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Exosomes/microvesicles as a mechanism of cell-to-cell communication

Giovanni Camussi[1], Maria C. Deregibus[1], Stefania Bruno[2], Vincenzo Cantaluppi[1] and Luigi Biancone[1]

[1]*Department of Internal Medicine, Centre for Molecular Biotechnology and Centre for Research in Experimental Medicine (CeRMS), Torino, Italy and* [2]*Sis-Ter, Palazzo Pignano, Italy*

Microvesicles (MVs) are circular fragments of membrane released from the endosomal compartment as exosomes or shed from the surface membranes of most cell types. An increasing body of evidence indicates that they play a pivotal role in cell-to-cell communication. Indeed, they may directly stimulate target cells by receptor-mediated interactions or may transfer from the cell of origin to various bioactive molecules including membrane receptors, proteins, mRNAs, microRNAs, and organelles. In this review we discuss the pleiotropic biologic effects of MVs that are relevant for communication among cells in physiological and pathological conditions. In particular, we discuss their potential involvement in inflammation, renal disease, and tumor progression, and the evidence supporting a bidirectional exchange of genetic information between stem and injured cells. The transfer of gene products from injured cells may explain stem cell functional and phenotypic changes without the need of transdifferentiation into tissue cells. On the other hand, transfer of gene products from stem cells may reprogram injured cells to repair damaged tissues.

Kidney International (2010) **78,** 838–848; doi:10.1038/ki.2010.278; published online 11 August 2010

KEYWORDS: exosomes; microvesicles; renal injury; stem cells

Correspondence: *Giovanni Camussi, Dipartimento di Medicina Interna, Ospedale Maggiore S. Giovanni Battista, Corso Dogliotti 14, Torino 10126, Italy. E-mail: giovanni.camussi@unito.it*

Received 9 April 2010; accepted 9 June 2010; published online 11 August 2010

Cell-to-cell communication is required to guarantee proper coordination among different cell types within tissues. Cells may communicate by soluble factors,[1] adhesion molecule-mediated cell-to-cell interactions including cytonemes that connect neighboring cells enabling ligand–receptor-mediated transfer of surface-associated molecules, or by tunneling nanotubules that establish conduits between cells, allowing the transfer of not only surface molecules but also cytoplasmic components.[2,3] Recent studies have suggested that cells may also communicate by circular membrane fragments named microvesicles (MVs).[4] For a long time, MVs were considered to be inert cellular debris, and the frequently observed vesicles by electron microscopy in the interstitial space of tissues or in blood were considered the consequence of cell damage or the result of dynamic plasmamembrane turnover.[5] De Broe *et al.*[6] first suggested that circular plasmamembrane fragments released from human cells may result from a specific process and showed that they may carry functional membrane enzymes in the same ratio as the membrane of the cells of origin. However, only recent studies have assigned a defined function to the vesicles/exosomes released in the microenvironment by various cell types. Two distinct processes of vesicle release from the cells have been described. MVs may derive from the endosomal membrane compartment that after fusion with the plasma membrane are extruded from the cell surface of activated cells as exosomes.[7,8] Otherwise, MVs may take origin by direct budding from the cell plasma membrane as shedding vesicles.[9] As the vesicle population detectable both *in vitro* and *in vivo* is a mixed population of exosomes and shedding vesicles, we will refer to them collectively as MVs. Released MVs may remain in the extracellular space in proximity of the place of origin or may enter into the biological fluids reaching distant sites. This may explain the presence of MVs in the plasma, urine, milk, and cerebrospinal fluid. The bulk of MVs present in the circulation is derived from platelets,[10] and in less extent from other blood cells and endothelial cells.[11] The MVs derived from platelets are also designed as microparticles,[10] whereas those derived from polymorphonuclear leukocytes are also named ectosomes.[12] Finally, MVs released during morphogenesis of multicellular organisms are indicated as argosomes.[13] Besides normal cells, tumor cells may also release MVs, and in patients suffering from neoplastic diseases, tumor-derived MVs may be

detected within the biological fluids.[14,15] Therefore, MVs are an assorted population, differing in cellular origin, number, size, and antigenic composition,[16] that are shed by various cell types in physiological and pathological conditions.

FORMATION OF MVs

The release of MVs may be constitutive or consequent to cell activation by soluble agonists, by physical or chemical stress such as the oxidative stress and hypoxia, and by shear stress.[4]

(i) Exosomes have an endosome origin and are a rather homogenous population with a size ranging from 30 to 120 nm.[7] They are stored as intraluminal vesicles within multivesicular bodies of the late endosome and are released when these multivesicular bodies fuse with the cell membrane (Figure 1a). Our knowledge on the mechanism of assembly and sorting of the exosomes is only partial, because of the fact that a common sorting signal for all cell types has not so far been identified.[17] They are released by exocytosis through a mechanism dependent on cytoskeleton activation and under the regulation of p53 protein.[18]

(ii) Shedding vesicles are usually larger than exosomes with size ranging from 100 nm to 1 μm. Formation of shedding vesicles takes place from the budding of small cytoplasmic protrusions followed by their detachment from the cell surface (Figure 1b). This process is dependent on calcium influx, calpain, and cytoskeleton reorganization.[9] Schara *et al.*[19] describe two physical mechanisms involved in the formation of MVs and nanotubes: the curvature-mediated lateral redistribution of membrane components with the formation of membrane nanodomains and the plasma-mediated attractive forces between membranes. The intracellular levels of calcium ions modify the asymmetric phospholipid distribution of plasmamembranes by specific enzymes named flippase, floppase, and scramblase.[20] The increase in calcium ions inhibits translocase and induces activation of scramblase that translocates phosphatydilserine from the inner leaflet of the cell membrane bilayer to the outer. Therefore, MVs expose on their surface large amounts of phosphatydilserine and are enriched in proteins associated with membrane lipid rafts.[21] Moreover, the intracellular pathways that activate reorganization of cytoskeleton induce the detachment of plasmamembrane protrusions from the cortical actin. Calcium ions by activation of calpain that cleaves tallin and activin and of gelsolin that cleaves actin-capping proteins also favor the reorganization of cytoskeleton.[22] Therefore, depending on the cell of origin and on the mechanism of formation, MVs vary on size and molecular composition.

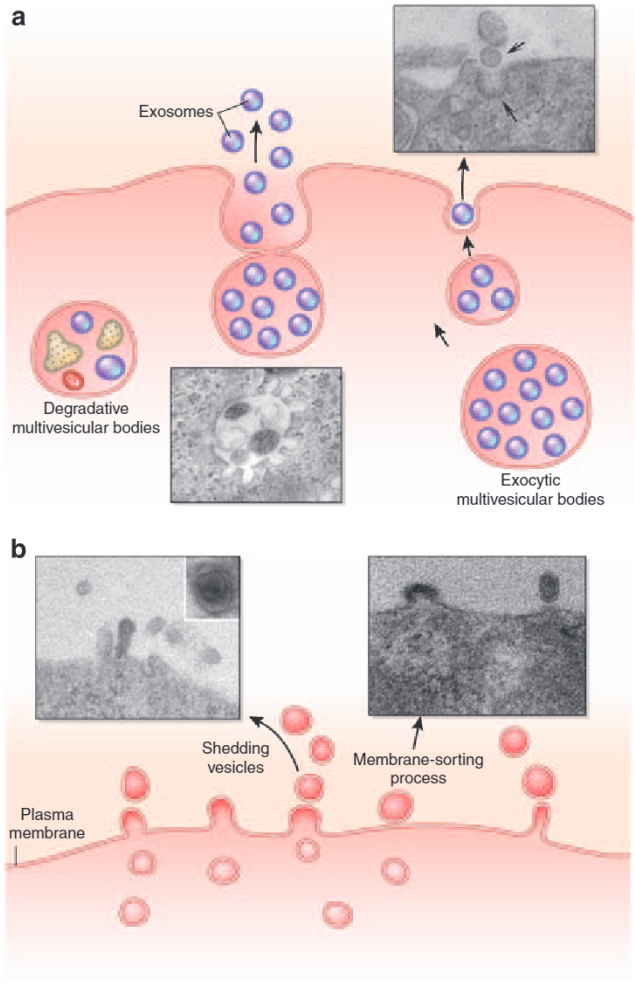


Figure 1 | Schematic representation of exosome and shedding vesicle formation. (**a**) Release of exosomes. Exosomes are accumulated within the multivesicular bodies as a result of endosome compartmentalization. The vesicles present in multivesicular bodies may undergo degradation or exocytosis. The exocytic multivesicular bodies fuse with membrane after cell stimulation and release exosomes. (Upper inset) Representative transmission electron microscopy showing exocytosis of exosomes from the surface of a mesenchymal stem cell (original magnification × 15,000). (Lower inset) Representative transmission electron microscopy showing a multivesicular body within a mesenchymal stem cell (original magnification × 10,000). (**b**) Production of shedding vesicles from the cell surface. Shedding vesicles are sorted out from cytoplasm by budding of cell plasmamembrane in response to cell stimulation. (Left micrograph) Transmission electron microscopy panel showing vesicles shed from the surface of an endothelial progenitor cell (original magnification × 10,000); the inset shows the high magnification ultrastructure of a vesicle shed from an endothelial progenitor (original magnification × 25,000). (Right micrograph) Transmission electron microscopy panel showing an aspect of cell membrane budding in an endothelial progenitor cell during microvesicle (MV) formation (original magnification × 15,000). The mechanisms involved in MV cargo as well as those involved in membrane-sorting processes remain at present largely unknown.

MV BIOLOGICAL ACTIVITIES

It is now recognized that MVs are an integral part of the intercellular microenvironment and may act as regulators of cell-to-cell communication. This concept is based on the observation that MVs released from a given cell type may interact through specific receptor ligands with other cells, leading to

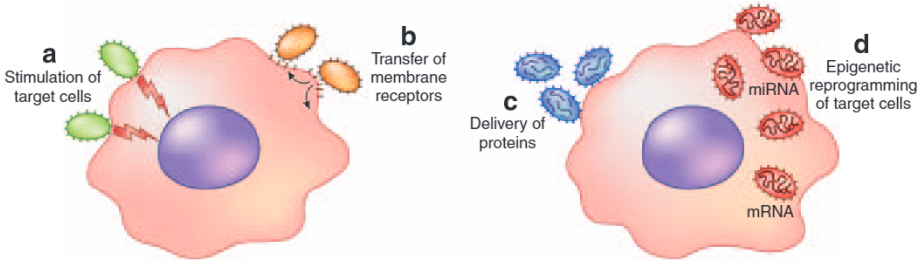


Figure 2 | Schematic representation of mechanisms involved in microvesicle (MV)-mediated cell-to-cell communication. (**a**) MVs may act as a 'signaling complex' through surface-expressed ligands that directly stimulate the target cells. (**b**) MVs may transfer receptors between cells. (**c**) MVs may deliver functional proteins or infectious particles to target cells. (**d**) MVs may transfer genetic information via mRNA, microRNA (miRNA), or transcription factors from one cell to another.

target cell stimulation directly or by transferring surface receptors.[23,24] This implicates that MVs interact only with target cells that specifically recognize rather than just with any cell present in the microenvironment.[25] This interaction may either be limited to a receptor-mediated binding to the surface of target cells forming a platform for assembly of multimolecular complexes or leading to cell signaling, either to be followed by internalization as a result of direct fusion or endocytic uptake by target cells.[9] Once internalized, MVs can fuse their membranes with those of endosomes, thus leading to a horizontal transfer of their content in the cytosol of target cells. Alternatively, they may remain segregated within endosomes and be transferred to lysosomes or dismissed by the cells following the fusion with the plasmamembrane, thus leading to a process of transcytosis.[9]

Ratajczak *et al.*[4] proposed that MV-mediated cell-to-cell communication emerged very early during evolution as a template for the development of further more refined mechanisms of cell communication. MVs may influence the behavior of target cells in multiple ways (Figure 2).

MVs may act as signaling complexes by direct stimulation of target cells

MVs derived from platelets, for instance, have an important role in coagulation as their phosphatydilserine-enriched membranes provide a surface for assembly of clotting factors.[4,9,26] The coagulation defects seen in Scott syndrome depend on defective scrambling of membrane phospholipids with an impaired formation of MVs.[26] After activation, platelets shed MVs coated with tissue factor that may interact with macrophages, neutrophils, and other platelets by ligation with molecules expressed on the surface of these cells such as P-selectin.[27] On the other hand, MVs released from neutrophils express activated leukocyte integrin alpha M beta2 (Mac-1) that is able to induce platelet activation.[28] Moreover, platelet-derived MVs, besides coagulation, trigger various cell responses as they activate endothelial cells,[29] polymorphonuclear neutrophils,[30] and monocytes,[31] and influence the functions of normal and malignant human hemopoietic cells.[4]

MVs may act by transferring receptors between cells

The transferring of receptors between cells is supported by the observation that bystander B cells rapidly acquire antigen receptors from activated B cells by a membrane transfer.[32] This allows an amplified expansion of the antigen-binding B cells with the ability to present a specific antigen to CD4 T cells. A number of other receptors were found to be transferred from one to another cell type. For instance, MVs can transfer the adhesion molecule CD41 from platelets to endothelial cells[33] or to tumor cells,[23] conferring pro-adhesive properties to them. MV-mediated transfer of Fas ligand from tumor cells induces apoptosis of activated T cells favoring tumor immune escape.[34] On the other hand, formation of shedding vesicles may be protective for cells that dismiss from their membranes to the extracellular compartment the potentially harmful molecules such as Fas or the membrane attack complex.[35,36] It has also been postulated that MVs may contribute in spreading certain infective agents such as human immunodeficiency virus type 1.[37,38] Indeed, the transfer by MVs of CXCR4 (chemokine (CXC motif) receptor 4) and CCR5 (chemokine (CC motif) receptor 5) chemokine co-receptors for human immunodeficiency virus type I may favor the entry of the virus in cells other than the lympho-hemopoietic lineage.[8,39] However, the viral transfer by MVs may also occur by the so-called 'Trojan exosome hypothesis' involving a direct delivery.[40]

MVs may deliver proteins within the target cells

An example of this mechanism is the recently reported MV-mediated transfer of a cell death message via encapsulated caspase-1.[41] It has been found that endotoxin-stimulated monocytes induce the cell death of vascular smooth muscle cells by releasing MVs containing caspase-1. This trans-cellular apoptosis induction pathway depends on the function of the delivered caspase-1 within the target cells. It has also been suggested that MVs may contribute to dissemination of certain infective agents, such as human immunodeficiency virus or prions.[42,43]

MVs may mediate a horizontal transfer of genetic information

The occurrence of epigenetic changes has been frequently reported in co-culture conditions. An explanation of this phenomenon is the transfer of genetic information between cells. It has been shown that tumor-derived MVs may transfer

not only surface determinants but also mRNA of tumor cells to monocytes.[44] Ratajczak *et al.*[45] demonstrated that MVs derived from murine embryonic stem cells (ESCs) may induce an epigenetic reprogramming of target cells. ES-derived MVs were shown to improve survival of hematopoietic stem/progenitor cells, to induce upregulation of early pluripotent and early hematopoietic markers, and to induce phosphorylation of mitogen-activated protein kinase p42/44 and Akt. In addition, ES-derived MVs were shown to express mRNAs for several pluripotent transcription factors that can be delivered to target cells and translated to the corresponding proteins. As RNase inhibited MV-mediated biological effect, the involvement of mRNA in the observed biological effects was suggested.[45] We demonstrated that MVs derived from human endothelial progenitor cells can also act as a vehicle for mRNA transport among cells.[46] MVs generated from endothelial progenitor cells were incorporated in normal endothelial cells by interaction with α4 and β1 integrins expressed on their surface and activated an angiogenic program.[46] This effect was also proved *in vivo* in severe combined immunodeficient mice, where MV-stimulated human endothelial cells subcutaneously implanted within Matrigel organized in a patent vessel network connected with the murine vasculature. RNase pretreatment of MVs abrogated their angiogenic activity even though they were internalized by endothelial cells, suggesting a critical role for RNA transfer following MV incorporation. The molecular analysis of mRNA indicated that MVs derived from endothelial progenitor cells were shuttling a specific subset of cellular mRNA, including mRNA associated with pathways relevant for angiogenesis such as the *PI3K/AKT* and endothelial nitric oxide synthase signaling pathways. Protein expression and functional studies demonstrated that phosphatidylinositol 3-kinase and endothelial nitric oxide synthase were upregulated in target cells after MV incorporation. As a proof of transduction in target cells of mRNA delivered from MVs, we used the green fluorescent protein (GFP) mRNA as reporter. Endothelial cells targeted with MVs carrying GFP mRNA produced the GFP proteins.[46] More recently, we demonstrated that MVs derived from human stem cells may also deliver *in vivo* human mRNA to mouse cells, resulting in protein translation.[47,48] Yuan *et al.*[49] have recently shown that besides mRNA, MVs may transfer in target cells microRNA. They demonstrated that MVs derived from ESCs contain abundant microRNA and that they can transfer a subset of microRNAs to mouse embryonic fibroblasts *in vitro*. As microRNAs are naturally occurring regulators of protein translation, this observation opens the possibility that stem cells can alter the expression of genes in neighboring cells by transferring microRNAs contained in MVs.

ROLE OF MVs IN INFLAMMATION AND IN CARDIOVASCULAR AND RENAL DISEASES

Inflammation is sustained by multiple interactions among cells. In this context, MVs may act at different stages of the process by carrying either anti-inflammatory or pro-inflammatory factors.[50] MVs derived from platelets and macrophages were found to be accumulated in the lipid core of the atherosclerotic plaques with the potential of triggering pro-inflammatory, angiogenic, and thrombotic signals.[51] These observations rise the possibility that targeting MVs may be a therapeutic strategy in atherosclerosis.[9,50] Indeed, increased levels of MVs of mainly endothelial origin were observed in cardiovascular pathology.[52] Endothelial dysfunction is an initial event in the development of atherosclerosis and correlate with an unfavorable cardiovascular prognosis.[53] Injured endothelial cells may release MVs, which are considered as markers of endothelial dysfunction.[54] Moreover, MVs have been implicated in the modulation of inflammation, as at early stages neutrophil-derived MVs may stimulate the production of anti-inflammatory cytokines[55,56] and at later stages MVs released from fibroblasts may induce the production of pro-inflammatory cytokines such as interleukin-6 and the monocyte chemotactic protein 1 and metalloproteinases.[57]

In experimental membranous glomerulonephritis, we found that the vesicular shedding of terminal components of complement from the cell plasma membrane protect podocytes from lyses.[35] This, by reducing their surface and activating the cytoskeleton, may favor retraction of foot processes and disruption of the slit pore thus favoring proteinuria.[35]

Although in healthy subjects, circulating MVs are mainly derived from platelets, in pathological conditions MVs may derive from other cell types such as endothelial and inflammatory cells and erythrocytes. Augmented blood levels of MVs have been found in various diseases such as pre-eclampsia,[58] diabetes,[59] acute coronary syndrome,[60] severe hypertension,[61] multiple sclerosis,[62] vasculitis,[63] as well as in patients with chronic renal failure.[64,65]

Circulating levels of MVs derived from endothelial cells correlate with arterial stiffness in hemodialysed patients.[64,66,67]

The recent discovery of exosomes/MVs in normal urine opens the possibility of obtaining information on the cell of origin in physiological and pathological conditions. It is conceivable that the analysis of urinary MVs may provide protein biomarkers for the involvement of different cellular components of the nephron.[68] Indeed, it has been recently shown that fetuin-A present in urine exosomes is a novel biomarker of structural renal injury in experimental models of cisplatin-induced nephrotoxicity and in intensive care unit patients developing acute kidney injury (AKI).[69] Moreover, a reduction in urinary exosomal levels of aquaporin-1 has been associated with renal ischemia–reperfusion injury in rats.[70] Zhou *et al.*[71] described the presence of transcription factors in urinary exosomes in different experimental models of AKI (cisplatin and ischemia–reperfusion) and of podocyte injury (puromycin-treated rats or podocin-V transgenic mice). In particular, the

transcription factor activating transcription factor 3 was associated with AKI and the Wilms tumor 1 with an early podocyte injury.[71]

In the setting of transplantation, it has been shown that the exchange of exosomes between dendritic cells in lymphoid organs may constitute a potential mechanism by which passenger leukocytes transfer alloantigens to recipient antigen-presenting cells, leading to an increased generation of donor-reactive T cells.[72] However, other studies showed that dendritic cell-derived exosomes may induce tolerance rather than immune stimulation. In particular, exosomes isolated from bone marrow-derived dendritic cells administered before transplantation can modulate heart allograft rejection, prolonging survival.[73] Moreover, dendritic cell-derived exosomes administered after heart transplantation in combination with short-term immunosuppression can induce regulatory responses that are able to modulate allograft rejection and to induce donor-specific allograft tolerance.[74]

On the other hand, MVs derived from cytomegalo virus-infected endothelial cells can stimulate allogenic CD4+ memory T cells, providing a new potential mechanism by which cytomegalovirus can exacerbate allograft rejection.[75]

ROLE OF MVs IN TUMOR BIOLOGY

MVs derived from activated platelets were found to be able to induce metastasis and angiogenesis in lung cancer.[76] Tumor

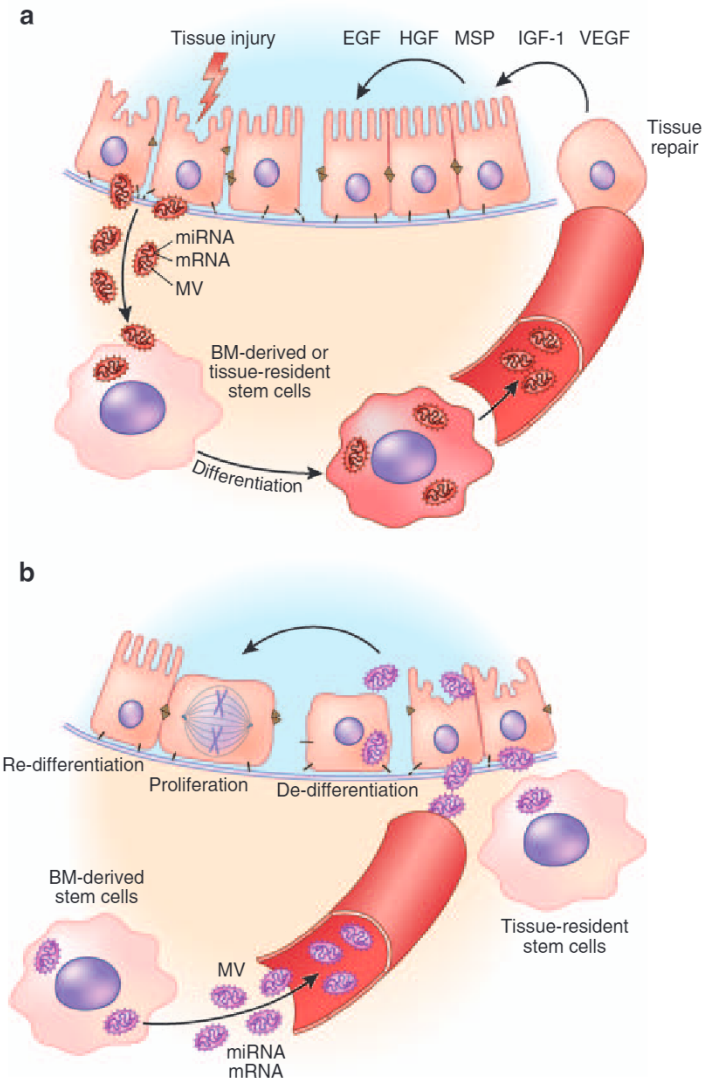


Figure 3 | Schematic representation of bidirectional exchange of genetic information between stem cells and tissue-injured cells mediated by microvesicles (MVs). (**a**) MVs released from tissue-injured cells may reprogram the phenotype of stem cells to acquire tissue-specific features by delivering to stem cells the mRNAs and/or microRNAs (miRNAs) of tissue cells. (**b**) MVs produced by stem cells recruited from the circulation or from resident stem cells may reprogram tissue-injured cells by delivering mRNA and/or miRNA that induce the de-differentiation, the production of soluble paracrine mediators, and the cell cycle re-entry of these cells favoring tissue regeneration. BM, bone marrow; EGF, epiotermal growth factor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; MSP, macrophage stimulating protein; VEGF, vascular endothelial growth factor.

cells were also found to release large amount of MVs. The number of circulating MVs is increased in patients with cancer and correlate with poor prognosis.[14] It has been suggested that the release of MVs may protect tumor cells from apoptosis by extrusion from the cell of apoptosis-inducing proteins.[77,78] In addition, cancer cells resistant to chemotherapy were found to release significant more MVs than those sensitive to chemotherapy.[79] It has been suggested that chemotherapeutic agents may be extruded from cells via MVs.[80] Moreover, it was found that MVs may favor the escape of tumor cells from immune surveillance. This may occur either by a mechanism called complement resistance related to vesicular shedding of terminal components of complement from the cell plasma membrane,[35,81] or by shedding of Fas ligand that reduces sensitivity to T-cell Fas-mediated apoptosis.[82] In addition, it was found that tumor-derived MVs can induce apoptosis in activated antitumor T cells, impairment of monocyte differentiation into dendritic cells, and induction of myeloid-suppressive cells.[15,83] By carrying active metalloproteinases, MVs may contribute to stromal remodeling and favor tumor cell invasion.[84] Moreover, MVs may carry pro-angiogenic signals that favor the tumor vascularization.[85,86] Recently, it has been shown that tumor-derived MVs may form the pre-metastatic niche that allow the development of lung metastasis.[87] Finally, it has been suggested that MVs may act by transferring oncogenes from tumor cells to stromal cells.[88]

On the other hand, exosomes derived from mature dendritic cells have been used as vaccines to stimulate efficient antitumor cytotoxic T-lymphocyte response.[89]

ROLE OF MV-MEDIATED CELL-TO-CELL INTERACTION IN STEM CELL BIOLOGY

Stem cells are characterized by an unlimited self-renewal and by high multilineage differentiation potential. Stem cells have essential roles in organogenesis during the embryonic development and in many adult tissues are responsible for the growth, homeostasis, and repair. Depending on the developmental status and origin, stem cells are classified as embryonic and adult stem cells. The ESCs are derived from the inner cell mass of the blastocyst-stage mammalian embryo few days after fertilization. ESCs are pluripotent as they generate the germ line during development and virtually all tissues.

The adult stem cells are undifferentiated cells resident in tissues, with a more limited self-renewal and differentiation capabilities.[90–92] When partially committed to differentiate in a defined cell lineage, they are named progenitor cells. Adult stem/progenitor cells are present in most tissues and organs such as bone marrow, liver, pancreas, heart, kidney, brain, lung, digestive tract, retina, breast, ovaries, prostate, testis, dental pulp, hair follicles, skin, skeletal muscle, adipose tissue, and blood.[93] It was assumed that stem cell self-renewal and differentiation may depend on an asymmetric division with a regulation that is hierarchical in nature, leading to a progressive loss of proliferative potential when they gain differentiated characteristics.[94] As an alternative to hierarchical model, a continuum model of stem cell biology has been recently proposed.[95,96] According to this theory, the phenotype of stem cells may vary with cell cycle state and may be reversible. Therefore, the phenotype of stem cells is reversibly changing during the cell cycle transit until a terminal-differentiating stimulus is encountered at a cycle-susceptible time.[95,96] Recently, Quesenberry and Aliotta[97] proposed that the interaction of stem cells with the microenvironment, also named niche, have a critical role in defining the stem cell phenotypes. In this context, MVs may have a regulatory task by transfer of genetic information between cells. These researchers proposed that a continuous genetic modulation through MV transfer between cells is a critical determinant of stem cell phenotype variation. Indeed, stem cells are an abundant source of MVs. It has been suggested that MVs derived from ESCs may represent one of the critical components supporting self-renewal and expansion of stem cells.[4,45] In fact, Ratajczak *et al.*[45] demonstrated that MVs released from ESCs may reprogram hematopoietic progenitors by a horizontal transfer of mRNA and by delivery of specific proteins.

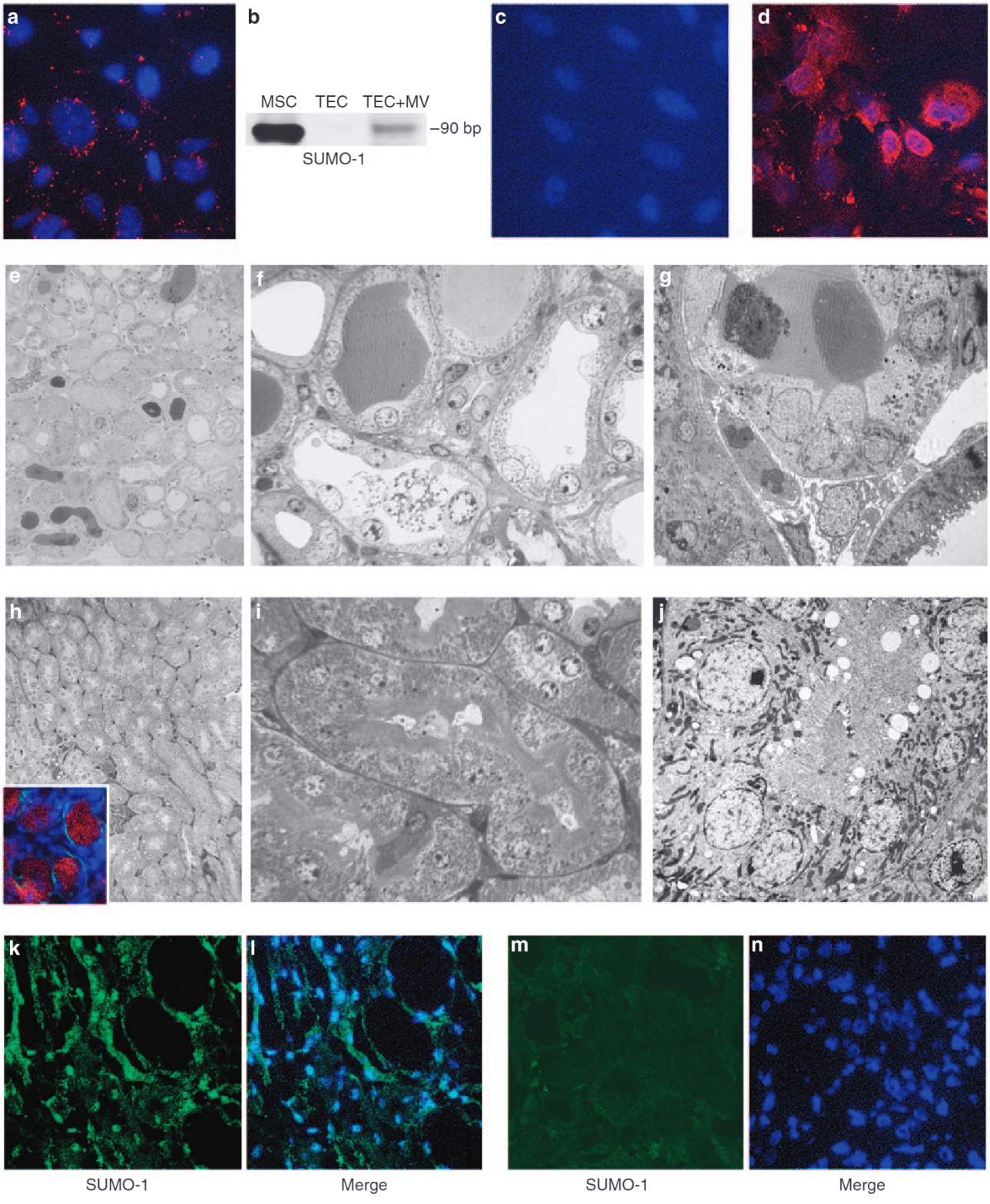
MVs, by transferring selected patterns of proteins, mRNAs and microRNAs, may also act as paracrine mediators of signaling between stem cells and differentiated cells. We can envisage a bidirectional exchange of genetic information from injured cells to bone marrow-derived or resident stem cells (Figure 3). In the first scenario, MVs released from injured tissue may reprogram the phenotype of stem cells to acquire tissue-specific features, whereas in the second, MVs derived from stem cells may induce cell cycle re-entry of cells survived to injury allowing tissue regeneration.

MVs derived from injured tissue may reprogram the phenotype of bone marrow or resident stem cells

It is still debated whether bone marrow-derived stem cells have the capacity to generate tissue-specific cells after their engraftment in injured tissues.[98,99] Poulsom *et al.*[100] demonstrated that bone marrow-derived cells could contribute to regeneration of the renal tubular epithelium, and in subsequent studies Fang *et al.*[101] suggested that the hematopoietic stem cells rather than the mesenchymal stem cells (MSCs) contribute to the repair of AKI. However, transdifferentiation as a mechanism of stem cell plasticity has never been conclusively proved and several studies challenged the ability of bone marrow-derived stem cells to differentiate in tubular epithelial cells.[99,102,103] Fusion studied with cross-sex transplantation experiments has been suggested as a mechanism of bone marrow stem cell plasticity in some reports but not in others.

As an alternative to transdifferentiation and fusion, Quesenberry and Aliotta[97] suggested that stem cell differentiation depends on epigenetic cell changes mediated by signals received from injured cells and delivered by MVs.[104] Co-culture of bone marrow cells with injured lung cells induced

the expression of lung-specific genes and proteins such as Clara cell-specific protein, surfactant B, and surfactant C in bone marrow cells.[105] It was found that changes in bone marrow stem cell phenotype depend on MVs released from injured cells that contain high levels of lung-specific mRNAs and deliver these mRNAs to bone marrow cells. This may also explain the observation that the conditioned medium derived from renal tubular epithelial cells initiates differentiation of human MSCs.[106] Indeed, in preliminary experiments we found that MVs derived from injured renal tubular epithelial cells



may induce expression of tubular cell markers in human MSCs. One can speculate that MVs released from injured tissue may reprogram not only bone marrow-derived stem cells, but also resident stem cells. Several studies indicate the presence of resident stem cell populations within the kidney that may contribute to renal repair.[47,107,108]

Taken together, these results suggest that MVs derived from injured tissues mediated transfer of genetic information that could explain not only the plasticity and phenotypic changes of stem cells, but also the functional effects without the need of their transdifferentiation into tissue cells.

MVs derived from stem cells may reprogram cells survived to injury and favor tissue regeneration

Experiments based on exogenous MSC administration in AKI demonstrate a functional and morphological recovery from acute tubular injury induced by toxic and ischemia–reperfusion injury[99,109,110] and a functional improvement in chronic renal failure.[111] As these beneficial effects are associated only with a transient recruitment of MSC within the renal vasculature with a minimal incorporation within the regenerating tubules,[102,103] it has been suggested that MSC may provide a paracrine support to the repair of injured tissue.[112] On the other hand, many studies on tubular repopulation after acute injury indicate a prominent contribution of renal tubular cells.[112,113] Strong support of a paracrine/endocrine mechanism for tissue repair comes from experiments of Bi *et al.*,[114] showing that the administration of conditioned medium from MSC is able to mimic the beneficial effects of the stem cell therapy. They demonstrated that MSC may favor renal regeneration independently from engraftment within tubules by producing factors that limit apoptosis and enhance proliferation of tubular cells. A growing body of evidence supports the hypothesis of a paracrine mechanism in bone marrow-derived stem cell therapy in other organs also, such as infarcted hearts.[115] Indeed, the frequency of stem cell engraftment and transdifferentiation or fusion to generate new cardiomyocytes and vascular cells appear too low to explain the beneficial effects observed. Conversely, several studies indicate that stem cell-released soluble factors may contribute to cardiac repair and regeneration.[116]

The paracrine mediators involved in the beneficial effect of exogenous stem cell administration may include not only growth factors,[103,114] but also the MVs released from stem cells. We envisage the possibility that MVs released from stem cells recruited at the site of tissue injury may induce de-differentiation of resident cells survived to injury with re-entry to cell cycle and activation of tissue regenerative programs (Figure 3b). Indeed, human MVs released from MSCs are able to enter in the epithelial cells, delivering their mRNA cargo (Figure 4). This stimulates *in vitro* proliferation and apoptosis resistance of tubular epithelial cells that acquire a mesenchymal phenotype. *In vivo*, MVs accelerate the functional and morphological recovery of glycerol-induced acute kidney injury in severe combined immunodeficient mice (Figure 4).[47] As the efficacy of MVs is comparable to that of MSC administration in inducing renal repair, our own bias is that the beneficial effect of MSCs is largely due to the release of MVs. RNA inactivation in MVs abrogated both the *in vitro* and the *in vivo* effects of MVs, suggesting a mechanism dependent on RNA delivery. Indeed, MVs contain a defined subset of transcripts representative of the multiple differentiative and functional properties of MSCs.[47] Preliminary results indicate that MSC-derived MVs also contain defined patterns of microRNAs that may serve as molecular signature and suggest a specific rather than a random accumulation in MVs.[117] A stimulus-dependent variation of RNA species packed within MVs suggests a tightly regulated process in their generation within the cells. We are currently investigating whether mRNA and microRNA entry in target cells activates translational control mechanisms or specific checkpoints for the transcripts. Whether MVs produced by stem cells may provide a

Figure 4 | Effect of mesenchymal stem cell (MSC)-derived microvesicles (MVs) *in vitro* on cultured mouse tubular epithelial cells (TECs) and *in vivo* on glycerol-induced acute kidney injury (AKI) in severe combined immunodeficient (SCID) mice (see Bruno *et al.*[47]). (**a**) Representative confocal micrograph showing the internalization by mouse TECs (30 min at 37 °C) of 30 μg/ml MVs labeled with PKH26 (red). Nuclei were stained by Hoechst dye (blue; original magnification × 400). The mRNA horizontal transfer and human protein translation by mouse TECs treated with human MSC-derived MVs was shown by reverse transcriptase-PCR (RT-PCR) for a specific human mRNA using small ubiquitin-like modifier-1 (SUMO-1) as target mRNA and by immunofluorescence using anti-human SUMO-1 antibodies. (**b**) A band of PCR products specific for human SUMO-1 of the expected size (90 bp) was detected in a 4% agarose gel electrophoresis in TECs cultured in the presence of 30 μg/ml MVs, whereas it was absent in TEC alone. As positive control, the extract of human bone marrow-derived MSC (BM-MSC) was used. (**c**, **d**) Representative micrographs showing the expression of human SUMO-1 proteins by mouse TECs cultured in the absence or in the presence of 30 μg/ml MVs for 24 h. SUMO-1 was detectable in the cytoplasm and nuclei of TECs incubated with MVs (**d**) but not in untreated TECs (**c**). Nuclei were counterstained with Hoechst dye (blue; original magnification × 400). (**e**–**g**) Representative micrographs of semifine sections (**e**, **f**) and transmission electron microscopy (**g**) showing the diffuse tubular injury characterized by blebbing, loss of brush border, and necrosis of TECs and by the presence of intraluminal tubular casts in mice 5 days after glycerol-induced AKI. (**h**–**j**) Representative micrographs of semifine sections (**h**, **i**) and transmission electron microscopy (**j**) showing the morphological recovery induced by treatment with 10 μg MSC-derived MVs in mice 5 days after glycerol-induced AKI. The inset in (**h**) shows the accumulation of PKH26-labeled MVs within the TECs (original magnification **e** and **h** × 150; **f**, **g**, and **i** × 600; and **j** × 3000). (**k**–**n**) The detection of human protein expression in kidneys of mice treated with human MSC-derived MVs indicated the translation of human proteins by the horizontally transferred mRNA into TECs *in vivo*. Representative confocal micrographs showing the presence of staining for human SUMO-1 protein with cytoplasmic and nuclear expression in kidney sections of AKI mice treated with MVs and killed 48 h later (**k**, **l**) or in control mice untreated with MVs (**m**, **n**). Nuclei were counterstained with Hoechst dye (original magnification × 400).

potential therapeutic strategy to avoid the possible maldifferentiation of stem cells once engrafted in the kidney in the long term[118] requires further investigations. We recently showed that MV-mediated transfer of RNA-based information from human liver stem cells stimulates liver regeneration in a model of 75% hepatectomy.[48]

CONCLUSION

The main function of MVs is signaling through specific interactions with target cells and transferring gene products. Therefore, they may participate in physiological and pathological processes. Gaining further insights into the molecular specificity of MVs may allow the identification of the cellular source and may provide new diagnostic tools. Indeed, an increasing body of evidence indicates that MVs may offer prognostic information in various diseases such as chronic inflammation, cardiovascular and renal diseases, pathological pregnancy, and tumors. The presence of MVs in body fluid makes them readily accessible, and their number, cellular origin, composition, and function can be disease state dependent. Cancer cells, for example, shed MVs that might not only help tumor and metastasis development but also represent an important non-invading diagnostic tool especially with regard to the fact that they contain genetic material under the form of RNA, which could be easily screened for cancer genetic markers. In addition, the recognition of the signals delivered by MVs may open new therapeutic strategies. The removal from plasma of harmful MVs may be beneficial in pathological conditions where MVs deliver thrombogenic and inflammatory signals or in tumors. On the other hand, MVs derived from stem cells may reprogram altered functions in target cells, suggesting that they could be exploited in regenerative medicine to repair damaged tissues. Moreover, MV-mediated transfer of genetic information could explain the observed plasticity and the functional effects of stem cells without the need of their transdifferentiation into tissue cells. Many points require further investigation: (1) the stimuli and the molecular pathways that regulate the assembly within MVs of the biologically active molecules that they shuttle; (2) the stimuli that trigger their release; (3) the surface receptors that may confer selective specificity; (4) the full diagnostic potential of MVs in different pathological conditions; (5) the strategy to inhibit formation or to remove from circulation potentially harmful MVs; and (6) the therapeutic exploitation in regenerative medicine of the ability of MVs to modify the phenotype and function of target cells. The recognition of the importance of MVs may open new perspectives of investigation.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

Our research was supported by grants from Regione Piemonte, Piattaforme Biotecnologiche, progetto PiSTEM, and from Oncoprot.

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