Cell

Leading Edge
Review

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

Mercedes Tkach[1] and Clotilde Théry[1,*]
[1]Institut Curie, PSL Research University, INSERM U932, 75005 Paris, France
*Correspondence: clotilde.thery@curie.fr
http://dx.doi.org/10.1016/j.cell.2016.01.043

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

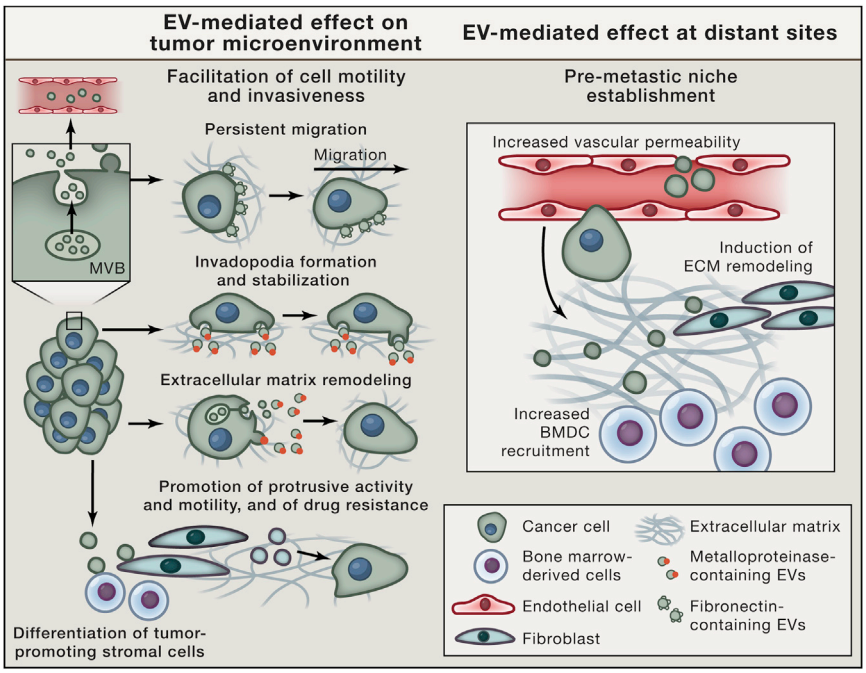

Figure 1. EV-Mediated Effects Promoting Tumor Growth, Invasiveness, and Metastasis

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