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BIOGENESIS OF EXOSOMES - A REVIEW OF CURRENT LITERATURE

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ABSTRACT
EXOSOMES WERE FIRST REPORTED A FEW DECADES AGO AND THEY HAVE BEEN THE CENTER OF INTEREST OF RESEARCHERS DUE TO THEIR VARIOUS FUNCTIONS, THEIR CAPACITY TO MEDIATE INTERCELLULAR COMMUNICATION AND TO PROVIDE INFORMATION ABOUT THE ORIGIN CELL. EXOSOMES ARE EXTRACELLULAR VESICLES DERIVED FROM THE CELL MEMBRANE AND CONTAIN A WIDE VARIETY OF MOLECULES SUCH AS PROTEINS, RNAS, LIPIDS, AND FRAGMENTS OF GENOMIC DNA. STUDIES REPORT THAT DIFFERENT EXOSOME- DERIVED PROTEINS CAN SERVE AS BIOMARKERS FOR THE ISOLATION AND QUANTIFICATION OF EXOSOMES. ALL CELL TYPES SECRETE EXOSOMES AND DATA SUGGESTS THAT EXOSOMES RELEASED FROM METABOLICALLY ACTIVE CELLS CAN REPROGRAM THE TARGET CELL.

KEYWORDS: EXOSOMES, CELL, PROTEINS, INFORMATION, BIOMARKERS

1. INTRODUCTION

"Exosomes" were discovered in 1983 by Harding and Pan and belong to a large family of extracellular vesicles, secreted by a majority of cells[7]. The researchers grew reticulocytes with labeled transferrin receptors in order to analise the passage of transferrin

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receptors from plasma membranes into the reticulocytes. They discovered that the labeled transferrin receptors were internalized and then packaged into vesicles (~50 nm) inside the reticulocytes. Furthermore, these vesicles were secreted out of the maturing blood reticulocytes into the extracellular space[8]. Vesicle populations can be classified depending on their origin, their morphology and mode of secretion into the extracellular space in three cathegories: apoptotic bodies, microvesicles (MV or ectosomes) and exosomes. Ectosomes are vesicles generated by the external budding of plasma membranes and have approximately 50-1000 nm.

2. BIOGENESIS OF EXOSOMES

Exosomes are generated by a process that involves double invagination of the plasma membrane with the formation of primary sorting endosomes (early endosomes- EE), late sorting endosomes (LE) and multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) inside. Most ILVs are released into the extracellular space after fusion with the plasma membrane and exocytosis, generating exosomes.

Exosomes are nano-sized vesicles (50–120 nm in diameter) with a lipid bilayer with an average thickness of ~5 nm. This lipidic bilayer includes ceramide, cholesterol, phosphoglycerides and saturated fatty-acyl chains. Its outer surface is rich in saccharide chains, such as mannose, alpha-2,6 sialic acid, and N-linked glycans. Exosomes have a cup-shaped morphology, when examined under an electron microscope.

The best way to define exosomes biochemically is to identify specific markers. Data suggests that cargos (such as MHC II from B cells) and other cell type-specific antigens may help to differentiate exosomes from other extracellular vesicles. These markers can be proteins that are specific to the endosomal pathway, for example proteins related to MVB biogenesis (such as Tsg101, Alix), tetraspanins (CD-63, CD-9, and CD-81), membrane fusion proteins (RAB GTPases and Annexins), signaling molecules (cell adhesion molecules, growth factor receptors) and heat shock proteins (HSP-70 and HSP-90).

Studies suggest an involvement of numerous mechanisms in the generation of exosomes: Ras-related small GTP-ase protein(Rab protein), ALIX(apoptosis- linked gene 2interacting protein X), syndecan-1, ESCRT(endosomal sorting complexes required for transport), proteins, phospholipids, tetraspanins, ceramides, sphingomelinases, SNARE (soluble N ethylenamide-sensitive factor (NSF) receptor protein attachement.

2.1 MATURATION OF ENDOSOMES

EE – the early sorting endosome- is defined as an organnel formed by the fusion of primary endocytic vesicles and represents the first sorting station in the endocytic pathway[9]. Endocytic vesicles appear through clathrin-mediated respectively clathrin-independent membrane mechanisms that direct their contents to EE in similar ways[10].

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EE receives vesicles for about 10 minutes, during which time membrane and fluid structures are directed to the fast recycling path. Some of the content is retained and accumulated through the life of EE, to be subsequently included in LE.

EE functions are defined by a series of cytosolic proteins on the membrane surface of EE. Rab5 and its effector Vps 34/p150 (a phosphoinositide 3- kinase PI3K) that generates phosphatidylinositol- 3- phosphate, mark EE and help to identify them. Structures like RAB5 and EE-A1 (EE- antigen 1) have a role in generating and maintaining EE. EE communicates with the trans-Golgi network through bidirectional vesicles exchange. Other structures like RAB5 and EE-A1 (EE- antigen 1) have a role in generating and maintaining EE.

EE communicates with the trans-Golgi network through bidirectional vesicles exchange. The traffic between the endosomes and the trans- Golgi network is a continuous studied process because it is responsible for the delivery of endolysosomes and the removal of endosomal components during endosomal maturation[11]. These processes take place at the level of EE, LE maturation and possibly after the fusion of LE with lysosomes. At the endosomal level, the sorting and formation of vesicles for transport to the trans-Golgi network depends on Rab7 and Rab9[12].

The clathrin-dependent content can be recycled to the cell surface by a fast path that requires RAB4 and RAB35[13]. EE can also transfer the contents to the ERC (endocytic recycling compartment) from which recycling endosomes can emerge. Traffic regulators and recycling include GTP-ases (Rab and Arf proteins) and their effectors (RME1- receptor mediated endocytosis 1, Eps15- epidermal growth factor receptor substrate 15, EHD1-EHD4, tubular proteins, motor proteins). From ERC, content recycling requires RAB11.

Recycling of clathrin-independent content involves the generation of separate tubes, dependent on RAB8 and RAB22A. The recycling tubular endosome requires the participation of: RAB 10, RAB11, RAB22A, RAB35, the complex consisting of EHD1 and ALIX, and RAB11FIP2, for its recycling function,

In the periphery, the tubular endosome divides into vesicles before merging with the cell surface, a process that requires PAR3 (partioning defective protein 3), CDC42 and ARF 6 (ADP-ribosylation factor 6), RAB11 and cortical actin.

Late sorting endosomes (LE) are formed from the vacular domains of EE that have a different content than the tubules. The slightly acidic medium in the EE lumen (pH 6.8-5,9) favors conformational changes in proteins, which cause the release of ligands from receptors. Thus, LE contains ligands, internalized solvents, ILV and various particles (i.e viruses). The membrane contains most cholesterol, lipid rafts rich in sphingolipids, membrane protein aggregates, V-ATP-ase, ESCRT membrane proteins intended for degradation..

After LE formation and a series of changes that occur during their maturation (RAB5 is replaced by RAB7, RAB4, RAB11, RAB22 are lost and RAB 9 is added, ubiquinated proteins recruit ESCRT and other factors that induce internal budding of the membrane with ILV formation, decrease in luminal pH) after 10-40 minutes they fuse with lysosomes[14].

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The maturation process involves changes in membrane components, migration into the perinuclear area, acquisition of lysoomal components, generation of MVB. These processes are regulated by surface factors recruited from the cytosol.

LE also mediates the transport of lysosomal components form the trans-Golgi network to lysosomes, maintaining the diversification, expansion of recycling and degradation systems.

2.2 BIOGENESIS OF INTRALUMINAL VESICLES

One of the most important processes in LE biogenesis is the formation of ILV but the process begins in EE. The cytosolic membrane surface of EE has specific areas containing clathrin and components of the ESCRT system (the machine responsible for sorting ubiquinated membrane proteins in ILV)[15]. Membrane proteins that have been monoubiquinated are coated by ESCRT and enter the MVB pathway along with the luminal content of EE. The vacuolar lumen of EE contains several ILVs.

There are several reasons why ILV is formed: the signaling receptors included in ILV are inactivated because they lose contact with the cytosol; lipids and membrane proteins are delivered to lysosomes in a form more accessible to hydrolases. A major role in ILV formation is played by the ESCRT complex (ESCRT 0, I, II, III) and a number of accessory proteins, AAA- type ATP-ase Vps4 and ALIX. They are recruited from the cytosol, at the surface of the endosomes. ESCRT is found as either heterodimer or heterotetrames composed of Hrs (hepatocyte growth factor-regulated tyrosine kinase substrates) and STAM (signal transducing adapter molecules), which are linked to each other.

The role of ESCRT is to sort the contents entering the EE, labeled membrane proteins and their ligands, their segregation into membrane domains and the generation of vesicles formed by internal budding.

Sorting is based on the presence of ubiquitin marker in the cytosolic domain of membrane proteins, recognized by ESCRT-0, which interacts with the membrane regions rich in phosphatidyl inositol 3 phosphate (PIP3).

ESCRT-0 links ubiquitin content through Zinc Finger Domains (ZFD) and Ubiquitin-interacting Motifs (UIMs)[16]. Up to 8 different ubiquinated molecules can be easily associated using Hrs Double Ubiquitin Interacting Motif as well as STAM Vps27/Hrs/STAM and UIM domain, in the case of heterotetramer. A domain at the C-terminus of the Hrs subunit of ESCRT-0 recruits ESCRT-1. This is a heterotetramer of TSG101 (Tumor Suppressing Gene 101), Vps 28 (Vacuolar protein sorting associated proteins) Vps37 and MVB 12(multivesicular body sorting factor 12), crossed to form a stem structure with domains for ESCRT-0 and ESCRT-II at opposite ends. ESCRT-I and ESCRT-II appear as a complex that induces the budding of endosomes in the cytoplasm. During the budding process, the ESCRT -0 content is relocates to the bud along with another content selected by the loading system. After bud formation and content selection, CHMP 6 (Charged Multivesicular body, Protein) of the ESCRT-III complex binds directly to ESCRT-II. This protein polymerises as a coil around the budged ILV neck and serves as a cord for the ILV pocket, which is able to close by adding CHMP3 to the ESCRT-III complex. This is possilble due to the high affinity of

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ESCRT-III for the plasma membrane. ESCRT-III rapidly recruits deubiquination enzymes that break the weak link between the ESCRT-0 complex and the luminal content of ILV.

Studies have shown that typical ALIX exosomal protein, which are associated with various ESCRT complex proteins (TSG 101, CHMP4) participate in the process of endosomal membrane budding and selection of exosomal content by interaction with syndecan.

Recent studies have suggested a mechanism for sorting exosomal content in MVB through an ESCRT- independent mechanism. It involves shelf microdomains for lateral segregations of endosomal membrane contens.

These microdomains are rich in sphingomyelinases that form ceramides. Ceramides induce the phase of lateral separation and coalescence of microdomains in the membrane model[17]. Because ceramides have a conical shape, endosomal membrane negativity can be created and this promotes domain-dependent budding.

Another mechanism involved in the biogenesis of exosomes is that of tetraspanins. Tetraspanin-rich microdomains (TEMs) are membrane platforms specializing in the compartimentalization of receptors and signaling proteins in the plasma membrane. Studies have shown that TEM and CD81 tetraspanin play a role in sorting target receptors and intracellular components to exosomes[18].

After ILV formation, MVB is either transported to lysosomes for digestion or directed through the cytoskeletal microtubules to the plasma membrane for fusion. The mechanism by which MVB is selected for degradation is unknown. An increase in ISGylation (ISG 15- interferon- stimulated 15- ubiquitin- like protein that is expressed and conjugated to the target) of TSG 101 on MVB, decreases the number of MVBs and consequently the number of exosomes release. Subsequently, cortactin (a protein responsible for stabilizing actin) is closely related to MVB secretion, without altering the content[19].

Studies have shown that MVBs marked for degradation have lower cholesterol levels compared to those that fuse with the plasma membrane. Rab 27, Rab11, Rab 35 and calcium play a role in the delivery of MVB to the plasma membrane[20]. Down-regulation of the components involved in MVB trafficking to the plasma membrane decreases the number of exocytosed exosomes. The release of exosomes into the extracellular medium is mediated by Rab GTPases and occurs by the fusion of the matured MVB with the plasma membrane.

3. CONCLUSIONS

Biogenesis of exosomes is a very complex process that involves double invagination of the plasma membrane with the formation of primary sorting endosomes (early endosomes-EE), late sorting endosomes (LE) and multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) inside and their exocytosis generating exosomes.

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Exosomes are nano-sized vesicles with a lipid bilayer rich in ceramide, cholesterol, phosphoglycerides and saturated fatty-acyl chains.

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There is a need of more research in this domain in order to understand all the mechanisms involved in exosomes biogenesis.

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