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Review

Communication by Extracellular Vesicles: Where We Are and Where We Need to Go

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In multicellular organisms, distant cells can exchange information by sending out signals composed of single molecules or, as increasingly exemplified in the literature, via complex packets stuffed with a selection of proteins, lipids, and nucleic acids, called extracellular vesicles (EVs; also known as exosomes and microvesicles, among other names). This Review covers some of the most striking functions described for EV secretion but also presents the limitations on our knowledge of their physiological roles. While there are initial indications that EV-mediated pathways operate in vivo, the actual nature of the EVs involved in these effects still needs to be clarified. Here, we focus on the context of tumor cells and their microenvironment, but similar results and challenges apply to all patho/physiological systems in which EV-mediated communication is proposed to take place.

Introduction

Cells can communicate with neighboring cells or with distant cells through the secretion of extracellular vesicles (EVs). EVs are composed of a lipid bilayer containing transmembrane proteins and enclosing cytosolic proteins and RNA. Cells can secrete different types of EVs that have been classified according to their sub-cellular origin (Colombo et al., 2014). On one hand, EVs can be formed and released by budding from the cells' plasma membrane. These EVs display a diverse range of sizes (100–1,000 nm in diameter) and are generally known in the literature as microvesicles, ectosomes, or microparticles. Other types of vesicles, the exosomes, are generated inside multivesicular endosomes or multivesicular bodies (MVBs) and are secreted when these compartments fuse with the plasma membrane. Exosomes are vesicles smaller than 150 nm in diameter and are enriched in endosome-derived components. All EVs bear surface molecules that allow them to be targeted to recipient cells. Once attached to a target cell, EVs can induce signaling via receptor-ligand interaction or can be internalized by endocytosis and/or phagocytosis or even fuse with the target cell's membrane to deliver their content into its cytosol, thereby modifying the physiological state of the recipient cell.

In this Review, we highlight and discuss the more recent studies on cancer-derived EVs, with a special focus on the latest discoveries on the role of EVs in cancer metastasis. The term "exosomes" is often used in these articles to designate the EVs analyzed. However, we now know that the most popular exosome purification protocols used historically in the literature (differential ultracentrifugation, 220 nm filtration [Thery et al., 2006])—and the recently released commercial kits—co-isolate different types of EVs. Thus, the term exosomes is generally used to refer to a mixed population of small EVs (sEVs) without further demonstration of their intracellular origin. In fact, functions assigned to exosomes may either reflect generic EV activities or truly exosome-specific ones; however, the published data cannot be used to determine the precise specificity. We thus chose here to use the generic term EVs when vesicles are isolated without specific attention to their size or sEVs when the method used selects vesicles smaller than 200 nm, independent of the term used in the article referred to.

EV-Borne Proteins Promote Cancer Progression and Metastasis

EVs have been shown to participate in the dissemination of cancer cells, and many groups have described how tumor- and stroma-derived EVs are involved in the different steps of the metastatic cascade (Figure 1). Tumor sEVs can directly modify tumor cells' intrinsic motility and invasiveness capacity. In particular, sEVs can promote directional cell motility through ECM components, such as fibronectin, which bind to integrins present on sEVs and thus provide a substrate favoring cell adhesion and enhancing cell speed (Sung et al., 2015). Moreover, sEVs participate in the biogenesis and activity of an invasive structure called invadopodia through the MVB-dependent delivery of metalloproteinases such as MT1-MMP and other cargo molecules (Hoshino et al., 2013), thus promoting cell motility. EVs can directly contribute to extracellular matrix (ECM) degradation by spreading matrix metalloproteinases present either on sEVs (Yue et al., 2015) or in tumor-shed large EVs (Clancy et al., 2015). The latter also have been shown to facilitate amoeboid movement and facilitate invasion. Not only tumor-derived EVs, but also sEVs from cancer-associated fibroblasts can stimulate invasiveness of recipient breast cancer cells, in this case by activating the planar cell polarity signaling pathway (Luga et al., 2012).

Tumor sEVs can alter the cellular physiology of both surrounding and distant non-tumor cells to allow dissemination and growth of cancer cells, i.e., by triggering vascular permeability

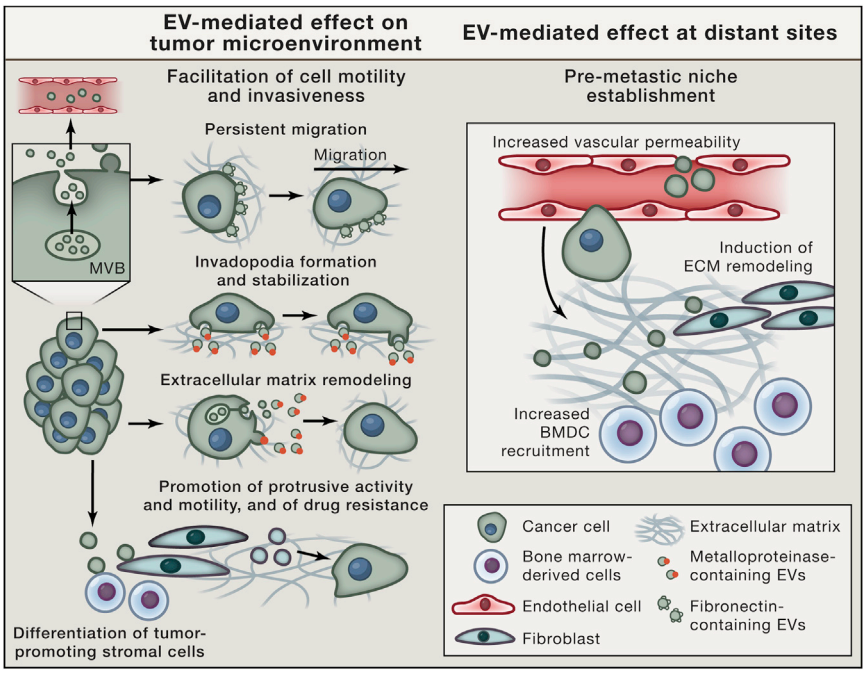

Figure 1. EV-Mediated Effects Promoting Tumor Growth, Invasiveness, and Metastasis

Tumor-derived EVs can have several effects on recipient cells. At the site of the primary tumor (left), EVs can enhance cancer cell motility by stabilizing cellular protrusions promoting an effective and directionally persistent migration via deposition of ECM cargoes, such as fibronectin, into sEVs. The secretion of EVs containing metalloproteinases also directly participates in ECM remodeling and promotes function of specialized cell protrusions endowed with degradative activity, called the invadopodia. ECM remodeling supports tumor cell motility through the tissues. EVs can also promote differentiation or recruitment of pro-tumoral stromal cells (fibroblasts and bone-marrow-derived cells). Reciprocally, tumor cell motility, but also acquisition of drug resistance, can be enhanced via a complex interplay with EVs secreted by surrounding fibroblasts. In addition, sEVs can enter the circulation and travel to distant sites from the primary tumor (right). Various sEV cargoes promote vascular permeability, and EVs can enter the distant tissue, where they may generate a pre-metastatic niche by inducing ECM remodeling and promoting the recruitment of bone-marrow-derived cells and eventually, tumor cells. This figure schematizes the effects of EVs demonstrated by mixed in-vivo-/in-vitro-based experiments. See the text for discussion on the evidence for fully physiological in vivo occurrence of these functions.

(Peinado et al., 2012; Zhou et al., 2014) or by conditioning pre-metastatic sites in distant organs (Costa-Silva et al., 2015; Hoshino et al., 2015; Peinado et al., 2012). In particular, melanoma tumor sEVs bearing a tyrosine-kinase receptor can promote migration of bone marrow progenitor cells to future sites of metastasis, whereas sEVs secreted by a less-aggressive version of the same tumor, devoid of the relevant receptor, do not display this effect (Peinado et al., 2012). Alternatively, sEVs from pancreatic cancer cells themselves migrate to distant organs and promote the formation of a pre-metastatic niche by creating a fibrotic environment enriched in TGFβ, fibronectin, and a macrophage-attracting chemokine (Costa-Silva et al., 2015). Interestingly, sEVs from different tumor types bear integrins (ITGs) that target these sEVs to specific organs and trigger signaling pathways, thereby initiating pre-metastatic niche formation (Hoshino et al., 2015). For example, sEVs expressing ITG$\alpha_v\beta_5$ bind specifically to Kupffer cells, mediating liver tropism, while ITG$\alpha_6\beta_4$ and ITG$\alpha_6\beta_1$ on sEVs bind to lung-resident fibroblasts and epithelial cells, leading to lung tropism (Hoshino et al., 2015). Modifications induced by sEVs in these distant organs then attract metastatic tumor cells.

This observation has been recently used in an innovative way to redirect tumor cell dissemination in a non-deleterious location (de la Fuente et al., 2015). An artificial pre-metastatic niche generated by embedding tumor sEVs in a 3D scaffold and then implanted in mouse peritoneum was able to capture ovarian tumor cells present in the peritoneum and divert them from their normal organ target for dissemination, resulting in strikingly increased survival of the animal. The possible application of this device in human patients could represent a very promising approach to suppress metastasis.

However, despite being extremely appealing, we must stress that the working model of circulating tumor-derived sEVs fostering pre-metastatic niche formation has not been demonstrated in a fully physiological in vivo context. In published articles to date, animals were subjected to sustained injections of in-vitro-purified tumor-derived sEVs, resulting in this enhanced metastasis. Whether sEV secretion in vivo by tumor cells is able to achieve this function is still not clear. One possible way to address this is by interfering in vivo with sEV biogenesis in cancer cells. Some studies have attempted to do this by inhibiting Ras-related RAB proteins. RAB27A or RAB35 have been first shown to be required for sEV secretion in HeLa cervical carcinoma (Ostrowski et al., 2010) and Oli-Neu oligodendroglial precursor cell lines (Hsu et al., 2010), respectively. Consistently, knocking down RAB27A in melanoma (Peinado et al., 2012), breast (Bobrie et al., 2012), fibrosarcoma (Sung et al., 2015), or prostate cancer cell lines (Webber et al., 2015) reduces the secretion of sEVs. Cells lacking RAB27A, when injected in vivo, displayed reduced local migration (Sung et al., 2015) or reduced growth due to impaired recruitment of bone-marrow-derived pro-tumoral immune cells (Bobrie et al., 2012), or impaired modification of co-injected fibroblasts into pro-tumoral myofibroblasts (Webber et al., 2015) (Figure 1). Lower incidence of metastasis was also observed (Bobrie et al., 2012; Peinado et al., 2012). However, RAB27A does not exclusively regulate EV secretion. Loss of the protein also decreases EV-independent secretion of soluble factors, such as some growth factors and metalloproteinases that are also involved in tumor metastasis (Bobrie et al., 2012; Peinado et al., 2012). The same problem has arisen with the other molecules proposed so far to regulate specifically sEV secretion, such as sphingomyelinases

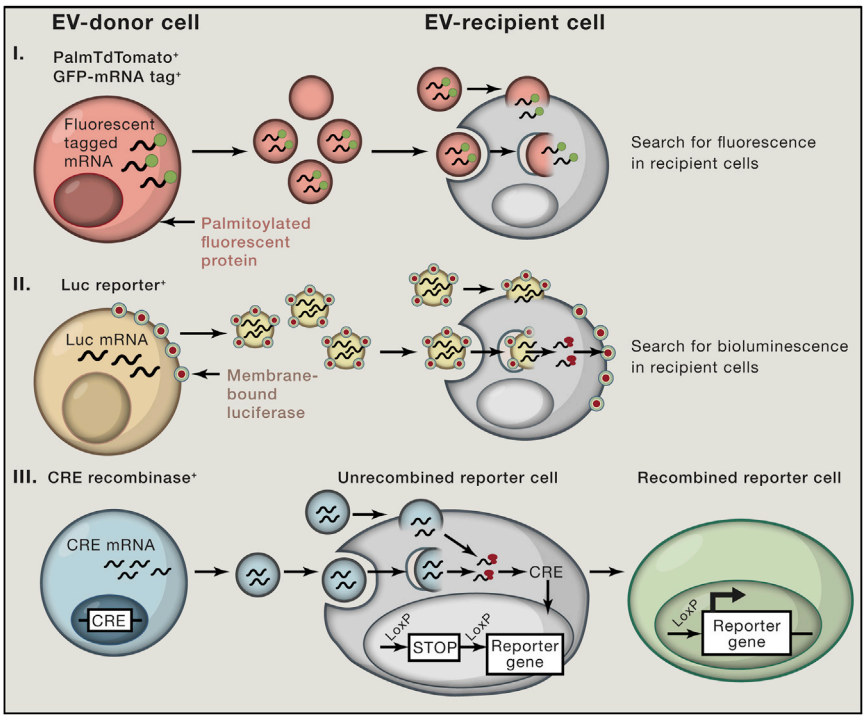

Figure 2. Approaches Used to Analyze EV-Mediated Transfer In Vivo

Recent novel approaches allowing the visualization of EV transfer to recipient cells in vivo involve genetic modification of the secreting cells, which then secrete EVs containing labeled components. (I) Genetic fusion of fluorescent proteins to a consensus palmitoylation sequence, enabling whole-cell membrane labeling, and to an mRNA allows tracking of cells with bound or internalized EVs by their newly acquired fluorescence. (II) Expression of a membrane-bound luciferase allows analysis of distant bioluminescent cells in vivo, resulting from EV-bound luciferase protein capture and/or luciferase mRNA neo-expression. (III) To demonstrate specifically neo-expression of an EV-associated mRNA, the CRE recombinase can be expressed in EV-secreting cells. Endogenous nuclear localization of CRE results in absence of the protein but presence of the mRNA in the secreted EVs. Transfer of the CRE mRNA contained within an EV to a cell carrying a fluorescent or enzymatic reporter gene expressed only after DNA recombination and excision of a STOP signal is detected by fluorescence or colorimetric changes.

(SMases) (Trajkovic et al., 2008). Knockdown or inhibition of SMases by small-molecule inhibitors, which results in impaired ceramide formation, has often been used to inhibit exosome/sEV secretion but without demonstration of the specificity of this effect for sEV secretion, as opposed to other secretions or other physiological features of the cells. Thus, to understand the functions of EVs in vivo, the development of complementary methodologies will be required.

Catching Communication in Action

A major challenge for the EV field and, more broadly, for understanding how EVs may support both physiological and pathophysiological processes is being able to demonstrate in vivo EV transfer between cells. To address this problem, a few groups have recently developed clever modifications of EVs, allowing tracking of their behavior and their target cells in vivo (Figure 2). Either proteins or mRNA cargoes of EVs have been thus modified. For instance, fusing a fluorescent protein to a palmitoylation sequence induces its localization at the plasma membrane as well as in secreted EVs of all sizes. The EVs could then be visualized in the tumor microenvironment by intravital microscopy on animals bearing tumors expressing this fusion protein (Lai et al., 2015). Fusion of luciferase to a protein transmembrane domain also allowed its secretion in EVs and subsequent enzymatic measurement of luciferase activity in distant cells (Lai et al., 2014). These authors also designed an intracellular probe to fluorescently label mRNA secreted in EVs and tools to measure EV-borne mRNA encoding luciferase signal (Lai et al., 2015). While these tools are promising, studies so far have only demonstrated EV-borne mRNA transfer between cultured cells in vitro.

Another elegant approach to address EV transfer has now demonstrated functional EV-mediated transfer in vivo of an mRNA into target cells in the absence of any ex vivo manipulation (Ridder et al., 2014). Transgenic mice expressing the CRE recombinase specifically in immune cells and a LacZ reporter gene, expressed only upon excision of a STOP sequence, were used for this purpose (Ridder et al., 2014). Ridder et al. showed that EVs containing the CRE mRNA, but devoid of the CRE protein, are present in blood circulation of these transgenic mice. Strikingly, LacZ expression was observed in some neurons and other non-immune cells throughout the animals. Leakiness of the immune cell-specific promoter and fusion between CRE-expressing and reporter-expressing cells were carefully excluded, leading to the conclusion that the CRE mRNA carried by immune cell EVs was transferred in the recipient non-immune cells and translated into functional CRE protein. Although the number of recombined neural cells was very low, suggesting a limited efficiency of this transfer in normal conditions, induction of systemic inflammation increased it, opening up the possibility that in vivo EV-mediated transfer may be particularly relevant to some pathological conditions.

This methodology has also been recently used to visualize cancer-derived EV transfer to other cancer cells (Zomer et al., 2015) and to immune cells (Ridder et al., 2015) in living mice and to study the effect of this transfer. In Zomer et al., the authors designed a reporter system based on the conversion of DsRed+ tumor cells to eGFP+ tumor cells upon uptake of tumor EVs containing CRE mRNA and analyzed the behavior of these latter cells without any manipulation of the EV-releasing cells. Using this system, intravital imaging revealed the EV transfer of functional mRNA from a malignant human tumor cell to a less malignant

one and demonstrated that the uptake of these EVs can alter the migratory behavior and metastatic capacity of the recipient cell (Zomer et al., 2015). It will be interesting to study this transfer of EVs among tumor cells in immunocompetent mice. In Ridder et al., a similar strategy highlighted the transfer of vesicle-enclosed CRE mRNA to non-tumor cells and showed that myeloid-derived suppressor cells that took up tumor EVs displayed increased immunosupressive functions (Ridder et al., 2015). In these two studies, the actual nature of the EVs involved in mRNA transfer was not investigated, nor was the transfer mechanism. It is therefore not clear whether transfer involves a direct fusion of EVs with the recipient cells or phagocytosis of live or apoptotic cell-derived EVs by the recipient cell. To more solidly support the pathway for EV-mediated transfer of CRE mRNA, inhibition of EV biogenesis in vivo would be ideal; however, as mentioned before, these experiments are quite difficult to accomplish and are not completely specific. Other transfer mechanisms, like formation of gap junctions or of nanotubes connecting two adjacent cells, were also not formally excluded. However, it was also observed that transfer occurred at a distance between two tumors localized in different parts of the animal, excluding the possibility of local communication between cells and supporting the idea of mRNA being transferred through a long-range extracellular carrier. Overall, this visualization system allows identification and isolation of cells that are targets of EVs in vivo and will be very important for understanding how tumor- and stromal-derived EVs affect their environment.

A Role for EV-Mediated Small RNA Transfer?

In addition to proteins and mRNAs, miRNAs and other non-coding RNAs are also possible active EV cargoes. The idea that miRNA secreted in sEVs can be functionally delivered to target cells, resulting in direct modulation of their mRNA targets, has become one of the most actively explored hypotheses in the EV field, especially in cancer. This idea was initially demonstrated for Epstein-Barr virus-infected cells, where secreted sEVs transferred viral miRNAs into neighboring non-infected cells, leading to repression of virus-target genes (Pegtel et al., 2010). Following this path, several groups have recently reported that EV-mediated secretion of a given miRNA in the tumor microenvironment is responsible for tumor metastasis. Various mechanisms have been proposed, involving either an effect on the local or distant tumor stroma (including modulating endothelial cell permeability, metabolism, or the pre-metastatic niche) or on the tumor cells themselves (by increasing oncogenic properties and/or invasiveness).

However, even if involvement of the described miRNA is generally well supported, direct demonstration that functional EV-mediated miRNA transfer is the relevant mechanism is still difficult to achieve. Importantly, carriers other than EVs could mediate miRNA transfer. All EV isolation techniques potentially co-isolate other RNA-binding structures, such as large protein complexes (Palma et al., 2012) and lipoproteins (Vickers et al., 2011). Lipoprotein-associated RNAs have been shown to be resistant to RNase treatment and to deliver miRNAs into host cells, and there is currently no reason for excluding that transfer of naked protein-miRNA complexes into the cytosol of host cells can occur. Therefore, as the International Society for Extracellular Vesicles recently highlighted (Lötvall et al., 2014), additional steps of separation of EVs from other structures, e.g., by floatation into density gradients or by immuno-isolation via specific antibodies, are necessary before claiming specific EV-mediated miRNA transfer.

The miRNA-dependent effect observed could, in fact, be mediated by induction of endogenous miRNA expression in the target cell by other EV components, rather than by the EV-enclosed miRNA. Such a mechanism can be excluded if the recipient cell is incapable of expressing the studied miRNA—for instance, when the transferred miRNA is encoded only by a foreign genome, e.g., viral (Pegtel et al., 2010) or parasitic (Buck et al., 2014). Similarly, if the recipient cell comes from a mouse engineered to lack the miRNA (Alexander et al., 2015), the dependence on EV-delivered miRNAs is more certain. However, this control has not yet been used to our knowledge in cancer studies. For example, recently, Zhang et al. have proposed that sEV-mediated transfer of astrocyte-derived miRNAs targeting PTEN leads to the loss of expression of this tumor suppressor in brain metastatic tumor cells, enhancing metastasis outgrowth (Zhang et al., 2015). Even though the hypothesis proposed in this work is very appealing, conclusive demonstration that miRNAs are being transferred through sEVs and that this is the mechanism responsible for PTEN downregulation in brain tumor metastasis is lacking. While the effects appear dependent on expression of the miRNAs in astrocytes and are lost upon global deletion of Rab27a in the brain, both controls potentially alter many other aspects than miRNA-containing sEV secretion by astrocytes; thus, specific EV-dependent transport of endogenous miRNA remains a model to be tested.

Other studies have explored similar questions, and it is clear that the field is working toward a suite of more definitive controls. For example, studies showing that co-treatment of recipient cells with an anti-miRNA together with miRNA-carrying EVs established that the analyzed miRNA is necessary for the functional effect observed (Le et al., 2014; Zhou et al., 2014). But since the anti-miRNA can inhibit expression of both the endogenous and the EV-transported molecule, this control does not actually demonstrate EV-mediated miRNA transfer. In this experimental setting, absence of upregulation of the endogenous pre- or pri-miRNA while the mature miRNA is increased in EV-recipient cells (Basu and Bhattacharyya, 2014; Le et al., 2014; Zhou et al., 2014) may be the strongest observation arguing for acquisition of the mature miRNA.

An important mechanistic aspect remains, however, mysterious. To achieve silencing of their mRNA targets, miRNAs must be associated with the RNA-induced silencing complex (RISC) containing the argonaute 2 (AGO2) endonuclease (Wilson and Doudna, 2013). For endogenous miRNAs, this association is formed following processing of the double-stranded pre-miRNA into miRNA/miRNA* duplex by DICER and subsequent incorporation into the AGO2-RISC-loading complex. Thus, how naked mature miRNAs brought in by EVs can associate with endogenous AGO2 and compete with an overwhelming amount of endogenous miRNA will remain unclear unless new molecular mechanisms of miRNA transfer into the RISC-loading complex are discovered. The form of miRNA present in EVs (i.e., as mature single-strand, miRNA/miRNA* duplex or other form,

naked or bound with AGO or other proteins) is generally not reported, and it will be important to answer this question in different cell types (e.g., tumoral or non-tumoral) and physiological contexts to elucidate the relevance of EVs as efficient miRNA carriers.

In that line, a recent study has brought an unexpected and possibly controversial turn to this field (Melo et al., 2014). Melo et al. have observed that pre-miRNAs loaded into the RISC machinery are secreted by tumor (but not by non-tumor) cell lines in sEVs and that miRNA maturation takes place extracellularly when sEVs are incubated at 37°C. The implication is that transferred AGO2-associated miRNA could thus be directly functional in a recipient cell. It will be interesting to see whether these results are borne out in other types of tumors. Furthermore, the actual nature of the RISC/miRNA carrier will have to be determined more precisely since another study reported that AGO2 secreted in tumor sEVs is not recovered in the same fractions as classical sEV markers (Van Deun et al., 2014). In conclusion, whether EV-mediated miRNA transfer is a functionally relevant communication mechanism in cancer, especially in vivo in the absence of artificial overexpression of a miRNA, is still an exciting but not yet fully demonstrated hypothesis.

In addition to miRNAs, EVs are now known to contain several other species of small non-coding RNA or RNA fragments (Nolte-'t Hoen et al., 2012). A recent report shows that miRNA may even be a minor form of RNA in all types of EVs, whereas tRNA fragments and Y-RNAs are specifically secreted in EVs (Tosar et al., 2015). It will be very interesting to determine now whether these comparatively poorly studied non-coding RNAs display some of the gene-regulatory functions so far attributed to miRNAs in EVs. Indeed, a recent report showed that tRNA fragments present in sperm regulate gene expression in the embryo and that the levels of these short tRNAs can be altered in response to paternal diet (Sharma et al., 2016). Interestingly, the tRNA fragments are not generated in spermatozoa themselves but are acquired during their transit through the epididymis, possibly via EVs named epididymosomes, which are secreted by cells forming the epithelium of this canal. The transfer of EVs to sperm had been previously documented in *Drosophila* in vivo (Corrigan et al., 2014) and in large mammals in vitro (Caballero et al., 2013). The novel idea that parental exposure can affect progeny through mechanisms involving transfer of information within EVs is a very promising and exciting hypothesis.

Ties to Immunity

A different consequence of EV-associated RNA transfer in the tumor microenvironment has been recently described (Boelens et al., 2014). EV-borne RNAs bearing 5′-triphosphate ends are recognized in the recipient cell cytosol by the RIG-I sensor, which induces development of an interferon response (including expression of genes like STAT1) similar to that induced upon viral infection. This response was shown to participate in cancer cell resistance to radiation or chemotherapy.

This observation provides one example of a growing appreciation of the similarities between sEVs and enveloped viruses (Assil et al., 2015). Indeed, enveloped viruses have recently been shown to carry a nucleotide-based compound, cGAMP, which can reach the cytosol of infected cells and induce an interferon response (Bridgeman et al., 2015; Gentili et al., 2015). In Gentili et al., we observed that cGAMP was present in non-viral sEVs, which could also transfer this signal to recipient cells, but only if they bore a fusogenic viral protein. This observation raises questions about the molecular mechanism involved in fusion of tumor sEVs with recipient cells: what surface molecules allow fusion with the membrane of recipient cells for delivery of the RNA or small-molecule content into the cytosol? Or can other mechanisms contribute, such as formation of channels between the apposed membranes of an EV and the recipient cell? These questions will be important to address.

Conclusions

With the functional implications proposed for EVs, it is now vital to understand these vesicles themselves. As mentioned above, most studies published so far analyze mixed EV populations, and we think that some of the most important steps the field must take are to comprehensively compare the different subtypes of EVs and to determine whether some of their functions are specific or prominent in a given subtype, e.g., exosomes, but not other EVs. This knowledge is necessary to identify which EVs should be targeted for any therapeutic approach. Indeed, EV research is now at the stage where the immunology field was in the 1950s. At that time, researchers could only claim that circulating white blood cells were capable of very different functions, such as killing other cells or making antibodies, simply because there were no means to distinguish what we know now as B versus T lymphocytes! For EVs, a recent article reported a calculation that the number of copies of a given miRNA present per EV, in a mixed sEV preparation, is below one (Chevillet et al., 2014), suggesting that either very few miRNA molecules are present within each sEV or, more likely, that only a restricted subtype of EVs contain significant amounts of miRNA molecules and thus are capable of transferring miRNA-based information.

This situation may apply to other transfer functions of EVs, whether they involve mRNA or proteins: if only a minor subtype of EVs carries the relevant activity, its actual efficiency will be difficult to detect, as it will be undermined by an abundance of non-functional EVs present in the same preparation. One of the challenges is therefore to re-define methods that allow discrimination between sEVs, exosomes, and other EVs. It is impossible to distinguish them on the basis of a single property, such as size, structure, buoyant density, or presence of a given protein, and the community is seeking novel methods of isolation leading to better enrichment of a specific subtype. For this, however, better knowledge of specific markers of EV subtypes is required. We have recently performed a quantitative comparison of the protein composition of several subtypes of EVs secreted simultaneously by human primary dendritic cells, which were separated by a combination of differential ultracentrifugation, floatation in a density gradient, and immuno-isolation (Kowal et al., 2016). Although a large majority of the proteins were shared between all isolated EV subtypes, including some generally used as "exosome markers" (e.g., heat shock proteins, flotillins, major histocompatibility complex molecules), we are able to propose a few new specific markers of medium and large EVs (e.g., actinins), of endosome-derived

exosomes (co-expressing three tetraspanins CD9/CD63/CD81 and including TSG101 and syntenin-1), and of non-endosomal sEVs (some ITGs), whose validity as specific markers can be tested in all EV sources, which will hopefully enable further functional studies. Indeed, knowing which EV markers to follow will eventually allow identification of molecular tools to specifically affect secretion of a given subtype of EVs and thus understand the patho/physiological function of a particular subtype. We hope that the near future will thus provide the necessary technical advances and subsequent understanding of the various and fundamental roles of each type of EV. The potential development of these delivery packets for efficient therapeutic strategies in cancer and in many other diseases depends on these next steps.

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