

## Review Article

# Exosome/microvesicle-mediated epigenetic reprogramming of cells

Giovanni Camussi<sup>1</sup>, Maria-Chiara Deregibus<sup>1</sup>, Stefania Bruno<sup>2</sup>, Cristina Grange<sup>1</sup>, Valentina Fonsato<sup>1</sup>, Ciro Tetta<sup>3</sup>

<sup>1</sup>Department of Internal Medicine, Centre for Molecular Biotechnology and Centre for Research in Experimental Medicine (CeRMS), Torino, Italy and <sup>2</sup>Sis-Ter, Palazzo Pignano, Italy and <sup>3</sup>Fresenius Medical Care, Bad Homburg, Germany.

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

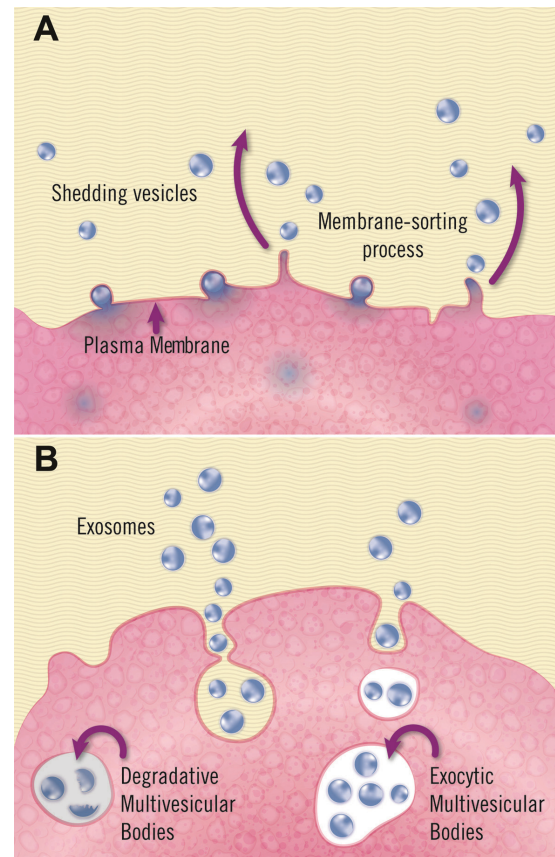
MVs and in cancer patients tumor-derived MVs are detectable within the biological fluids [9, 10].

#### 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  $\mu$ m. 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

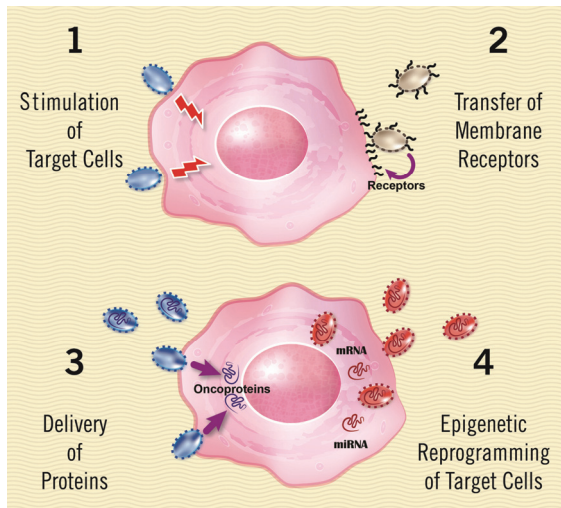


**Figure 1.** Production and release of shedding vesicles and exosomes. (A) Schematic representation of production and release from the cell surface of the shedding vesicles. Shedding vesicles are produced by budding of cell plasmamembrane. (B) Schematic representation of exosome release. The exocytic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

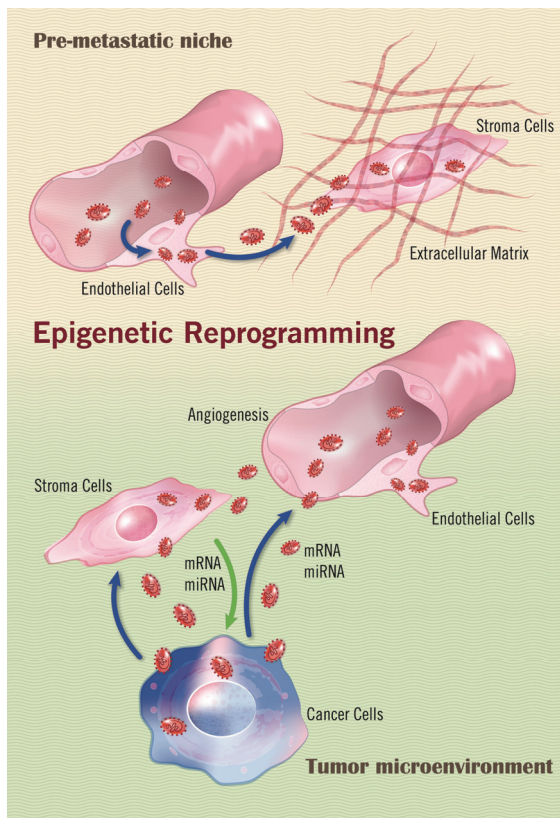
#### *Tumor induced blood coagulation and MVs*

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 procoagulant 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  $\alpha 4$ - and  $\beta 1$ -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.

**Please address correspondence to:** Giovanni Camussi, PhD, Dipartimento di Medicina Interna, Ospedale Maggiore S. Giovanni Battista, Corso Dogliotti 14, 10126, Torino, Italy; Phone +39-011-6336708, Fax +39-011-6631184, E-mail: [giovanni.camussi@unito.it](mailto:giovanni.camussi@unito.it)

## References

- [1] Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for intercellular organelle transport. *Science* 2004;303: 1007-1010.
- [2] Sherer NM, Mothes W. Cytonemes and tunneling nanotubules in cell-cell communication and viral pathogenesis. *Trends Cell Biol* 2008;18: 414-420.
- [3] 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;20:1487-1495.



- [4] 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; 94:3791-3799.
- [5] Rozmyslowicz T, Majka M, Kijowski J, Murphy SL, Conover DO, Poncz M, Ratajczak J, Gaulton GN, Ratajczak MZ. Platelet- and megakaryocyte-derived microparticles transfer CXCR4 receptor to CXCR4-null cells and make them susceptible to infection by X4-HIV. *AIDS* 2003; 17: 33-42.
- [6] Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. *Trends Cell Biol* 2008;19:43-51.
- [7] George JN, Thoi LL, McManus LM, Reimann TA.. Isolation of human platelet membrane microparticles from plasma and serum. *Blood* 1982; 60: 834-840.
- [8] Martinez MC, Tesse A, Zobairi F, Andriantsitohaina R. Shed membrane microparticles from circulating and vascular cells in regulating vascular function. *Am J Physiol Heart Circ Physiol* 2005; 288: H1004-H1009.
- [9] Kim HK, Song KS, Park YS, Kang YH, Lee YJ, Lee KR, Kim HK, Ryu KW, Bae JM, Kim S. Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor. *Eur J Cancer* 2003; 39: 184-91.
- [10] Iero M, Valenti R, Huber V, Filipazzi P, Parmiani G, Fais S, Rivoltini L. Tumour-released exosomes and their implications in cancer immunity. *Cell Death Differ* 2008;15:80-8.
- [11] Schara K, Jansa V, Sustar V, Dolinar D, Pavlic JI, Lokar M, Kralj-Iglic V, Veranic P, Iglic A. Mechanismss for the formation of membranous nanostructures in cell-to-cell communication. *Cell Mol Biol Lett* 2009;14:636-656.
- [12] Del Conde I, Shrimpton CN, Thiagarajan P, López JA. Tissue-factor-bearing microvesicles arise from lipids rafts and fuse with activated platelets to initiate coagulation. *Blood* 2005; 106: 1604-1611.
- [13] Hugel B, Martinez MC, Kunzelmann C, Freyssinet JM. Membrane microparticles: two sides of the coin. *Physiology (Bethesda)* 2005;20:22-27.
- [14] 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.
- [15] Thery C, Amigorena S, Raposo G, Clyton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol*. Wiley Online Library, 2006 Apr;Chapter 3:Unit 3.22.
- [16] Johnstone RM. Exosomes biological significance: A concise review. *Blood Cells Mol Dis* 2006;36:315-321.
- [17] Lösche W, Scholz T, Temmler U, Oberle V, Claus RA. Platelet-derived microvesicles transfer tissue factor to monocytes but not to neutrophils. *Platelets* 2004; 15:109-15.
- [18] Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. *J Thromb Haemost* 2005; 3: 1590-1596.
- [19] Barry OP, Pratico D, Lawson JA, FitzGerald GA. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *J Clin Invest* 1997; 99: 2118-2127.
- [20] Miyamoto S, Kowalska MA, Marcinkiewicz C, Marcinkiewicz MM, Mosser D, Edmunds LH Jr, Niewiarowski S. Interaction of leukocytes with platelet microparticles derived from outdated platelet concentrates. *Thromb Haemost* 1998; 80: 982-988.
- [21] Barry OP, Kazanietz MG, Praticò D, FitzGerald GA. Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2-dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. *J Biol Chem* 1999; 274: 7545-7556.
- [22] Barry OP, Praticò D, Savani RC, FitzGerald GA. Modulation of monocyte-endothelial cell interactions by platelet microparticles. *J Clin Invest* 1998; 102:136-144.
- [23] Janowska-Wieczorek A, Majka M, Kijowski J, Baj-Krzyworzeka M, Reza R, Turner AR, Ratajczak J, Emerson SG, Kowalska MA, Ratajczak MZ. Platelet-derived microparticles bind to hematopoietic progenitor cells and enhance their engraftment. *Blood* 2001; 98:3143-3149.
- [24] 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; 11:1010-1020.
- [25] Mack M, Kleinschmidt A, Brühl H, Klier C, Nelson PJ, Cihak J, Plachý J, Stangassinger M, Erfle V, Schlöndorff D. Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immunodeficiency virus 1 infection. *Nat Med* 2000; 6:769-775.
- [26] Sarkar A, Mitra S, Mehta S, Raices R, Wewers MD. Monocyte derived microvesicles deliver a cell death message via encapsulated caspase-1. *PLoS One* 2009; 4: e7140.
- [27] Facler OT, Peterlin BM. Endocytic entry of HIV-1. *Curr Biol* 2000;10:1005-1008.
- [28] Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, Laude H, Raposo G. Cells release prions in association with exosomes. *Proc. Natl Acad Sci USA* 2004; 101:9683-9688.
- [29] Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J. Intercellular transfer of the oncogenic receptor EGFRvIII by Microvesi-

- cles derived from tumour cells. *Nat Cell Biol* 2008;10:619-24.
- [30] Baj-Krzyworzeka M, Szatanek R, Weglarczyk K, Baran J, Urbanowicz B, Brański P, Ratajczak MZ, Zembala M. Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes. *Cancer Immunol Immunother* 2006; 55:808-818.
- [31] Collino F, Deregibus MC, Bruno S, Sterpone L, Aghemo G, Viltono L, Tetta C, Camussi G. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. *PLoS One* 2010;5:e11803.
- [32] Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, Ratajczak MZ. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* 2006; 20:847-856.
- [33] Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007; 9:654-659.
- [34] Deregibus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, Bruno S, Bussolati B, Camussi G. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood* 2007; 110:2440-2448.
- [35] Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, Morando L, Busca A, Falda M, Bussolati B, Tetta C, Camussi G. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J Am Soc Nephrol* 2009; 20:1053-1067.
- [36] Herrera MB, Fonsato V, Gatti S, Deregibus MC, Sordi A, Cantarella D, Calogero R, Bussolati B, Tetta C, Camussi G. Human liver stem cell-derived Microvesicles accelerate hepatic regeneration in hepatectomized rats. *J Cell Mol Med* 2010; 14:1605-1618.
- [37] Aliotta JM, Pereira M, Johnson KW, de Paz N, Dooner MS, Puente N, Ayala C, Brilliant K, Berz D, Lee D, Ramratnam B, McMillan PN, Hixson DC, Josic D, Quesenberry PJ. Microvesicle entry into marrow cells mediates tissue-specific changes in mRNA by direct delivery of mRNA and induction of transcription. *Exp Hematol* 2010; 38:233-45.
- [38] Yuan A, Farber EL, Rapoport AL, Tejada D, Deniskin R, Akhmedov NB, Farber DB. Transfer of microRNAs by embryonic stem cell microvesicles. *PLoS One* 2009; 4:e4722.
- [39] Quesenberry PJ, Aliotta JM. The paradoxical dynamism of marrow stem cells: considerations of stem cells, niches, and microvesicles. *Stem Cell Rev* 2008; 4:137-147.
- [40] Quesenberry PJ, Dooner MS, Aliotta JM. Stem cell plasticity revisited: the continuum marrow model and phenotypic changes mediated by microvesicles. *Exp Hematol* 2010; 38:581-592.
- [41] Deregibus MC, Tetta C, Camussi G. The dynamic stem cell microenvironment is orchestrated by microvesicle-mediated transfer of genetic information. *Histol Histopathol* 2010; 25: 397-404.
- [42] Camussi G, Deregibus MC, Tetta C. Paracrine/endocrine mechanism of stem cells on kidney repair: role of microvesicle-mediated transfer of genetic information. *Curr Opin Nephrol Hypertens* 2010; 19:7-12.
- [43] Zwicker JI, Liebman HA, Neuberg D, Lacroix R, Bauer KA, Furie BC, Furie B. Tumor-derived tissue factor-bearing microparticles are associated with venous thromboembolic events in malignancy. *Clin Cancer Res* 2009; 15:6830-6840.
- [44] Yu JL, May L, Lhotak V, Shahrzad S, Shirasawa S, Weitz JI, Coomber BL, Mackman N, Rak JW. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. *Blood* 2005; 105:1734-1741.
- [45] Yu JL, Rak JW. Shedding of tissue factor (TF)-containing microparticles rather than alternatively spliced TF is the main source of TF activity released from human cancer cells. *J Thromb Haemost* 2004; 2:2065-2067.
- [46] Aharon A, Brenner B. Microparticles, thrombosis and cancer. *Best Pract Res Clin Haematol* 2009; 22: 61-69.
- [47] Castellana D, Toti F, Freyssinet JM. Membrane microvesicles: macromessengers in cancer disease and progression. *Thromb Res* 2010; 125 Suppl 2: S84-88.
- [48] Rak J, Milsom C, Yu J. Tissue factor in cancer. *Curr Opin Hematol*. 2008; 15:522-528.
- [49] Abid Hussein MN, Böing AN, Biró E, Hoek FJ, Vogel GM, Meuleman DG, Sturk A, Nieuwland R. Phospholipid composition of in vitro endothelial microparticles and their in vivo thrombogenic properties. *Thromb Res* 2008; 121: 865-871.
- [50] Milsom C, Yu J, May L, Meehan B, Magnus N, Al-Nedawi K, Luyendyk J, Weitz J, Klement P, Broze G, Mackman N, Rak J. The role of tumor- and host-related tissue factor pools in oncogene-driven tumor progression. *Thromb Res* 2007;120 Suppl 2: S82-91.
- [51] Muralidharan-Chari V, Clancy JW, Sedgwick A, D'Souza-Schorey C. Microvesicles: mediators of extracellular communication during cancer progression. *J Cell Sci* 2010; 123:1603-1611.
- [52] Sidhu SS, Mengistab AT, Tauscher AN, LaVail J, Basbaum C. The microvesicle as a vehicle for EMMPRIN in tumor-stromal interactions. *Oncogene* 2004; 23:956-963.

- [53] Castellana D, Zobairi F, Martinez MC, Panaro MA, Mitolo V, Freyssinet JM, Kunzelmann C. 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: 785-793.
- [54] Lima LG, Chamas R, Monteiro RQ, Moreira ME, Barcinski MA. Tumor-derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner. *Cancer Lett* 2009; 283:168-175.
- [55] Jung T, Castellana D, Klingbeil P, Cuesta Hernández I, Vitacolonna M, Orlicky DJ, Roffler SR, Brodt P, Zöller M. CD44v6 dependence of premetastatic niche preparation by exosomes. *Neoplasia* 2009; 11:1093-10105.
- [56] Muralidharan-Chari V, Clancy J, Plou C, Romao M, Chavrier P, Raposo G, D'Souza-Schorey C. ARF6-regulated shedding of tumor cell-derived plasma membrane microvesicles. *Curr Biol* 2009; 19: 1875-1885.
- [57] Wysoczynski M, Ratajczak MZ. Lung cancer secreted microvesicles: underappreciated modulators of microenvironment in expanding tumors. *Int J Cancer* 2009; 125: 1595-603.
- [58] McCready J, Sims JD, Chan D, Jay DG. Secretion of extracellular hsp90alpha via exosomes increases cancer cell motility: a role for plasminogen activation. *BMC Cancer* 2010; 10: 294-304.
- [59] Millimaggi D, Mari M, D'Ascenzo S, Carosa E, Jannini EA, Zucker S, Carta G, Pavan A, Dolo V. Tumor vesicle-associated CD147 modulates the angiogenic capability of endothelial cells. *Neoplasia* 2007; 9: 349-357.
- [60] Kim CW, Lee HM, Lee TH, Kang C, Kleinman HK, Gho YS. Extracellular membrane vesicles from tumor cells promote angiogenesis via sphingomyelin. *Cancer Res* 2002; 62: 6312-6317.
- [61] Nazarenko I, Rana S, Baumann A, McAlear J, Hellwig A, Trendelenburg M, Lochnit G, Preissner KT, Zöller M. Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation. *Cancer Res* 2010; 70: 1668-1678.
- [62] Marhaba R, Klingbeil P, Nuebel T, Nazarenko I, Buechler MW, Zoeller M. CD44 and EpCAM: cancer-initiating cell markers. *Curr Mol Med*; 8: 784-804.
- [63] 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: 3794-3799.
- [64] Sheldon H, Heikamp E, Turley H, Dragovic R, Thomas P, Oon CE, Leek R, Edelmann M, Kessler B, Sainson RC, Sargent I, Li JL, Harris AL. New mechanism for notch signaling to endothelium at a distance by Delta-like 4 incorporation into exosomes. *Blood* 2010; 116:2385-2394.
- [65] Taraboletti G, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. *Am J Pathol* 2002; 160: 673-680.
- [66] Angelucci A, D'Ascenzo S, Festuccia C, Gravina GL, Bologna M, Dolo V, Pavan A. Vesicle-associated urokinase plasminogen activator promotes invasion in prostate cancer cell lines. *Clin Exp Metastasis* 2000;18: 163-170.
- [67] Graves LE, Ariztia EV, Navari JR, Matzel HJ, Stack MS, Fishman DA. Proinvasive properties of ovarian cancer ascites-derived membrane vesicles. *Cancer Res* 2004; 64: 7045-7049.
- [68] Peters BA, Diaz LA, Polyak K, Meszler L, Romans K, Guinan EC, Antin JH, Myerson D, Hamilton SR, Vogelstein B, Kinzler KW, Lengauer C. Contribution of bone marrow-derived endothelial cells to human tumor vasculature. *Nat Med* 2005; 11: 261-262.
- [69] Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999; 85: 221-228.
- [70] Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajar KA, Manova K, Benezra R, Rafii S. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med* 2001: 1194-1201.
- [71] O'Neill TJ 4th, Wamhoff BR, Owens GK, Skalak TC. Mobilization of bone marrow-derived cells enhances the angiogenic response to hypoxia without transdifferentiation into endothelial cells. *Circ Res* 2005; 97: 1027-1035.
- [72] Ziegelhoeffer T, Fernandez B, Kostin S, Heil M, Voswinckel R, Helisch A, Schaper W. Bone marrow-derived cells do not incorporate into the adult growing vasculature. *Circ Res* 2004; 94: 230-238.
- [73] De Palma M, Venneri MA, Galli R, Sergi L, Politi LS, Sampaiolesi M, Naldini L. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell* 2005; 8:211-226.
- [74] Hong BS, Cho JH, Kim H, Choi EJ, Rho S, Kim J, Kim JH, Choi DS, Kim YK, Hwang D, Gho YS. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that

- promote proliferation of endothelial cells. *BMC Genomics* 2009; 10:556.
- [75] Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 2008;10:1470-1476.
- [76] Bussolati B, Deregibus MC, Camussi G. Characterization of molecular and functional alterations of tumor endothelial cells to design anti-angiogenic strategies. *Curr Vasc Pharmacol* 2010; 8: 220-232.
- [77] Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol* 2009; 9: 581-593.
- [78] Sprague DL, Elzey BD, Crist SA, Waldschmidt TJ, Jensen RJ, Ratliff TL. Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles. *Blood* 2008; 111: 5028-5036.
- [79] Hao S, Bai O, Li F, Yuan J, Laferte S, Xiang J. Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity. *Immunology* 2007;120: 90-102.
- [80] Viaud S, Théry C, Ploix S, Tursz T, Lapierre V, Lantz O, Zitvogel L, Chaput N. Dendritic cell-derived exosomes for cancer immunotherapy: what's next? *Cancer Res* 2010; 70:1281-1285.
- [81] Valenti R, Huber V, Iero M, Filipazzi P, Parmiani G, Rivoltini L. Tumor-released microvesicles as vehicles of immunosuppression. *Cancer Res* 2007; 67:2912-2915.
- [82] Sims PJ, Faioni EM, Wiedmer T, Shattil SJ. Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. *J Biol Chem* 1988; 263: 18205-18212.
- [83] Huber V, Fais S, Iero M, Lugini L, Canese P, Squarcina P, Zaccacheddu A, Colone M, Arancia G, Gentile M, Seregini E, Valenti R, Ballabio G, Belli F, Leo E, Parmiani G, Rivoltini L. Human colorectal cancer cells induce T-cell death through release of proapoptotic microvesicles: role in immune escape. *Gastroenterology* 2005; 128: 1796-1804.
- [84] Safaei R, Larson BJ, Cheng TC, Gibson MA, Otani S, Naerdemann W, Howell SB. Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. *Mol Cancer Ther* 2005; 4:1595-1604.
- [85] Shedden K, Xie XT, Chandaroy P, Chang YT, Rosania GR. Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles. *Cancer Res* 2003; 63: 4331-4337.
- [86] Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. *Expert Rev Proteomics* 2009; 6: 267-283.
- [87] Orozco AF, Lewis DE. Flow cytometric analysis of circulating microparticles in plasma. *Cytometry A* 2010; 77: 502-514.
- [88] van Doormaal FF, Kleinjan A, Di Nisio M, Büller HR, Nieuwland R. Cell-derived microvesicles and cancer. *Neth J Med* 2009; 67: 266-273.
- [89] Tesselaar ME, Romijn FP, Van Der Linden IK, Prins FA, Bertina RM, Osanto S. Microparticle-associated tissue factor activity: a link between cancer and thrombosis? *J Thromb Haemost* 2007; 5:520-527.
- [90] Smalley DM, Ley K. Plasma-derived microparticles for biomarker discovery. *Clin Lab* 2008; 54: 67-79.
- [91] Smalley DM, Sheman NE, Nelson K, Theodorescu D. Isolation and identification of potential urinary microparticle biomarkers of bladder cancer. *J Proteome Res* 2008; 7: 2088-2096.
- [92] Logozzi M, De Milito A, Lugini L, Borghi M, Calabrò L, Spada M, Perdicchio M, Marino ML, Federici C, Iessi E, Brambilla D, Venturi G, Lozupone F, Santinami M, Huber V, Maio M, Rivoltini L, Fais S. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. *PLoS One* 2009; 4: e5219.
- [93] Helley D, Banu E, Bouziane A, Banu A, Scotte F, Fischer AM, Oudard S. Platelet microparticles: a potential predictive factor of survival in hormone-refractory prostate cancer patients treated with docetaxel-based chemotherapy. *Eur Urol* 2009; 56: 479-484.
- [94] Baran J, Baj-Krzyworzeka M, Weglarczyk K, Szatanek R, Zembala M, Barbasz J, Czupryna A, Szczepanik A, Zembala M. Circulating tumour-derived microvesicles in plasma of gastric cancer patients. *Cancer Immunol Immunother* 2010; 59: 841-850.
- [95] Friel AM, Corcoran C, Crown J, O'Driscoll L. Relevance of circulating tumor cells, extracellular nucleic acids, and exosomes in breast cancer. *Breast Cancer Res Treat* 2010; 123:613-625.
- [96] Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci* 2010; 101: 2087-2092.
- [97] Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem* 2010; 285: 17442-17452.
- [98] Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen

- TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One* 2008; 3: e3694.
- [99] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol* 2008; 110:13-21.
- [100] Rabinowits G, Gerçel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. *Clin Lung Cancer* 2009; 10:42-46.
- [101] Renzulli JF 2nd, Tatto MD, Dooner G, Aliotta J, Goldstein L, Dooner M, Colvin G, Chatterjee D, Quesenberry P. Microvesicle Induction of Prostate Specific Gene Expression in Normal Human Bone Marrow Cells. *J Urol* 2010, in press.