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Biology and biogenesis of shed microvesicles

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ABSTRACT

The ability of cells to transmit bioactive molecules to recipient cells and the extracellular environment is a fundamental requirement for both normal physiology and disease pathogenesis. It has traditionally been thought that soluble factors released from cells were responsible for this cellular signaling but recent research has revealed a fundamental role for microvesicles in this process. Microvesicles are heterogeneous membrane-bound sacs that are shed from the surface of cells into the extracellular environment in a highly regulated process. They are shed following the selective incorporation of a host of molecular cargo including multiple types of proteins and nucleic acids. In addition to providing new insight into the etiology of complex human diseases, microvesicles also show great promise as a tool for advanced diagnosis and therapy as we move forward into a new age of personalized medicine. Here we review current status of the rapidly evolving field of microvesicle biology, highlighting critical regulatory roles for several small GTPases in the biology and biogenesis of shed microvesicles.

Introduction

Microvesicles are heterogeneous, membrane bound sacs, shed from the surface of myriad cell types.[1] Throughout the scientific literature, microvesicles have also been referred to as shedding vesicles, ectosomes, oncosomes, shedding bodies, and microparticles. In recent years, investigators have come to appreciate their important roles in altering the extracellular environment, intercellular signaling, and facilitating cell invasion through cell-independent matrix proteolysis.[2-4] For example, through their ability to transfer bioactive molecules, including proteins, DNA,[5] mRNA, and miRNA,[6] microvesicles are able to modify the extracellular milieu, proximal, and distal recipient cells. Additionally, the identification of microvesicles in multiple bodily fluids[7,8] has heightened interest in research aimed at elucidating their functions in both healthy and diseased tissues. Together with recent advances in techniques for isolating microvesicles from peripheral bodily fluids,[9,10] these developments suggest that microvesicles may play an important role in future diagnostic and therapeutic strategies, and thus make them an important focus of continued biomedical research.

Microvesicle biogenesis involves vertical trafficking of molecular cargo to the plasma membrane, a redistribution of membrane lipids, and the use of contractile machinery at the surface to allow for vesicle pinching.[3] Shed microvesicles are distinct from another population of cell-derived extracellular vesicles known as exosomes. The two populations differ in in size, cargo, and mechanism of formation. Unlike microvesicles (described in detail below), exosomes are formed by the inward invagination of late endosomal membranes to form what has come to be known as a multivesicular body (MVB). The fusion of a mature MVB with the cell's limiting membrane then releases its exosomal contents into the extracellular space.[11] Because MVBs and their enclosed exosomes are derived from endosomal membranes, exosomes are observed to be similar in size to their endosomal precursors the intraluminal vesicle (ILV). That is, exosomes range from 30–100 nm in diameter when observed by electron microscopy.[12] However, since microvesicles are formed by direct budding from the plasma membrane, they lack a similar upper size limit and may be as much as several microns in diameter.[2,4,12,13] Exosome cargo includes proteins from endosomes, the plasma membrane, the cytosol, and specific subsets of cellular protein depending on the cell type as would be expected given their mechanism of biogenesis.[12] While much has been learned regarding the content of both exosomes, microvesicles, and apoptotic bodies (another form of extracellular vesicle released specifically from cells undergoing apoptosis), the identification of cargo specifically expressed in either type of vesicle remains an ongoing area of intense research.[3,12,14] Both

microvesicles and exosomes are capable of encapsulating and transferring multiple forms of cargo including proteins, RNA transcripts, and miRNAs. It was, however, recently reported that there are general differences in the ability to deliver macromolecules from transiently transfected cells. Microvesicles and exosomes can, for example, efficiently incorporate ectopically expressed reporter proteins, mRNA, and siRNA, however, the researchers found that only microvesiclces could transfer reporter function to recipient cells in the form of plasmid DNA.[15]

A spectrum of eukaryotic cell types release microvesicles under both physiological and disease conditions.[16] The onset of disease states, such as the development of multiple cancers, can perturb this highly regulated process leading to aberrant shedding.[3] Microvesicles comprise a heterogeneous population with their function ultimately determined by vesicle cargo content, which is in turn dependent upon the cell type from which they are shed. Macromolecular cargoes contained within microvesicles participate in a wide range of biological processes. Utilizing the Vesiclepedia[17] database, we have depicted 10 of the most common functions associated with cargo reported in the literature to date (Fig. 1). Furthermore, studies have documented that cargo content also varies with the particular profile of gene expression at the time of release.[3] Both cargo sorting and microvesicle shedding are tightly regulated by several small GTPases, including members of the ARF (ARF6[2,18] and ARF1[19]), Rab,[20] and Rho (Rac1 and RhoA[18,21]) families. Several small GTPases discussed in this review are also highlighted in Figure 1. Finally, given the plethora of macromolecular cargo content found within microvesicles, it is worth noting that similar to what has been described for exosomes,[22] there are likely heterogeneous pools containing distinct cargo within the total microvesicle population.[23,24]

When released into the extracellular environment, shed microvesicles can subsequently release their cargo, which can itself alter the extracellular milieu, or microvesicles can interact with recipient cells via endocytosis, fusion, or activation of signaling pathways through receptor interactions.[6] Microvesicle activities, for example, play important roles in coagulation; inflammation; stem cell expansion and renewal; and tumor progression. Here, we will review recent advances in our understanding of the biogenesis and biological activity of microvesicles as well as the future perspectives and applications for this research moving forward.

Biogenesis

Microvesicle biogenesis occurs via the direct outward blebbing and pinching of the plasma membrane releasing the nascent microvesicle into the extracellular space.[8,25] Membrane blebbing is accompanied by distinct, localized changes in plasma membrane protein and lipid components, which modulate changes in membrane curvature and rigidity (Table 1).[26,27] Changes in plasma membrane components are complemented by a vertical redistribution of microvesicle cargo components which are selectively enriched within microvesicles.[3] As described below, these processes represent a unique mechanism of extracellular vesicle formation in comparison to exosomes, which are formed intracellularly within MVBs, or apoptotic bodies, which result from indiscriminate surface blebbing. This novel mechanism of

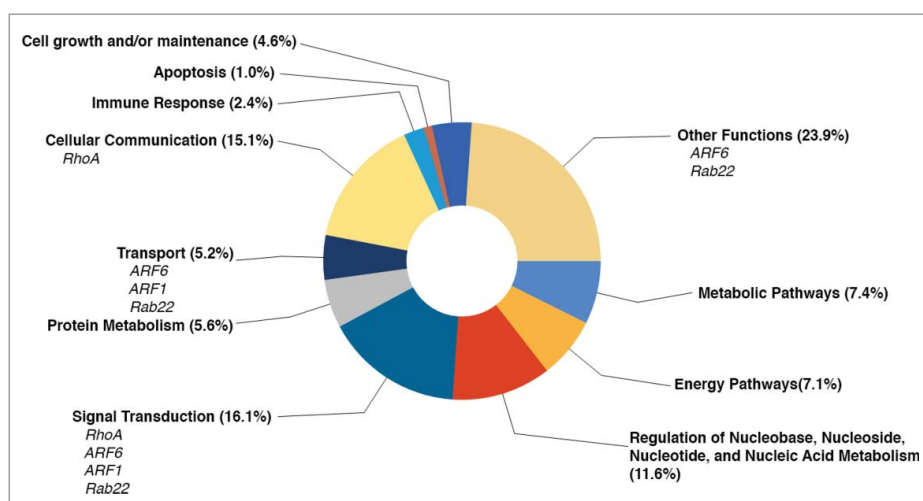**Figure 1.** Common biological functions of microvesicle protein cargo identified in Vesiclepedia database. Vesiclepedia cargo content was analyzed using the FunRich Functional Enrichment Analysis Tool to examine the biological processes associated with each of the database entries. The 10 most common activities are displayed, and those associated with small GTPases discussed in the text are highlighted.

Table 1. Proteins with identified functional roles in microvesicle biogenesis.

Aminophospholipid translocases (flippase and floppases)	(Stachowiak et al., 2013;[30] Yang et al., 2008[31])
ARF1 *(Activation of contractile machinery via MLCK)*	(Schlienger et al., 2014)[19]
ARF6 *(Regulates the selective recruitment of proteins into microvesicles and activates contractile machinery via MLCK)*	(Muralidharan-Chari et al., 2009)[2]
ARRDC1, TSG101	(Nabhan et al., 2012)[28]
DIAPH3	(Brown et al., 2014;[80] Kim et al., 2014[81])
Glutaminase	(Li et al., 2012)[38]
Hyaluronan synthase	(Rilla et al., 2013)[33]
Localized protein enrichment	(Stachowiak et al., 2012)[29]
Myosin-1a	(McConnell et al., 2009)[32]
Rab22a *(Selective recruitment of proteins to microvesicles under hypoxic conditions)*	(Wang et al., 2014)[20]
RhoA *Links ARF6 activation to MLC phosphorylation via ROCK signaling pathway; also involved in actin cytoskeletal rearrangements via RhoA-coflin pathway. Rho family proteins including Rac1 and RhoA are involved in an antagonistic relationship that determines switching between microvesicle shedding amoeboid, and invadopodia utilizing mesenchymal phenotypes*	(Sedgwick et al., 2015;[18] Schlienger et al., 2014;[19] Li et al., 2012[38])

formation results in the regulated release of extracellular microvesicles containing specifically enriched molecular cargoes.

Membrane lipids and dynamics

Despite growing understanding and appreciation of extracellular microvesicles as a novel means of cell-cell communication, the mechanisms governing their formation and release remain, as of this writing, incompletely understood. Microvesicle budding from the limiting plasma membrane, though a distinct process from exosome formation which occurs in late-endosomal compartments, does utilize endosomal machinery to allow for vesicle formation. This includes the Ras-related GTPase ADP-ribosylation factor 6 (ARF6), discussed in greater detail below, and components of the ESCRT system.[28] It was recently reported that a process similar to viral budding results from the interaction of arrestin domain-containing protein-1 (ARRDC1) with the late endosomal protein TSG101 leading to the relocalization of TSG101 from endosomal membranes to the plasma membrane likely resulting in localized changes in membrane curvature in an epithelial cell model. This shift in TSG101 localization subsequently leads to the release of membrane microvesicles which contain both TSG101 and ARRDC1 along with other cellular components but

lacking known markers of late endosomal compartments.[28]

Researchers have recently outlined another mechanism through which proteins, even those unrelated to the generation of membrane curvature, can result in bending of the plasma membrane due to crowding at the cell periphery and lateral pressure generation through protein-protein interactions, even in a cell-free lamellar vesicle system.[29] With these results indicating that the crowded protein environment on the surface of cellular membranes can contribute to membrane shape changes it is tempting to speculate that the enrichment of protein cargo alone at sites of nascent microvesicle formation could be sufficient to drive *de novo* microvesicle formation.

In addition to reports outlining multiple roles for protein composition in the regulation of plasma membrane bending, alterations in lipid composition may also perturb membrane rigidity and curvature, acting at times to stabilize membrane-bending forces.[30] The unequal distribution of plasma membrane lipid components can result in changes to local membrane curvature consistent with the events of microvesicle budding. For example, phospholipids comprised of large head groups and small hydrocarbon tails (or vice versa) can take on a roughly conical shape and the uneven distribution of these irregularly shaped components between plasma membrane leaflets used to impose discreet membrane curvature. Additionally, the local recruitment of aminophospholipid translocases (flippase and floppases) could readily contribute to the formation of membrane curvature during microvesicle formation as has previously been reported during formation and fission of Golgi vesicles *in vitro*.[31] Furthermore, research has implicated the buildup of extraneous membrane at microvillar tips as a source of microvesicles shed into the gut lumen in a myosin-1a dependent fashion.[32] Similarly, researchers have also reported that increased production of hyaluronan can lead to the release of microvesicles from the ends of long, microvilli-like projections.[33] Taken together, these reports suggest that under certain conditions pinching of microvilli or other cell protrusions may be yet another mechanism for microvesicle release.

Cargo trafficking

Microvesicles are not simply random samplings of cellular components; protein and nucleic acid cargos are selectively recruited into micrcovesicles while others are excluded.[3] One protein identified as a regulator of selective recruitment of proteins, is ARF6. Specialized ARF6 recycling endosomes target specific protein cargo such as VAMP3, β-1 integrin, and MHC-I for incorporation into tumor cell-derived microvesicles.[2] Interestingly, although transferrin receptor is trafficked via ARF6 positive endosomes, it is

not incorporated into microvesicles.[2] This result suggests that there are multiple mechanisms for selective cargo recruitment at the budding vesicle in addition to the selection that occurs by virtue of being trafficked via ARF6 positive endosomes, with the nascent vesicle being a convergence point for multiple membrane trafficking pathways. In addition to ARF6-regulated endosomal trafficking, for example, it has recently been demonstrated that in breast cancer cells, Rab22a co-localizes to shedding microvesicles and overexpression of Rab22a leads to increased microvesicle shedding.[20] Furthermore, it is likely that Rab22a is responsible for selectively recruiting proteins utilized for microvesicle formation under hypoxic conditions as Rab22a knockdown abrogates hypoxia-induced increases in microvesicle generation, but has only a small effect on microvesicle biogenesis in non-hypoxic conditions.[20] Furthermore, additional cargo trafficking that converges on vesicle blebbing and shedding is mediated by interaction with specific v-SNAREs. It was previously demonstrated that when the surface protease (and known component of shed microvesicles) MT1-MMP associates with VAMP7, the protease is delivered to invadopodia.[34] However, the same MT1-MMP cargo, when associated with VAMP3 is trafficked to shedding microvesicles in a CD-9 dependent fashion where it will facilitate matrix invasion by tumor cells.[4] With nucleic acids, very few mechanisms for regulated trafficking into nascent microvesicles have been elucidated. One possible mechanism by which mRNAs are selectively incorporated into microvesicles was uncovered in glioblastoma cells where it was demonstrated that conserved zip code RNA sequence motifs in the 3′ untranslated regions function to enrich mRNA in microvesicles.[35,36] miRNA have also been shown to be associated with RNA trafficking proteins such as T-cell internal antigen 1 as well as proteins involved in RNA stability such as Argonaute2.[37] Furthermore, CSE1L, a functional nuclear export protein that has been implicated in chromatin binding, was recently shown to be included in melanoma microvesicles.[38] Taken together, these studies highlight the likelihood that nucleic acids are co-trafficked with protein cargos into budding microvesicles. Finally, it is also worth noting the possibility that this vertical redistribution of cargo is tightly regulated and dependent on the physiological context and a wide variety of extracellular stimuli. For example, in endothelial cells, TNF-α signaling leads to vesicle shedding with distinct populations of protein cargo and different levels of miRNA inclusion.[39]

Contractile machinery and vesicle fission

Microvesicle fission and release from the cell surface is dependent upon the interaction of actin and myosin together with a subsequent ATP-dependent contraction.[3,32]

Interestingly, ARF6, with its known roles in peripheral actin remodeling, cell invasion, and endocytic trafficking,[40,41] has been shown to be a key regulator of microvesicle shedding. ARF6-mediated activation of extracellular signal-regulated kinase (ERK) via phospholipase D (PLD) leads to localized myosin light chain kinase (MLCK) activity at the neck of budding vesicles, phosphorylation of myosin light chain (MLC), and activation of the aforementioned contractile machinery.[2] This phosphorylation of MLC has been shown to occur down stream of ARF6 as well as ARF1 occurring via activation of the Rho GTPases, in particular RhoA, which phosphorylates MLC via a Rho-associated protein kinase (ROCK) signaling pathway.[18,19] In addition to phosphorylation of MLC, RhoA has long been known to regulate actin cytoskeletal rearrangements via the RhoA-cofilin pathway[42] which may also play a role in vesicle fission as actin rearrangements are likely essential to effective fission.[18,42]

Biological roles of microvesicles

Blood cell derived microvesicles (coagulation, inflammation, and immunity)

Microvesicles found in the circulatory system with roles in coordinating the pro-coagulatory response are shed from a variety of cell types, among them platelets, macrophages, and neutrophils.[6] Much of the work on these pro-coagulatory microvesicles has focused on vesicles released by platelets but vesicles from other blood mononuclear cells have also been shown to promote coagulation.[43] A pro-coagulant response can be produced via microvesicles due to 2 key properties of circulatory system microvesicles: tissue factor, a transmembrane protein that acts as a cofactor for factor VIIa (FVIIa), and exposure of anionic phospholipids on the external leaflet of the vesicle membrane. Surface exposure of tissue factor, the primary biological initiator of the coagulation cascade[44] has been documented in microvesicles shed from platelets, and platelet derived microvesicles have a 50–100 fold higher pro-coagulant activity than activated platelets alone. These results indicate that vesicles shed by platelets may be even more important to clotting than the platelets themselves.[45,46] This difference in clotting activity is due in large part to the externalization of anionic phospholipids like phosphotidylserine as the anionic microvesicle membrane can then serve as a catalytic surface for the cationic domains of clotting proteins.[47,48] It is likely that numerous other coagulation mechanisms are activated by blood cell-derived microvesicles as well, for example the FXII dependent coagulation pathway initiated predominantly in erythrocyte and platelet derived microvesicles.[49]

The link between coagulation and the innate immune system has long been appreciated[50] and it follows that microvesicles released from circulatory cells would have functional roles in immunity as well as coagulation. It was recently shown that *Staphylococcus aureus* infection leads to tissue factor positive microvesicle release.[51] Microvesicles released by peripheral blood mononuclear cells in response to infection function both to aid in combatting infection as they can opsonize bacteria and to promote coagulation.[43] In particular, microvesicles from neutrophillic granulocytes contribute to immunity as they contain antibacterial proteins such as PMN granule proteins.[52] Neutrophil derived microvesicles generated in response to the presence of bacteria contain more antibacterial proteins and are more effective at combatting infection than neutrophil derived vesicles generated in response to other stimuli.[52,53] This differential response in shed vesicle content depending on whether or not the cells were exposed to bacteria indicates that cargo is selectively recruited into vesicles and can vary in response to stimuli. Together with the reports describing the impact of TNF-α signaling these results highlight a variation in cargo recruitment in response to stimuli that is likely an interesting property of microvesicles in general.[39] Furthermore, a hypercoagulant state has been identified in cancer patients where tumor cells have been shown to release TF positive microvesicles[54] subsequently associated with the increased likelihood of distant metastases in lung cancer.[55]

It has long been known that inflammation is a result of the interactions between various cell types in the blood[56] and it has since become clear that microvesicles are involved in this crosstalk. Microvesicles can exert both pro-inflammatory[57] and anti-inflammatory[58,59] effects on their environment principally through the horizontal transfer of cytokines and chemokine receptors to recipient cells; and by inducing recipient cells to subsequently release cytokines. It has been suggested that toggling between pro- and anti-inflammatory vesicle release changes throughout the inflammation response[6] likely as a result of extracellular stimuli being integrated with activation of intracellular signaling pathways in shedding cells. For example, a recent report demonstrated that exposure to cigarette smoke induces pro-inflammatory vesicle shedding in mononuclear cells via intracellular Ca^{2+} mobilization.[60] It is also important to note that microvesicles may regulate the inflammatory response in cytokine independent mechanisms including apoptotic induction of immune cells through vesicle associated FasL signaling.[61] This, together with the fact that inflammation itself stimulates coagulation and the crosstalk between these 2 systems can be accomplished by microvesicles,[62,63] further solidifies that the roles of blood cell

derived microvesicles in coagulation, immunity, and inflammation are interconnected and interdependent. This is of particular importance because the coordination of these physiological functions as regulated by microvesicles has a crucial role in the development of cardiovascular disease.[64-66]

Tumor cell derived microvesicles

Tumor cells of varied origins release significant numbers of microvesicles.[8] The amount of shed vesicles has been documented to correlate with increased invasiveness and disease progression.[8] Tumor microvesicles (TMVs) have many postulated roles during the onset and spread of disease. These roles, including transfer of growth factor receptors, increased cell motility, induction of angiogenesis, evasion of immune detection, and development of drug resistance; are often attributed to the ability of shed TMVs to condition the extracellular milieu.[3] The mechanisms through which TMVs mediate disease progression are varied. Tumor microvesicles can, for example, participate in the horizontal transfer of bioactive cargo, including oncogenic growth factor receptors such as EGFRvIII from aggressive glioma cells to naïve non-aggressive cells.[67] Transfer of the oncogenic variant of the EGF receptor in turn leads to activation of transforming signaling pathways (Akt and ERK) and an increase in the expression of EGFRvIII related genes. In addition to cell surface receptors, TMVs also contain multiple pro-angiogenic cargos such as VEGF,[68] TGF-β, and miRNA 1246.[69] These vesicles also contain cargo such as indoleamine-2,3-dioxygenase which suppresses T-cell proliferation and enables tumor cells to evade detection and elimination by the immune system.[70,71] Furthermore, TMVs are reported to contribute to the development of chemotherapeutic resistance via the transfer of P-glycoprotein and Multidrug Resistance-Associated Protein 1 which serve as a plasma membrane multidrug efflux transporters to clear drugs from the intracellular space.[72,73] Although the influence of tumor derived microvesicles on these hallmarks of cancer has been understood for a number of years, much of the recent work has succeeded in putting previous results into context whether in different disease states such as multiple myeloma;[74] physiological conditions such as hypoxia;[20] exposure to environmental insults, for example, tobacco smoke extract;[75] or the induction of senescence.[76] Interestingly microvesicles have also recently been implicated in cancer cachexia via induction of myoblast apoptosis resulting from exposure to miR-21 positive microvesicles.[77] Finally, TMVs are known to contain a host of proteases, including MMPs, which act to digest matrix components. This degradation has multiple pro-

tumorigenic qualities including the release of sequestered cytokines and growth factors; and the formation of paths of least resistance to facilitate tumor cell invasion.[4,18,78] Furthermore, the release of preotolytically active, MT1-MMP containing TMVs was recently demonstrated to be necessary to support the amoeboid-type invasion of tumor cells.[4]

Invading tumor cells can interchangeably adopt distinct morphologies: mesenchymal cells are characterized by their elongated morphology and robust utilization of invadopodia-mediated cell invasion while amoeboid cells are characterized by their rounded appearance with dynamic plasma membrane blebbing and the release of protease-loaded microvesicles. The switch between modes is regulated in part by an antagonistic relationship between Rac1 and RhoA[79] which is in turn governed by the physical characteristics of the extracellular matrix.[18] For example, when tumor cells encounter compliant matrices, this plasticity of invasive mechanisms results in high levels of active RhoA and increased microvesicle shedding from cells that are amoeboid in morphology.[18] Interestingly, membrane blebbing and microvesicle shedding is further linked to the amoeboid phenotype as both rely on increased intracellular tension and contractility based on phospho-MLC and actin interactions.[80] Additional molecular pathways linking shedding and the amoeboid phenotype include the Rho effector DIAPH3. Depletion of DIAPH3 has been shown to both increase shedding from prostate cancer cells[81] and lead to adoption of the amoeboid invasive/migratory phenotype in multiple tumor cell lines.[80] This link between shedding and the amoeboid phenotype is important in disease contexts because tumor cells assuming the amoeboid phenotype are equipped with a 2 pronged method of invasiveness since the increased cell contractility of the amoeboid phenotype allows cells to deform in order to force themselves through small voids in the ECM,[80] while increased microvesicle shedding may allow cells to create paths of least resistance through protease-mediated ECM degradation.[3,4] Taken together, these results indicate that tumor derived microvesicles and the increased shedding that occurs during the development of cancer affect disease onset, spread, progression and co-morbidities.

Stem cell microvesicles

Investigators have come to appreciate that much of the efficacy of stem cell therapies such as bone marrow transplants, come not from the implanted stem cells replacing cells of the injured target tissue, but rather through the effects of soluble factors acting on injured cells.[82,83] Microvesicles have been implicated as one of the crucial factors involved in this paracrine signaling between stem and recipient cells as early as 2006.[63] Microvesicles play critical roles in the crosstalk between stem and injured cells in part through their facilitating a reciprocal interaction between the 2 cell populations.[37] Microvesicles shed from damaged cells may facilitate the differentiation-dependent repair associated with stem cell based therapies. Microvesicle-mediated transmission of bioactive signals released from injured tissue results in stem cell differentiation in order to compensate for the loss of cells following injury.[84,85] Interestingly, it has been shown that tumor cells use microvesicles to influence mesenchymal stem cells (MSCs) leading the MSCs to acquire a more malignant phenotype with increased proliferation, migration, and protease secretion.[86] This, together with the fact that mesenchymal stem cells are recruited to tumors *in vivo*,[87] may indicate that stem cell-based therapies may be an ill-advised treatment plan for cancer patients. Cell free therapy using microvesicles, however, may avoid these adverse effects. Microvesicles released from stem cells have been shown to activate regenerative programs in injured recipient cells and induce functional changes including enhanced proliferation and inhibition of apoptosis.[88,89] MSC derived microvesicles have also been shown to have anti-inflammatory properties via suppression of the Akt and STAT3 signaling pathways[90] and by inhibiting T-lymphocyte functions as well as modifying the cytokine production of dendritic cells, naïve and effector T-cells, and natural killer cells.[37] Endothelial precursor stem cells also release microvesicles containing pro-angiogenic mRNA and miRNA which upon receipt can activate quiescent endothelial cells facilitating vascularization and thus injury healing.[91,92] More recent work has identified the use of stem cell-derived microvesicles as a general method for rescuing the phenotype of dying organs and tissues after various injuries ranging from acute and chronic renal fibrosis,[88] acute lung injury, and pulmonary edema.[93] Taken together these findings indicate that use of stem cell derived microvesicles may provide a future efficacious cell free approach to regenerative therapies.

Neurodegeneration

Protein aggregation is a hallmark feature of many neurodegenerative disorders with accumulation of B-amyloid and tau in Alzheimer disease; α-synuclein in Parkinson disease, TDP-43 in ALS, and mutant huntingtin in Huntington's disease.[94] These proteins can be spread indiscriminately following cell death or may be selectively released and shed in extracellular vesicles and subsequently taken up by naïve recipient cells.[95,96] Much of the work on the cell-to-cell transmission of these

aggregating proteins has focused on exosomes but investigators are beginning to appreciate the critical role of microvesicles in this process. For example, tau has recently been detected in the interior of shed microvesicles and is shed to a greater extent in microvesicles than exosomes.[97] Additionally, microglia derived microvesicles also contribute to Alzheimer disease pathogenesis by converting extracellular β-amyloid from an insoluble form to its more toxic soluble form.[98] Further study is needed to determine whether these and other neurodegenerative proteins are present in microvesicle fractions. Finally, recent research has shown that myeloid-derived microvesicles contribute to neuroinflammation, a characteristic of many neurodegenerative disorders, and it was very recently demonstrated that microglial microvesicles carry N-arachidonoy-lethanolamine (AEA) which is able to stimulate presynaptic type-1 cannabinoid receptors, inhibit spontaneous gamma-aminobutyric acid release, and likely represent a mechanism through which microglia participate in inflammation associated excitatory phenomena.[99-101]

Future perspectives

The continued study of microvesicles has led to an increased appreciation of their value as biomarkers in disease, potential therapeutic targets, and platforms for drug delivery. Using microvesicles as biomarkers has distinct advantages in that they are easily accessible in various biological fluids (as opposed to repeated tissue biopsies) and their isolation simultaneously isolates and concentrates the molecular changes occurring in the pathologically relevant tissue thereby increasing sensitivity.[3] For example, this enrichment was recently demonstrated in a recent study wherein isolated TMVs isolated from the ascites, serum, or intraperitoneal washings of ovarian cancer patients were enriched with VAMP3, MT1-MMP, ARF-6 and CA-125 (a commonly used biomarker for ovarian cancer) relative to the unfractionated fluids.[4] Together these advantages increase the likelihood of early detection and diagnosis of pathological conditions and thus have the potential to positively affect patient outcomes. The combination of disease specific markers such as matrix metalloproteases in cancer;[102] and tissue factor in thromboembolism and disseminated intravascular coagulation;[103] as well as tissue specific markers including cytokines in microglia;[99] and myeloperoxidase and lactoferrin in neutrophils[52] could allow for defining a unique and specific microvesicle biosignature for multiple diseases. Given that vesicles are shed by most cell types under normal physiological conditions, critical to this diagnostic capacity would be the establishment of standardized protocols to isolate diseased cell vesicles from other shed vesicle populations.

Since genomic and proteomic expression profiles can change with disease progression or in response to treatment, microvesicles can also be used for disease staging and monitoring treatment efficacy and/or response. This prognostic capacity is especially important for risk assessment and determining patient management options and has the potential to be paradigm shifting for patient care. For example, a patient with a non-invasive, slowly progressing form of breast cancer that is responding to treatment may opt to avoid invasive procedures such as lumpectomy or mastectomy in favor of other treatment options. Profiling microvesicle cargo to determine what genomic and proteomic abnormalities are present and driving disease progression also will allow for personalized and individual treatment plans, for example, targeting a specific signaling pathway as opposed to targeting the disease or its symptoms more generally.

Although microvesicles participate in normal growth, development, and homeostasis,[16] microvesicle shedding, and in particular altered levels of shedding,[8] play important roles in the progression of various diseases as discussed above. As such, inhibiting the horizontal transfer of biomolecules accomplished by microvesicles may be an important and valuable therapeutic goal. As outlined by Andaloussi and colleagues, there are 4 principle mechanisms that can be targeted to accomplish this: the biogenesis and formation of microvesicles; the shedding and release of microvesicles; the uptake of microvesicles by recipient cells; and the trafficking to microvesicles of specific cargoes involved in disease progression.[104] However, in order to turn these hypothetical targeting possibilities into bona fide clinical treatment options, more study is needed on the mechanisms of molecular sorting and plasma membrane budding and fission as we have yet to completely elucidate the regulatory mechanisms of either process though research progresses on these fronts. Take, for example, RhoA signaling which has been shown to be both necessary and sufficient for microvesicle shedding in multiple tumor cell lines. Cerione et al. have shown that glutaminase inhibition, which has been shown to block Rho GTPase-dependent growth of cancer cells in culture and mouse xenografts,[105] also potently blocks microvesicle shedding in vitro.[106] This work provides a powerful example of how insight into the process of microvesicle biogenesis and the GTPases regulating that process can lead to the identification of therapeutic targets for inhibition of microvesicle shedding.

Because of their ability to horizontally transfer biomolecules, microvesicles can be harnessed for targeted drug

and therapeutic delivery. In addition to unmodified microvesicles, discussed in more detail below, drug delivery may also be accomplished using engineered microvesicles. This has been accomplished, for example, through the overexpression of miRNA-143 in THP-1 cells followed by intravenous injection of transduced cells into nude mice where they shed miRNA-143 positive vesicles.[107] These genetically engineered vesicles have more recently been shown to be viable delivery vehicles using additional cell culture model systems including their ability to deliver suicide genes to cancerous schwannoma recipient cells[108] and anti-obesity miRNA-130b to adipocytes.[109] Microvesicles are particularly exciting in terms of drug delivery as they offer several advantages over other delivery vectors. Microvesicles are biocompatible, can be immunologically inert, and can, if necessary, be patient derived.[104] Additionally, research has shown that these vesicles may be able to target specific recipient cell tissues through proteins on the vesicle surface such as CD44 (isoform 10).[73] Similarly, microvesicles have been shown to increase the effectiveness of adeno-associated virus based gene therapy as association with microvesicles increases viral capsid transduction efficiency and protects capsids from anti-adeno associated virus antibodies.[110]

As discussed above, unmodified microvesicles released from stem cells have the ability to activate angiogenic programs in endothelial cells, suppress apoptosis, stimulate cell proliferation, and ultimately activate regenerative programs in injured tissue.[104] Additionally, it is conceivable that microvesicles with the capacity to modulate inflammation could be exploited to induce or inhibit the immune response. For this potential to be realized it will be necessary to specifically isolate and capture microvesicles with the desired functionality, illustrating the importance of establishing standardized protocols for isolating specific populations of microvesicles.

Conclusions

Despite a growing understanding of their roles in both normal and disease physiology, little remains known about the mechanisms regulating cargo enrichment and microvesicle release. Further investigation in these areas has the ability to radically change our understanding of how cells interact with the surrounding environment during normal tissue homeostasis or the onset and progression of many disease states. The ability to readily purify microvesicle populations from bodily fluids can be paradigm shifting in the way in which we diagnose, monitor, and treat multiple diseases. Research into functional applications of shed microvesicles has only scratched the surface and the future of this research

hinges upon further refinement of enrichment and isolation techniques. The mounting evidence reviewed here points to microvesicles becoming an increasingly appreciated mechanism of intercellular communication and vital components of both laboratory and clinical research.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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