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Review Article

Diagnostic and Prognostic Potential of Extracellular Vesicles in Peripheral Blood

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ABSTRACT

Purpose: Extracellular vesicles (EVs) are small, membrane-enclosed entities released from cells in many different biological systems. These vesicles play an important role in cellular communication by virtue of their protein, RNA, and lipid content, which can be transferred among cells. The complement of biomolecules reflects the parent cell, and their characterization may provide information about the presence of an aberrant process. Peripheral blood is a rich source of circulating EVs, which are easily accessible through a blood sample. An analysis of EVs in peripheral blood could provide access to unparalleled amounts of biomarkers of great diagnostic and prognostic value. The objectives of this review are to briefly present the current knowledge about EVs and to introduce a toolbox of selected techniques, which can be used to rapidly characterize clinically relevant properties of EVs from peripheral blood.

Methods: Several techniques exist to characterize the different features of EVs, including size, enumeration, RNA cargo, and protein phenotype. Each technique has a number of advantages and pitfalls. However, with the techniques presented in this review, a possible platform for EV characterization in a clinical setting is outlined.

Findings: Although EVs have great diagnostic and prognostic potential, a lack of standardization regarding EV analysis hampers the full use of this potential. Nevertheless, the analysis of EVs in peripheral blood has several advantages compared with traditional analyses of many soluble molecules in blood.

Implications: Overall, the use of EV analysis as a diagnostic and prognostic tool has prodigious clinical potential. (*Clin Ther*. 2014;36:830–846) © 2014 The Authors. Published by Elsevier HS Journals, Inc.

Key words: Extracellular vesicles, microvesicles, exosomes, diagnostics, phenotyping, RNA cargo, enumeration.

INTRODUCTION

In recent years, interest in the characterization, biogenesis, and function of extracellular vesicles (EVs) has increased immensely. These membrane-derived vesicles play vital roles in a plethora of processes in several biological systems. In humans, EVs are pivotal to cellular communication for the maintenance of homeostasis and the development and progression of pathologic conditions, such as cancer. Consequently, this communication forms the basis for the use of EV analysis in a clinical setting because EVs seem to be a promising source of biomarkers of diagnostic and prognostic value. EV analysis can likely be used as one component of treatment surveillance. In addition, EVs have the potential of being used as drug therapy entities, delivering a tailored pharmacologic cargo to a specific target.

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Classification of EVs

In general, EVs are a heterogeneous population of membrane-enclosed vesicles released from a variety of cells into the extracellular space *in vivo* and *in vitro*. One general feature for these vesicles is that they are enclosed by a membrane that consists of a phospholipid bilayer. However, the EVs can be divided into a number of subpopulations each with specific characteristics, including their biogenesis, size, cellular origin, protein composition, mRNA and microRNA (miRNA) content, and/or biological function. With biogenesis as a classification tool, the EVs can be divided into 3 major groups: exosomes, microvesicles (MVs), and apoptotic bodies. Many of the properties of EVs, and in particular exosomes, have been reviewed extensively elsewhere[1–9]; therefore, the following section states the overall characteristics of these 3 EV groups.

Exosomes

Exosome is the vesicle type that has been studied most intensely. They are approximately 30 to 100 nm in diameter and originate from inward budding of the limiting membrane of multivesicular bodies, which are late endosomal compartments present in the cytosol of the cell.[4,6,10,11] When the multivesicular bodies fuse with the plasma membrane, the release of the exosomes to the extracellular space is facilitated. The biogenesis of the exosomes causes the orientation of the membrane proteins to be similar to that of the plasma membrane. The exosomal membrane is enriched in cholesterol, ceramide, and sphingomyelin and exposes the phospholipid phosphatidylserine.[6,11] In addition, exosomes contain several proteins that are currently used as markers to identify exosomes. These makers are not ubiquitously expressed on all exosomes but are found in a large proportion of these vesicles. Therefore, they are generally accepted as exosomal markers. These markers include TSG101, Alix, and the tetraspanins CD9, CD63, and CD81.[2,4] Along with these hallmark proteins, the phenotype of exosomes often reflects a molecular signature of the cell from which they originate. This cell-specific signature may provide some indication about the functionality of the exosomes because some of these signature molecules could ensure the delivery of the exosomes to the correct target cell,[12] point to a signal being transduced by a receptor-ligand interaction of exosomes and recipient cell,[13] or simply indicate which cells are the active exosome producers.[14] The composition of the exosome cargo can also be related to the potential biological function of this vesicle type. They have been known to contain proteins from both the plasma membrane and cytosol along with mRNA and the non–protein-coding miRNAs and small interfering RNAs.[2,15–17]

Microvesicles

The size of MVs ranges from 100 to 1000 nm. They are formed from outward budding of the plasma membrane, thus releasing the MVs directly into the extracellular space.[2,4,18] Hence, the membrane proteins of MVs retain the topologic features of those found in the plasma membrane. Generally, most MVs incorporate phosphatidylserine in the outer leaflet of the membrane.[4,6,10,19] This feature has frequently been used to isolate and identify MVs from biological samples along with a combination of cell-specific protein markers to determine their cellular origin.[19,20] However, several studies indicate that phosphatidylserine may only be present in some subpopulations of MVs.[21–24] Currently, there is a less extensive list of markers to identify MVs when compared with exosomes. Nonetheless, CD40 ligand, adenosine diphosphate ribosylation factor 6, and several integrins and selectins have been proposed as MV markers.[2,5,6,18,25] Like exosomes, the phenotype of MVs reflects their parent cell, and the content of the vesicle cargo also includes membrane and cytosolic proteins, mRNA, and miRNAs.[2,5]

Apoptotic Bodies

Apoptotic bodies are the largest vesicle type of the 3 major EV classification groups, with a size ranging from approximately 500 to 4000 nm.[26,27] They are formed from blebbing of the plasma membrane in cells undergoing apoptosis, releasing the apoptotic bodies straight into the extracellular space.[4] Similar to both the membrane of exosomes and MVs, phosphatidylserine can be found in the outer leaflet of the lipid bilayer of apoptotic bodies.[2,6,10,28] In terms of identifying apoptotic bodies, thrombospondin and complement component C3b are in many cases accepted apoptotic body markers.[1] Unlike the 2 other vesicle types, apoptotic bodies are distinguished by containing organelles, DNA fragments, and histones as a part of the vesicular cargo in addition to proteins and other molecules from the cytosol of the parent cell.[2,4,27,28]

EVs and the Immune System

Because some of the first reports of vesicular release were published 3 decades ago,[29,30] an