

Review Article

Exosome/microvesicle-mediated epigenetic reprogramming of cells

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Received October 19, 2010; accepted October 21, 2010; Epub October 22, 2010; Published January 1, 2011

Abstract: Microvesicles (MVs) are released by different cell types and may remain in the extracellular space in proximity of the cell of origin or may enter the biological fluids. MVs released by tumor cells are detectable in patients with cancer and their number in the circulation correlates with poor prognosis. Recent studies demonstrated that MVs may act as mediator of cell-to-cell communication thus ensuring short- and long-range exchange of information. Due to their pleiotropic effects, MVs may play a role in the prothrombotic state associated with cancer as well as in cancer development and progression. It has been recently shown that MVs may induce epigenetic changes in target cells by transferring genetic information. This finding suggests that tumor and stromal cells may talk each other via MVs to establish a favorable tumor niche and to promote tumor growth, invasiveness and progression. Moreover, MVs contain genetic material under the form of mRNA and microRNA, that may allow an easy screening for cancer genetic markers and offer new diagnostic and prognostic information. This review presents an overview of the many biological actions of MVs and of the potential role of MV-mediated exchange of genetic information among cells in tumor biology.

Keywords: Microvesicles, exosomes, angiogenesis, tumor niche, stem cells

Introduction

Exchange of information between cells may involve soluble factors or direct cell-to-cell contact. This may include cytonemes and tunneling nanotubules. Cytonemes connect neighboring cells enabling ligand-receptor-mediated transfer of surface-associated molecules. Tunneling nanotubules, by establishing conduits between cells, allow the transfer not only of surface molecules but also of cytoplasmic components [1, 2]. In addition, cells may communicate through membrane transfer by the secretion of exosomes/microvesicles [3]. Such membrane transfer is associated with the acquisition of functional properties in the recipient cells related to the transferred proteins, receptors and/or bioactive lipids. Recent studies indicate that exosomes/microvesicles shuttle mRNAs and microRNAs, raising the possibility that the trans-

fer of genetic information might alter the function of recipient cells. The vesicles detectable both *in vitro* and *in vivo* are a mixed population of exosomes derived from the endosomal membrane compartment [4, 5] and of shedding vesicles, originated by direct budding from the cell plasma membrane [6]. For this reason in the present review we will call them collectively microvesicles (MVs). Once secreted, MVs may remain in the extracellular space in proximity of the cell of origin or may enter the biological fluids such as plasma, urine, milk, cerebrospinal fluid, amniotic fluid and tumor effusions, thus allowing long-range exchange of MV-mediated information. In normal subjects, the majority of MVs present in the circulation, designed also as microparticles, are derived from platelets [7], and in a smaller amount from other blood cells and endothelial cells [8]. However, many cell types including tumor cells are able to release

MVs and in cancer patients tumor-derived MVs are detectable within the biological fluids [9, 10].

Intracellular origin and characteristics of released MVs

Irrespective from their origin, MVs are circular membrane fragments retaining the characteristics of the cell of origin and containing cytosol. Depending on their intracellular origin and the mechanisms of formation, MVs may be distinguished in shedding vesicles or exosomes.

Shedding vesicles, also named as ectosomes, microparticles or exovesicles, are rather heterogeneous with a size ranging from 100 nm to 1 µm. They are formed by budding of the cell membrane producing small cytoplasmic protrusions that undergo detachment from the cell surface (**Figure 1A**). This process depends on calcium influx, cytoskeleton reorganization and curvature-mediated lateral redistribution of membrane components with the formation of membrane nanodomains [11]. Shedding vesicles expose on their surface large amounts of phosphatidylserine and are enriched in proteins associated with membrane lipid rafts [12]. Their formation involves an increase of calcium ions that inhibits translocase and induces activation of scramblase that translocates phosphatidylserine from the inner leaflet of the cell membrane bilayer to the outer [13]. Moreover, calcium ions by activation of calpain favor the reorganization of cytoskeleton, leading to detachment of plasmamembrane protrusions from the cortical actin [14].

Exosomes are more homogenous and smaller than shedding vesicles, with a size ranging from 30 to 120 nm and have an endosomal origin [4]. The exosomes share the biochemical characteristics with the internal vesicles of the multivesicular bodies. It has been suggested that they are stored as intraluminal vesicles within multivesicular bodies of the late-endosome. The release of exosomes follows the fusion of multivesicular bodies with the cell membrane (**Figure 1B**). Tetraspanins, Alix and TSG101 are considered markers of exosomes [15]. The mechanism of assembly and sorting of the exosomes is still largely unknown since a common sorting signal for all cell types has not been yet identified [16]. Therefore, MVs differ on size and molecular composition depending on the

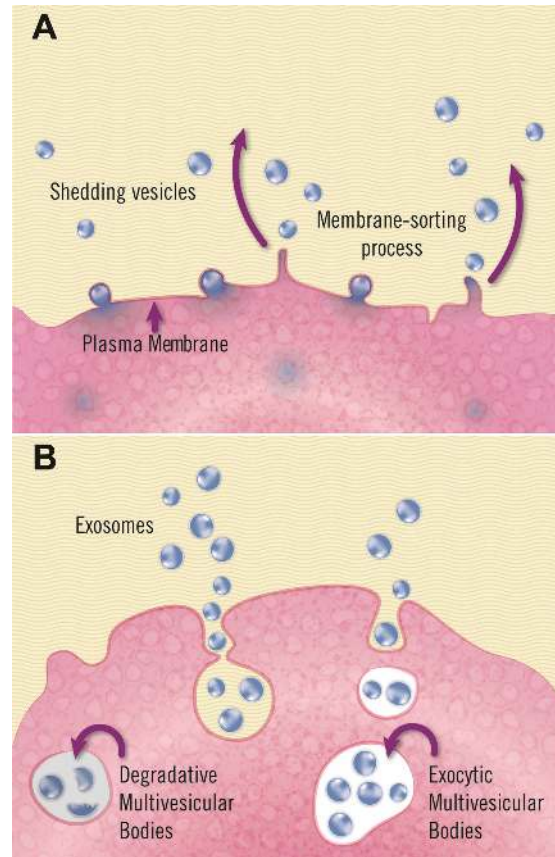


Figure 1. Production and release of shedding vesicles and exosomes. **(A)** Schematic representation of production and release from the cell surface of the shedding vesicles. Shedding vesicles are produced by budding of cell plasmamembrane. **(B)** Schematic representation of exosome release. The exocytic multivesicular bodies may fuse with membrane and release exosomes.

cell of origin and on the mechanism of formation. In addition, the content of released MVs may vary depending on whether the secretion is constitutive or consequent to cell activation.

MVs as mediators of intercellular communication

MVs released from a given cell type express on their surface the adhesion molecules of the cell of origin. Therefore, MVs may be captured through specific receptor-ligand interaction by target cells that specifically recognize them rather than just by any cell present in the micro-environment [17]. Following interaction, MVs

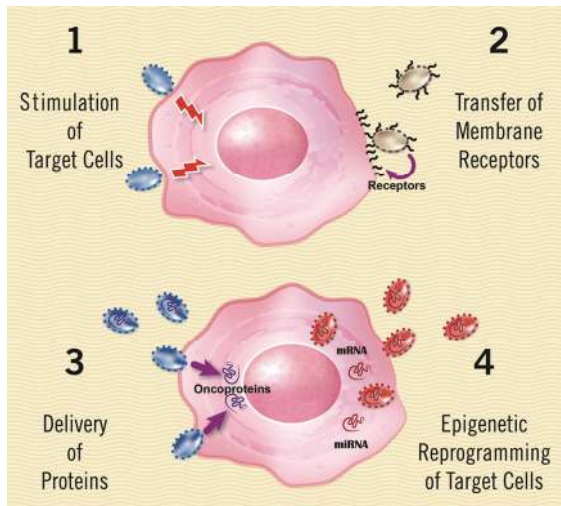


Figure 2. Schematic representation of MV-mediated cell-to-cell interaction. (1) MVs may signal through surface expressed receptors leading stimulation of target cells. (2) MVs may transfer receptors from the cell of origin to the target cell. (3) MVs may transfer oncogene products, transcription factors or infectious particles to target cells. (4) MVs may mediate a horizontal transfer of mRNA and microRNA (miRNA) inducing epigenetic changes in the target cell.

may influence the behavior of the recipient cells in different ways (**Figure 2**).

MVs may directly stimulate the cells by a surface interaction

For instance, after activation, platelet-shed MVs coated with tissue factor (TF) are able to interact with molecules, such as P-selectin, expressed on the surface of macrophages, polymorphonuclear neutrophils and platelets [18]. Platelet-derived MVs remain on the surface of these cells and their phosphatidylserine enriched membranes provide a surface for the assembly of clotting factors.

Moreover, platelet-derived MVs, may directly activate endothelial cells [19], polymorphonuclear neutrophils [20] and monocytes [21] and influence the functions of normal and malignant human hemopoietic cells [3].

MVs may act by transferring receptors between cells

For instance, MVs can transfer the adhesion

molecule CD41 from platelets to endothelial cells [22] or to tumor cells [23] conferring them pro-adhesive properties. Other receptors that have been shown to be transferred by MVs include: Fas ligand that after transferring from tumor cells to activated T cells may induce T cell apoptosis [24]; CXCR4 and CCR5 chemokine receptors that may act as co-receptors for HIV1 virus favoring the entry of the virus into cells other than the lympho-haemopoietic lineage [5, 25].

MVs may deliver proteins to target cells

For instance, it has been found that endotoxin-stimulated monocytes induce the cell death of vascular smooth muscle cells by transfer of a cell death message via encapsulated caspase-1 [26]. It has been also suggested that MVs may contribute to dissemination of certain infective agents, such as HIV or prions [27, 28]. In addition tumor-derived MVs may transfer the products of oncogenes to neighboring cells [29].

MVs may induce epigenetic changes in target cells by transferring genetic information

Tumor-derived MVs may transfer not only surface determinants but also mRNA of tumor cells to monocytes [30]. Indeed, MVs contain selected patterns of mRNA and microRNA (miRNA) associated with ribonucleoproteins involved in the intracellular traffic of RNA, suggesting a dynamic regulation of RNA compartmentalization in MVs [31].

An epigenetic reprogramming of adult haematopoietic stem/progenitor cells by MVs derived from murine embryonic stem cells was demonstrated by Ratajczak J *et al.* [32]. In these cells, MVs induce an up-regulation of early pluripotent and early hematopoietic markers and phosphorylation of MAPKp42/44 and Akt. This biologic effect has been attributed to a horizontal transfer of mRNA mediated by MVs [32]. Similarly, Valadi *et al.* [33] demonstrated that exosomes from a mouse and a human mast cell line shuttle RNAs that can be transferred to other mouse and human mast cells. After transfer to human mast cells, new mouse proteins become detectable in the recipient cells, indicating that transferred exosomal mRNA can be translated after entering the target cells. We demonstrated that MVs derived from human endothelial progenitor cells (EPC) can also act

as a vehicle for mRNA transport among cells [34]. Indeed, EPC-derived MVs activate an angiogenic program in recipient quiescent endothelial cells by transferring selected patterns of mRNA. Evidence for MV-mediated transfer of genetic information has been provided by experiments showing translation into protein of reporter mRNA such as the green fluorescence protein mRNA (GFP) [34]. Endothelial cells targeted with MVs carrying GFP mRNA produce the GFP proteins [34]. In addition, we demonstrated that MVs derived from human stem cells may deliver also *in vivo* human mRNA to mouse cells, resulting in protein translation [35, 36]. Recently Aliotta *et al.* [37] demonstrated that MV entry into bone marrow cells induces tissue-specific changes in mRNA not only by direct delivery of mRNA but also by induction of tissue specific mRNA. Besides mRNA, MVs may transfer miRNA into target cells [31, 33, 38]. Yuan *et al.* [38] demonstrated that MVs derived from mouse embryonic stem cells contain abundant miRNA and that they can transfer a subset of miRNA to mouse embryonic fibroblasts *in vitro*. We, recently, characterized miRNA shuttled by MVs released by adult human mesenchymal stem cells [31]. Hierarchical clustering and similarity analysis of miRNA showed that some miRNA are selectively accumulated within MVs and absent in the cells after MV release whereas other are retained within the cells and not secreted in MVs. This suggests a regulated process of miRNA compartmentalization and secretion by MVs. Gene ontology analysis of predicted and validated targets showed that the highly expressed miRNA in MVs derived from mesenchymal stem cells may be involved in multi-organ development, cell survival, differentiation and immune system regulation. Moreover, we demonstrated that miRNA carried by in MVs may be transferred to target cells after MV incorporation. These observations open the possibility that the MV-mediated transfer of miRNA, which are naturally occurring regulators of protein translation, can alter the expression of gene products in neighboring cells. Quesenberry and Aliotta [39] suggested that MV-mediated transfer of genetic information may play a critical role also in stem cell biology. It has been suggested that transfer of genetic information by MVs play a pivotal role in stem cell plasticity and tissue regeneration [37, 41]. This mechanism possibly contributes to the paracrine action of stem cells in the repair of tissue injury [42].

Role of MVs in tumor biology

Tumor cells release large amount of MVs and the number of circulating MVs is increased in patients with cancer and correlates with poor prognosis [9]. This may depend on the pleiotropic effects of MVs.

Tumor induced blood coagulation and MVs

Thrombotic events are common among different cancer types and stages. In this context TF is emerging as one of the main mediators involved in the hypercoagulability of cancer patients. MVs expressing TF have a central role in triggering the coagulation cascade. It has been shown that the majority of TF-bearing MVs are of tumor origin [43]. Indeed, cancer cells overexpress TF [44] and may shed it from their surface [45]. A correlation between the presence of TF-bearing MVs and a increased risk of thromboembolic events has been established [43]. The pro-coagulant properties of MVs depend also on their over-expression of phosphatidylserine providing a catalytic site for the coagulation cascade [8, 46]. The surface expression of phosphatidylserine facilitates the assembly and activation of tenase and pro-thrombinase complexes, thus propagating the coagulation process [47]. Besides tumor cells, MVs in cancer patients may derive from other cells of the host, such as monocytes, erythrocytes, endothelial cells, platelets and tumor stromal cells [48]. Monocyte-derived TF-bearing MVs originate from lipid rafts and fuse with activated platelets to initiate coagulation [12]. Indeed, MVs expressing P-Selectin glycoprotein ligand 1 may interact with P-Selectin-bearing platelets and increase TF-FVIIIa activation. Also MVs released from activated endothelial cells may induce thrombosis by a TF-dependent mechanism [49]. The role of MV-triggered coagulation has been implicated not only in the thromboembolic events but also in delivering signals within the tumor microenvironment, leading to proliferation of dormant cancer stem cells [50, 51] or to activation of the angiogenic shift of tumors [47].

Tumor niche, angiogenesis, metastasis and MVs

Tumor growth involves a coordinated effort of different cell types to establish a favourable microenvironment. In this context, tumor cells are thought to orchestrate the behaviour of en-

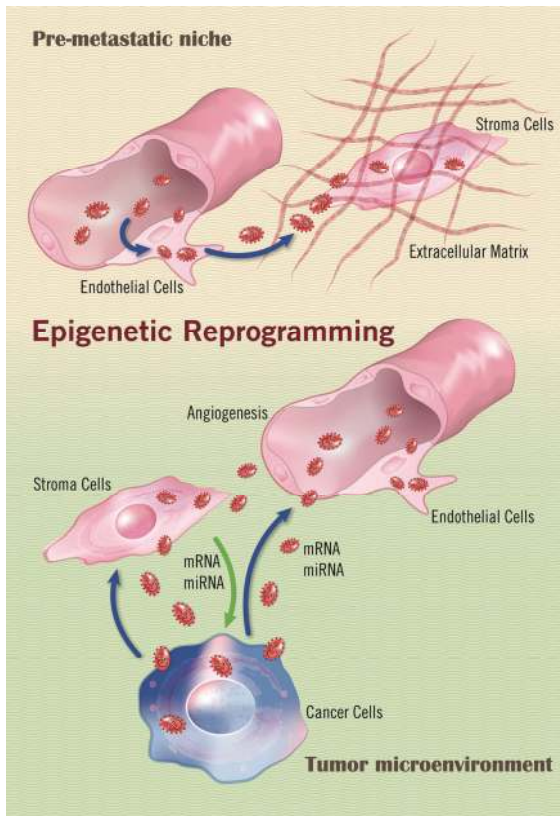


Figure 3. Schematic representation of exchange of genetic information among tumor and stroma cells. Tumor and normal stroma cells talk each other to establish a favorable tumor niche and to promote tumor growth, invasiveness and progression via mutual shedding of MVs. This induces changes in the behaviour of normal cells and increases tumorigenicity. The MVs released from cancer cells may reprogram the phenotype of cells present in the tumor microenvironment by delivering to cells the mRNA and/or microRNA (miRNA). This may induce epigenetic alterations in tumor endothelial cells promoting the angiogenic shift of the tumor. Moreover, tumor-derived MVs, by entering the circulation and biological fluids, may allow long distance cell-to-cell communication favoring the development of a pre-metastatic niche.

endothelial cells, fibroblasts, monocytes, adipocytes and immune cells [47]. Besides soluble factors, MVs emerged in recent years as potential candidates of cell-to-cell communication (**Figure 3**). An example of such role of MVs is the release by tumors of EMMPRIN a transmembrane glycoprotein identified as a tumor-derived factor that can stimulate matrix metalloproteinase expression in fibroblasts and conse-

quently facilitate tumor invasion and metastasis [52]. It has been shown that EMMPRIN is released from the surface of tumor cells via MV shedding by a pathway dependent on protein kinase C, calcium mobilization and mitogen-activated protein kinase. These results outline a feature of tumor-stromal interaction whereby degradation of extracellular matrix by fibroblasts is controlled through the microvesicular release of EMMPRIN from tumor cells. Recently, Castellana *et al.* [47] provided evidence that tumor and normal stroma cells communicate via mutual shedding of MVs. This induces changes in the behaviour of normal cells and increases tumorigenicity [53]. MVs derived from prostate carcinoma cell lines express matrix metalloproteinases (MMP) and extracellular MMP inducer at their surface, suggesting a role in extracellular matrix degradation. Moreover, MVs induce the activation of fibroblasts increasing motility and resistance to apoptosis and promote MV shedding from activated fibroblasts. In turn, MVs derived from activated fibroblasts increase migration and invasion of highly metastatic prostate carcinoma cells by a mechanism, at least in part, dependent on membrane-bound CX3CL1/fractalkine ligand for chemokine receptor CX3CR1 [53]. Lima *et al.* [54] demonstrated that tumor-derived MVs modulate the establishment of metastatic melanomas by a mechanism dependent on phosphatidylserine, possibly by down-regulating the host's inflammatory and immune responses.

It has been also shown that the rat pancreatic adenocarcinoma-derived MVs may form the pre-metastatic niche that allows the development of lung metastasis [55]. In this study Jung *et al.* [55] demonstrated that *in vivo* pre-metastatic changes result from cooperation of both, exosomes and soluble matrix. Recently, Al-Nedawi *et al.* [29] demonstrated that tumor derived MVs may act as intercellular transfer of an oncogene. Some cells of aggressive human brain tumours express a truncated and oncogenic form of the epidermal growth factor receptor, known as EGFRvIII. This oncogene receptor can be 'shared' between glioma cells by intercellular transfer of membrane-derived MVs, leading to the transfer of oncogenic activity [29]. These experiments suggest that tumor derived MVs may allow a horizontal propagation of oncogenes among different subsets of cancer cells thereby transforming their phenotype. The shedding of MVs from tumor cells into the sur-

rounding environment is regulated by a small GTP-binding protein ARF6. Inhibition of ARF6 activation is associated with PKC-mediated phosphorylation of myosin light-chain, which blocks MV shedding [56]. Released MVs contain selected cellular components involved in cell adhesion and motility [56]. Wysoczynski and Ratajczak [57] demonstrated that lung cancer cells once secreted MVs in response to non-apoptotic doses of hypoxia and irradiation are able to activate and chemoattract stroma fibroblasts and endothelial cells thus inducing the expression of several pro-angiopoietic factors in stromal cells. Moreover, stroma cells stimulated by tumor-derived MVs enhance the metastatic potential of lung cancer cells *in vivo* suggesting that MVs are important constituents of tumor microenvironment with a pivotal role in tumor progression, metastasis and angiogenesis. Tumor-derived MVs carrying hsp90a may also favor cancer cell invasion by activating plasmin [58].

Moreover, tumor-derived MVs may modulate tumor angiogenesis. MVs shed by ovarian cancer cells may induce endothelial cell activation and angiogenesis by a CD147-mediated mechanism [59]. Human colorectal cancer cells express TF-bearing MVs under control of activation of K-ras oncogene and inactivation of the p53 tumor suppressor, in a manner dependent on MEK/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3'-kinase (PI3K) [44]. This study established a link between the levels of MV-associated TF activity and the genetic status of cancer cells. Moreover, it suggested that TF-bearing MVs are an important effector of the K-ras-dependent tumorigenic and angiogenic phenotype *in vivo*. Beside TF, tumor-derived MVs may stimulate tumor growth and metastasis by promoting endothelial cell migration, invasion, and tube formation, and inducing *in vivo* neovascularization by a mechanism dependent on sphingomyelin expression [60]. Tetraspanin, a constitutive component of exosomes released by tumor cells, may also contribute to induce endothelial cell activation and angiogenesis [61]. Tetraspanin-enriched MVs have been suggested to play a central role in pre-metastatic niche preparation [62].

Al-Nedawi *et al.* [63] suggested that the angiogenic switch in tumors may be induced by MV-mediated transfer of the oncogenic EGFR that in turn activate an autocrine expression of VEGF.

Moreover, activated endothelial cells may communicate at distance by transferring Delta-like 4 Notch ligand *via* MVs, thus propagating the angiogenic signal [64].

Metalloproteinases harboured by endothelial MVs regulate the proteolytic activity on matrix required to elicit angiogenesis [65]. MVs shed by activated endothelial cells express on their surface matrix metalloproteinases that may allow focal proteolytic activity favoring endothelial cell invasion [65]. MV-associated urokinase plasminogen activator also favors the invasion of prostate cancer cells [66]. By carrying active metalloproteinases, MVs may contribute to stromal remodelling leading to tumor cell invasion [67]. It has been shown that CD147/extracellular MMP inducer is expressed in MVs derived from epithelial ovarian cancer cells and that CD147-positive MVs may promote an angiogenic phenotype in endothelial cells *in vitro*. The treatment of ovarian cancer cells with small interfering RNA against CD147 suppresses the angiogenic potential of MVs suggesting that vesicles shed by ovarian cancer cells may induce proangiogenic activities by a CD147-mediated mechanism [59].

Data relative to the role of EPC in tumor angiogenesis remain controversial [68]. Some experimental studies have shown that EPC are capable of incorporating and differentiating in vessel-like structures contributing to tumor angiogenesis [69, 70]. However, other studies suggested that EPC promote angiogenesis without a direct contribution in the formation of vessels with only a perivascular localization without incorporation into the vessel wall [71, 72]. In the human tumor vasculature, co-expression of Y chromosome and endothelial markers varies from 1 up to 12%, depending on the tumor type in patients submitted to bone marrow transplantation from donors of opposite sex [73]. Therefore, it has been suggested that EPC stimulate in a paracrine manner resident endothelial cells [68, 73]. In line with this possibility, we found that MVs released by human EPC activate an angiogenic program in normal endothelial cells by a horizontal transfer of mRNA [34]. *In vitro*, after incorporation in normal endothelial cells by interaction with α 4- and β 1-integrins expressed on MV surface, MVs promote endothelial cell survival, proliferation and organization in capillary-like structures [34]. This effect also occurs *in vivo* in SCID mice, where MV-

stimulated human endothelial cells implanted subcutaneously within Matrigel organize in a patent vessel network connected with the murine vasculature. Pre-treatment of MVs with elevated concentration of RNase reduces their angiogenic activity suggesting a critical role for RNA transfer following MV incorporation. The angiogenic effect correlates with transfer of mRNA following the MV incorporation within the normal endothelial cells after adhesion receptor-mediated interaction. The molecular analysis of mRNA indicates that MVs derived from EPC shuttle a specific subset of cellular mRNA, including mRNA associated with pathways relevant to angiogenesis such as the PI3K/AKT and eNOS signaling pathways. Protein expression and functional studies demonstrated that PI3K and eNOS are up-regulated in target cells after MV incorporation. The transfer of genetic information by MVs has been also shown for MVs derived from tumor cells (**Figure 3**). Indeed, MVs released by colorectal cancer cells are enriched in cell cycle-related mRNAs and promote endothelial cell proliferation, suggesting that tumor derived MVs may be involved in the angiogenic shift and in tumor progression [74]. Skog *et al.* [75] demonstrated that MVs released by glioblastoma tumour contain selected patterns of mRNA, miRNA and angiogenic proteins. These MVs after incorporation by normal host cells, such as brain microvascular endothelial cells, transfer mRNA for a reporter protein that can be translated by the recipient cells. Moreover, these MVs carry angiogenic proteins and *in vitro* induce tube formation by endothelial cells. This study confirms that MVs released by cancer cells may deliver specific and functional RNAs and proteins to recipient cells in the tumor microenvironment. In fact, recent studies suggest epigenetic mutations in stroma and tumor-derived endothelial cells [76].

Tumor-derived MVs in immuno-escape and chemoresistance

Recently, the role of MVs in immune response has been extensively reviewed by Thery C *et al.* [77]. Depending on the cell of origin and the molecular composition MVs may either stimulate or inhibit the immune response. Indeed, MVs may act as direct peptide-MHC complex presentation to T cell, may transfer antigen or peptide-MHC complex to dendritic cells leading to indirect antigen presentation, may activate natural killer cells and macrophages or confer

protection of T cells against “activation-induced cell death”. Moreover, CD40L bearing MVs may stimulate B cell activation and antibody production [78]. Based on these properties MVs derived from mature dendritic cells have been used as vaccines to stimulate efficient antitumor cytotoxic T-lymphocyte response [79, 80].

On the other hand MVs may inhibit immune response favoring escape of tumor cells from immune surveillance. This may depend on the ability of tumor-derived MVs to induce apoptosis in activated anti-tumor T cells, impairment of monocyte differentiation into dendritic cells and induction of myeloid suppressive cells [10, 81]. Moreover, vesicular shedding of terminal components of complement from the cell plasma membrane [82] by a mechanism called “complement resistance” may confer protection to tumor cells from antibody mediated immune response. Similarly shedding of Fas ligand from tumor cell surface reduces sensitivity to T cell Fas-mediated apoptosis [83].

MVs may also facilitate tumor cell survival by expulsion of therapeutic drugs from cancer cells. A correlation between MV release and multidrug resistance has been established. Cancer cells resistant to chemotherapy were found to release significant more MVs than those sensitive to chemotherapy [84]. In addition, these MVs contained significant more chemotherapeutic drugs than those derived from cancer cells sensitive to chemotherapy. Based on this observation it has been suggested that chemotherapeutic agents may be extruded from cells *via* MVs [85].

MVs as diagnostic tool in cancer

The proteomic and the cytofluorimetric analysis [86, 87] of MVs present in the circulation may provide qualitative and quantitative information on MVs present in blood. It has been suggested that mucin-bearing MVs may be useful in the early detection of adenocarcinomas [88, 89]. In glioblastomas the tumor-specific EGFRvIII marker is expressed by MVs and may provide diagnostic information [75]. The proteomic analysis on urinary MVs allowed identification of potential biomarkers of bladder cancer [90, 91]. Using a sandwich ELISA to capture and quantify exosomes in plasma Logozzi *et al.* [92] demonstrated high levels of exosomes expressing CD63 and caveolin-1 in plasma of patients with

melanoma. Several studies have suggested that the level of circulating MVs correlate with the prognosis and survival of patients with cancer [9, 93].

The recent identification that MVs carry specific patterns of mRNA and miRNA stimulate research aimed to find a molecular signature of circulating MVs in cancer patients that may be relevant for diagnostic or prognostic purpose. Cancer-specific mRNA has been detected in circulating MVs in patients with glioblastoma [75], in gastric cancer [94] and in breast cancer patients [95]. The profile of miRNA carried by MVs may also be exploited for diagnostic purposes [96]. miRNA in plasma would be degraded by RNase if not protected by a membrane envelope. This envelope is provided by MVs that therefore may allow detection of miRNA in the circulation. The secretory mechanisms and intercellular transfer of miRNA via MVs have been recently studied by Kosaka N *et al.* [97]. Hunter *et al.* [98] defined the miRNA expression in circulating plasma MVs in normal subjects. Cancer-specific miRNA have been detected in patients with ovarian cancer showing that miRNA profile may vary with the disease stage [99]. Studies on lung cancer demonstrated that the profile of circulating miRNA present in MVs is similar to that of tumor-derived miRNA, suggesting that miRNA detectable in MVs might be useful as a screening test for lung adenocarcinoma [100]. miR-21 which is over-expressed in glioblastoma is also detectable in the circulating MVs [75]. Overall these studies suggest that gaining more information on the molecular composition of MVs may offer new diagnostic and prognostic information. Moreover, Renzulli *et al.* [101], on the base of the observation that prostate cancer tumor cells may induce via MVs prostate specific gene expression in circulating monocytes, stem cells or other cells, altering their phenotype, proposed novel therapeutic strategies to block the MV release from cancer cells or the MV entry in recipient cells.

Conclusion

In conclusion, MVs have pleiotropic biological actions implicated in signalling among cells and in transferring gene products. The biological action of MVs may take place either in a defined microenvironment either in long distance as they may enter the circulation and other biological

fluids. The presence of MVs in body fluids, their number, cellular origin, composition and function, can depend on disease state and represents a new non-invasive potential diagnostic tool. The finding that MVs may exchange genetic information among cells suggests that tumor and stromal cells talk each other to establish a favorable tumor niche and to promote tumor growth, invasiveness and progression.

Moreover, the fact that MVs contain genetic material under the form of RNA, may allow an easy screening for cancer genetic markers. The identification of signals delivered by MVs may also open new therapeutic strategies. In particular, the removal from plasma of harmful MVs may be beneficial in tumors where MVs deliver thrombogenic and cell transforming signals.

Disclosure

Stefania Bruno is a full time employee of Sister, Palazzo Pignano; Ciro Tetta is a full time employee of Fresenius Medical Care. All the other authors declared no competing interests.

Acknowledgements

Our research is supported by grants from Regione Piemonte, Piattaforme Biotecnologiche, project PiSTEM and Oncoprot and from Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) project PRIN08.

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