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REVIEW

Information transfer by exosomes: A new frontier in hematologic malignancies

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ABSTRACT

Exosomes are small (30–150 mm) vesicles secreted by all cell types and present in all body fluids. They are emerging as vehicles for delivery of membrane-tethered signaling molecules and membrane enclosed genes to target cells. Exosome-mediated information transfer allows for crosstalk of cells within the hematopoietic system and for interactions between hematopoietic cells and local or distant tissue cells. Exosomes carry physiological signals essential for health and participate in pathological processes, including malignant transformation. In hematologic malignancies, exosomes reprogram the bone marrow microenvironment, creating a niche for abnormal cells and favoring their expansion. The molecular and genetic mechanisms exosomes utilize to shuttle information between cells are currently being examined as are the potential roles exosomes play as biomarkers of disease or future therapeutic targets.

1. Introduction

The means by which cells within tissues, organs, and bodies communicate has been of interest for years.

There has been little doubt that inter-cellular communication exists and plays a key role in shaping physiologically-relevant cellular responses. Biochemical and molecular evidence for receptor-ligand interactions and for directed signaling via complementary protein structures on cell surfaces, respectively, has provided a solid basis for the concept of molecular networks which operate in neural, endocrine, hematologic and immune systems to name a few. The first indications of the existence of extracellular vesicles (EV) that might facilitate these cellular interactions date back to late 1960s, when it was demonstrated that human platelet-free plasma contained a 20–50 nm sized vesicles carrying a clotting factor later identified as tissue factor (TF) [1,2]. In the late 1970s, it was confirmed that these 50 nm vesicles were present in plasma as well as all other body fluids of many animal species and that similar EV, identified by electron microscopy, were also present in culture supernatants of maturing sheep reticulocytes [3,4]. In now seminal series of studies, Johnstone's and Stahl's laboratories showed in 1980s that 50 nm EV were released from maturing reticulocytes when intracellular storage vesicles and multivesicular bodies (MVB) fused with the plasma membrane [3,4]. At the time, this process was considered to be responsible the elimination of cellular waste. It took several more years to realize that EV might represent a new way of extracellular communication (reviewed in [5,6]). Today, EV are envisioned as vehicles for the horizontal transfer of molecular signals as well as genes [7]. EV carry and transfer to recipient cells proteins, lipids and nucleic acids in a functionally-active form [8]. They regulate gene expression in target cells and can thus alter their fate [9]. Depending on the cargo they carry, EV can induce target cell activation, proliferation and differentiation or death and thus play a key role in the regulation of physiological as well as pathological processes. It has been suggested, although not proven so far, that EV carry information dispatched by the parental cell to only reach and be delivered to specifically targeted recipients. In this sense, transfer of information by EV might be directed and specific, depending on the program of the parental cell. Another attribute of EV is related to their vesicular structure, which guarantees that the molecular cargo enclosed by the membrane is protected from extracellular enzymes and will be delivered to target cells in its original state. Because EV carry a broad variety of membrane-associated as well soluble factors, they can simultaneously exert stimulatory as well as inhibitory effects on a broad array of different cell types. EV are produced by bacteria, plants and animals, including man [10,11]. Thus, EV represent an evolutionarily conserved, remarkably well organized system for information transfer in multicellular as well as unicellular organisms.

EVs are highly heterogeneous, consisting of vesicles with different sizes originating from distinct subcellular compartments and with a diverse molecular make up. They have been categorized into three main groups: (a) microvesicles (MVs), which are formed by outward budding

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from the plasma membrane. These are larger vesicles, ranging in diameter from 200 to 1000 nm, and they are variously referred to as ectosomes, shedding vesicles, or microparticles; (b) exosomes, which are smaller than MV with a diameter of 30–150 nm, have an endocytic origin. They are formed inside the multivesicular bodies (MVBs) by intraluminal budding of the endosomal membrane; (c) apoptotic bodies (ABs) which are large clumps of material (>1um in diameter) originating from cells undergoing apoptosis [6,12]. This nomenclature does not adequately discriminate exosomes from MVs, as an overlap in size and the ability of the same cell to produce both types of vesicles confuse the classification. Furthermore, both types of vesicles are commonly found in the same extracellular fluids and may exert similar biological effects. Consequently, exosomes are often confused with MV and, for many years prior to the development of techniques capable of sizing microparticles and their physical separation, all EVs were lumped together and studied without recognition of their distinct biogenesis.

Today, the criteria for definition of different types of EVs have improved and exosomes have emerged as particularly interesting information-carrying vehicles because of their potential use as non-invasive biomarkers and putative importance in bioengineering and therapeutics. Many aspects of exosome biology still remain to be discovered. Nevertheless, considerable insights into their biogenesis and interactions exist with diverse cells, including cells of the hematopoietic system. In this review, we hope to address the role of exosomes in providing information to, and maintaining cross-talk with, immune cells and bone marrow stromal cells, thus serving as communication conduits for promoting progression of hematologic malignancies and their resistance to therapy.

2. Exosome biogenesis

The process of exosome biogenesis by various normal and pathological cells has been intensively investigated by cell biologists and has recently been reviewed [6,13,14]. Cellular biogenesis of exosomes begins with early endosomes and involves inward budding of the endosomal membrane. Intraluminal vesicles which are thus formed enclose various endoplasmic components and are coated with clathrin and other components of the plasma membrane [14]. Late endosomes, also referred to as MVB, are characterized by the presence in their lumen of multiple exosomes. When MVBs fuse with the plasma membrane, exosomes are released in an ATP-dependent manner into extracellular space as virus-size membranous vesicles, whose molecular cargo contains proteins/glycoproteins expressed on surface membranes of parental cells [6,14,15]. The vesicular content of exosomes includes nucleic acids, nucleoproteins, enzymes, soluble factors, and a variety of molecules derived from the cytosol of the parental cell [14,15]. This biogenesis process produces exosomes with the profile of membrane molecules that resembles that of the parental cell and gives rise to the concept of exosomes as putative non-invasive biomarkers which upon recovery from body fluids, can provide information about pathological cellular content based on their molecular profile [16].

The molecular machinery involved in exosome biogenesis consists of an endosomal sorting complex responsible for transport (ESCRT) and various accessory proteins such as ALIX and tumor-susceptibility gene 101 (TSG101) as recently reviewed [6,13]. A sorting mechanism that operates independently of the ESCRT machinery has also been described [17]. Tetraspanins organized into tetraspanin enriched microdomains (TEMs) play an important role in exosome biogenesis, sorting of proteins and nucleic acids to exosomes, and exosome release involving fusion of MVBs with the surface membrane of producer cells (reviewed in [18]). The molecular components of the release process are still under investigation but are known to require the cytoskeleton (actin and microtubules), associated kinesins and myosins acting as molecular motors, Rab GTPases representing molecular switches and molecules that drive MVB fusion with the plasma membrane, such as the SNARE complex [6,13]. The identity of molecular components of this complex pathway and their interactions in different cell types are not yet established and require further studies.

3. Exosome release and up-take by recipient cells

While exosome secretion occurs under physiologic conditions, and all live cells produce and release exosomes, cells under stress are especially avid exosome producers. Whether this represents an attempt to remove factors damaging cellular homeostasis or an attempt to signal the neighboring cells in the microenvironment is a matter of speculation. It has been reported that inducible formation and release of exosomes depends on the DNA damage-inducing p53-dependent secretory pathway [19]. Also, hypoxic conditions are thought to favor exosome release [20]. Consequently, tumor cells, including leukemic blasts, release copious quantities of exosomes [21,22]. For example, plasma of patients with acute myeloid leukemia (AML) collected at diagnosis were found to contain a 60-fold greater quantity of exosomes (measured in ug protein/mL plasma) than plasma of normal donors [21].

Once released from the producer cell, exosomes have to interact with recipient cells to be able to deliver their cargos. Here again, different mechanisms could be involved. Exosomes, armed with a wide range of membrane-bound activating or inhibitory molecules, can directly signal via the relevant receptors on responder cells, delivering signals to downstream pathways which specify either cellular activation or suppression. Alternatively, exosomes could be internalized by the recipient cells via endocytosis/phagocytosis or membrane fusion, and thus transfer a spectrum of biologically-active molecules, including nucleic acids, which can alter functions of these cells [23,24]. Exosomes can deliver functioning surface receptors or ectoenzymes from one cell to another and thus restore or augment missing functions, as described by us recently for ectonucleotidases responsible for exogenous adenosine production in human Treg [25]. Various mRNA and miRNA species carried by exosomes can also be transferred and translated into functional protein by exosomes [23,26]. This type of information transfer by exosomes has been under considerable scrutiny, largely because of speculations it may be cell-type specific. This is based on the finding that exosomes derived from B cells selectively bound to follicular dendritic cells (DC) [27], while those produced by human intestinal epithelial cells bound to DC rather than B or T lymphocytes [28]. However, neither the molecular mechanisms nor cellular interactions regulating the specificity of exosome binding to pre-designed target cells have been defined so far. The image of an exosome that emerges today is that of miniature, nano-sized vesicle bound by the membrane decorated with highly biologically-active molecules, and the lumen filled with genetic materials, enzymes, cytokines and other soluble factors, all of which are potentially transferable to target cells [29]. As would be expected, the exosome lumen content is protected from enzymes present in body fluids, ensuring its safe delivery to recipient cells.

4. Isolation of exosomes from cell supernatants or body fluids

Many different commercially-developed procedures are currently available for exosome enrichment and isolation from supernatants of cultured cells or body fluids. Caution is necessary, however, because these methods might not distinguish exosomes from larger EVs or might interfere with exosome integrity or functions. These methods largely aim at the isolation of exosomal nucleic acids, especially miRNA. To be able to use exosomes from body fluids for functional studies or as biomarkers, it is necessary to minimize losses in their content and to isolate exosomes as purified, biologically-active vesicles. Fig. 1 presents a multi-step schema used in the authors' laboratory for the isolation of exosomes from plasma or supernatants of cultured cells [30]. Differential centrifugation of plasma at various speeds and ultrafiltration remove microorganisms, cell fragments and large EVs. Ultrafiltration and exclusion chromatography are especially important in processing human plasma and other body fluids to remove non-