Extracellular vesicles: communication, coercion, and conditioning

David A. Shifrin, Jr.[a], Michelle Demory Beckler[b], Robert J. Coffey[a,b], and Matthew J. Tyska[a]
[a]Department of Cell and Developmental Biology and [b]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232

ABSTRACT **Cells communicate with neighboring cells and condition their local environment by secreting soluble factors into the extracellular space. These well-studied facets of cell biology are essential for the establishment and maintenance of physiological homeostasis. However, accumulating evidence has revealed that specific ligands, enzymes, and macromolecules are distributed into the extracellular space by virtue of their association with small vesicles, which are released by a variety of cell types. Although the biological significance of such vesicles was initially debated, purification and subsequent functional studies have shown that these extracellular vesicles are bioactive organelles carrying a wide range of protein and nucleic acid cargoes. In many cases these vesicles are laden with molecules that are involved in cell signaling, although other diverse functions are being revealed at a rapid pace. In this Perspective, we discuss recent developments in the understanding of the major pathways of extracellular vesicle biogenesis and how these vesicles contribute to the maintenance of physiological homeostasis.**

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INTRODUCTION

Cells play an active role in shaping their local environment by releasing factors that either affect neighboring cells or manipulate the biochemical properties of the extracellular milieu. Although soluble protein ligands have received the most experimental attention in this regard, a rapidly growing field of investigation suggests that these events are also mediated by small extracellular vesicles (ECVs). Indeed, ECVs are now known to affect processes ranging from immune signaling to angiogenesis to detoxification of bacterial products (Thery *et al.*, 2009; Mause and Weber, 2010; Shifrin *et al.*, 2012). In these cases and many others, ECVs facilitate the distribution of specific cargoes that mediate communication with or coercion of other cells or the conditioning of the extracellular environment. ECVs range in size from ~50 to 500 nm and are typically enriched in specific proteins and lipids, which differentiates their composition from that of the plasma membrane (Mause and Weber, 2010). One challenge for investigators new to this field is the variable and confusing nomenclature that exists in the ECV literature; this is primarily a result of the many cellular sources, isolation procedures, and apparently distinct mechanisms of formation. For the purposes of this discussion, we group ECVs into two broad categories that are distinguished by their mechanism of formation: exosomes and ectosomes. Exosomes (40–100 nm) are likely produced through an exocytic pathway via the formation of multivesicular bodies (MVBs) and their subsequent fusion with the plasma membrane (Mathivanan *et al.*, 2010). In contrast, ectosomes range from 100 to 500 nm in diameter and are released through outward budding of the plasma membrane. Other names for ectosomes one might encounter in the literature are "microvesicles," "membrane particles," "microparticles," or "nanoparticles" (Bastida *et al.*, 1984; Thery *et al.*, 2001; Cocucci *et al.*, 2009; Mathivanan *et al.*, 2010; van der Pol *et al.*, 2012). Both exosomes and ectosomes are formed such that the original plasma membrane topology is maintained, that is, vesicles are released into the extracellular space with membranes "right-side out" (van der Pol *et al.*, 2012). Exosomes and ectosomes are produced by a vast array of cell types in a variety of physiological contexts, suggesting that they may contribute to many essential aspects of cell and tissue function, some of which will be discussed in more detail in what follows.

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Address correspondence to: Matthew J. Tyska (matthew.tyska@vanderbilt.edu).
Abbreviations used: ECV, extracellular vesicle; ESCRT, endosomal sorting complex required for transport; LPS, lipopolysaccharide; MVB, multivesicular body; Myo1a, Myosin-1a; PLAP, placental alkaline phosphatase; SCID, severe combined immunodeficiency; TNAP, tissue-nonspecific alkaline phosphatase.

MECHANISMS OF ECV FORMATION

Exosome biogenesis

Although the molecular details underlying exosome production remained unclear for many years, recent models have converged on a late-endosomal, MVB-dependent pathway (de Gassart *et al.*, 2004; Hurley and Odorizzi, 2012). How specific cargoes destined for exosomes sort into these membranes and how differentiated vesicles bud into the lumen of the MVB are still matters of debate. An initial lipid-centric model suggested that ceramide enrichment in endosomal membranes was a major driving force (Trajkovic *et al.*, 2008). Because of the small size of its head group, ceramide may directly influence the shape of the membrane to promote bending and downstream budding of vesicles. This model also invokes ceramide in the formation of raft-like domains that presumably function to sort specific cargo molecules into exosomal membranes, although this point has not been tested directly. A second model that has gained support more recently suggests that exosome formation follows a mechanism that parallels the endolysosomal degradation pathway, which relies on the function of endosomal sorting complex required for transport (ESCRT) machinery to generate MVBs (van Niel *et al.*, 2006). Indeed, RNA interference silencing of the key ESCRT components tumor susceptibility gene 101 (TSG101, ESCRT-I), vacuolar protein sorting (VPS) 22 (ESCRT-II), charged multivesicular body protein (CHMP) 2A, CHMP4A/B/C (ESCRT-III), VPS4A/B, or ALIX (an ESCRT-III–interacting protein) significantly reduces exosome production in MCF-7 cells (Baietti *et al.*, 2012). Other studies reported decreased exosome secretion from dendritic cells after silencing of the ESCRT-0 protein hepatocyte growth factor–regulated tyrosine kinase substrate (Hrs; Tamai *et al.*, 2010). Cargoes destined for endolysosomal degradation are selectively recruited to the limiting membrane of the MVB through a process that involves their ubiquitylation and subsequent ubiquitin-dependent interactions with ESCRT-0, -I, and -II (Raiborg and Stenmark, 2009). However, ALIX can bind directly to exosomal cargo molecules, which might represent a ubiquitin-independent recruitment mechanism and also provide a point of distinction between sorting into exosomes versus endolysosomes (Baietti *et al.*, 2012; Hurley and Odorizzi, 2012). Although the extent of functional overlap between the ceramide-dependent and ESCRT-dependent pathways outlined here is unclear, the mechanistic details will likely become the focus of future studies.

After MVB formation, a subset of these organelles fuse with the plasma membrane and release their constituent exosomes (Thery *et al.*, 2002). Although little is known about the direct regulation of MVB fusion with the plasma membrane, it appears that several Rab GTPases, including Rab5, Rab27, and Rab35, are involved in exosome secretion (Vanlandingham and Ceresa, 2009; Hsu *et al.*, 2010; Ostrowski *et al.*, 2010; Baietti *et al.*, 2012). Further investigation will be needed to clarify the role of various Rab proteins in MVB fusion with the plasma membrane and exosome release.

Ectosome biogenesis

Although comparatively little is known about the details of ectosome formation, evidence suggests that ectosomes are produced not from an endomembrane system as for exosomes, but by direct budding from the plasma membrane (Thery *et al.*, 2009). Despite this seemingly significant mechanistic distinction, proposed models of ectosome formation include elements that are common to the production of exosomes. For example, the topology of outward budding from the plasma membrane is equivalent to budding into the MVB lumen. Thus one might expect the ESCRT system to be involved in ectosome formation. Indeed, recent studies revealed that arrestin-domain containing-1 (ARRDC1) interacts with the ESCRT component TSG101 during ectosome shedding directly from the plasma membrane (Nabhan *et al.*, 2012). Another parallel with exosome production is related to the significance of specific lipids in vesicle budding from the plasma membrane. ECVs of both types are enriched in cholesterol, ceramide, and other lipids implicated in raft formation. In glial cells, for example, production of ceramide is believed to promote membrane curvature during ectosome formation (Bianco *et al.*, 2009).

An alternative pathway for ectosome formation might involve actin-based motors. In the context of the enterocyte brush border, the membrane-binding motor, myosin-1a (Myo1a), distributes along the length of the microvillus, where it exerts plus end (tipward)–directed force on the apical membrane (McConnell and Tyska, 2007). This activity leads to the accumulation of membrane at microvillar tips, which in turn drives the formation and release of ectosomes into the gut lumen (80–100 nm in diameter). Mice lacking Myo1a produce fewer ectosomes, and those that are released lack characteristic enrichment of cargo molecules, such as the host defense factor intestinal alkaline phosphatase (McConnell *et al.*, 2009). Although the involvement of Myo1a in this system is likely an adaptation linked to the unique morphology of the microvillus, these studies suggest that manipulation of plasma membrane mechanics is of central importance to the mechanism of ectosome formation.

PHYSIOLOGICAL ROLES OF ECVS

ECVs are released from a variety of "donor" cell types and contribute to an array of physiological processes. In this section, our discussion of ECV function is organized according to physiological context; key examples and relevant references are highlighted in Table 1.

Development

Although relatively little is known about ECVs during vertebrate development, several reports provide clues as to how these vesicles might contribute to normal morphogenesis. ECVs are detected in the peripheral circulation of pregnant women, and ECV cargoes vary based on the phase of gestation (Abrahams *et al.*, 2004; Frangsmyr *et al.*, 2005; Atay *et al.*, 2011; Clifton *et al.*, 2012). Although it is unknown how ECVs act during gestation, studies have shown that proteins with established functions in development are found in ECVs. For example, β-catenin, Wnt, GSK3, and the Wnt cargo receptor Evi are components of exosomes purified from cultured human (HEK293, Caco-2) and *Drosophila* (S2,Kc167) cells (Chairoungdua *et al.*, 2010; Taelman *et al.*, 2010; Gross *et al.*, 2012). The function of β-catenin– and Wnt-containing exosomes is not clear, but given the central role these proteins play in tissue and organ development (Petersen and Reddien, 2009), it is tempting to speculate that their function may be facilitated by distribution in ECVs. In addition, sonic hedgehog (SHH) is contained in ECVs from activating T lymphocytes, and these vesicles appear to function in promoting neovascularization (Soleti and Martinez, 2012). SHH has critical functions during embryogenesis, including patterning of the nervous system (Fuccillo *et al.*, 2006) and development of the gastrointestinal system and other endoderm-derived organs (van den Brink, 2007). Thus, with high concentrations of the morphogen, it seems likely that SHH-containing ECVs could exert effects on developing organs and tissues. Taken together, the results of these studies are suggestive of developmental roles for ECVs, but future studies are warranted to clarify functional roles and molecular mechanisms.

Immunity

ECVs are shed from immune cells, with up-regulated production occurring upon stimulation by inflammatory signaling (van der Pol *et al.*, 2012; Lo Cicero and Raposo, 2013). Conversely, infected cells

Category	Location/source	Function	Cargo	Reference[a]
Immunity	Enterocyte	Anti-inflammatory, antibacterial	Intestinal alkaline phosphatase	Shifrin *et al*. (2012)
	Macrophage, mast cell, B cell, dendritic cell	Immune cell activation, antigen presentation, MHC transfer	IL-1β, TNF-α, HMGB1	Thery *et al*. (2009); Lo Cicero and Raposo, (2013)*
	T-cell, polymorphonuclear cell	Immune modulation	FasL	van der Pol *et al*. (2012)*
	Microglial cell	Immunostimulation, increased neurotransmission	IL-1β	Turola *et al*. (2012)*
	Mono/lymphocyte	Promotion of *Trypanosoma cruzi* infection	TGF-β	Cestari *et al*. (2012)
	Syncytiotrophoblast	Immunosuppression	CD63, PLAP, TGF-β, FasL, ULBP, mRNAs, miRNAs	Mincheva-Nilsson and Baranov (2010)*
	Dendritic cell	T_H1 response activation, natural killer activation, anti-inflammatory	MHC proteins, antigens, T-cell stimulatory molecules	Zitvogel *et al*. (1998), Thery *et al*. (2001), van der Pol *et al*. (2012)*
Development	Stem cell	Tissue repair, plasticity, reprogramming	mRNA, miRNA	Camussi *et al*. (2011), Tetta *et al*. (2011)*
	Cardiomyocyte in culture	Gene transfer, gene expression changes	mRNA, chromosomal DNA	Waldenstrom *et al*. (2012)
	Endothelial progenitor cell	Angiogenesis	mRNA	Mause and Weber (2010)*
	Lung tissue	Fate determination, marrow cell phenotype	mRNA	Aliotta *et al*. (2007, 2010, 2012*)
	Experimentally induced in HEK293	Confer permissiveness for induced pluripotent stem cell generation	mCAT-1	Mangeot *et al*. (2011)
Neurological	Microglial cell	Immunostimulation, increased neurotransmission	IL-1β	Turola *et al*. (2012)*
	Primary neuron, especially somatodendritic compartment	Synaptic transmission and plasticity, neurodegeneration	GluR2	Schneider and Simons (2013)
Bone	Epiphysis	Cartilage calcification	TNAP	Golub (2009, 2011)*
Blood/ cardiovascular	Platelets	Thrombus formation, immune cell activation, angiogenesis/ neovascularization	Tissue factor, chemokines, IL-1β, signaling lipids	von Hundelshausen and Weber (2007), Mause and Weber (2010), Owens and Mackman (2011), Rautou *et al*. (2011), Zwicker *et al*. (2011)*
	Endothelial cell	Might promote atherosclerosis		Rautou *et al*. (2011)*
	Smooth muscle cells (aorta)	Might promote atherosclerosis		Bobryshev *et al*. (2012)
Cancer	MDA-MB-231	Increase tumor cell invasion	Amphiregulin	Higginbotham *et al*. (2011)
	Most cancer cell types	Increased invasiveness, antitumor immunosuppression, possibly T-cell priming	Oncogenic sequences, single-strand DNA, retrotransposons, tumor antigens, cytokines	D'Souza-Schorey and Clancy (2012)*, Iero *et al*. (2008)*
	Glioma cell	Transfer of EGFRvIII	EGFRvIII	Al-Nedawi *et al*. (2008)

HMGB1, high-mobility group protein B1; mCAT-1, murine cationic amino acid transporter 1; MHC, major histocompatibility complex; miRNA, micro RNA; PLAP, placental alkaline phosphatase;TGF-β, transforming growth factor β; TNAP, tissue-nonspecific alkaline phosphatase; TNF-α, tumor necrosis factor α; ULBP, UL16-binding protein.
[a]Asterisk indicates a review.

TABLE 1: Physiological roles of ECVs.

can also release ECVs, which target immune cells and activate innate responses (Dreux *et al*., 2012). For example, hepatitis C–infected cells release viral RNA packaged inside exosomes (genetic material is a common ECV cargo), which are then taken up by plasmacytoid dendritic cells (Dreux *et al*., 2012). Moreover, ectosomes shed from intestinal epithelial cells are highly enriched in intestinal alkaline phosphatase and serve to both detoxify soluble bacterial lipopolysaccharide and inhibit bacterial colonization of the intestinal

epithelial surface (Shifrin *et al.*, 2012). Lumenal ECVs are shed constitutively, as they can be isolated from the small intestine of uninfected rodents and sterile cell culture models. However, ectosome production increases dramatically in the presence of bacterial pathogens (Shifrin *et al.*, 2012). Similarly, ECV shedding from macrophages and microglial cells is dependent on activation of the ATP receptor and ligand-gated ion channel $P2\times_7$, which can be induced by exogenous ATP (e.g., as a result of cellular injury) or through normal ATP release into the extracellular environment of astrocyte–microglia cocultures (Bianco *et al.*, 2005; Thomas and Salter, 2010). $P2\times_7$ can also be activated during sterile inflammation (Thomas and Salter, 2010). ATP released from astrocytes in culture is sufficient to stimulate ECV release from microglia (Bianco *et al.*, 2005); these vesicles carry proinflammatory cytokines, including interleukin 1-β (IL1-β), and are capable of activating other macrophages and perhaps dendritic cells (Thomas and Salter, 2010). Activation of macrophages by ECVs suggests the initiation of a large-scale inflammatory response. However, it is possible that in some instances the targeted, local activation of ECV shedding through TLR4 signaling or other inflammatory mechanisms could actually prevent such a system-wide process. By releasing lumenal ECVs to combat bacterial attachment, for example, enterocytes could mitigate the spread of infection, thereby abrogating the need for mounting a systemic anti-inflammatory response (Shifrin *et al.*, 2012). In the case of virally infected cells, host cells use ECVs to communicate with neighboring uninfected cells, which serves to initiate an immune response (Dreux *et al.*, 2012). This allows an appropriate local-scale response without hyperactivating the immune system or further spreading the pathogen.

Bone formation

Matrix ECVs found in the epiphysis of long bones bear a strong resemblance to ECVs shed from enterocyte microvilli. Matrix ECVs are ectosomes released during the calcification process and are highly enriched in tissue-nonspecific alkaline phosphatase (Golub, 2009, 2011). This enzyme decreases the concentration of inorganic pyrophosphate, increases that of inorganic phosphate (P_i), and as a result promotes hydroxyapatite formation and bone mineralization (Golub, 2011). Although isolated from entirely different contexts, microvillus-derived ECVs and matrix ECVs share a common function in biochemically conditioning their local microenvironment. In the former case, dephosphorylation of lipopolysaccharide (LPS) by alkaline phosphatase significantly reduces LPS toxicity (Poelstra *et al.*, 1997); in the latter, production of P_i is critical for proper assembly of normal bone. In both systems, however, the ability to produce ECVs with specific, enriched catalytic activity is needed for maintaining homeostasis.

Distribution of catalytic activity is not the only function of EVCs in bone; however, as Aliotta *et al.* (2007, 2010) discovered that marrow cell phenotype is altered by lung-derived ECVs through transfer of mRNA, aiding in recovery of lung tissue after injury. This suggests that ECVs may be involved in regulating stem cell fate decisions during tissue repair by regulating gene expression.

Cardiovascular function

In the cardiovascular system, circulating ECVs are released from virtually every cell type, including platelets, immune cells (van der Pol *et al.*, 2012; Lo Cicero and Raposo, 2013), and endothelial and vascular smooth muscle cells. In some cases, these vesicles were shown to participate in the coagulation process (Cocucci *et al.*, 2009), whereas other work revealed that cardiovascular ECVs can distribute HSP60 (Gupta and Knowlton, 2007). Waldenstrom *et al.* (2012) "transfected" fibroblasts with nucleic acid–containing ECVs isolated from adult mouse cardiomyocytes and demonstrated that genetic material from ECVs is transferred into target cells, where it induces changes in gene expression. Similarly, ECVs isolated from endothelial progenitor cells were able to stimulate vessel formation in culture and in an SCID mouse model, a process dependent on mRNA contained in the ECVs (Deregibus *et al.*, 2007). In disease states, cardiovascular ECV production is upregulated, such as increased vesicle shedding observed in preatherosclerotic areas of the aorta (Bobryshev *et al.*, 2012) and in cases of prothrombotic anemias (Zwicker *et al.*, 2011). Bastida *et al.* (1984) described ECVs from isolated human glioblastoma cells that contain factors capable of activating clotting and platelet aggregation in vitro; this work is consistent with earlier findings of ECVs isolated from human breast cancer and guinea pig hepatocarcinoma cells (Dvorak *et al.*, 1983). A notable feature of glioblastoma ECVs is their potent activity, showing up to 10-fold greater procoagulant activity compared with cellular membrane fractions. Similar to other systems, this finding demonstrates the utility of ECVs as distributors of a highly concentrated biological activity, allowing source cells to propagate the relevant signal in a targeted, efficient manner.

Nervous system function

In neural tissue, exosomes are released from many cell types (Turola *et al.*, 2012), whereas ectosomes are shed by microglial cells (discussed earlier), neurons (Schiera *et al.*, 2007), astrocytes (Bianco *et al.*, 2005), and possibly oligodendrocytes (Turola *et al.*, 2012; Verderio *et al.*, 2012). Neuronal ECVs are enriched in fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF) and could function in regulating angiogenesis (Schiera *et al.*, 2007). Astrocytic ECVs carry IL-1β, implicating the organelles in the mediation of immune responses, and an examination of oligodendrocyte-derived ECVs from patients with multiple sclerosis also suggests a role in proinflammatory signaling (Verderio *et al.*, 2012). Faure *et al.* (2006) demonstrated the release of exosomes from neurons and astrocytes, which contained the GluR2 subunit of the glutamate receptor and other proteins. Although further work investigating the function of neuronal ECVs is needed, this and a subsequent study (Lachenal *et al.*, 2011) suggest that neuronal-derived ECVs play a regulatory role in the CNS for neuronal plasticity, perhaps by recycling or disposing of various receptors or by transferring cargo proteins to receiving cells. ECVs were long believed to be a general cellular "trash disposal," an idea that was mostly discarded as investigators discovered specific ECV functions. In the nervous system, however, removing cellular detritus is still considered a primary function of ECVs and one that may play a significant role in maintaining homeostasis by regulating receptors on the cell surface and removing pathology-inducing proteins targeted for degradation (Von Bartheld and Altick, 2011).

ECV FUNCTION IN DISEASE

Based on the broad contributions to normal physiology, it is perhaps not surprising that ECVs play roles in numerous diseases (atherosclerosis, coagulopathies, inflammation, infection and autoimmune disease; Thomas and Salter, 2010; Zwicker *et al.*, 2011; Bobryshev *et al.*, 2012; Dreux *et al.*, 2012; Verderio *et al.*, 2012). However, here we focus on their role in cancer.

A key finding that first implicated ECVs in cancer was the discovery that membrane vesicles shed from metastatic cells could induce lung metastasis (Poste and Nicolson, 1980). Early research on exosomes in the immune system (Chaput *et al.*, 2004) led to their eventual identification as vesicles secreted from both mouse and

human tumor cells (Wolfers *et al.*, 2001; Andre *et al.*, 2002). Although we do not fully understand the differences between ECV released from normal and cancer cells, recent work suggests that ECVs facilitate horizontal transfer of cargo and harness the activity of these cargoes to stimulate intracellular signaling pathways, promoting tumor progression (Muralidharan-Chari *et al.*, 2010; Lee *et al.*, 2011; Demory Beckler *et al.*, 2013). Although we focus on protein cargo of ECVs in the subsequent discussion, it is becoming increasingly apparent that these vesicles contain DNA and RNA, which may also confer important proneoplastic effects (Valadi *et al.*, 2007; Skog *et al.*, 2008; Hong *et al.*, 2009; Pisetsky *et al.*, 2011).

Oncogenes represent an important class of active ECV cargoes that modulate recipient cells. Tissue transglutaminase (tTG)-containing ECVs purified from cancer cells induced NIH 3T3 cell survival and anchorage-independent growth, effects that were linked to transfer of tTG via ECVs. Moreover, exosomes purified from colon cancer cells transfer mutant G13D KRAS and induce anchorage-independent growth and colony formation in three-dimensional collagen matrix, further suggesting that oncogene-containing exosomes promote cancer-related phenotypes (Demory Beckler *et al.*, 2013). In addition, treatment of mildly aggressive glioma cells with EGF receptor variant III (EGFRvIII)–containing ECVs increased observed levels of EGFRvIII in these cells, which may be partly responsible for the observed increases in VEGF production and anchorage-independent growth of recipient cells (Al-Nedawi *et al.*, 2008). Not surprising, given the variety of cargo and roles in intercellular communication, it seems that in addition to promoting cancer phenotypes, ECVs also carry tumor suppressors. A recent study showed functional PTEN in exosomes purified from fibroblast cells and that treatment of cells with these exosomes reduced cellular proliferation (Putz *et al.*, 2012). Combined, these data provide strong support for ECVs as vehicles for protein cargo transfer to recipient cells, allowing for promotion and suppression of tumor-related phenotypes.

In addition to inducing features of tumor initiation, data indicate a role for ECVs also in promoting angiogenesis, invasion, and metastasis. ECVs contain a wide variety of prometastatic cargo, including insulin-degrading enzyme, matrix metalloproteinases, tetraspanins, heat shock proteins, plasminogen, integrin family members, and growth factors (VEGF and FGF; Graner, 2011; D'Souza-Schorey and Clancy, 2012). Proangiogenic effects of ECVs include enhanced endothelial cell tubule formation and vascularization of in vivo Matrigel plugs (Hood *et al.*, 2009; Mineo *et al.*, 2012). Cathepsin B–containing ECVs and EGFR ligand–containing exosomes are two examples of ECVs that elicit recipient cell invasion in vitro (Giusti *et al.*, 2008; Higginbotham *et al.*, 2011). Luga *et al.*, (2012) recently reported that exosomes secreted from fibroblasts enhance breast cancer cell motility and metastasis, a mechanism that was suggested to require exosome association with Wnt11 and activation of recipient breast cancer planar cell polarity proteins. This study provides additional support for the cross-talk of stroma and tumor and implicates stromal fibroblast ECVs in cancer progression. The identity of cargoes packaged in ECVs and their ability to induce invasion both support a role for these vesicles in promoting metastatic phenotypes.

Several key in vivo studies demonstrated the ability of ECVs to prime metastatic niches. Melanoma exosomes selectively home to and prime sentinel lymph nodes for melanoma cell metastasis (Hood *et al.*, 2011). Similarly, pretreatment of mice with melanoma exosomes results in accelerated lung metastasis (Liu *et al.*, 2010). Finally, it was recently reported that melanoma exosomes reprogram bone marrow–derived cells to a more provasculogenic phenotype that supports enhanced tumor growth and metastasis (Peinado *et al.*, 2012). Taken together, these reports strongly indicate that

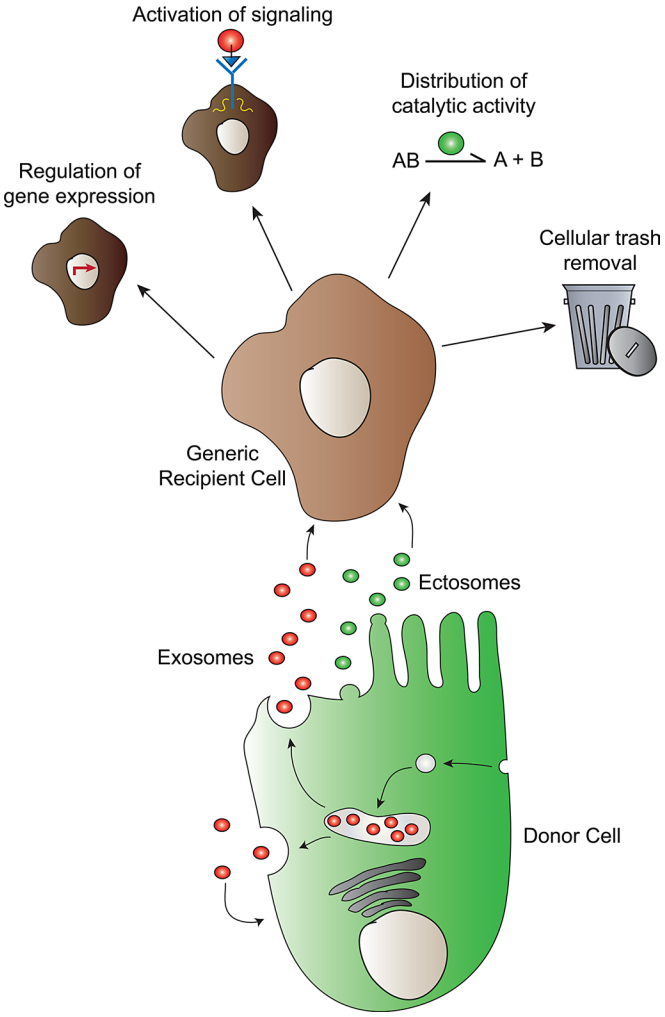

FIGURE 1: Extracellular vesicle biogenesis and function. Extracellular vesicles are formed via two known biogenesis pathways: exosomes via an endomembrane MVB intermediate, and ectosomes via direct budding from the plasma membrane. Once released into the extracellular environment, ECVs perform one of four general functions: 1) regulation of gene expression, 2) activation of cell signaling, 3) distribution of catalytic activity, and 4) cellular trash removal.

ECVs act to enhance metastatic properties of recipient cells. Future studies are needed, however, to identify the mechanisms by which ECVs exert these actions.

COMMON THEMES AND CONCLUSIONS

Despite the diverse physiological contexts highlighted here, ECV functions generally partition into one of four categories: regulation of gene expression, induction of signaling, distribution of catalytic activity, and disposal of cellular "trash" (Figure 1). Even more broadly, ECVs serve to protect, concentrate, or remove cargo. Genetic material must be protected from degradation as it is transported from source to target cell. Ligands or receptors may need to be concentrated in order to efficiently activate a signaling cascade, whereas catalytic activity may be enhanced by concentrating an enzyme on the surface of an ECV. All of the studies highlighted here suggest that ECVs are highly adaptable cargo-carrying platforms.

Given that discoveries from the last decade have demonstrated the near-ubiquity of ECVs, several new areas of investigation will be

important for developing a full understanding of these organelles. More detailed studies of exosome and ectosome formation will provide much needed clarity into the distinctions between these two pathways. Moreover, very little is known about how ECVs are captured by recipient/target cells. Initial studies implicate specific lipids (e.g., phosphatidylserine) and integrins in this process, but the details are only beginning to come into focus (Deregibus *et al.*, 2007; Iglesias *et al.*, 2012). Once precise mechanisms of ECV tethering, fusion, and/or internalization with target cells have been established, understanding the transfer of enriched cargo molecules and downstream effects can be clarified.

Translational applications of ECV biology are in their infancy but include their use as therapeutic delivery vehicles and disease biomarkers. Recent studies showed that stem cell ECVs are able to reprogram diseased cells (Iglesias *et al.*, 2012), and "custom" ECVs are capable of delivering experimental cargoes (Mangeot *et al.*, 2011). Other potential ECV-based therapies, such as tissue repair and revascularization, are being investigated (Lee *et al.*, 2012). ECVs also serve as attractive biomarkers of disease progression. In addition to blood and serum, ECVs can be purified from other biofluids, such as urine (Dear *et al.*, 2013), and their cargo composition and rate of production are altered in various pathologies. However, a better understanding of ECV formation, uptake, and function is needed to expand current studies of ECVs as therapeutic delivery systems (Mangeot *et al.*, 2011; Lee *et al.*, 2012), tailor synthetic ECVs for treatment of disease, or use these organelles as biomarkers.

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REFERENCES

Abrahams VM, Straszewski-Chavez SL, Guller S, Mor G (2004). First trimester trophoblast cells secrete Fas ligand which induces immune cell apoptosis. Mol Hum Reprod 10, 55–63.

Aliotta JM, Lee D, Puente N, Faradyan S, Sears EH, Amaral A, Goldberg L, Dooner MS, Pereira M, Quesenberry PJ (2012). Progenitor/stem cell fate determination: interactive dynamics of cell cycle and microvesicles. Stem Cells Dev 21, 1627–1638.

Aliotta JM *et al.* (2010). Microvesicle entry into marrow cells mediates tissue-specific changes in mRNA by direct delivery of mRNA and induction of transcription. Exp Hematol 38, 233–245.

Aliotta JM *et al.* (2007). Alteration of marrow cell gene expression, protein production, and engraftment into lung by lung-derived microvesicles: a novel mechanism for phenotype modulation. Stem Cells 25, 2245–2256.

Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J (2008). Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol 10, 619–624.

Andre F *et al.* (2002). Malignant effusions and immunogenic tumour-derived exosomes. Lancet 360, 295–305.

Atay S, Gercel-Taylor C, Kesimer M, Taylor DD (2011). Morphologic and proteomic characterization of exosomes released by cultured extravillous trophoblast cells. Exp Cell Res 317, 1192–1202.

Baietti MF *et al.* (2012). Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. Nat Cell Biol 14, 677–685.

Bastida E, Ordinas A, Escolar G, Jamieson GA (1984). Tissue factor in microvesicles shed from U87MG human glioblastoma cells induces coagulation, platelet aggregation, and thrombogenesis. Blood 64, 177–184.

Bianco F *et al.* (2009). Acid sphingomyelinase activity triggers microparticle release from glial cells. EMBO J 28, 1043–1054.

Bianco F, Pravettoni E, Colombo A, Schenk U, Moller T, Matteoli M, Verderio C (2005). Astrocyte-derived ATP induces vesicle shedding and IL-1 beta release from microglia. J Immunol 174, 7268–7277.

Bobryshev YV, Killingsworth MC, Orekhov AN (2012). Increased shedding of microvesicles from intimal smooth muscle cells in athero-prone areas of the human aorta: implications for understanding of the predisease stage. Pathobiology 80, 24–31.

Camussi G, Deregibus MC, Bruno S, Grange C, Fonsato V, Tetta C (2011). Exosome/microvesicle-mediated epigenetic reprogramming of cells. Am J Cancer Res 1, 98–110.

Cestari I, Ansa-Addo E, Deolindo P, Inal JM, Ramirez MI (2012). *Trypanosoma cruzi* immune evasion mediated by host cell-derived microvesicles. J Immunol 188, 1942–1952.

Chairoungdua A, Smith DL, Pochard P, Hull M, Caplan MJ (2010). Exosome release of beta-catenin: a novel mechanism that antagonizes Wnt signaling. J Cell Biol 190, 1079–1091.

Chaput N, Taieb J, Schartz NE, Andre F, Angevin E, Zitvogel L (2004). Exosome-based immunotherapy. Cancer Immunol Immunother 53, 234–239.

Clifton VL, Stark MJ, Osei-Kumah A, Hodyl NA (2012). Review: the feto-placental unit, pregnancy pathology and impact on long term maternal health. Placenta 33 (Suppl), S37–S41.

Cocucci E, Racchetti G, Meldolesi J (2009). Shedding microvesicles: artefacts no more. Trends Cell Biol 19, 43–51.

Dear JW, Street JM, Bailey MA (2013). Urinary exosomes: a reservoir for biomarker discovery and potential mediators of intra-renal signaling. Proteomics, DOI: 10.1002/pmic.201200285.

de Gassart A, Geminard C, Hoekstra D, Vidal M (2004). Exosome secretion: the art of reutilizing nonrecycled proteins. Traffic 5, 896–903.

Demory Beckler M, Higginbotham JN, Franklin JL, Ham AJ, Halvey PJ, Imasuen IE, Whitwell C, Li M, Liebler DC, Coffey RJ (2013). Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS. Mol Cell Proteomics 12, 343–355.

Deregibus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, Bruno S, Bussolati B, Camussi G (2007). Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. Blood 110, 2440–2448.

Dreux M, Garaigorta U, Boyd B, Decembre E, Chung J, Whitten-Bauer C, Wieland S, Chisari FV (2012). Short-range exosomal transfer of viral RNA from infected cells to plasmacytoid dendritic cells triggers innate immunity. Cell Host Microbe 12, 558–570.

D'Souza-Schorey C, Clancy JW (2012). Tumor-derived microvesicles: shedding light on novel microenvironment modulators and prospective cancer biomarkers. Genes Dev 26, 1287–1299.

Dvorak HF, Van DeWater L, Bitzer AM, Dvorak AM, Anderson D, Harvey VS, Bach R, Davis GL, DeWolf W, Carvalho AC (1983). Procoagulant activity associated with plasma membrane vesicles shed by cultured tumor cells. Cancer Res 43, 4434–4442.

Faure J *et al.* (2006). Exosomes are released by cultured cortical neurones. Mol Cell Neurosci 31, 642–648.

Frangsmyr L, Baranov V, Nagaeva O, Stendahl U, Kjellberg L, Mincheva-Nilsson L (2005). Cytoplasmic microvesicular form of Fas ligand in human early placenta: switching the tissue immune privilege hypothesis from cellular to vesicular level. Mol Hum Reprod 11, 35–41.

Fuccillo M, Joyner AL, Fishell G (2006). Morphogen to mitogen: the multiple roles of hedgehog signalling in vertebrate neural development. Nat Rev Neurosci 7, 772–783.

Giusti I, D'Ascenzo S, Millimaggi D, Taraboletti G, Carta G, Franceschini N, Pavan A, Dolo V (2008). Cathepsin B mediates the pH-dependent proinvasive activity of tumor-shed microvesicles. Neoplasia 10, 481–488.

Golub EE (2009). Role of matrix vesicles in biomineralization. Biochim Biophys Acta 1790, 1592–1598.

Golub EE (2011). Biomineralization and matrix vesicles in biology and pathology. Semin Immunopathol 33, 409–417.

Graner MW (2011). Brain tumor exosomes and microvesicles: pleiotropic effects from tiny cellular surrogates. In: Molecular Targets of CNS Tumors, ed. M Garami, New York: InTech, 43–78.

Gross JC, Chaudhary V, Bartscherer K, Boutros M (2012). Active Wnt proteins are secreted on exosomes. Nat Cell Biol 14, 1036–1045.

Gupta S, Knowlton AA (2007). HSP60 trafficking in adult cardiac myocytes: role of the exosomal pathway. Am J Physiol Heart Circ Physiol 292, H3052–H3056.

Higginbotham JN *et al.* (2011). Amphiregulin exosomes increase cancer cell invasion. Curr Biol 21, 779–786.

Hong BS *et al.* (2009). Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. BMC Genomics 10, 556.

Hood JL, Pan H, Lanza GM, Wickline SA (2009). Paracrine induction of endothelium by tumor exosomes. Lab Invest 89, 1317–1328.

Hood JL, San RS, Wickline SA (2011). Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. Cancer Res 71, 3792–3801.
Hsu C *et al*. (2010). Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. J Cell Biol 189, 223–232.
Hurley JH, Odorizzi G (2012). Get on the exosome bus with ALIX. Nat Cell Biol 14, 654–655.
Iero M, Valenti R, Huber V, Filipazzi P, Parmiani G, Fais S, Rivoltini L (2008). Tumour-released exosomes and their implications in cancer immunity. Cell Death Differ 15, 80–88.
Iglesias DM *et al*. (2012). Stem cell microvesicles transfer cystinosin to human cystinotic cells and reduce cystine accumulation in vitro. PloS One 7, e42840.
Lachenal G, Pernet-Gallay K, Chivet M, Hemming FJ, Belly A, Bodon G, Blot B, Haase G, Goldberg Y, Sadoul R (2011). Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. Mol Cell Neurosci 46, 409–418.
Lee TH, D'Asti E, Magnus N, Al-Nedawi K, Meehan B, Rak J (2011). Microvesicles as mediators of intercellular communication in cancer—the emerging science of cellular "debris." Semin Immunopathol 33, 455–467.
Lee Y, El Andaloussi S, Wood MJ (2012). Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet 21, R125–R134.
Liu Y, Xiang X, Zhuang X, Zhang S, Liu C, Cheng Z, Michalek S, Grizzle W, Zhang HG (2010). Contribution of MyD88 to the tumor exosome-mediated induction of myeloid derived suppressor cells. Am J Pathol 176, 2490–2499.
Lo Cicero A, Raposo G (2013). Emerging concepts of tumor exosome–mediated cell-cell communication. In: The Cell Biology of Exosomes: Historical and Perspectives, ed. H-G Zhang, New York: Springer, 1–32.
Luga V *et al*. (2012). Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell 151, 1542–1556.
Mangeot PE, Dollet S, Girard M, Ciancia C, Joly S, Peschanski M, Lotteau V (2011). Protein transfer into human cells by VSV-G-induced nanovesicles. Mol Ther 19, 1656–1666.
Mathivanan S, Ji H, Simpson RJ (2010). Exosomes: extracellular organelles important in intercellular communication. J Proteomics 73, 1907–1920.
Mause SF, Weber C (2010). Microparticles: protagonists of a novel communication network for intercellular information exchange. Circ Res 107, 1047–1057.
McConnell RE, Higginbotham JN, Shifrin DA Jr, Tabb DL, Coffey RJ, Tyska MJ (2009). The enterocyte microvillus is a vesicle-generating organelle. J Cell Biol 185, 1285–1298.
McConnell RE, Tyska MJ (2007). Myosin-1a powers the sliding of apical membrane along microvillar actin bundles. J Cell Biol 177, 671–681.
Mincheva-Nilsson L, Baranov V (2010). The role of placental exosomes in reproduction. Am J Reprod Immunol 63, 520–533.
Mineo M, Garfield SH, Taverna S, Flugy A, De Leo G, Alessandro R, Kohn EC (2012). Exosomes released by K562 chronic myeloid leukemia cells promote angiogenesis in a Src-dependent fashion. Angiogenesis 15, 33–45.
Muralidharan-Chari V, Clancy JW, Sedgwick A, D'Souza-Schorey C (2010). Microvesicles: mediators of extracellular communication during cancer progression. J Cell Sci 123, 1603–1611.
Nabhan JF, Hu R, Oh RS, Cohen SN, Lu Q (2012). Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. PNAS 109, 4146–4151.
Ostrowski M *et al*. (2010). Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol 12, 19–30, supp 11–13.
Owens AP 3rd, Mackman N (2011). Microparticles in hemostasis and thrombosis. Circ Res 108, 1284–1297.
Peinado H *et al*. (2012). Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med 18, 883–891.
Petersen CP, Reddien PW (2009). Wnt signaling and the polarity of the primary body axis. Cell 139, 1056–1068.
Pisetsky DS, Gauley J, Ullal AJ (2011). Microparticles as a source of extracellular DNA. Immunol Res 49, 227–234.
Poelstra K, Bakker WW, Klok PA, Kamps JA, Hardonk MJ, Meijer DK (1997). Dephosphorylation of endotoxin by alkaline phosphatase in vivo. Am J Pathol 151, 1163–1169.
Poste G, Nicolson GL (1980). Arrest and metastasis of blood-borne tumor cells are modified by fusion of plasma membrane vesicles from highly metastatic cells. Proc Natl Acad Sci USA 77, 399–403.
Putz U, Howitt J, Doan A, Goh CP, Low LH, Silke J, Tan SS (2012). The tumor suppressor PTEN is exported in exosomes and has phosphatase activity in recipient cells. Sci Signal 5, ra70.
Raiborg C, Stenmark H (2009). The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. Nature 458, 445–452.
Rautou PE, Vion AC, Amabile N, Chironi G, Simon A, Tedgui A, Boulanger CM (2011). Microparticles, vascular function, and atherothrombosis. Circ Res 109, 593–606.
Schiera G, Proia P, Alberti C, Mineo M, Savettieri G, Di Liegro I (2007). Neurons produce FGF2 and VEGF and secrete them at least in part by shedding extracellular vesicles. J Cell Mol Med 11, 1384–1394.
Schneider A, Simons M (2013). Exosomes: vesicular carriers for intercellular communication in neurodegenerative disorders. Cell Tissue Res 352, 33–47.
Shifrin DA Jr, McConnell RE, Nambiar R, Higginbotham JN, Coffey RJ, Tyska MJ (2012). Enterocyte microvillus-derived vesicles detoxify bacterial products and regulate epithelial-microbial interactions. Curr Biol 22, 627–631.
Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO (2008). Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 10, 1470–1476.
Soleti R, Martinez MC (2012). Sonic Hedgehog on microparticles and neovascularization. Vitam Horm 88, 395–438.
Taelman VF, Dobrowolski R, Plouhinec JL, Fuentealba LC, Vorwald PP, Gumper J, Sabatini DD, De Robertis EM (2010). Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. Cell 143, 1136–1148.
Tamai K *et al*. (2010). Exosome secretion of dendritic cells is regulated by Hrs, an ESCRT-0 protein. Biochem Biophys Res Commun 399, 384–390.
Tetta C, Bruno S, Fonsato V, Deregibus MC, Camussi G (2011). The role of microvesicles in tissue repair. Organogenesis 7, 105–115.
Thery C, Boussac M, Veron P, Ricciardi-Castagnoli P, Raposo G, Garin J, Amigorena S (2001). Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. J Immunol 166, 7309–7318.
Thery C, Ostrowski M, Segura E (2009). Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 9, 581–593.
Thery C, Zitvogel L, Amigorena S (2002). Exosomes: composition, biogenesis and function. Nat Rev Immunol 2, 569–579.
Thomas LM, Salter RD (2010). Activation of macrophages by P2×7-induced microvesicles from myeloid cells is mediated by phospholipids and is partially dependent on TLR4. J Immunol 185, 3740–3749.
Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brugger B, Simons M (2008). Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science 319, 1244–1247.
Turola E, Furlan R, Bianco F, Matteoli M, Verderio C (2012). Microglial microvesicle secretion and intercellular signaling. Front Physiol 3, 149.
Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9, 654–659.
van den Brink GR (2007). Hedgehog signaling in development and homeostasis of the gastrointestinal tract. Physiol Rev 87, 1343–1375.
van der Pol E, Boing AN, Harrison P, Sturk A, Nieuwland R (2012). Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev 64, 676–705.
van Niel G, Porto-Carreiro I, Simoes S, Raposo G (2006). Exosomes: a common pathway for a specialized function. J Biochem 140, 13–21.
Vanlandingham PA, Ceresa BP (2009). Rab7 regulates late endocytic trafficking downstream of multivesicular body biogenesis and cargo sequestration. J Biol Chem 284, 12110–12124.
Verderio C *et al*. (2012). Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. Ann Neurol 72, 610–624.
Von Bartheld CS, Altick AL (2011). Multivesicular bodies in neurons: distribution, protein content, and trafficking functions. Prog Neurobiol 93, 313–340.
von Hundelshausen P, Weber C (2007). Platelets as immune cells: bridging inflammation and cardiovascular disease. Circ Res 100, 27–40.
Waldenstrom A, Genneback N, Hellman U, Ronquist G (2012). Cardiomyocyte microvesicles contain DNA/RNA and convey biological messages to target cells. PloS One 7, e34653.
Wolfers J *et al*. (2001). Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nat Med 7, 297–303.
Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S (1998). Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med 4, 594–600.
Zwicker JI, Trenor CC 3rd, Furie BC, Furie B (2011). Tissue factor-bearing microparticles and thrombus formation. Arterioscler Thromb Vasc Biol 31, 728–733.