HHS Public Access

Author manuscript

Semin Cell Dev Biol. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:

Semin Cell Dev Biol. 2015 April ; 40: 41–51. doi:10.1016/j.semcdb.2015.02.010.

Extracellular Vesicles in Cancer: Exosomes, Microvesicles and the Emerging Role of Large Oncosomes

Valentina R. Minciacchi[1], **Michael R. Freeman**[1,2], and **Dolores Di Vizio**[1,2,*]

[1]Division of Cancer Biology and Therapeutics, Departments of Surgery, Biomedical Sciences and Pathology and Laboratory Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA

[2]The Urological Diseases Research Center; Boston Children's Hospital, Boston, MA; Department of Surgery, Harvard Medical School, Boston, MA.

Abstract

Since their first description, extracellular vesicles (EVs) have been the topic of avid study in a variety of physiologic contexts and are now thought to play an important role in cancer. The state of knowledge on biogenesis, molecular content and horizontal communication of diverse types of cancer EVs has expanded considerably in recent years. As a consequence, a plethora of information about EV composition and molecular pathways involved in the regulation of important biological processes has emerged, along with the notion that cancer cells rely on these particles to invade tissues and propagate oncogenic signals at distance. *In vivo* studies, designed to achieve a deeper understanding of the extent to which EV biology can be applied to clinically relevant settings, are increasing. This review will summarize recent studies on EVs functionally implicated in cancer, with a focus on a novel EV population referred to as large oncosomes, which originate from highly migratory, amoeboid tumor cells. Here we provide an overview about the biogenesis and composition of exosomes, microvesicles and large oncosomes, along with their cancer-specific and more general functions. We also discuss current challenges and emerging technologies that might improve EV detection in various systems. Further studies on the functional role of EVs in specific steps of cancer formation and progression will expand our understanding of the diversity of paracrine signaling mechanisms in malignant growth.

1. Introduction

The coexistence of many cell types within the same organism requires a high level of coordination, which is mediated by molecular mechanisms of intercellular communication. Historically, soluble factors have been considered the central players in this process[1] [2]. Soluble factors include secreted ligands that can bind plasma membrane receptors, thus

*Correspondence: Dolores Di Vizio, MD, PhD. Division of Cancer Biology and Therapeutics, Departments of Surgery, Biomedical Sciences & Pathology and Laboratory Medicine, Cedars-Sinai Medical Center 8700 Beverly Blvd. Los Angeles, CA 90048. Dolores.Divizio@cshs.org, Tel: 310-423-7709.

activating signaling cascades in target cells[3]. Depending on the distance between originating cell and target cell, the principal categories of intercellular communication are: autocrine, in which the target cell and the secreting cell are the same; paracrine, in which the target cell is in close proximity with the secreting one; and endocrine, in which the target is distant and the secreted factors travel great distances through the blood[4]. Cell communication can also be achieved by cell-to-cell contacts, as is the case for juxtacrine interactions[4]. More recently, a more complex, evolutionary conserved communication system has emerged. Cells are now known to exchange information through the release of membrane-enclosed particles called extracellular vesicles (EVs)[5-10]. EVs mediate the exchange of intricate intercellular messages comprised of classical soluble and insoluble signaling factors, as well as molecules of a different nature, including structural proteins, nucleic acids, and lipids. Additionally, EVs can travel through body fluids, thus conveying functional information to distant sites *in vivo*[11]. These and other findings have completely changed the concept of the nature intercellular communication, and have helped to clarify diverse cellular processes in pathological and physiological conditions.

The term “extracellular vesicle” has been introduced relatively recently and is currently used as a general term to describe virtually any type of membrane particle released by any type of cell, including microorganisms[12], into the extracellular space, regardless of differences in biogenesis and composition. Current criteria to distinguish between diverse EV populations are based on size, density, subcellular origin, function and molecular cargo[7]. Despite the many challenges in EV isolation and characterization, it is now clear that several types of EVs can be released from a single cell. We are also limited in selectively differentiating one EV population from another to fully study them individually, because current methods of purification often result in mixtures of particles. Despite the above limitations, the notion that EVs are products of many – and possibly all – cells, and are actively shed in a finely regulated manner, has completely ruled out the possibility that they represent an artifact of purification[6]. Notably, along with the demonstration that EVs derived from tumor cells represent specialized molecular and functional compartments, evidence that tumor cells may release different subtypes of EVs, together with those originating from normal cells, is emerging[13, 14].

This review focuses on the functional roles and clinical relevance of cancer EVs, which can be organized into several categories: exosomes (30-100 nm diameter), microvesicles (MVs) (100-1000 nm diameter), and a more recently identified cancer-derived EV population termed “large oncosomes,” which are much larger than most EV types characterized to date (1-10 μm diameter). Because of their atypical size (>1000 times larger than exosomes by volume), large oncosomes may have unique properties *in vivo* and may present new opportunities for tumor profiling. Finally, we discuss current methods and future opportunities for the study and characterization of different classes of EVs.

2. Exosomes and microvesicles

2.1. Biogenesis

Unraveling the mechanism of EV biogenesis is a biologically relevant goal that might shed light on extracellular communication and also result in clinically applicable tools, including

development of new therapies. The sorting of EV cargo seems to occur during EV formation, suggesting that the two processes might be interconnected, and molecules exported in EVs might also be functionally involved in their biogenesis. Filling the gaps of our understanding is imperative if we want to eventually be able to modulate this process in different cell types and diseases. Many different cells are capable of secreting both exosomes and MVs, including red blood cells[15] platelets[16], lymphocytes[17], dendritic cells[18], fibroblasts[19], endothelial cells[20], and epithelial and tumor cells[21]. Recent reports suggest that different types of EVs can originate from the same donor cells, and whether the various biogenetic pathways are completely independent or overlapping, and to what extent, needs further study[22].

2.1.1. Exosomes—It is now evident that exosomes can be produced by most organisms, including bacteria, and can be identified in diverse ecosystems, including in the ocean[23]. In the human body, exosomes can be produced by all cell types examined so far[8, 24]. Despite the demonstration in T cells that exosomes can originate by direct budding from the plasma membrane[25], they are generally thought to derive from the endosomal compartment. Exosomes form within the intraluminal vesicles (ILVs) that are generated within the multivesicular bodies (MVBs) as part of the endocytic machinery known as late endosomes[26, 27]. MVBs can either fuse with lysosomes for degradation or travel back to and fuse with the plasma membrane[28]. Thus, molecules can be recycled and released into the extracellular space within exosomes[6]. Sorting of EV cargo during the internal budding of the membrane that leads to ILV formation is an essential step in exosome biogenesis. The endosomal-sorting complex required for transport (ESCRT) is responsible for the accumulation and sorting of molecules channeled into the ILVs, as recently confirmed in reports from Morvan and Adell[26, 27]. Therefore ESCRT activity has been the topic of many studies directed to investigate the process of exosome formation [24]. The ESCRT, with its four main complexes (ESCRT-0, -I, -II and –III), is responsible for final delivery of ubiquitinated proteins to the degradation machinery. A recent study has demonstrated that depletion of specific ESCRT-family members can alter the exosome protein content and the rate of exosome release from cancer cells[29]. For example, Alix and the tumor susceptibility gene 101 (Tsg101) are involved in the formation of ILVs, which are then released as exosomes[24]. Alix was recently reported to interact with syndecans through the cytosolic adaptor syntenin, leading to exosome formation in MCF-7 and HeLa cells[30]. In addition, two independent studies have shown that inhibition of Alix impairs the ability of dendritic or muscle cells to secrete CD63-enriched exosomes[29, 31]. Proteins frequently found involved in exosome biogenesis in other systems, such as Tsg101, have been used as exosome markers in benign and cancer models. This suggests that these proteins are likely involved in exosome biogenesis in cancer cells. However, conclusive reports demonstrating their functional role in exosome formation are still lacking.

The ESCRT machinery is not the only mediator of ILV cargo sorting and formation, and other ESCRT-independent processes seem to functionally participate in exosome biogenesis[32]. For example, involvement of sphingomyelinase activity was first demonstrated in 2008, both by the high levels of ceramide found within purified exosomes, and by the reduction of EV release upon inhibition of sphingomyelinases[33]. Two other

lipids, cholesterol and phosphatidic acid (PA), have been implicated in this process[34, 35]. Furthermore, evidence suggests that syntenin can promote exosome formation in collaboration with the GTP-binding protein, ADP-ribosylation factor 6 (ARF6) and its effector phospholipase D2 (PLD2)[30, 36]. Once formed, ILVs containing MVBs can either fuse with the lysosomes or with the plasma membrane. Whether this is the result of the co-existence of two different populations of MVBs inside the cells, or the activation of specific signaling pathways, is still a subject of investigation. The involvement of the Rab family of small GTPases in vesicle trafficking and fusion with the plasma membrane[37, 38] suggests a role for these proteins in exosome release. How impaired activity of certain Rab family members, such as Rab7, Rab11, Rab27a/b and Rab35, affects exosome release has been the topic of several reports[30, 39-42]. While the importance of these proteins, as a class, in the regulation of exosome shedding, is unequivocal, specific roles for each of the above Rab family members in the process are still unclear[42]. For example, Rab27a appears to play a more specific function than Rab27b in regulating exosome release from metastatic tumor cells[43]. The observation that the GTPases that regulate exosome shedding vary from one cellular system to another suggests the existence of cell-type specific isoforms. The last and least characterized step of exosome biogenesis consists of the fusion of the MVBs with the plasma membrane, with consequent release of the EVs into the extracellular space. The soluble NSF-attachment protein receptor (SNARE) complex has been implicated in this process, and the Ca^{2+}-regulated vesicle-associated membrane protein 7 (VAMP7), a member of the SNARE complex, appears to be necessary for MVB fusion with the plasma membrane in leukemic cells[44]. However, while single SNARE complex members, like VAMP-1, 2 and 3, have been identified in cancer cell-derived exosomes by mass spectrometry in more than one study[45-47], the dependency of exosome biogenesis on the activity of the whole SNARE complex is unclear.

2.1.2. Microvesicles—MVs were originally characterized as products of activated blood platelets and erythrocytes[16, 48] and described for their involvement in coagulation[49]. More recently, MVs have been described in other diseases[50] including cancer[51-53], as tumor-derived microvesicles (TMVs)[54]. Unlike exosomes, MVs seem to originate directly from the plasma membrane, and are often classified as ectosomes[22, 55, 56]. The process that leads to MV generation starts from the formation of outward buds in specific sites of the membrane followed by fission and subsequent release of the vesicle into the extracellular space[53, 57]. The plasma membrane undergoes several molecular rearrangements at the sites of MV origin, which result in membrane budding, including changes in lipid and protein composition[58], and in Ca^{2+} levels[59]. The altered levels of Ca^{2+} result in the recruitment and activation of calcium-dependent enzymes like scramblase and floppase with subsequent modification of the plasma membrane lipid composition[58]. Externalization of phosphatidylserine (PS) appears to be one of the main features of MVs[5, 60], although the secretion of PS-negative MVs has been reported[61]. Moreover, lipid raft domains seem to be abundant in MVs, and MV formation can be impaired by cholesterol depletion[62]. In addition to rearrangements in the plasma membrane composition, proteins responsible for cell shape maintenance may be involved in MV biogenesis, by regulating actin dynamics[55, 63]. Li et al. recently identified RhoA, a member of the small GTPases family, together with its downstream targets Rho-associated coiled coil containing protein kinase

(ROCK) and the LIM kinase (LIMK) as a regulator of MV release[55]. While it is unclear whether the EV preparations described in this study were enriched with MV over exosomes, ectopic expression of a dominant negative form of CHMP3 protein (CHMP3 DN), a mammalian homolog of the yeast VPS24 protein that is essential for the secretion of exosomes, did not prevent MV shedding from MDAMB231 cells, suggesting that MVs and exosomes are distinct species. Whether RhoC, whose activated form is similar to RhoA[64], is involved in the process is still a matter of debate[55, 65]. Calpain, a calcium dependent enzyme, which regulates cytoskeletal proteins, has also been reported as a component of the MV biogenesis machinery in platelets[63]. In addition to this, the D'souza-Schorey group has demonstrated that ARF6 is a key protein in MV formation and shedding[56], and the ARF6-regulated endosomal complex seems to play an important role in the selective incorporation of molecular cargo into MVs[66]. ARF6 downstream targets include extracellular signal-regulated kinases (ERK) and myosin light-chain kinas (MLCK), key regulators of actin polymerization and myosin activity, both very important processes in MV release[56]. As a result, MV release in the extracellular space can be reduced by the inhibition of either ARF6 activity or activity of ARF6 targets[56].

2.2. Content

EV content is highly heterogeneous because EVs can accommodate proteins, nucleic acids and lipids, and protein cargo includes both surface molecules (possibly to mediate intercellular interactions) and intra-vesicular species [67]. EV cargo is not merely a reflection of the donor cell composition but rather the result of a regulated, but still largely unresolved, sorting mechanism. What seems to be clear is that the selection mechanism allows discrimination between molecules meant to be included in the intercellular message that EVs are delivering, and those that are not [68]. The nature and abundance of the molecular cargo is often influenced by the type and physiological or pathological condition of the donor cell, the stimuli that modulate EV production and release, and most likely the pathways that lead to the formation of different EV types. These different layers of regulation explain, at least in part, how the EV message can be precisely modulated. EVs can carry and consequently transfer into recipient cells tumor-derived molecules, including epidermal growth factor receptor vIII (EGFRvIII), mutant Ras family members or c-Met[11, 38, 69, 70], and other proteins or transcripts with oncogenic functions that are currently being proposed as biomarkers in cancer. Studies determining the number of copies of transcripts, miRNAs and proteins per single EV are still lacking. Understanding the stoichiometry of the various molecules in different EV types might, in the future, improve our ability to select better candidates for clinical investigations.

Large-scale profiling experiments are generating valuable information on different types of EV populations originating from single cell systems and on single classes of EVs from different cell types. These include mass spectrometry and miRNA arrays and sequencing[38, 71, 72], while lipidomics characterizations are still lagging behind[73, 74]. The resulting datasets have been collected into three main databases: Exocarta, Vesiclepedia and EVpedia[75-77]. These resources contain information on protein, mRNA and miRNA identified in at least one EV population, and EVpedia contains integrated datasets from prokaryotic and eukaryotic EVs. This information can be used as a means of comparison

with new datasets and as a valuable resource for computational studies to identify new candidate markers that can facilitate the understanding of EV origin and function. Gene Ontology and network analyses of protein and miRNA datasets are now achievable by using this tool[77]. A recent report that analyzed 16 different mass spectrometry studies identified almost 800 common vesicular proteins in exosomes derived from different cell lines[78, 79]. This is useful information that can help identify molecules functionally involved in EV biogenesis, which could result in the identification of tumor-enriched markers. Reports suggesting that given proteins are unique or enriched in specific EV populations from disease states are contributing to our understanding of disease markers generally[73, 80]. Recently, Muturi et al. have demonstrated that EV signatures can be donor cell specific[81], and Garnier et al. have identified EV signatures that discriminate cellular differentiation and transformation[82]. However, more extensive investigation on whether tumor cells can modulate their cargo in different phases of biological activity is missing. Future studies will likely provide additional categories of markers with significant mechanistic implications, along with disease-specific biomarkers that could help distinguish between pathophysiological conditions. We can also envision the discovery of new molecules to stratify diverse EV populations and attribute identified functions to them.

The selection of the proteins exported in EVs is not only affected by the status of the donor cell but also depends on the subcellular compartment of origin, as is the case for the above mentioned exosomes, derived from MVB and MVs and shed from the plasma membrane[56, 83, 84]. This choice influences both the intercellular interactions and the message delivered by these EVs. Proteins that are frequently used as exosome markers are often also involved in exosome biogenesis. These include Alix, Tgs101, ceramide, flotillin, Rab and tetraspannin family members[29-31, 33, 39-42, 85]. In particular, CD9, CD81 and CD63 have been shown to participate in endosomal vesicle trafficking[86, 87]. Additional reports suggest the involvement, in protein sorting within exosomes, of the ESCRT complex, which targets ubiquitinated proteins to the degradation route[88]. Moreover there are observations of an ESCRT-independent, CD63 dependent sorting process [89], suggesting that sorting can be regulated at multiple levels. In the case of MVs an important role in the cargo selection seems to be directed by the ARF6-regulated recycling pathway[53, 56]. This process can regulate the inclusion of proteins such as major histocompatibility complex (MHC) class I, β1 integrin receptors, vesicle associated protein 3 (VAMP3) and membrane type 1 matrix metalloproteinase (MT1MMP)[56].

The mechanism of sorting nucleic acids in exosomes is less understood. Exosomes can export miRNAs, long non-coding and other non-coding RNAs, and mRNA[68, 90, 91]. Importantly, EVs might represent a vehicle in which these nucleic acids can be preserved and analyzed in biological fluids, as well as delivered to their target cells without being degraded in the extracellular space. This is particularly important for mRNA, which is sensitive to RNAses. Once taken up by recipient cells, mRNAs could play specific functions upon translation into protein products[57, 68]. The first demonstration that exosomes can carry single-stranded DNA (ssDNA) and retrotrasposones was reported by Balaj et al. in 2011[92]. More recently, two independent studies have shown that exosomes also contain double-stranded DNA (dsDNA), which can be profiled in the circulation using next generation sequencing technologies[93, 94]. However, whether the DNA contained in EVs

is representative of the originating tumor cells has not been fully demonstrated. This revolutionary finding opens up the opportunity to use the dsDNA derived from exosomes and potentially other EVs as an alternative, more concentrated and better preserved source of cancer-derived genomic material than circulating DNA. From a more functional perspective, a recent study has shown that the oncogene H-*ras*, identifiable by DNA sequencing in brain tumor cell-derived EVs can be transferred to recipient cells where it transiently alters their biological behavior[95]. Further investigation into the molecular mechanisms by which EVs induce DNA-mediated transformation might open up enormous opportunities for cancer biology and therapeutics.

2.3. Internalization

The interaction of EVs with their target cells is not only mediated by membrane-membrane contact, but often results in EV uptake with subsequent transfer of EV cargo. Both fusion and active endocytosis have been proposed as mechanisms for exosome uptake. Exosome and MV binding and internalization can be regulated by adhesion molecules[96]. For example, the interaction between diverse combinations of tetraspannin complexes, highly represented on exosome membranes, and integrins on the target cell, might influence the selection of the recipient cell[97]. EV-cell interactions can also be mediated by the PS expressed on EVs and TIM4[98] or other PS-specific receptors on target cells. Finally, a relevant role in EV internalization seems to be played by heparan sulfate proteoglycans residing on the plasma membrane of the target cell, as recently reported by Christianson[99]. Whether these processes are truly selective or rather can occur randomly is still largely unclear. The EV-target cell interaction is the first step of EV uptake, and seems to be unavoidably followed by fusion or endocytosis[100]. And while the fusion is considered a more passive event in which the membrane of the exosome and the membrane of the recipient cell melt together, thereby forming a continuous structure after the merging of the two distinct lipid bilayers, endocytosis is an active process. The regulatory features of this active uptake is still largely undefined, although phagocytosis[98], as well as a lipid raft-dependent endocytosis, positively regulated by ERK1/2 and inhibited by Caveolin-1 (Cav-1) [101], could be alternatives to conventional endocytosis of soluble molecules. Finally, once inside the recipient cell, exosomes can release their content through fusion with the endosome membrane or can be targeted to lysosomes for degradation.

3. Large oncosomes

The term oncosome was first used by Janus Rak's group in 2008 to describe, in the context of brain tumors, the existence of EVs released from glioma cells and expressing EGFRvIII, a mutated form of the receptor. These vesicles were shown to be capable of transferring the oncoprotein EGFRvIII to the membrane of tumor cells lacking this receptor, thus propagating tumor-promoting material and inducing transformation[69]. Our group recently reported that prostate cancer cells release bioactive EVs with diameter of 1-10 μm (Fig. 1). We used the term “large oncosome” because of their atypically large size (Fig. 1) and because they appeared to be contain oncogenic material and to be cancer-specific[102].

3.1. Biogenesis – The amoeboid phenotype –

Metastasis occurs when tumor cells develop an ability to exit from the primary tumor, cross the basement membrane, enter and survive within the vasculature and colonize ectopic tissues. Toward these ends, disparate migratory modes are adopted, including collective cell migration as strands or sheets, or single-cell mesenchymal and amoeboid modes. Mesenchymal motility was long thought to be the main phenotype adopted by invasive tumor cells. One of the main features of mesenchymal migration is its reliance on pericellular proteolysis, and in some tumor cell contexts protease inhibition can inhibit cell motility. Disappointing results from clinical trials of metalloproteinase (MMP) inhibitors[103], however, argue that alternative modes of dissemination may also exist. The first evidence for such escape mechanisms came from Wolf et al.[104], who demonstrated that tumor cells invade in the face of hindering pericellular proteolysis by transitioning to an amoeboid phenotype. Amoeboid cancer cells display an elliptical, blebbing morphology induced by activation of the GTPase RhoA or its effector ROCK[105]. Importantly, the non-apoptotic membrane blebs typical of amoeboid cells are dynamically extruded and retracted thus enabling gliding and directional propulsion through matrices. Amoeboid migration can also be induced by the silencing of the cytoskeletal regulator Diaphanous related formin-3, DIAPH3. DIAPH3 loss induces, in different cancer cell types, a transition to a rapid migratory phenotype with increased metastatic potential, also known as mesenchymal to amoeboid transition (MAT)[106, 107]. Our group was the first to demonstrate that these non-apoptotic membrane blebs can be shed from amoeboid cancer cells in the form of large oncosomes. The phenomenon could be induced by stimulation of prostate cancer cells with EGF and observed by live microscopy for a long period of time[102]. Overexpression of a constitutively active form of Akt is another trigger that promotes the release of large oncosomes[13, 102]. Because large oncosomes, similar to MVs, originate from the plasma membrane, some of the pathways implicated in the biogenesis of MVs might also contribute to large oncosome biogenesis. For example, ARF6, which is involved in the abscission of MVs from the plasma membrane and is enriched within MVs[53, 56], is also highly expressed in large oncosomes[13]. Because large oncosomes originate from membrane shedding from aggressive cancer cells that have acquired an amoeboid phenotype, we believe that amoeboid cells may be associated with tumor progression, not only as a function of their adaptive plasticity, but also through oncosome-mediated progressive remodeling of their surroundings.

3.2. Association with cancer and molecular content

Studies from our group have determined the rate of non-apoptotic blebbing and oncosome shedding using multiple systems and cell lines with different degrees of aggressiveness. The conclusion is that only tumor cells release large oncosomes at a quantifiable rate, and the number of large oncosomes is directly correlated with aggressiveness[13, 14, 102]. A recent report identified large oncosome-like blebs in cancer-associated fibroblasts (CAFs) but not in fibroblasts from benign tissue[108]. However, a quantitative analysis to establish the extent of the phenomenon was not performed, therefore there is not conclusive evidence that this represents a common feature.

Large oncosomes harbor abundant bioactive molecules, including signaling factors involved in cell metabolism, mRNA processing and cell growth and motility[102]. They also contain miRNAs, metalloproteinases, and Cav-1[13]. Cav-1 is a serum biomarker of metastatic prostate cancer[109]. Interestingly the presence, in the circulation, of large oncosomes containing Cav-1 correlates with tumor progression in mice with autochthonous prostate tumors[13] and discriminates patients with metastatic disease from patients with organ-confined prostate cancer[14]. Large oncosome-like structures can be identified in formalin-fixed, paraffin embedded (FFPE) tumor sections by light microscopy, and this feature correlated with high Gleason score (GS) and the presence of metastases in two independent cohorts of prostate cancer patients[13]. Electron microscopy highlighted large membrane blebs directly exposed to the lumen of blood vessels in tumor tissues. Similar structures were detected in paraffin sections of tumors from mice with metastatic disease by staining with anti-ARF6 antibodies[13]. Large oncosomes resemble “giant vesicles” recently identified by an independent group in breast cancer lines and detected in tissue sections of human breast cancer[110]. The demonstrated correlation between the oncosome-like feature and tumor progression in tissues, albeit potentially significant, requires further investigation. Objective criteria of identification, based on specific markers, and automated quantitation of large oncosomes in tissues are necessary to obtain standardized procedures, clinically applicable. To date, it is not known how much overlap in terms of molecular cargo and function exists between tumor-derived MVs and large oncosomes. Additional investigation is required not only to better define both large oncosome content and function in tumor progression but also to identify markers to determine if they represent a unique EV population. Even if the characterization of large oncosomes is still in its infancy, miRNA profiling of the EV cargo from cancer cells suggests a selection of miRNA species in large oncosomes versus smaller EVs[14].

4. Horizontal communication

Whether EVs play a role in tumor development, survival and progression or other specific phase of tumorigenesis is object of active investigation. Tumor derived EVs can alter the homeostasis of the tumor microenvironment by directly targeting fibroblasts[111-113], endothelial[114, 115] and immune[116] cells or by altering the structure and composition of the extracellular matrix (ECM)[117] (Fig. 2). Along with several studies showing an EV-mediated horizontal propagation of tumor promoting molecules[11, 69], it is now emerging that EVs can also spread acquired phenotypes and functions including drug resistance[118, 119]. A few recent studies have demonstrated that EVs may mediate the transfer of nucleic acids, including mutated genetic material, a phenomenon that was previously thought to happen only vertically within clonal cells. The hypothesis that this might occur through direct transfer of DNA is attracting strong interest and has resulted in a few reports on the functional role of mutated DNA in recipient cells[95]. Additionally, evidence is slowly emerging that EV-enclosed DNAs can be transferred into cell nuclei, as demonstrated by acridine orange or lipophilic dye-labeled EVs, identified inside the nuclear membrane (*unpublished observations*) and[120]. Whether the transferred DNA can exert a functional role on target cells is still unresolved. Oncogene transcripts can be propagated through EV transfer and then translated into proteins in the recipient cells[114]. Analogously, EV

enclosed miRNAs can regulate gene expression, thus altering the behavior of the recipient cells[108, 119, 121] and increasing metastatic potential in poorly metastatic cells. An example is provided by recent findings on miR-200, in breast cancer metastasis, using a series of xenograft models [122].

4.1. ECM remodeling

The MMP family of proteins is implicated in ECM remodeling and in cancer cell protease-dependent migration and invasion. MT1-MMP, MMP9 and MMP2 or A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) have been identified in different EV types, including large oncosomes[13, 38, 56]. MMPs can be found inside or on the EV membrane and they are also functionally active as is the case for MT1-MMP, whose ability to remodel the ECM has been shown for melanoma derived exosomes[117]. Because MT1-MMP is a proteolytic activator of MMP9 and MMP2, and these three proteins are not always identified in the same type of EV, it might be possible that different EVs release different molecules that then regulate each other's activity in the extracellular milieu.

4.2. Fibroblasts

Tumor derived EVs can functionally modify fibroblasts[123], by reprogramming these cells to cancer associated fibroblasts (CAFs), which exhibit a myofibroblastic differentiation. Studies from Webber et al. demonstrated that exosomes can release an EV specific form of transforming growth factor beta1 (TGF-β1), which differs from the soluble form of this growth factor in that not only does it induce myofibroblast differentiation but also actively promotes tumor progression[111, 112]. Interestingly, disruption of EV mediated interactions of the tumor cells with the surrounding stroma *in vivo* significantly reduces tumor growth by impairment of either EV dependent signaling activation in target cells or exosome production[112]. This result might imply that EVs could be targeted at multiple levels, from biogenesis to interactions with target cells, alone or as combinatorial approaches. Parallel studies, from Antonyak et al., have demonstrated that MVs released from breast cancer or glioblastoma cell lines can induce transformation of fibroblasts and the process is mediated by fibronectin1 (FN1) and transglutaminase (tTG)[113]. Along with the notion that CAFs might release soluble factors that induce epithelial-mesenchymal transition or stemness in cancer cells, it is becoming evident that these processes might be also regulated by CAF-derived EVs. A previous report from Wrana's group had demonstrated the ability of exosomes released by fibroblasts to enhance breast cancer cell invasion[124]. A more recent report indicated that cancer cell motility is induced by exosomes released from CAFs silenced for tissue inhibitor of metalloproteinases (Timp). In this system, Timp knock down resulted in increased expression of ADAM10 in the exosomes, in the absence of increased intracellular levels[125]. This is yet an additional example of how important is to also consider and analyze the exosome content and function within the tumor cell and extracellular components, since they may contain information or alterations that are not necessarily manifest in the donor cell. More recently, fibroblast-derived exosomes have been shown to induce EMT in cancer cells by miRNA[108].

4.3. Angiogenesis

Studies on the role of EVs in angiogenesis have demonstrated that cancer cell-derived EVs contain interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF), potent pro-angiogenic factors, as well as other molecules able to enhance endothelial cell invasion and organization in tubule-like structures[114, 115]. Using similar mechanisms, exosomes expressing the neutral sphingomyelinase 2 (nSMase 2) can induce tubule formation and migration of HUVEC and promote tumor progression through *in vivo* stimulation of angiogenesis as result of their interaction with endothelial cells[126]. Formation of a vasculature within the tumor facilitates cancer cell entry in the circulation thus promoting metastasis. The demonstration of a direct involvement of tumor derived EVs in this process might provide additional targets for the development of anti-angiogenic drugs that may be used in combination with the ones already developed. In addition to the direct transfer of canonical pro-angiogenic proteins, recent studies are identifying novel EV-enclosed molecules that participate in angiogenesis. These include EGFR[127], miR-210[121] and miR-9[128]. Angiogenesis is strongly stimulated by hypoxia, in which an increased release of exosomes stimulates tubule formation in different tumor types[121].

4.4. Tolerogenic Immune Response

Tumor cells can acquire immunotolerance either through induction of T regulatory (T reg) proliferation or cytotoxic T cell death, and both mechanisms can be mediated by EVs[129]. In fact ovarian cancer-derived EVs can enhance Treg proliferation and activity[116], and EVs originating from tumor cell lines can induce a FasL or TNF-related apoptosis-inducing ligand (TRAIL) dependent cell death in $CD8^+$ T cells[130, 131]. Similar results have been elicited by EVs obtained *ex vivo* from patients with oral squamous cell carcinoma[132]. As an additional mechanism of EV-mediated immunotolerance, EVs containing TGF-β can impair Natural Killer (NK) and T cells activation through downregulation of NKG2D[133]. However, exosomes may not always be involved in the regulation of the adaptive immune response *in vivo*[43]. This finding highlights the difficulties in translating *in vitro* findings to animal and human models due to large intercellular diversity *in vivo*.

4.5. Drug Resistance

The involvement of EVs in drug resistance is a novel area of investigation. EVs from breast cancer cells and other tumor types can transfer resistance to docetaxel in cells that are sensitive to the drug[118, 134]. In breast cancer the process seems to be specifically mediated by P-glycoprotein[118], or by its activator transient receptor potential channel 5 (TrpC5)[135]. EV-mediated docetaxel resistance is also regulated by miR-222[119, 136]. Further evidence demonstrates that the transfer of the prosurvival Akt/mTOR complex in EVs results in propagating resistance to gefitinib in non-small cell lung carcinoma cells (NSCLC)[134].

4.6. Metastatic niche

The demonstrated EV ability to enter the circulation and potentially travel far from the site of their origin has generated the hypothesis of a role for EVs in the education of the metastatic niche. *In vivo* results in this area are still largely limited. However, Peinado et al.

have reported, in a very elegant study, the capacity of melanoma-derived exosomes within the bone marrow to condition the metastatic niche [11]. Moreover CD105 positive MVs, which can promote angiogenesis *in vitro*, may stimulate lung metastasis *in vivo* in renal cancer[137].

4.7. Large oncosomes in extracellular communication

Evidence that large oncosomes can modulate diverse features of different cell types, including endothelial cells, fibroblast and tumor cells[13, 14], suggests a functional role in the tumor microenvironment. Large oncosome-induced migration of CAFs can be potentiated by EVs derived from tumor cells in which miR-1227 has been overexpressed. Interestingly, this forced expression of the miRNA intracellularly results in a 3-fold greater export of it in large oncosomes in comparison to exosomes, in line with their significantly larger size. Large oncosomes can also potently stimulate expression of metastasis-associated factors, such as brain-derived neurotrophic factor (BDNF), C-X-C motif chemokine 12 (CXCL12) and osteopontin, in stromal cells[13], confirming a role for these EVs as potent mediators of the communication between tumor cells and stroma. Moreover, large oncosomes can induce migration of tumor and endothelial cells. Importantly, migration of normal endothelial cells was induced by large oncosomes purified from the circulation of mice with metastatic disease[13]. Furthermore, tumor cell migration was also enhanced by fibroblasts pre-treated with large oncosomes and used as tumor cell attractants. Notably, large oncosomes are ~1000 times the size by volume of exosomes and therefore can theoretically accommodate a much larger number of tumor-derived molecules with a distinct impact on the tumor microenvironment than exosomes. This hypothesis is supported by a recent report demonstrating that most of the exosomes in exosome preparations obtained by standard methods of isolation only contain a few miRNA molecules[138]. The results further suggest that, if functional experiments were performed with exosomes released in physiologically relevant conditions, the effect of the various single molecules would be diluted. One interesting consequence of these findings is that larger EVs including large oncosomes might contain more abundant miRNA molecules, thus serving as an enriched source of biomarkers and for the identification of functionally relevant EV cargo. The molecular mechanisms underlying the function of the large oncosomes in horizontal communication are still largely unknown, and additional studies are necessary to determine, in a comparative manner, whether large oncosome functions exploit specific pathways (Fig. 2).

5. EV isolation and detection methods

EVs can be isolated from conditioned media of cultured cells as well as from virtually any type of body fluid, including blood and derivatives, urine, ascites, bronchoalveolar lavage (BAL), saliva, and cerebrospinal fluid (CSF). The attempts to perfect current methods of EV isolation have recently lead to increased understanding of the biological function and nature of diverse types of EVs. Along with much progress, unexpected complexity has emerged. Despite the numerous reports published on comparative methods for EV isolation, we are still far from having standard protocols applicable to clinical practice. One of the most commonly employed methods of EV isolation is based on differential centrifugation[139].

In conventional protocols, after pelleting down cells and cell debris at low speed, EVs are purified at 10,000-20,000 × g, as is the case for MVs and large oncosomes[13, 14, 56, 102, 139], or at 100,000-120,000 × g as for exosomes[47, 139]. However, different variations of this protocol have been employed, resulting in confusing results. Recent studies have also highlighted that differential centrifugation, known to result in heterogeneous preparations, is not sufficient to isolate pure populations of EVs[47]. EVs can be better purified and cleared from free proteins and protein complexes or other contaminants by centrifugation gradients, typically used to separate different intracellular organelles based on their sedimentation coefficient[47]. Discontinuous cushion gradients represent a viable alternative to continuous gradients that can be cumbersome. With these methodologies, EVs can be purified through flotation of the different EV populations at known concentrations of sucrose, iodixanol or other agents[47]. Size exclusion methods based on the use of filters with specific pore size are often used in combination with other isolation techniques. This is frequently the first step in exosome purification from serum and plasma[140]. Our group recently developed a size retention rather than exclusion isolation method able to select EVs larger than 200 nm, which include large oncosomes, while allowing smaller EVs to flow through the filter. Using this method, large oncosomes positive for Cav-1 were shown to discriminate patients with locally confined prostate cancer from patients with castration resistant and metastatic disease[14].

Immunoaffinity capture is emerging as a new tool to purify specific EV populations based on the expression of certain membrane proteins. For example, microbeads coated with glycoprotein A33 and epithelial cell adhesion molecule precursor (EpCAM) have been successfully used to immunocapture different EVs from colon cancer cells[46, 47, 141]. Novel and promising methodologies include microfluidic systems that allow EV immunocapture using specific antibodies. One elegant system, with high potential for clinical applicability, has been used to quantitatively analyze EVs in the serum of patients with glioblastoma using general exosome markers and EGFRvIII[142]. As a result of the increasing technical ability to stratify heterogeneous EV populations, several markers that have been considered exosome-specific, including CD63, CD81, CD9 are now being identified in other types of EVs, whereas proteins such as Alix and Tsg101 appear to be more consistent markers[143]. PS, ARF6 and Rho family members have been proposed as MV markers[52, 56, 60]. However, cross-reactivity with other EVs has been demonstrated, limiting the current understanding of potentially different functions and clinical significance for these two classes of EVs[75-77]. Whether large oncosomes represent a discrete population of EV is still unresolved, and whether MVs and large oncosomes express different markers has not yet been explored. Identification of sets of markers rather than single ones by multiplexing techniques and large scale mass spectrometry, including targeted proteomics will improve our ability to purify EVs species with different signatures *in vitro* and *in vivo*. If performed under well-controlled conditions, these experiments, followed by extensive validation, can improve the sensitivity and specificity of currently available methods.

5.1. EV imaging

A range of imaging methodologies has been applied to EVs, contributing to the conclusion that EVs are discrete, particulate structures with a lipid bilayer. Nano-sized EVs and MVs require the resolution power of electron microscopy (EM) to be visualized. Larger EVs, such as large oncosomes, can be visualized by confocal or optical microscopy in tissue plasma membranes, and measured using imaging software. Immufluorescence imaging allows identification of large oncosomes also in cell media and body fluids[13, 14]. Notably, in FFPE sections, large oncosomes can be highlighted by chromogenic immunohistochemistry[13, 14], supporting a high potential for clinical translation in cancer.

5.2. EV fluidic based analysis

Flow cytometry analysis is often employed for EV detection, although most instruments cannot analyze particles smaller than 500 nm. A recent study from van der Vlist demonstrated that nano-sized particles can be accurately quantified[144]. Interestingly, using a multicolor labeling strategy, this approach could be used to stratify subsets of heterogeneous EVs. Recent studies further demonstrate that nano-sized EVs can be enumerated by flow cytometry with the support of antibody-coated beads larger than exosomes[46, 47, 141]. Given their atypically large size, large oncosomes can be quantified in cell secretions, mouse and human plasma, with and without staining with fluorescently-labeled antibodies, using 1 and 10 μm size beads[13, 14]. More frequently used for the study of nano-sized EVs, the nanoparticle tracking analysis (NTA) system the light scattered by the particles can be captured and analyzed by computer software, resulting in a measurement of the size distribution and concentration of the EVs in the samples[145]. This method is not suitable for quantitative analysis of EVs larger than 400 nm. Newer systems, based on dynamic as well as electrophoretic and static light scattering in combination, seem to allow quantitative analysis of EVs of several microns in diameter. However, their application has been limited to the use of liposomes in drug delivery experiments[146].

6. Clinical implications

EVs represent a potentially rich source of information obtainable from body fluids through non-invasive approaches. Filtering and interpreting the meaning of this information is challenging. Several studies have attempted to identify signatures of cancer and cancer progression and response to the therapy using proteome and miRNome profiling. For example reduced levels of miR-34a in EVs derived from docetaxel resistant prostate cancer cells and of miR-192 in EVs derived from lung adenocarcinoma have been recently proposed as possible indicators of cancer progression and metastasis[147, 148]. In addition, the recent demonstration that next generation sequencing technologies can be applied to the genomic DNA and to miRNA enclosed in EVs[93-95] suggests the opportunity to derive tumor-specific genomic alterations from EVs. With the rapid progress of targeted sequencing, it might also be possible, by analyzing the DNA content of circulating EVs, to periodically monitor tumor progression and response to therapy, as well as investigate tumor heterogeneity, tumor evolution and clonality. More challenging is the study of the whole transcriptome in EVs. In fact, current studies rely on the identification of specific mRNAs by polymerase chain reaction (PCR)[114], whereas whole transcriptome profiling of EVs

has not been optimized. Reports on RNA sequencing often describe the relative proportion of different RNA species that can populate EVs rather than identifying specific mRNA reads[149, 150]. Furthermore, because the predominant species seem to be ribosomal RNA and miRNA and other non-coding RNA[90], the levels of mRNA might be extremely low. Therefore, for clinical applications of RNA profiling, it is important to develop protocols specific for EVs and to achieve the deep coverage than that is commonly used to sequence tumor tissues. Large oncosomes might represent a population of EVs with advantages for translational studies. The detection of oncosome-like feature in tumor sections of patients with advanced disease[13] can allow direct comparisons between tissue features and circulating large oncosomes in matched samples. As previously mentioned, large oncosomes might also contain more abundant tumor-derived molecules, possibly including actionable molecules, resulting in candidates for a non-invasive source of information useful in precision medicine. Importantly, analyses performed in circulating large oncosomes could be validated directly on tumor tissues.

7. Conclusion

Creative experimentation is gradually elucidating what is still largely unknown about the plethora of functional and clinical applications of EVs in cancer diagnostics and therapeutics. A deeper understanding of the heterogeneity of EVs, of the molecular pathways exploited by EVs in cancer, and of their responsiveness to specific therapeutic agents will result in a better understanding of the mechanisms of drug resistance. It will be important to identify “druggable” targets with specific extracellular functions involved in tumor dissemination. These molecules include not only proteins and lipids but also nucleic acids and DNA. EVs might even contain driver mutations of known clinical significance that could be analyzable directly in body fluids in the near future. Finally, the fact that DNA, RNA and proteins are packaged together in EVs suggests the untested hypothesis that these nano or micro particles might function as complex, mobile machines for transcription and/or translation.

Acknowledgments

The authors apologize for not having cited many original research articles on this topic, and thank Samantha Morley for helpful suggestions and critical reading of the manuscript. M.R.F. and D.D.V. are supported by the National Institutes of Health as NCI NIH R00 CA131472 (to D.D.V.), NIH P50 CA092131 (to D.D.V.), R01 CA143777, CA112303, NIDDK R3747556 (to M.R.F.); by the Avon Foundation Fund 02-2013-043 (D.D.V.); by the Martz Translational Breast Cancer Research Fund (to M.R.F. and D.D.V.), and by the Steven Spielberg Discovery Fund in Prostate Cancer Research (to M.R.F. and D.D.V.).

References

1. Douglas RM, Goddard GV. Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. Brain Res. 1975; 86:205–15. [PubMed: 163667]
2. Edlund M, Sung SY, Chung LW. Modulation of prostate cancer growth in bone microenvironments. J Cell Biochem. 2004; 91:686–705. [PubMed: 14991761]
3. Mikels AJ, Nusse R. Wnts as ligands: processing, secretion and reception. Oncogene. 2006; 25:7461–8. [PubMed: 17143290]
4. Singh AB, Harris RC. Autocrine, paracrine and juxtacrine signaling by EGFR ligands. Cell Signal. 2005; 17:1183–93. [PubMed: 15982853]

5. Al-Nedawi K, Meehan B, Rak J. Microvesicles: messengers and mediators of tumor progression. Cell Cycle. 2009; 8:2014–8. [PubMed: 19535896]
6. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. Trends Cell Biol. 2009; 19:43–51. [PubMed: 19144520]
7. Gould SJ, Raposo G. As we wait: coping with an imperfect nomenclature for extracellular vesicles. J Extracell Vesicles. 2013; 2
8. Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. J Proteomics. 2010; 73:1907–20. [PubMed: 20601276]
9. Tetta C, Ghigo E, Silengo L, Deregibus MC, Camussi G. Extracellular vesicles as an emerging mechanism of cell-to-cell communication. Endocrine. 2013; 44:11–9. [PubMed: 23203002]
10. Turturici G, Tinnirello R, Sconzo G, Geraci F. Extracellular membrane vesicles as a mechanism of cell-to-cell communication: advantages and disadvantages. Am J Physiol Cell Physiol. 2014; 306:C621–33. [PubMed: 24452373]
11. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med. 2012; 18:883–91. [PubMed: 22635005]
12. Jang SC, Kim SR, Yoon YJ, Park KS, Kim JH, Lee J, et al. In vivo Kinetic Biodistribution of Nano-Sized Outer Membrane Vesicles Derived from Bacteria. Small. 2014
13. Di Vizio D, Morello M, Dudley AC, Schow PW, Adam RM, Morley S, et al. Large oncosomes in human prostate cancer tissues and in the circulation of mice with metastatic disease. Am J Pathol. 2012; 181:1573–84. [PubMed: 23022210]
14. Morello M, Minciacchi VR, de Candia P, Yang J, Posadas E, Kim H, et al. Large oncosomes mediate intercellular transfer of functional microRNA. Cell Cycle. 2013; 12:3526–36. [PubMed: 24091630]
15. Danesh A, Inglis HC, Jackman RP, Wu S, Deng X, Muench MO, et al. Exosomes from red blood cell units bind to monocytes and induce proinflammatory cytokines, boosting T-cell responses in vitro. Blood. 2014; 123:687–96. [PubMed: 24335232]
16. Aatonen MT, Ohman T, Nyman TA, Laitinen S, Gronholm M, Siljander PR. Isolation and characterization of platelet-derived extracellular vesicles. J Extracell Vesicles. 2014; 3
17. Yu X, Huang C, Song B, Xiao Y, Fang M, Feng J, et al. CD4+CD25+ regulatory T cells-derived exosomes prolonged kidney allograft survival in a rat model. Cell Immunol. 2013; 285:62–8. [PubMed: 24095986]
18. Thery C, Duban L, Segura E, Veron P, Lantz O, Amigorena S. Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. Nat Immunol. 2002; 3:1156–62. [PubMed: 12426563]
19. Luga V, Wrana JL. Tumor-stroma interaction: Revealing fibroblast-secreted exosomes as potent regulators of Wnt-planar cell polarity signaling in cancer metastasis. Cancer Res. 2013; 73:6843–7. [PubMed: 24265274]
20. Ju R, Zhuang ZW, Zhang J, Lanahan AA, Kyriakides T, Sessa WC, et al. Angiopoietin-2 secretion by endothelial cell exosomes: regulation by the phosphatidylinositol 3-kinase (PI3K)/Akt/endothelial nitric oxide synthase (eNOS) and syndecan-4/syntenin pathways. J Biol Chem. 2014; 289:510–9. [PubMed: 24235146]
21. Riches A, Campbell E, Borger E, Powis S. Regulation of exosome release from mammary epithelial and breast cancer cells - a new regulatory pathway. Eur J Cancer. 2014; 50:1025–34. [PubMed: 24462375]
22. Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. Blood. 1999; 94:3791–9. [PubMed: 10572093]
23. Biller SJ, Schubotz F, Roggensack SE, Thompson AW, Summons RE, Chisholm SW. Bacterial vesicles in marine ecosystems. Science. 2014; 343:183–6. [PubMed: 24408433]
24. Kowal J, Tkach M, Thery C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol. 2014; 29C:116–25. [PubMed: 24959705]
25. Booth AM, Fang Y, Fallon JK, Yang JM, Hildreth JE, Gould SJ. Exosomes and HIV Gag bud from endosome-like domains of the T cell plasma membrane. J Cell Biol. 2006; 172:923–35. [PubMed: 16533950]

26. Morvan J, Rinaldi B, Friant S. Pkh1/2-dependent phosphorylation of Vps27 regulates ESCRT-I recruitment to endosomes. Mol Biol Cell. 2012; 23:4054–64. [PubMed: 22918958]
27. Adell MA, Vogel GF, Pakdel M, Muller M, Lindner H, Hess MW, et al. Coordinated binding of Vps4 to ESCRT-III drives membrane neck constriction during MVB vesicle formation. J Cell Biol. 2014; 205:33–49. [PubMed: 24711499]
28. Buschow SI, Nolte-'t Hoen EN, van Niel G, Pols MS, ten Broeke T, Lauwen M, et al. MHC II in dendritic cells is targeted to lysosomes or T cell-induced exosomes via distinct multivesicular body pathways. Traffic. 2009; 10:1528–42. [PubMed: 19682328]
29. Colombo M, Moita C, van Niel G, Kowal J, Vigneron J, Benaroch P, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J Cell Sci. 2013; 126:5553–65. [PubMed: 24105262]
30. Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, et al. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. Nat Cell Biol. 2012; 14:677–85. [PubMed: 22660413]
31. Romancino DP, Paterniti G, Campos Y, De Luca A, Di Felice V, d'Azzo A, et al. Identification and characterization of the nano-sized vesicles released by muscle cells. FEBS Lett. 2013; 587:1379–84. [PubMed: 23523921]
32. Marsh M, van Meer G. Cell biology. No ESCRTs for exosomes. Science. 2008; 319:1191–2. [PubMed: 18309064]
33. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science. 2008; 319:1244–7. [PubMed: 18309083]
34. Kirby RL, McLean AD, Eastwood BJ. Influence of caster diameter on the static and dynamic forward stability of occupied wheelchairs. Arch Phys Med Rehabil. 1992; 73:73–7. [PubMed: 1729979]
35. Laulagnier K, Motta C, Hamdi S, Roy S, Fauvelle F, Pageaux JF, et al. Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J. 2004; 380:161–71. [PubMed: 14965343]
36. Ghossoub R, Lembo F, Rubio A, Gaillard CB, Bouchet J, Vitale N, et al. Syntenin-ALIX exosome biogenesis and budding into multivesicular bodies are controlled by ARF6 and PLD2. Nat Commun. 2014; 5:3477. [PubMed: 24637612]
37. Stenmark H. Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol. 2009; 10:513–25. [PubMed: 19603039]
38. Tauro BJ, Mathias RA, Greening DW, Gopal SK, Ji H, Kapp EA, et al. Oncogenic H-ras reprograms Madin-Darby canine kidney (MDCK) cell-derived exosomal proteins following epithelial-mesenchymal transition. Mol Cell Proteomics. 2013; 12:2148–59. [PubMed: 23645497]
39. Savina A, Vidal M, Colombo MI. The exosome pathway in K562 cells is regulated by Rab11. J Cell Sci. 2002; 115:2505–15. [PubMed: 12045221]
40. Savina A, Fader CM, Damiani MT, Colombo MI. Rab11 promotes docking and fusion of multivesicular bodies in a calcium-dependent manner. Traffic. 2005; 6:131–43. [PubMed: 15634213]
41. Hsu C, Morohashi Y, Yoshimura S, Manrique-Hoyos N, Jung S, Lauterbach MA, et al. Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. J Cell Biol. 2010; 189:223–32. [PubMed: 20404108]
42. Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G, Savina A, et al. Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol. 2010; 12:19–30. sup pp 1-13. [PubMed: 19966785]
43. Bobrie A, Krumeich S, Reyal F, Recchi C, Moita LF, Seabra MC, et al. Rab27a supports exosome-dependent and -independent mechanisms that modify the tumor microenvironment and can promote tumor progression. Cancer Res. 2012; 72:4920–30. [PubMed: 22865453]
44. Fader CM, Sanchez DG, Mestre MB, Colombo MI. TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. Biochim Biophys Acta. 2009; 1793:1901–16. [PubMed: 19781582]

45. Choi DS, Lee JM, Park GW, Lim HW, Bang JY, Kim YK, et al. Proteomic analysis of microvesicles derived from human colorectal cancer cells. Journal of proteome research. 2007; 6:4646–55. [PubMed: 17956143]
46. Mathivanan S, Lim JW, Tauro BJ, Ji H, Moritz RL, Simpson RJ. Proteomics analysis of A33 immunoaffinity-purified exosomes released from the human colon tumor cell line LIM1215 reveals a tissue-specific protein signature. Mol Cell Proteomics. 2010; 9:197–208. [PubMed: 19837982]
47. Tauro BJ, Greening DW, Mathias RA, Ji H, Mathivanan S, Scott AM, et al. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods. 2012; 56:293–304. [PubMed: 22285593]
48. Fourcade O, Simon MF, Viode C, Rugani N, Leballe F, Ragab A, et al. Secretory phospholipase A2 generates the novel lipid mediator lysophosphatidic acid in membrane microvesicles shed from activated cells. Cell. 1995; 80:919–27. [PubMed: 7697722]
49. Muller I, Klocke A, Alex M, Kotzsch M, Luther T, Morgenstern E, et al. Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2003; 17:476–8. [PubMed: 12514112]
50. Hulsmans M, Holvoet P. MicroRNA-containing microvesicles regulating inflammation in association with atherosclerotic disease. Cardiovasc Res. 2013; 100:7–18. [PubMed: 23774505]
51. Wang T, Gilkes DM, Takano N, Xiang L, Luo W, Bishop CJ, et al. Hypoxiainducible factors and RAB22A mediate formation of microvesicles that stimulate breast cancer invasion and metastasis. Proc Natl Acad Sci U S A. 2014; 111:E3234–42. [PubMed: 24938788]
52. Antonyak MA, Cerione RA. Microvesicles as mediators of intercellular communication in cancer. Methods Mol Biol. 2014; 1165:147–73. [PubMed: 24839024]
53. Muralidharan-Chari V, Clancy JW, Sedgwick A, D'Souza-Schorey C. Microvesicles: mediators of extracellular communication during cancer progression. J Cell Sci. 2010; 123:1603–11. [PubMed: 20445011]
54. Whiteside TL. Tumour-derived exosomes or microvesicles: another mechanism of tumour escape from the host immune system? Br J Cancer. 2005; 92:209–11. [PubMed: 15702078]
55. Li B, Antonyak MA, Zhang J, Cerione RA. RhoA triggers a specific signaling pathway that generates transforming microvesicles in cancer cells. Oncogene. 2012; 31:4740–9. [PubMed: 22266864]
56. Muralidharan-Chari V, Clancy J, Plou C, Romao M, Chavrier P, Raposo G, et al. ARF6-regulated shedding of tumor cell-derived plasma membrane microvesicles. Curr Biol. 2009; 19:1875–85. [PubMed: 19896381]
57. Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia. 2006; 20:1487–95. [PubMed: 16791265]
58. Piccin A, Murphy WG, Smith OP. Circulating microparticles: pathophysiology and clinical implications. Blood Rev. 2007; 21:157–71. [PubMed: 17118501]
59. Pap E, Pallinger E, Pasztoi M, Falus A. Highlights of a new type of intercellular communication: microvesicle-based information transfer. Inflamm Res. 2009; 58:1–8. [PubMed: 19132498]
60. Lima LG, Chammas R, Monteiro RQ, Moreira ME, Barcinski MA. Tumor-derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner. Cancer Lett. 2009; 283:168–75. [PubMed: 19401262]
61. Barteneva NS, Fasler-Kan E, Bernimoulin M, Stern JN, Ponomarev ED, Duckett L, et al. Circulating microparticles: square the circle. BMC Cell Biol. 2013; 14:23. [PubMed: 23607880]
62. Del Conde I, Shrimpton CN, Thiagarajan P, Lopez JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood. 2005; 106:1604–11. [PubMed: 15741221]
63. Crespin M, Vidal C, Picard F, Lacombe C, Fontenay M. Activation of PAK1/2 during the shedding of platelet microvesicles. Blood Coagul Fibrinolysis. 2009; 20:63–70. [PubMed: 20523167]

64. Cussac D, Leblanc P, L'Heritier A, Bertoglio J, Lang P, Kordon C, et al. Rho proteins are localized with different membrane compartments involved in vesicular trafficking in anterior pituitary cells. Mol Cell Endocrinol. 1996; 119:195–206. [PubMed: 8807639]

65. Schlienger S, Campbell S, Claing A. ARF1 regulates the Rho/MLC pathway to control EGF-dependent breast cancer cell invasion. Mol Biol Cell. 2014; 25:17–29. [PubMed: 24196838]

66. D'Souza-Schorey C, Chavrier P. ARF proteins: roles in membrane traffic and beyond. Nat Rev Mol Cell Biol. 2006; 7:347–58. [PubMed: 16633337]

67. D'Asti E, Garnier D, Lee TH, Montermini L, Meehan B, Rak J. Oncogenic extracellular vesicles in brain tumor progression. Front Physiol. 2012; 3:294. [PubMed: 22934045]

68. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007; 9:654–9. [PubMed: 17486113]

69. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, et al. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol. 2008; 10:619–24. [PubMed: 18425114]

70. Demory Beckler M, Higginbotham JN, Franklin JL, Ham AJ, Halvey PJ, Imasuen IE, et al. Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS. Mol Cell Proteomics. 2013; 12:343–55. [PubMed: 23161513]

71. Camacho L, Guerrero P, Marchetti D. MicroRNA and protein profiling of brain metastasis competent cell-derived exosomes. PLoS One. 2013; 8:e73790. [PubMed: 24066071]

72. Hessvik NP, Phuyal S, Brech A, Sandvig K, Llorente A. Profiling of microRNAs in exosomes released from PC-3 prostate cancer cells. Biochim Biophys Acta. 2012; 1819:1154–63. [PubMed: 22982408]

73. Choi DS, Kim DK, Kim YK, Gho YS. Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. Proteomics. 2013; 13:1554–71. [PubMed: 23401200]

74. Llorente A, Skotland T, Sylvanne T, Kauhanen D, Rog T, Orlowski A, et al. Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. Biochim Biophys Acta. 2013; 1831:1302–9. [PubMed: 24046871]

75. Mathivanan S, Simpson RJ. ExoCarta: A compendium of exosomal proteins and RNA. Proteomics. 2009; 9:4997–5000. [PubMed: 19810033]

76. Kalra H, Simpson RJ, Ji H, Aikawa E, Altevogt P, Askenase P, et al. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS Biol. 2012; 10:e1001450. [PubMed: 23271954]

77. Kim DK, Kang B, Kim OY, Choi DS, Lee J, Kim SR, et al. EVpedia: an integrated database of high-throughput data for systemic analyses of extracellular vesicles. J Extracell Vesicles. 2013; 2

78. Choi DS, Kim DK, Kim YK, Gho YS. Proteomics of extracellular vesicles: Exosomes and ectosomes. Mass Spectrom Rev. 2014

79. D'Souza-Schorey C, Di Vizio D. Biology and proteomics of extracellular vesicles: harnessing their clinical potential. Expert Rev Proteomics. 2014; 11:251–3. [PubMed: 24476411]

80. D'Souza-Schorey C, Clancy JW. Tumor-derived microvesicles: shedding light on novel microenvironment modulators and prospective cancer biomarkers. Genes Dev. 2012; 26:1287–99. [PubMed: 22713869]

81. Muturi HT, Dreesen JD, Nilewski E, Jastrow H, Giebel B, Ergun S, et al. Tumor and endothelial cell-derived microvesicles carry distinct CEACAMs and influence T-cell behavior. PLoS One. 2013; 8:e74654. [PubMed: 24040308]

82. Garnier D, Magnus N, Meehan B, Kislinger T, Rak J. Qualitative changes in the proteome of extracellular vesicles accompanying cancer cell transition to mesenchymal state. Exp Cell Res. 2013; 319:2747–57. [PubMed: 23954818]

83. Corrado C, Raimondo S, Chiesi A, Ciccia F, De Leo G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. Int J Mol Sci. 2013; 14:5338–66. [PubMed: 23466882]

84. Tauro BJ, Greening DW, Mathias RA, Mathivanan S, Ji H, Simpson RJ. Two distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids. Mol Cell Proteomics. 2013; 12:587–98. [PubMed: 23230278]

85. Braschler U, Shiner J, Streit J, Spenger C, Luscher HR. Simulation of action potential propagation in terminal arborizations of cultured sensory ganglia. Schweiz Arch Neurol Psychiatr. 1989; 140:13–8.

86. Pols MS, Klumperman J. Trafficking and function of the tetraspanin CD63. Exp Cell Res. 2009; 315:1584–92. [PubMed: 18930046]

87. Abache T, Le Naour F, Planchon S, Harper F, Boucheix C, Rubinstein E. The transferrin receptor and the tetraspanin web molecules CD9, CD81, and CD9P-1 are differentially sorted into exosomes after TPA treatment of K562 cells. J Cell Biochem. 2007; 102:650–64. [PubMed: 17407154]

88. Holleman J, Marchese A. The ubiquitin ligase deltex-3l regulates endosomal sorting of the G protein-coupled receptor CXCR4. Mol Biol Cell. 2014; 25:1892–904. [PubMed: 24790097]

89. van Niel G, Charrin S, Simoes S, Romao M, Rochin L, Saftig P, et al. The tetraspanin CD63 regulates ESCRT-independent and -dependent endosomal sorting during melanogenesis. Developmental cell. 2011; 21:708–21. [PubMed: 21962903]

90. Cheng L, Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. J Extracell Vesicles. 2014; 3

91. Gezer U, Ozgur E, Cetinkaya M, Isin M, Dalay N. Long non-coding RNAs with low expression levels in cells are enriched in secreted exosomes. Cell Biol Int. 2014; 38:1076–9. [PubMed: 24798520]

92. Balaj L, Lessard R, Dai L, Cho YJ, Pomeroy SL, Breakefield XO, et al. Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. Nat Commun. 2011; 2:180. [PubMed: 21285958]

93. Kahlert C, Melo SA, Protopopov A, Tang J, Seth S, Koch M, et al. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. J Biol Chem. 2014; 289:3869–75. [PubMed: 24398677]

94. Thakur BK, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, et al. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res. 2014; 24:766–9. [PubMed: 24710597]

95. Lee TH, Chennakrishnaiah S, Audemard E, Montermini L, Meehan B, Rak J. Oncogenic ras-driven cancer cell vesiculation leads to emission of double-stranded DNA capable of interacting with target cells. Biochemical and biophysical research communications. 2014; 451:295–301. [PubMed: 25086355]

96. Morelli AE, Larregina AT, Shufesky WJ, Sullivan ML, Stolz DB, Papworth GD, et al. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. Blood. 2004; 104:3257–66. [PubMed: 15284116]

97. Wei Y, Lai X, Yu S, Chen S, Ma Y, Zhang Y, et al. Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. Breast Cancer Res Treat. 2014; 147:423–31. [PubMed: 25007959]

98. Feng D, Zhao WL, Ye YY, Bai XC, Liu RQ, Chang LF, et al. Cellular internalization of exosomes occurs through phagocytosis. Traffic. 2010; 11:675–87. [PubMed: 20136776]

99. Christianson HC, Svensson KJ, van Kuppevelt TH, Li JP, Belting M. Cancer cell exosomes depend on cell-surface heparan sulfate proteoglycans for their internalization and functional activity. Proc Natl Acad Sci U S A. 2013; 110:17380–5. [PubMed: 24101524]

100. Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles. 2014; 3

101. Svensson KJ, Christianson HC, Wittrup A, Bourseau-Guilmain E, Lindqvist E, Svensson LM, et al. Exosome uptake depends on ERK1/2-heat shock protein 27 signaling and lipid Raft-mediated endocytosis negatively regulated by caveolin-1. J Biol Chem. 2013; 288:17713–24. [PubMed: 23653359]

102. Di Vizio D, Kim J, Hager MH, Morello M, Yang W, Lafargue CJ, et al. Oncosome formation in prostate cancer: association with a region of frequent chromosomal deletion in metastatic disease. Cancer Res. 2009; 69:5601–9. [PubMed: 19549916]

103. Zucker S, Cao J, Chen WT. Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. Oncogene. 2000; 19:6642–50. [PubMed: 11426650]
104. Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, et al. Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. J Cell Biol. 2003; 160:267–77. [PubMed: 12527751]
105. Oppel F, Muller N, Schackert G, Hendruschk S, Martin D, Geiger KD, et al. SOX2-RNAi attenuates S-phase entry and induces RhoA-dependent switch to protease-independent amoeboid migration in human glioma cells. Molecular cancer. 2011; 10:137. [PubMed: 22070920]
106. Yilmaz M, Christofori G. Mechanisms of motility in metastasizing cells. Mol Cancer Res. 2010; 8:629–42. [PubMed: 20460404]
107. Hager MH, Morley S, Bielenberg DR, Gao S, Morello M, Holcomb IN, et al. DIAPH3 governs the cellular transition to the amoeboid tumour phenotype. EMBO Mol Med. 2012; 4:743–60. [PubMed: 22593025]
108. Josson S, Gururajan M, Sung SY, Hu P, Shao C, Zhau HE, et al. Stromal fibroblast-derived miR-409 promotes epithelial-to-mesenchymal transition and prostate tumorigenesis. Oncogene. 2014; 0
109. Tahir SA, Ren C, Timme TL, Gdor Y, Hoogeveen R, Morrisett JD, et al. Development of an immunoassay for serum caveolin-1: a novel biomarker for prostate cancer. Clin Cancer Res. 2003; 9:3653–9. [PubMed: 14506154]
110. Wright PK, Jones SB, Ardern N, Ward R, Clarke RB, Sotgia F, et al. 17beta-estradiol regulates giant vesicle formation via estrogen receptor-alpha in human breast cancer cells. Oncotarget. 2014; 5:3055–65. [PubMed: 24931391]
111. Webber J, Steadman R, Mason MD, Tabi Z, Clayton A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. Cancer Res. 2010; 70:9621–30. [PubMed: 21098712]
112. Webber JP, Spary LK, Sanders AJ, Chowdhury R, Jiang WG, Steadman R, et al. Differentiation of tumour-promoting stromal myofibroblasts by cancer exosomes. Oncogene. 2014
113. Antonyak MA, Li B, Boroughs LK, Johnson JL, Druso JE, Bryant KL, et al. Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. Proc Natl Acad Sci U S A. 2011; 108:4852–7. [PubMed: 21368175]
114. Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol. 2008; 10:1470–6. [PubMed: 19011622]
115. Thompson CA, Purushothaman A, Ramani VC, Vlodavsky I, Sanderson RD. Heparanase regulates secretion, composition, and function of tumor cell-derived exosomes. J Biol Chem. 2013; 288:10093–9. [PubMed: 23430739]
116. Szajnik M, Czystowska M, Szczepanski MJ, Mandapathil M, Whiteside TL. Tumor-derived microvesicles induce, expand and up-regulate biological activities of human regulatory T cells (Treg). PLoS One. 2010; 5:e11469. [PubMed: 20661468]
117. Hakulinen J, Sankkila L, Sugiyama N, Lehti K, Keski-Oja J. Secretion of active membrane type 1 matrix metalloproteinase (MMP-14) into extracellular space in microvesicular exosomes. J Cell Biochem. 2008; 105:1211–8. [PubMed: 18802920]
118. Lv MM, Zhu XY, Chen WX, Zhong SL, Hu Q, Ma TF, et al. Exosomes mediate drug resistance transfer in MCF-7 breast cancer cells and a probable mechanism is delivery of P-glycoprotein. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014
119. Chen WX, Liu XM, Lv MM, Chen L, Zhao JH, Zhong SL, et al. Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of microRNAs. PLoS One. 2014; 9:e95240. [PubMed: 24740415]
120. Cai J, Han Y, Ren H, Chen C, He D, Zhou L, et al. Extracellular vesicle-mediated transfer of donor genomic DNA to recipient cells is a novel mechanism for genetic influence between cells. Journal of molecular cell biology. 2013; 5:227–38. [PubMed: 23580760]
121. Tadokoro H, Umezu T, Ohyashiki K, Hirano T, Ohyashiki JH. Exosomes derived from hypoxic leukemia cells enhance tube formation in endothelial cells. J Biol Chem. 2013; 288:34343–51. [PubMed: 24133215]

122. Le MT, Hamar P, Guo C, Basar E, Perdigao-Henriques R, Balaj L, et al. miR-200-containing extracellular vesicles promote breast cancer cell metastasis. The Journal of clinical investigation. 2014; 124:5109–28. [PubMed: 25401471]

123. Castellana D, Zobairi F, Martinez MC, Panaro MA, Mitolo V, Freyssinet JM, et al. Membrane microvesicles as actors in the establishment of a favorable prostatic tumoral niche: a role for activated fibroblasts and CX3CL1-CX3CR1 axis. Cancer Res. 2009; 69:785–93. [PubMed: 19155311]

124. Luga V, Zhang L, Viloria-Petit AM, Ogunjimi AA, Inanlou MR, Chiu E, et al. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell. 2012; 151:1542–56. [PubMed: 23260141]

125. Shimoda M, Principe S, Jackson HW, Luga V, Fang H, Molyneux SD, et al. Loss of the Timp gene family is sufficient for the acquisition of the CAF-like cell state. Nat Cell Biol. 2014; 16:889–901. [PubMed: 25150980]

126. Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis. J Biol Chem. 2013; 288:10849–59. [PubMed: 23439645]

127. Al-Nedawi K, Meehan B, Kerbel RS, Allison AC, Rak J. Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. Proc Natl Acad Sci U S A. 2009; 106:3794–9. [PubMed: 19234131]

128. Zhuang G, Wu X, Jiang Z, Kasman I, Yao J, Guan Y, et al. Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway. The EMBO journal. 2012; 31:3513–23. [PubMed: 22773185]

129. Taylor DD, Gercel-Taylor C. Exosomes/microvesicles: mediators of cancer-associated immunosuppressive microenvironments. Seminars in immunopathology. 2011; 33:441–54. [PubMed: 21688197]

130. Abusamra AJ, Zhong Z, Zheng X, Li M, Ichim TE, Chin JL, et al. Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis. Blood cells, molecules & diseases. 2005; 35:169–73.

131. Huber V, Fais S, Iero M, Lugini L, Canese P, Squarcina P, et al. Human colorectal cancer cells induce T-cell death through release of proapoptotic microvesicles: role in immune escape. Gastroenterology. 2005; 128:1796–804. [PubMed: 15940614]

132. Kim JW, Wieckowski E, Taylor DD, Reichert TE, Watkins S, Whiteside TL. Fas ligand-positive membranous vesicles isolated from sera of patients with oral cancer induce apoptosis of activated T lymphocytes. Clin Cancer Res. 2005; 11:1010–20. [PubMed: 15709166]

133. Clayton A, Mitchell JP, Court J, Linnane S, Mason MD, Tabi Z. Human tumor-derived exosomes down-modulate NKG2D expression. Journal of immunology. 2008; 180:7249–58.

134. Choi DY, You S, Jung JH, Lee JC, Rho JK, Lee KY, et al. Extracellular vesicles shed from gefitinib-resistant nonsmall cell lung cancer regulate the tumor microenvironment. Proteomics. 2014; 14:1845–56. [PubMed: 24946052]

135. Ma X, Chen Z, Hua D, He D, Wang L, Zhang P, et al. Essential role for TrpC5-containing extracellular vesicles in breast cancer with chemotherapeutic resistance. Proc Natl Acad Sci U S A. 2014; 111:6389–94. [PubMed: 24733904]

136. Chen WX, Cai YQ, Lv MM, Chen L, Zhong SL, Ma TF, et al. Exosomes from docetaxel-resistant breast cancer cells alter chemosensitivity by delivering microRNAs. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014

137. Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, et al. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res. 2011; 71:5346–56. [PubMed: 21670082]

138. Chevillet JR, Kang Q, Ruf IK, Briggs HA, Vojtech LN, Hughes SM, et al. Quantitative and stoichiometric analysis of the microRNA content of exosomes. Proc Natl Acad Sci U S A. 2014; 111:14888–93. [PubMed: 25267620]

139. Cvjetkovic A, Lotvall J, Lasser C. The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. J Extracell Vesicles. 2014; 3

140. Witwer KW, Buzas EI, Bemis LT, Bora A, Lasser C, Lotvall J, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. J Extracell Vesicles. 2013; 2

141. Kalra H, Adda CG, Liem M, Ang CS, Mechler A, Simpson RJ, et al. Comparative proteomics evaluation of plasma exosome isolation techniques and assessment of the stability of exosomes in normal human blood plasma. Proteomics. 2013; 13:3354–64. [PubMed: 24115447]

142. Shao H, Chung J, Balaj L, Charest A, Bigner DD, Carter BS, et al. Protein typing of circulating microvesicles allows real-time monitoring of glioblastoma therapy. Nat Med. 2012; 18:1835–40. [PubMed: 23142818]

143. Yoshioka Y, Konishi Y, Kosaka N, Katsuda T, Kato T, Ochiya T. Comparative marker analysis of extracellular vesicles in different human cancer types. J Extracell Vesicles. 2013; 2

144. van der Vlist EJ, Nolte-'t Hoen EN, Stoorvogel W, Arkesteijn GJ, Wauben MH. Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry. Nature protocols. 2012; 7:1311–26. [PubMed: 22722367]

145. Wright M. Nanoparticle tracking analysis for the multiparameter characterization and counting of nanoparticle suspensions. Methods Mol Biol. 2012; 906:511–24. [PubMed: 22791460]

146. Kang MH, Park MJ, Yoo HJ, hyuk KY, Lee SG, Kim SR, et al. RIPL peptide (IPLVVPLRRRRRRRRC)-conjugated liposomes for enhanced intracellular drug delivery to hepsin-expressing cancer cells. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV. 2014; 87:489–99.

147. Corcoran C, Rani S, O'Driscoll L. miR-34a is an intracellular and exosomal predictive biomarker for response to docetaxel with clinical relevance to prostate cancer progression. The Prostate. 2014; 74:1320–34. [PubMed: 25053345]

148. Valencia K, Luis-Ravelo D, Bovy N, Anton I, Martinez-Canarias S, Zandueta C, et al. miRNA cargo within exosome-like vesicle transfer influences metastatic bone colonization. Molecular oncology. 2014; 8:689–703. [PubMed: 24593875]

149. Cheng L, Sun X, Scicluna BJ, Coleman BM, Hill AF. Characterization and deep sequencing analysis of exosomal and non-exosomal miRNA in human urine. Kidney international. 2014; 86:433–44. [PubMed: 24352158]

150. Li M, Zeringer E, Barta T, Schageman J, Cheng A, Vlassov AV. Analysis of the RNA content of the exosomes derived from blood serum and urine and its potential as biomarkers. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2014; 369

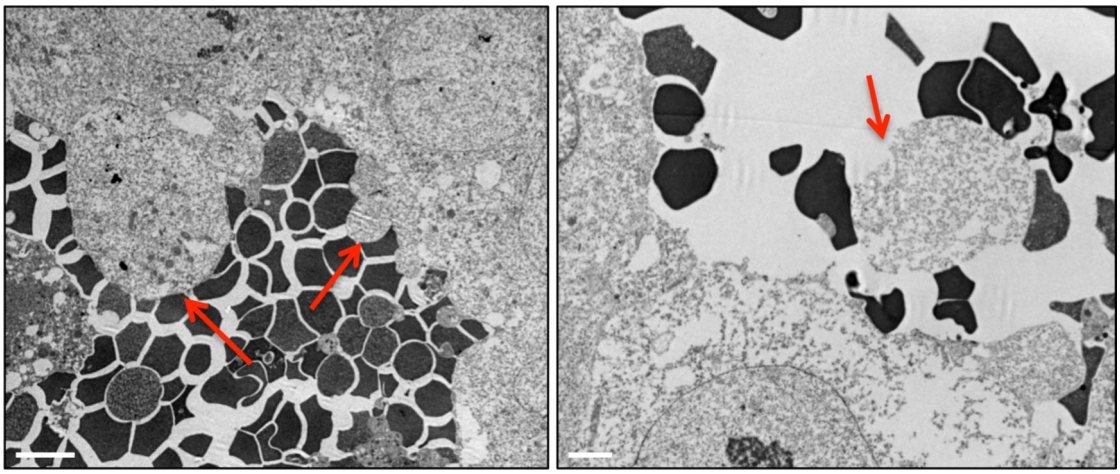

Figure 1. Tumor cells release large oncosomes

Tumor sections of PC xenografts imaged by EM showing large oncosome-like features (white arrows). Size bars are 5 mm (left) and 2 mm (right).

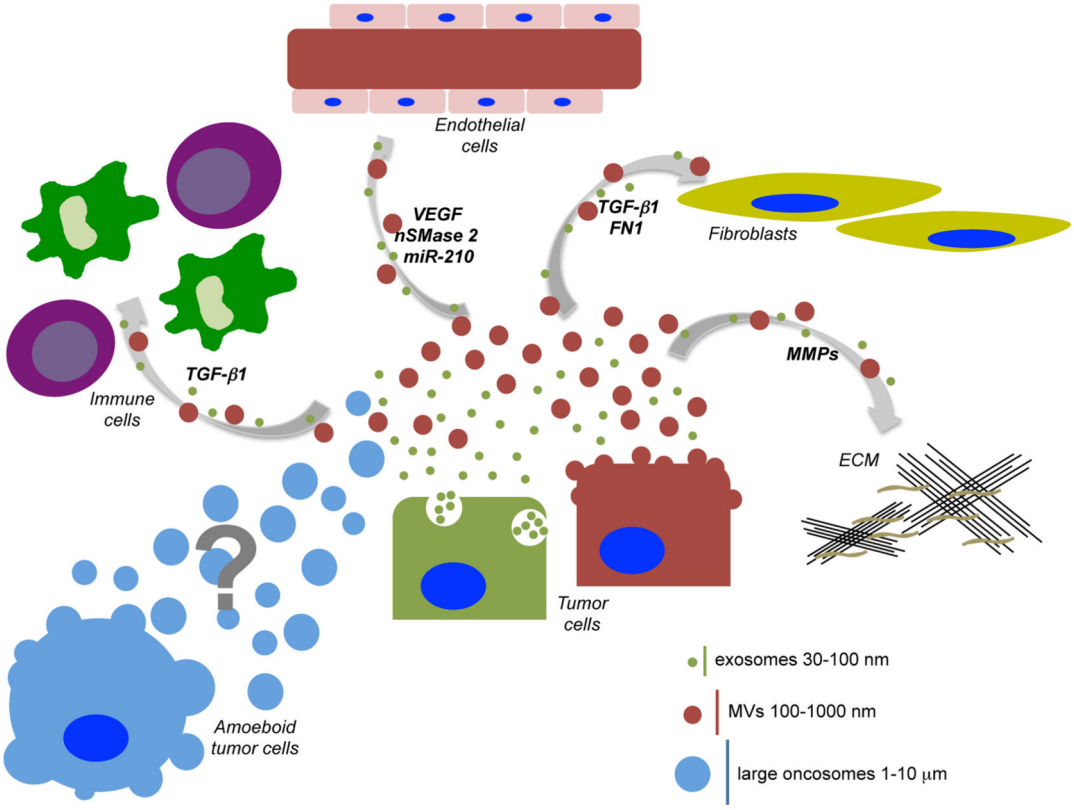

Figure 2. Large oncosomes as new players in intercellular communication

Tumor cells communicate with various components of the tumor microenvironment by EVs. While some of the most common mechanisms of interaction between tumor-derived MV (red dots) and exosomes (green dots) with target cells have been described, those that govern the cross talk between large oncosomes and the microenvironment are still largely unknown.