu/projects.htm) and the pathways in which exosomal proteins were involved were identified [17]. Impact factor was calculated by the web-based software (Pathway-Express module) using both a statistically significant number of differentially expressed genes and biologically meaningful changes on a given pathway. Pathway-Express provides two types of *p*-values for each pathway: (i) *p*-value obtained using the classical statistics (referred to as classical *p*-value) and (ii) *p*-value obtained using the impact analysis (referred to as gamma *p*-value). The classical *p*-value is calculated as an over-representation analysis using hypergeometric test. The gamma *p*-value is the *p*-value provided by the impact analysis [18].

Exosomal proteins involved in pathways with the high impact factor (cutoff >10), were selected for studying protein–protein interactions (PPIs) network and hubs. Protein–protein interactions (PPIs) were commonly understood as physical contacts with molecular docking between proteins that occur in a cell or in a living organism in vivo [19]. Each of these interactions is specifically adapted to carry out certain biological functions. A PPI network is represented with proteins as nodes and interactions between nodes as the edges. The common proteins among top 9 pathways were further studied by a web-based software, POINeT (poinet.bioinformatics/tw). The number of iterations used was one i.e., only first degree neighbors of queried nodes were searched. The protein–protein interaction (PPI) information collected is integrated by various protein databases to provide PPI filtering and network

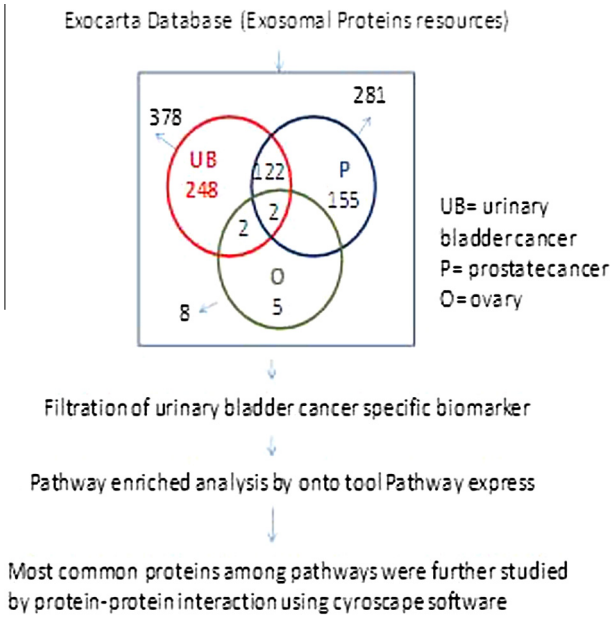

Figure 1 Study design for identification of exosomal proteins in bladder cancer.