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Short Communication

EXOSOMES: BIOLOGY, FUNCTION AND DIAGNOSTIC POTENTIAL

Nishant Kumar
Virology Group, ICGEB, Aruna Asaf Ali Marg, New Delhi, India

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Abstract: Exosomes are small (30-100nm) vesicles that are generated intracellularly through the inward invagination of multivesicular bodies (MVBs) and released from cells when MVBs fuse with the plasma membrane. The exosomes contain mRNAs, miRNAs, proteins and various other signaling molecules. These fuse with other cells, release their contents in the recipient cells, where the mRNAs, miRNA and other exosome contents exert their functional effect. The molecular contents of exosomes depend on the patho-pyhysiological status of the cells and this property, nowadays, is utilized in the discovery of novel biomarkers of diseases.

Key Words: Exosomes, Multivesicular bodies (MVBs), miRNAs, mRNAs, Biomarkers.

INTRODUCTION

Exosomes are small vesicles, size ranging from 30-100nm in diameter, released from the cells into the culture media, *in vitro*; or body fluids such as blood, urine, spinal fluids and amniotic fluid, *in vivo*. Initially, the secretion of vesicles as exosomes were described to be a mechanism for the removal of cellular waste outside the cells, but subsequent works of many researchers in this field have shown an important role of exosomes in intercellular communication [1, 2].

Biogenesis, secretion and composition of exosomes

Exosomes are formed within endosomes by invagination of the endosomal limiting membrane, resulting in the formation of multivesicular bodies (MVBs). The MVBs have two fates: either they fuse with the lysosome, resulting in degradation of the intraluminal content of the MVBs; or it fuse with the plasma membrane, resulting in the secretion of their intraluminal vesicles as exosomes outsides the cells [3]. Structurally, exosomes are microvesicles enclosed by a lipid bilayer enriched in sphingolipids and ceramide. The lipid membrane of the exosomes contains various integral proteins like CD81, alix, CD63, MHC-I, MHC-II, CD9, calnexin, clathrin, transferring receptor and tsg101. The exact constellation of the integral membrane proteins of the exosomes depends on the source of exosomes, but some of the proteins, particularly CD81, CD63, and tsg101, are present in most of the exosomes irrespective of the source of cells producing it and are used as characteristic markers to differentiate exosomal vesicles from other microvesicles [4].

The lumen of the exosomal vesicles contains miRNA, mRNA, soluble proteins and various others signaling molecules. The characteristic feature of the RNA content of exosomes is that it lacks ribosomal RNA [5].

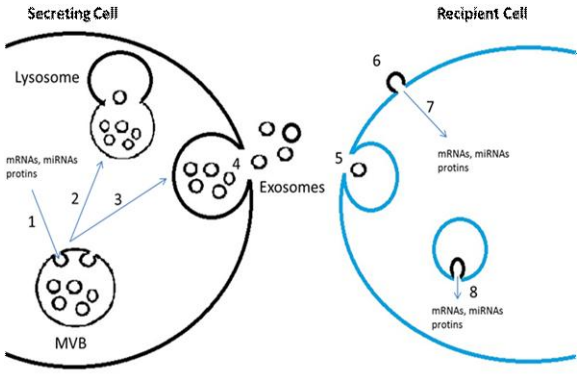

Figure 1: Biogenesis of exosomes and their targeting to the recipient cell.

Packaging of mRNAs, miRNAs and proteins into the intraluminal vesicles of MVBs (step 1). MVB has two fates; either it fuses with the lysosome for degradation of its intraluminal contents (step 2), or it fuses with the plasma membrane and releases its contents as exosomes outside the cells (step 3 and 4). The exosomes, present in the extracellular spaces, are internalized by the recipient cells, either by endocytosis (step 5), or directly fuse with the plasma membrane of the recipient cells (step 6). Finally, exosomes release their intaluminal content in the recipient cell as shown in step 7 and 8.

Isolation of exosomes

Exosomes are isolated from cell culture or body fluids using differential centrifugation. The first few steps in the isolation of exosomes involves removal of dead cells and cell debris in which samples are first centrifuged at 300xg to remove the cells followed by centrifugation at 3000xg for 10 min and 10,000xg for 30 min to remove larger and smaller cells debris respectively. Second step involves centrifugation of sample at 100,000xg for 70 min to pellet the exosomes fraction followed by washing the pellet in phosphate buffered saline (PBS), and

***Corresponding Author:**
Nishant Kumar,
Senior Research Fellow,
Virology Group, ICGEB,
Aruna Asaf Ali Marg, New Delhi 110067, India.

centrifugation at 100,000xg for 70 min. Finally, the pellet is dissolved in required amount of PBS [6].

Biological functions of exosomes

Exosomes, present in the extracellular fluids, are internalized by the recipient's cells where the exosomal contents upon their release into the recipient cells perform their respective functions. As exosomes composition and their contents widely vary and depends on the types of exosomes secreting cells, so the types of response that exosomes will elicit in the recipient cells depends on the source of exosomes. Here, we are describing some of the functions of exosomes in intercellular communication [7].

As carrier of genetic information

Valadi *et al.,* (2007) has shown the presence of mRNAs and miRNAs in exosomes and that the mRNAs, present in the exosomes, are functional using *in vitro* translation system. They have also shown that exosomes deliver its mRNAs and miRNAs contents in other cells when treated the recipient cells with purified exosomes. They treated human cells with exosomes isolated from mouse cell line and found new proteins of mouse origin in the recipient human cells. Subsequent research in other labs has also shown the exosomes mediated transfer of mRNAs and miRNAs in recipient cells. Thus, exosomes acts as a carrier of genetic exchange between cells [8].

As carrier of cell signaling molecules

Exosomes, in addition to mRNAs and miRNAs, carry various protein signaling molecules. The signaling molecules, present in the exosomes, upon their delivery into the recipient cells, modulate their respective signaling pathways in recipient cells. Putz *et al.,* (2012) has demonstrated that phosphatase and tensin homolog (PTEN), a tumor suppressor protein normally localized in the cytoplasm and nucleus, is secreted in exosomes. They found that PTEN, present in the exosomes, was internalized by recipient cells with resultant functional activity, which resulted in reduced phosphorylation of the serine and threonine kinase Akt and reduced cellular proliferation [9].

As regulator of cellular pool of various molecules

Exosomes, in some instances, not only acts as carrier to deliver various molecules to recipient cells but also regulate the cellular pool of various molecules by discarding the unwanted one e.g., exosomal secretion down regulates proteins such as transferrin receptor or integrins, which are known to have no role in differentiated red blood cells, from reticulocytes [10, 11]. Chairoungdua *et al.,* (2010) has shown that CD82 and CD9 down regulate the Wnt-signaling pathway through the exosomal discharge of β-catenin thereby depleting its cellular pool. Thus, exosomal packaging and discharge of cytosolic and membrane proteins can modulate the activity of cellular signaling pathways [12].

As modulator of immune response

Exosomes released from antigen presenting cell play an important role in the activation of immunological responses. Exosmes derived from antigen bearing dendritic cells have been shown to induce antigen specific T cell activation [13]. Mittelbrunn *et al.,* (2011) has demonstrated that at immunological synapse there is a unidirectional transfer of miRNA-loaded exosomes from T-cells to antigen presenting cells in antigen dependent fashion [14].

Exosomes in diagnosis of diseases

Recent developments in the field of exosomes biology have shown that all body fluids (e.g. blood, urine, saliva, milk etc.) contains exosomes, and the fact that exosomal contents widely vary depending on the source i.e., cell type producing the exosomes and patho-physiological state of the cells, analysis of the exosomal contents could be an early diagnostic markers for various disease [15]. The table below summarizes the characteristic signature of some exosomal molecules that are altered in diseased conditions:

Table: Characteristic signature of some exosomal molecules that are altered in diseased conditions

Disease	Samples	Molecular signature of exosomal contents (proteins / miRNAs)	Levels	References
Prostate cancer	Urine	PCA-3, TMPRSS2: ERG and PSA	Up	16
Acute kidney injury	Urine	Fetuin-a	Up	17
Breast cancer	Plasma	miR-141 and miR195	Up	18

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