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Regulatory function of membrane trafficking components in exosome secretion and cancer progression

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Abstract Cancer cell-derived exosomes have recently been implicated in contributing to metastasis. It is expectable that specific membrane trafficking factors would participate in the regulation of exosome formation, transport and release from cells. Recent investigations have revealed certain members belonging to the SNARE, sorting factor and Rab GTPase protein families as being crucial in governing the exosome life cycle. These trafficking components have therefore been primed as new targets potentially modulating cancer progression. This mini-review is focused on the involvement of membrane trafficking components in regulating exosome-related transport and signaling, and in turn influencing clinical outcomes in cancer.
Keywords: SNARE, membrane trafficking, exosomes, cancer progression

Introduction

Multicellular organisms rely on intercellular communication for information exchange in order to execute appropriate development and functioning of tissues, and promote survival. This communication occurs either through direct physical contact via nanotubes [1], secreted chemical signals like cytokines, chemokines and metabolites [2], or via exosomes [3].

Exosomes are membrane-bound nanovesicles (40 - 100 nm in diameter) released from various cell types into the extracellular space [4]. They are present in almost all biological fluids including blood, lymph, urine, saliva, cerebrospinal fluid, breast milk and semen. Lipids and proteins are the main components of exosome membranes, which are enriched with lipid rafts [5]. In addition to proteins, various nucleic acids have recently been identified in the exosomal lumen, including mRNAs, microRNAs (miRNAs), and other non-coding RNAs (ncRNAs) [6]. Under normal physiological conditions, exosomes have been primarily tasked with the removal of unnecessary proteins from cells [7]. They can be thought of as intercellular communicasomes by transferring signals to their target cell via surface ligands, and delivering receptors and functional molecules. Exosomes appear to be intended for sustaining normal cellular metabolism even under unfavorable conditions such as starvation and oxidative stress [8]. Their complete functional relevance in normal as well as disease physiology is still under investigation.

Accumulating evidence indicates that exosomes play important roles in cancer progression [9] by contributing to the escape from immune surveillance and the formation of tumor niche. The majority of deaths from cancer are attributed to metastasis. Metastasis consists of a series of successive and interrelated steps mainly including invasion of malignant cells into surrounding tissue, intravasation, circulation, adhesion to and extravasation from capillaries into target organs [10]. Studies have shown a significant difference in total exosome and exosomal miRNA between cancer patients and healthy control individuals, and a similarity between the circulating exosomal miRNA and the tumor-derived miRNA patterns [11]. This suggests that the exosome might be useful as a screening test for various cancer types. These exosomes transfer oncogenic proteins and nucleic acids, especially microRNAs, to

modulate the behavior of neighboring or distant recipient cells and play decisive roles in malignant transformation, growth, metastasis, and drug resistance.

In this mini-review, the role of membrane trafficking components in the regulation of exosome release from cancer cells, and its potential influence on clinical outcomes is discussed. Membrane fusion events underlie crucial physiological functions such as synaptic transmission and endocrine secretion involving vesicle fusion with the plasma membrane and subsequent release of neurotransmitter/ hormone, as well as organelle maturation associated with immune responses, apoptosis and cell division. Soluble N-ethylmaleimide sensitive factor Attachment Protein Receptors (SNAREs) are central players mediating membrane fusion reactions. Tethering factors and Rab GTPases are the other typically essential factors regulating cargo sorting and fidelity of membrane fusion events within the cell.

Discussion

Exosome biogenesis, transport and release is thought to be precisely regulated by several proteins, including SNAREs, tethering complexes and rab GTPases. Ykt6, belonging to the longin family of R-SNAREs, has been known to be involved in vesicular transport between secretory compartments such as in the endoplasmic reticulum-Golgi step, within early/ recycling endosomes, in endocytic trafficking to the lysosome, and in autophagocytosis [12-14]. Over the last seven years, there is emerging evidence from multiple model systems on Ykt6 being implicated in exosome release [15-17]. For instance, Ykt6 was identified as a key molecule, and arguably the only member of the SNARE family, functioning in the release of Wnt3a-containing exosomes from HEK293 cells [15]. A microarray analysis of invasive phenotypes in a breast cancer model revealed that Ykt6 overexpression was associated with (i) an aggressive phenotype in an *in vitro* local invasion assay and (ii) a metastatic phenotype in an *in vivo* tumorigenesis assay [16]. Interestingly, in breast cancer patient samples, Ykt6 was found to be upregulated in p53-mutant tumors that were resistant to Docetaxel, suggesting that elevated Ykt6 levels might contribute to taxane therapy resistance [17]. This hypothesis is reinforced by the finding that Docetaxel-induced apoptosis was enhanced by the *in vitro* silencing of Ykt6 [17]. In the non-small cell lung cancer (NSCLC) cell line A549, siRNA-mediated knockdown of Ykt6 produced a remarkable downregulation in the level of secreted exosomes [18]. Moreover, the same study also reported that NSCLC patients with high levels of Ykt6 protein expression had shorter overall survival (OS) and progression-free survival (PFS). In this small subset of patients, those with higher Ykt6 expression had significantly more exosomes in blood plasma compared to those with lower Ykt6 levels. These preliminary results provide the first indication that Ykt6 levels in the tumor may act as a surrogate for exosome levels in the plasma and thus could serve as a biomarker for NSCLC prognosis.

Gross et al. have demonstrated, through elegant biochemical and genetic approaches in Drosophila, that a portion of functional Wnts (Wg is the Drosophila Wnt1 homolog) is secreted on exosomes both *ex vivo* and *in vivo* [15]. To systematically identify proteins involved in secretion of Wnts on exosomes, the authors carried out an *in vivo* RNA interference (RNAi) screen of exosome-associated proteins in Drosophila. Depletion of Ykt6 by RNAi showed accumulation of Wg and other exosomal markers inside the producing cells. In addition, depletion of Ykt6 in Wg-producing cells reduced the expression of the Wg target gene senseless in the neighboring target cells when compared with control, consistent with a defect in Wg signaling. Strikingly, knockdown of Ykt6 did not impair other transport pathways or secretion through non-exosomal routes that it is involved in. Their results strongly indicate the selective requirement of Ykt6 not only in Wg secretion but also in Wg signaling in target cells.

One of the mechanisms of segregation of exosomal proteins into intraluminal vesicles (ILVs) in multi-vesicular bodies (MVBs) involves recognition of cargo receptors by the ESCRT (endosomal sorting complex required for transport) machinery which acts as a multisubunit tethering factor [19]. RNAi-mediated depletion of the ESCRT subunit HGS in HEK293 cells reduced the level of Wnt3A in the exosomal fraction and reduced Wnt activity in the supernatant by 40% as compared with control [15, 20]. This study proposed that an ESCRT-mediated sorting step is required for Wnt complex trafficking into MVBs. It remains to be seen whether interfering with other cargo recruitment components can affect cargo incorporation into exosomes and subsequent signaling, which would be of tremendous potential in designing newer anti-cancer therapies to combat metastasis and drug resistance. There is increasing interest in exploring the identity of microRNAs captured within exosomes and their corresponding recruitment protein complexes, due to their potential to be developed as therapeutic targets. MicroRNA-223 (miR-223) has been found to be commonly repressed in various cancers [21, 22] and is selectively enriched in the exosome fraction relative to cellular levels [23]. While identifying proteins that are directly involved in packaging miR-223 in exosomes, Shurtleff et al. [23] demonstrated that the Y-box protein I (YBX1) co-precipitated with miR-223 isolated from a cell-free reaction. The authors concluded that the RNA-binding protein YBX1 binds to and is required for the sorting of miR-223 in exosomes in the cell-free reaction, and serves an important role in the secretion of miRNAs in exosomes by HEK293T cells.

An independent study performed on A549 cells described the involvement of Rab27A in exosome secretion [24]. In this study, the reduction of Rab27A by Rab27A-specific shRNA was reported to significantly decrease the secretion of exosomes from A549 cells. The authors also found that EPI64, acting as a specific GTPase Activating Protein (GAP) for Rab27A and not Rab27B, participated in exosome secretion from A549 cells. Overexpression of EPI64 was found to enhance exosome secretion. In general, Rabs function as molecular switches by cycling between two nucleotide bound states: a GTP-bound active state and a GDP-bound inactive state. Activated Rabs promote various steps in membrane trafficking. Further studies might employ Rab27A as a molecular switch to control exosome secretion and force this exosome secretion in a direction that benefits human health. Furthermore, inhibition of Rab35 and its GAPs TBC1D10A-C, has been shown to block exosome secretion in a cell-type-dependent manner [25]. High levels of Rab27B, possibly contributing to higher exosome release and in turn increasing the likelihood of generating malignant transformation in exosome recipient or target cells, have been linked to poorer prognosis in liver, bladder and pancreatic cancer [26-28]. However, there is conflicting evidence regarding the correlation between Rab27B expression levels and its prognostic value in colorectal cancer [29, 30] and further studies would be required to resolve this and associated conundrums.

Conclusion

Along with regulating the well-characterized membrane transport pathways, many of the evolutionarily conserved trafficking factors belonging to the SNARE, tethering complex and Rab GTPase families have also recently been implicated in the control of exosome biogenesis and secretion. Further studies identifying coding and non-coding genes involved in the regulation of exosomal transport would be necessary to understand their utility in determining cancer diagnosis and prognosis. Identifying and targeting genes encoding membrane trafficking components may be a more simplistic yet comprehensive anti-cancer therapeutic strategy, in comparison to a similar process for a potentially much larger abundance and diversity of exosomal oncogenes/ oncoproteins. Increasing knowledge of the protein and lipid

compositions of exosomes in addition to their resilient physicochemical properties will facilitate engineering or manipulating these extracellular vesicles as nanodevices for the development of new diagnostic and therapeutic applications. Before protein, lipid and nucleic acid components from exosomal origin may be employed as diagnostic agents not only in the context of cancer but also cardiovascular and neurodegenerative disorders, many challenges need to be addressed. These include isolating highly pure exosome extracts, understanding exosome biogenesis and function in cell-cell communication, designing well-controlled clinical trials, obtaining large multicenter validation of biomarkers and standardizing data interpretation.

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