Advances in Experimental Medicine and Biology 998

Junjie Xiao
Sanda Cretoiu *Editors*

Exosomes in Cardiovascular Diseases

Biomarkers, Pathological and Therapeutic Effects

Springer

Advances in Experimental Medicine and Biology

Advances in Experimental Medicine and Biology presents multidisciplinary and dynamic findings in the broad fields of experimental medicine and biology. The wide variety in topics it presents offers readers multiple perspectives on a variety of disciplines including neuroscience, microbiology, immunology, biochemistry, biomedical engineering and cancer research. Advances in Experimental Medicine and Biology has been publishing exceptional works in the field for over 30 years and is indexed in Medline, Scopus, EMBASE, BIOSIS, Biological Abstracts, CSA, Biological Sciences and Living Resources (ASFA-1), and Biological Sciences. The series also provides scientists with up to date information on emerging topics and techniques. 2015 Impact Factor: 1.953

More information about this series at http://www.springer.com/series/5584

Junjie Xiao • Sanda Cretoiu
Editors

Exosomes in Cardiovascular Diseases

Biomarkers, Pathological and Therapeutic Effects

Springer

Editors
Junjie Xiao
Shanghai University
Shanghai
China

Sanda Cretoiu
Victor Babeş National Institute of Patho
Bucharest
Romania

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-981-10-4396-3 ISBN 978-981-10-4397-0 (eBook)
DOI 10.1007/978-981-10-4397-0

Library of Congress Control Number: 2017952039

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

Part I
Overview

Chapter 1
The Multifaceted Functions of Exosomes in Health and Disease: An Overview

Claudia Arenaccio and Maurizio Federico

1.1 Introduction

Cytoplasm of eukaryotic cells contains several compartments, including *trans*-Golgi network, mitochondria, peroxisomes, endoplasmic reticulum, having different functions. Transport of macromolecules among these dynamic structures is mediated by vesicles moving in a densely populated microenvironment [1, 2]. In some instances, part of these vesicles are released into the extracellular milieu. Extracellular vesicles (EVs) are part of mechanism of intercellular communication, a function of vital importance for multicellular organisms. For decades, intercellular communication has been thought to be solely regulated by cell-to-cell contact and release of soluble molecules into the extracellular space. These molecules transmit the signal through their uptake or binding to specific receptors on target cells. However, the discovery of vesicular structures released into the extracellular space containing a multitude of factors including signaling molecules, proteins and nucleic acids, has opened a new frontier in the study of signal transduction, thereby adding a new level of complexity to our understanding of cell-to-cell communication.

Body fluids (e.g., blood, urine, saliva, amniotic fluid, bronchoalveolar lavage fluid, synovial fluid, breast milk) contain various types of membrane-enclosed vesicles [3] recognizing different pathways of biogenesis. These vesicles possess different biophysical features and functions in health, e.g., protein clearance [4], immune regulation [5], cell signaling [6–8], as well as in disease, such as in infections [9–12] and cancer [13, 14]. Originally, EVs were thought to be garbage bags through which cells eject their waste. Today, it is widely accepted that EVs are key components of the intercellular communication network.

C. Arenaccio (✉) • M. Federico
National Center for Global Health, Istituto Superiore di Sanità,
Viale Regina Elena 299, 00161 Rome, Italy
e-mail: claudia.arenaccio@iss.it

J. Xiao, S. Cretoiu (eds.), *Exosomes in Cardiovascular Diseases*, Advances in Experimental Medicine and Biology 998, DOI 10.1007/978-981-10-4397-0_1

All EV subtypes are limited by a lipid bilayer membrane surrounding a specific cargo of molecules, and having different sizes and buoyant densities. The variety of vesicles released from cells as well as the methods used to isolate them led to some confusion in their nomenclature. Current research mainly considers two types of EVs according to their biogenesis, i.e., ectosomes and exosomes. The term ectosomes indicates vesicles of 150–1000 nm in diameter directly budding from plasma membrane, whereas exosomes refer to vesicles of 30–150 nm in diameter generated intracellularly by inward invagination of endosome membranes leading to formation of intraluminal vesicles (ILVs). ILVs became part of multivesicular bodies (MVBs) which are released in the extracellular space upon fusion with plasma membrane [15]. The term exosomes was coined in 1981 by Trams and coll. who described the release from various normal and neoplastic cell lines of EVs with an average diameter of 500–1000 nm accompanied by a vesicle sub-population having a diameter of ~40 nm [16]. Some years later, it had been reported that reticulocytes actively secrete vesicles of 50–100 nm in diameter through a process mediated by fusion events of multivesicular endosomes with the plasma membrane [4].

Exosomes contain DNA, RNA, proteins, lipids, and metabolites of producing cells, and are released into the extracellular space under both physiological and pathological conditions. In recent years, the effects of exosomes are being studied in several pathological conditions, such as neurodegenerative, viral, cancer, and cardiovascular diseases. Their presence in many biological fluids prompted many research groups to investigate their possible use as disease biomarkers and tools for the development of new therapies.

In this introductive chapter, an overview about biogenesis, structure, and functions of exosomes in both physiological and pathological conditions is provided. In addition, some clues about current and future utilizations of exosomes in both diagnostic and therapy are summarized.

1.2 Biogenesis of Exosomes

Cell vesiculation can be induced by multiple stimuli, including cell differentiation, activation, senescence, hypoxia, transformation, and viral infections. Among the different types of EVs, exosomes are the best characterized. They have a buoyant density of 1.10–1.14 g/mL, and display either a round spherical shape (Fig. 1.1), or a cup-like morphology depending on the transmission electron microscopy technique used [17]. Exosomes are the only known secreted cellular vesicles originating from internal membranes. They are essentially ILVs generated by inward budding of endosomal MVBs and targeted to plasma membrane [18].

The processes leading to generation of ILVs in MVBs and their fusion with plasma membrane are not completely known. Two independent pathways have been proposed (Fig. 1.2). The first one involves the endosomal sorting complex required for transport (ESCRT). This multi-molecular machinery comprises ESCRT0, ESCRTI, ESCRTII and ESCRTIII, and is recruited to the endosomal membranes

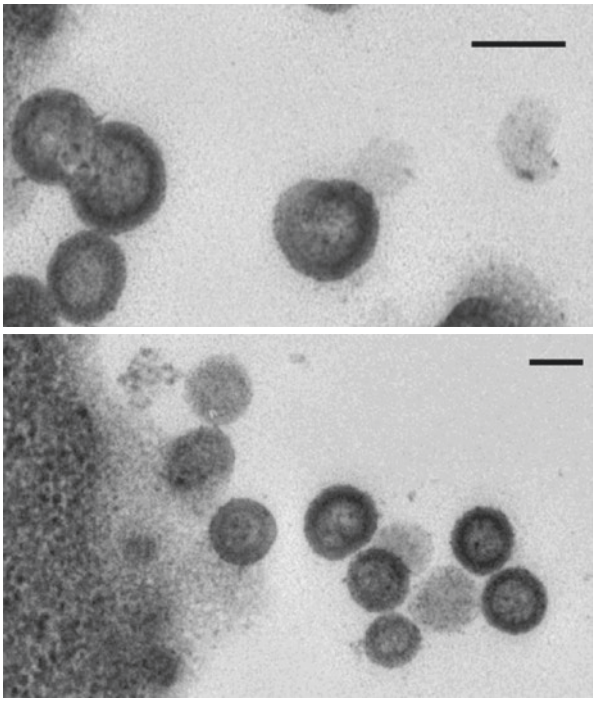

Fig. 1.1 Exosomes as detected by transmission electron microscopy upon negative staining. Bar: 0.1 μm

where ILVs are generated. In detail, ESCRT0, ESCRTI and ESCRTII recognize ubiquitinated proteins, whereas ESCRTI and ESCRTII induce, together with additional factors, the invagination of the late endosomal membrane [7, 19]. Afterwards, ESCRTIII binds ESCRTII thereby leading to the deubiquitination of cargo proteins, the promotion of vesicle abscission and, ultimately, the generation of ILVs [20].

Recently published evidences describe the existence of an ESCRT-independent pathway based on the specific lipid composition of the endosomal membranes. This hypothesis stemmed from the evidence that MVB can be formed in cells depleted of the four ESCRT components [21]. Membranes of endosomal compartments include lipid rafts comprising high quantities of sphingolipids, which are substrates for the neutral sphingomyelinase 2 (nSMase2) [22]. This enzyme converts sphingolipids to ceramide, whose accumulation induces microdomain coalescence thereby triggering ILV budding. As a matter of fact, ILV formation and exosome release are reduced when nSMase2 is inhibited [23].

Once ILVs are released into MVBs, they are either forwarded to degradation through the lysosomal pathway, or transferred to the cell periphery for the secretory pathway. Both processes are regulated by RabGTPases. While Rab7 mediates the ILV degradation through the fusion of MVBs with lysosomes, several other Rab proteins (i.e., Rab27a, Rab27b, and Rab11) are responsible, together with

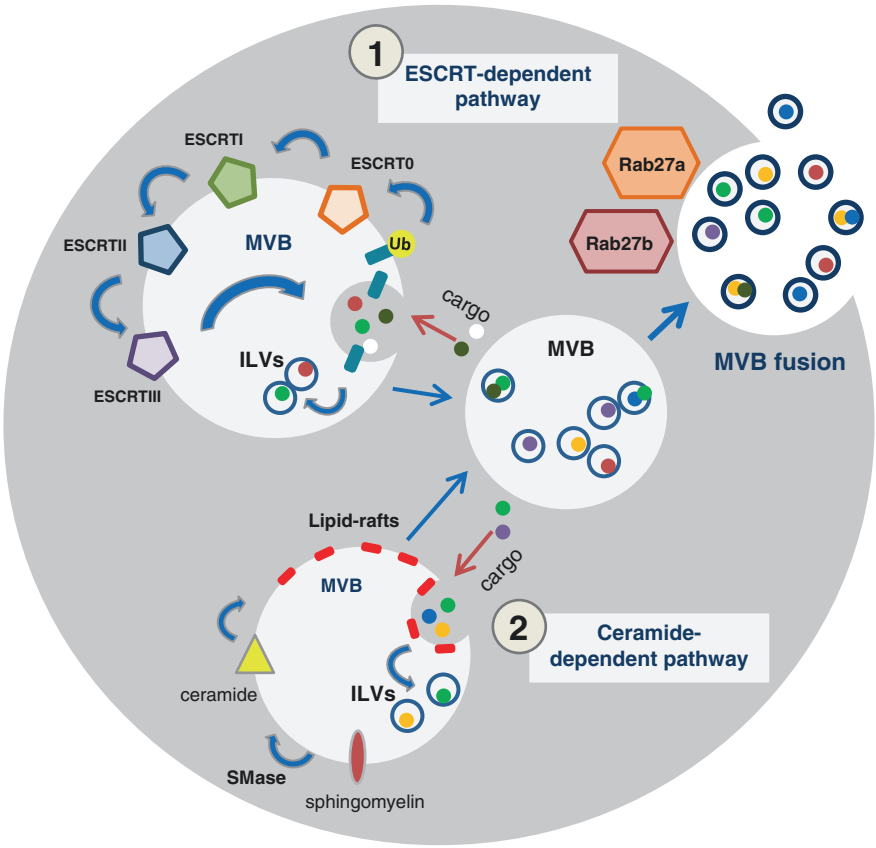

Fig. 1.2 Biogenesis and secretion of exosomes**.** Exosome biogenesis is mediated by ESCRT (*1*) and/or ceramide (*2*)-dependent pathways. In ESCRT depend pathway, sequential recruitment of ESCRT0, ESCRT I, ESCRT II to the endosomal membrane induces membrane curvature, as well as recruitment of ubiquitinated (Ub) proteins for sorting into the vesicles. Binding of ESCRTII to ESCRTIII leads to deubiquitination of cargo proteins, promotion of vesicle abscission, and thereby generation of ILVs. In ceramide dependent pathway, nSMase2 converts sphingolipids to ceramide whose accumulation leads to ILV budding. After ILV formation, MVBs fuse with plasma membrane. ILVs released into extracellular space are referred to as exosomes

tetraspanins, for intracellular MVB trafficking and secretion [22, 24]. In detail, Rab27b induces the mobilization of MVBs to the actin-rich cortex beneath the plasma membrane to which MVBs contact and fuse as consequence of the action of Rab27a. In cells defective for Rab27a functions, the fusion of MVBs with plasma membrane is induced by Rab11 in response to increased cytosolic calcium [25].

Endosome-like domains rich in exosomal proteins, lipids, and carbohydrates have been found within the plasma membrane of certain cell types [26]. These domains are supposed to be involved in either trafficking of cargo from plasma membrane back to MVBs, or in vesicle formation and budding from the plasma

membrane [20]. For instance, it was shown that vesicles with the typical size of exosomes bud from the plasma membrane of both lymphocytes [27] and muscle cells [28, 29].

1.3 Structure and Composition of Exosomes

In recent years, many research groups have focused their efforts on the identification of the content of EVs and exosomes. These works led to the development of two constantly updated databases, i.e., Vesiclepedia (http://microvesicles.org), a compendium where the characteristics of all EVs are summarized [30], and ExoCarta (http://www.exocarta.org), a manually updated list of proteins, RNAs, and lipids identified in exosomes [31, 32].

Exosomes are formed by a lipid bilayer membrane enclosing a small organelle-free cytosol containing a heterogeneous array of macromolecules defined luminal cargo [33, 34]. It includes proteins, RNA, DNA, and lipid-derivatives, such as ceramide, cholesterol, phosphatidylserine and sphingolipids. Similarly to plasma membrane, the composition of lipid bilayer of these vesicles includes lipid rafts, i.e., detergent-resistant microdomains enriched in specific proteins such as flotillins and caveolins [35, 36]. At the same time, exosome membrane comprises components not present in plasma membrane of the exosome-producer cells and vice versa. For instance, exosome membranes do not contain lysobisphosphatidic acid (LBPA) [37] which, on the contrary, has been isolated from both plasma membrane and ILVs [38]. Starting from this evidence, it was hypothesized that LBPA has an a role exclusively in the formation of MVBs targeted to lysosomes [39].

Recent studies based on mass spectrometry highlighted two key aspects regarding the protein contents of exosomes. First, some exosome proteins are cell type-specific, while others are invariable part of exosomes independently from the cell of origin. Second, the exosome protein composition does not necessarily reflect the proteome of the parental cell. Typical proteins found in exosomes include those involved in MVB formation (e.g., Alix, TSG101), membrane transport and fusion (e.g., annexins, flotillins, GTPases), adhesion (e.g., integrins), tetraspanins (e.g., CD9, CD63, CD81, CD82), and antigen presentation (MHC class I and II molecules). Heat shock proteins (e.g., HSP70, HSP90) and lipid-related proteins [17, 40] were also found in exosomes. Some proteins are preferentially uploaded in exosomes, but it is still unclear how proteins are targeted specifically to exosomes. More studies are needed to unravel possible mechanisms of exosome sorting/incorporation, hence addressing the question of selectivity versus randomness. In particular, current research aimed at improving the methods of vesicle isolation, protein purification and detection will allow to identify the vesicle proteome more precisely [41].

Exosomes contain both short and long RNAs [42]. When transferred to target cells, mRNAs are translated into proteins [43, 44], and microRNAs (miRs) can silence target genes [45]. These findings have given way to study the role of

exosome-delivered extracellular RNA in different biological processes, such as immune response, cancer, viral infections, formation of immunological synapse, and angiogenesis. Besides mRNAs and miRs, other RNA species have been found within exosomes, such as viral RNAs, Y-RNAs, fragments of tRNAs, small nuclear RNA, small nucleolar RNA, piwi-interacting RNAs, and long non-coding RNAs [46–48]. However, mechanisms controlling the specific loading of RNA species into exosomes are only partly known. Recently, it has been identified a short nucleotide motif regulating the sorting of RNA into exosomes through binding with the heterogeneous nuclear ribonucleoprotein (hnRNP)-A2B1, i.e., a ubiquitously expressed RNA-binding protein [49]. Interestingly, an additional short nucleotide sequence has been identified as binding motif for the hnRNP–Q-mediated delivery of miRs into exosomes released by hepatocytes [50].

Exosomes also incorporate genomic DNA through unknown mechanism. Likely, this process is mediated by the release of DNA fragments in cytoplasm during mitosis after breaking of nuclear membrane. Genomic DNA has been found in a panel of tumor cell lines of nervous and gut origin [51]. They can contain oncogenes as well as transposable elements of the genomic DNA [52, 53]. However, the function of the DNA incorporated into exosomes is still unclear, and further studies are needed to understand its possible role in physiological and pathological processes.

1.4 Interaction of Exosomes with Bystander Cells

Experimental evidences indicate that exosomes can transfer their contents into the cytoplasm of target cells. Since exosomes have been isolated from many biological fluids [34], it is likely that these vesicles can reach very distant recipient cells while protecting their cargo from enzymatic degradation during transit into the extracellular environment [54–56]. Exosome contents can be delivered through fusion of exosome lipid membrane with either plasma or endosomal membrane, in the latter case upon endocytosis. After release of luminal cargo inside the recipient cells, exosome macromolecules can induce pre- and/or post-translational alterations of gene expression [57].

Given the emerging role of exosomes in both physiological and pathological conditions, as well as their therapeutic potential, understanding the molecular processes by which they are taken up by recipient cells is relevant. Exosome uptake has been monitored mainly using both flow cytometry and confocal microscopy. These techniques allowed to analyze the dynamic localization of exosomes through the labeling with fluorescent lipid membrane dyes. Examples of such dyes include PKH67 [58], PKH26 [59], rhodamine B [60], DiI [61] and DiD [62]. The use of GFP-tagged exosomal proteins also (e.g., GFP-CD63) allowed direct vesicle visualization, confirming their rapid incorporation into recipient cells [58, 63]. The treatment of target cells with either acidic buffers [63] or trypsin [64] allowed to discriminate between internalized and surface-bound fluorescent vesicles.