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Role of Microvesicles in Acute Kidney Injury

Giovanni Camussi[a] · Vincenzo Cantaluppi[a] ·
Maria Chiara Deregibus[a] · Emanuele Gatti[b,c] · Ciro Tetta[b–d]

[a]Department of Internal Medicine, Centre for Molecular Biotechnology and Centre for Research in Experimental Medicine (CeRMS), Torino, Italy; [b]International Research and Development, Fresenius Medical Care, Bad Homburg, Germany; [c]Danube University, Center for Biomedical Technology, Krems, Austria; [d]Sis-Ter, Palazzo Pignano, Italy

Abstract

The main function of microvesicles (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 has allowed identifying the cellular source and may provide new diagnostic tools in the future. 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, tumors, and sepsis. The presence of MVs in body fluids makes them readily accessible. Their number, cellular origin, composition and function can be dependent on the state of the disease. In sepsis for example, activated endothelial cells may shed MVs that might trigger leukocyte and monocyte production and release pro-oxidant and inflammatory mediators. MVs from platelets may trigger disseminated intravascular coagulopathy. MVs are no doubt also involved in modulating immunity and future studies will clarify their functional role in negatively modulating the cell response. In addition, the recognition of the signals delivered by MVs may open new therapeutic strategies. The removal of harmful MVs from plasma may be beneficial in pathological conditions where MVs deliver thrombogenic and inflammatory signals. 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. We will discuss the role of stem cell-derived MVs in the repair of acute kidney injury.

First described as platelet derivatives of less than 0.1 μm with procoagulant activity [1], extracellular vesicles such as exosomes, apoptotic blebs, microvesicles (MVs), microparticles, prostasomes and prominosomes were recognized from a large number of cell types, e.g. epithelial, hematopoietic, immune, placental, tumor and stem cells, and fibroblasts [2–4]. For a long time, the investigation on MVs was merely descriptive as MVs were considered to be inert cellular debris. The vesicles frequently observed by electron microscopy in the interstitial space or in blood were considered as the consequence of cell damage or as the result of dynamic plasma membrane turnover [5]. Only recently, however, have studies assigned specific functions to MVs released in the microenvironment by various cell types and in biological fluids such as blood, urine and exudates. The role of MVs is now evoked in regenerative medicine (multiorgan development, cell survival, differentiation), immune system regulation and several diseases [6].

In this review, we will discuss how MVs should be seen as two sides of the same coin and their potential role in the complex scenario of sepsis, the systemic response to infection associated with the development of multiple organ failure (MOF) and high mortality rates.

Biogenesis of Microvesicles

There has been a rather confusing nomenclature and some clarification is in order. Two terms that identify two different vesicles based on their size and mode of their release from the cell of origin are most commonly used. Exosomes are stored as intraluminal vesicles within multivesicular bodies of the late endosome and are released when these multivesicular bodies fuse with the cell membrane. Exosomes have an endosomal origin [7]. They are released by exocytosis through a mechanism dependent on cytoskeleton activation and under the regulation of p53 protein [8]. While exosomes are a rather homogenous population with a size of 30–120 nm, shedding vesicles are usually larger with a size ranging from 100 nm to 1 μm. Shedding vesicles originate from the budding of small cytoplasmic protrusions followed by their detachment from the cell surface, a process dependent on calcium influx, calpain and cytoskeleton reorganization [9]. MVs expose on their surface large amounts of phosphatidylserine from the inner leaflet to the outer bilayer of the cell membrane and are enriched in proteins associated with membrane lipid rafts [10] by calcium-dependent mechanisms that modify the asymmetric phospholipid distribution of plasma membranes by activation of specific enzymes named flippase, floppase and scramblase and by inhibition of translocase [11]. Moreover, the intracellular pathways that activate the reorganization of the cytoskeleton induce the detachment of plasma membrane protrusions from the cortical actin.

The release of MVs occurs in cells in a resting state or upon activation by soluble agonists, physical or chemical stress such as oxidative stress as well as

hypoxia, or shear stress [12]. Blood levels of MVs are increased in various diseases including acute coronary syndrome [13], chronic renal failure [14] and sepsis [15]. MVs are a source of phospholipids, a substrate for phospholipase A2, which facilitates platelet aggregation, inflammation and chemotaxis of platelets and/or leukocytes to the endothelium, thus triggering production of monocyte cytokines (IL-1β, IL-8 and TNF-α). Upon stimulation, the plasma membrane of endothelial cells and monocytes express phosphatidylserine and tissue factor, allowing activation of coagulation factors (factor VII and thrombin at the cell surface). It is also evident that MVs may downregulate the procoagulant activity in response to the modulatory expression of the endothelial protein C receptor that binds protein C. Future studies will have to clarify the role of MVs in the amplification as well as in the control of feedback loops.

Modes of Action of Microvesicles

MVs may influence the behavior of target cells in multiple ways, such as signaling complexes by direct stimulation of target cells by transferring receptors between cells, delivery of proteins within the target cells or by a horizontal transfer of genetic information.

The most recent discovery includes the horizontal transfer of genetic information by MVs. For years, epigenetic changes were frequently reported in co-culture conditions. To explain this phenomenon, the transfer of genetic information between cells was shown [16]. MVs derived from human endothelial progenitors (EPC) shuttle mRNA to endothelial cells via interaction with α_4- and β_1-integrins expressed on their surface, thus activating an angiogenic program [17]. The molecular analysis of mRNA indicated that MVs derived from EPC were the cargo of 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. Furthermore, protein expression and functional studies demonstrated that PI3K and endothelial nitric oxide synthase were upregulated in target cells after MV incorporation. 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 [18, 19].

Besides mRNA, MVs may transfer microRNAs (miRNA) to target cells [20]. Since miRNAs 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 miRNAs contained in MVs. We recently characterized miRNA shuttled by MVs released by human adult mesenchymal stem cells (MSCs) [21]. Hierarchical clustering and similarity analysis of miRNAs showed that miRNA compartmentalization and secretion by MVs are both highly regulated processes. Gene ontology analysis of predicted and validated

targets showed that the highly expressed miRNAs in MVs derived from MSCs may be involved in multiorgan development, cell survival, differentiation and immune system regulation. It has been suggested that transfer of genetic information by MVs play a pivotal role in stem cell plasticity and tissue regeneration [22].This mechanism possibly contributes to the paracrine action of stem cells in the repair of tissue injury [23].

Sepsis: The As Yet Unraveled Complexity of 'Soluble Factors'

Sepsis, septic shock and MOF occur either as a response to infection or in the context of a noninfectious systemic inflammatory reaction syndrome, and are the main causes of mortality in intensive care units. The heart and the kidney are often acutely involved. The pathophysiology of the cellular and humoral alterations is complex and seems to be related not only to the ischemic response to hypoperfusion, but also to direct detrimental activity induced by circulating mediators with both pro- and anti-inflammatory properties able to interact in a dynamic manner and induce MOF. Sepsis-induced cardiac dysfunction has been known for many years, but the mechanism appears to be complex, including both 'intrinsic' cardiomyopathy and direct and/or indirect effects of circulating depressing factors [24].

Acute kidney injury (AKI) occurs very frequently in burn patients with MOF. We demonstrated that plasma derived from septic patients with severe burns induced apoptosis and functional alterations of glomerular podocytes and tubular epithelial cells [25]. Plasma collected from septic patients induced granulocyte adhesion, apoptosis and altered polarity in tubular cells – all biological events correlated to the onset of AKI. By using different adsorptive matrixes, the unselective removal of circulating 'soluble mediators' and MVs could limit plasma-induced tubular cell injury.

We recently demonstrated that adsorption significantly decreased plasma-induced tubular alterations and abated the concentrations of several soluble mediators such as TNF-α, Fas ligand and the CD40 ligand [26]. The inhibition of granulocyte adhesion to tubular cells was associated with the downregulation of ICAM-1 and CD40. Adsorption inhibited tubular cell apoptosis induced by septic plasma by downregulating the activation of caspase-3, -8, -9 and of Fas/death receptor-mediated signaling pathways. The alteration of cell polarity, morphogenesis, protein reabsorption and the downregulation of the tight junction molecule ZO-1, sodium transporter NHE3, glucose transporter GLUT-2 and of endocytic receptor megalin (all induced by septic plasma) were significantly reduced by resin adsorption.

The role of MVs in sepsis is presently under close scrutiny. Much evidence exists to support the contention that MVs may trigger the proinflammatory and pro-oxidant activities. In an experimental model of septic peritonitis in the rat,

Mortaza et al. [27] inoculated MVs from septic rats in healthy ones and reproduced hemodynamic septic inflammatory patterns associated with oxidative and nitrosative stresses. Of interest, the authors recognized a certain phenotype of MVs capable of exhibiting such an activity. Meziani et al. [15] reviewed the role of MVs in the pathogenesis of sepsis through multiple ways. MVs regulate vascular tone and are potent vascular proinflammatory and procoagulant mediators.

Perspectives in Microvesicle-Targeted Therapies

In the context of severe shock and MOF, the removal of MVs from the blood or plasma has so far had no proof-of-concept. While standard hemodialysis techniques, e.g. hemofiltration or hemodiafiltration, are not suitable, plasmapheresis and adsorption modalities would be most appropriate. The recent availability of diagnostics capable of quantifying MVs of a specific cell origin could be useful in understanding the cells/organs where injury is occurring. This approach would overlap with what has been done in oncology. The mRNA from MVs is enriched and differentiated to obtain a result that is indicative of the condition of tissue or organ from which the MVs originated. In tumors, these diagnostics could identify and stage by differential analysis of one or more distinct mRNAs, optionally together with identification and analysis of a non-RNA component of the MVs (Microvesicle-Based Compositions and Methods, United States Patent Application 20100075315). Adsorbents in extracorporeal therapies work by either direct adsorption of a given molecule, retention of a given target molecule, or adsorption/retention of both the molecules of interest and their carrier molecules (i.e. α_2-macroglobulin).

Adsorbent technologies have been intensely studied and have made tremendous advances in the last 20–30 years. These advances have been driven to a great extent by new developments in chromatography. A potential focus for future biotechnological research could be the application of selective removal of MVs expressing specific biomarkers. Antibodies or synthetic ligands immobilized on stationary matrix could be selected to act as acceptors for various proteins: Fas ligand, MHC I, MHC II, CD44, placental alkaline phosphatase, TSG-101, MHC I-peptide complex, MHC II-peptide complexes and in general any protein found to be expressed by MVs contributing to proinflammatory and/or anti-inflammatory biological activities.

Role of Stem Cell-Derived Microvesicles in the Repair of Acute Kidney Injury

The mechanism of stem cell-induced kidney repair after AKI is still controversial. The engraftment of stem cells derived from bone marrow and fusion are

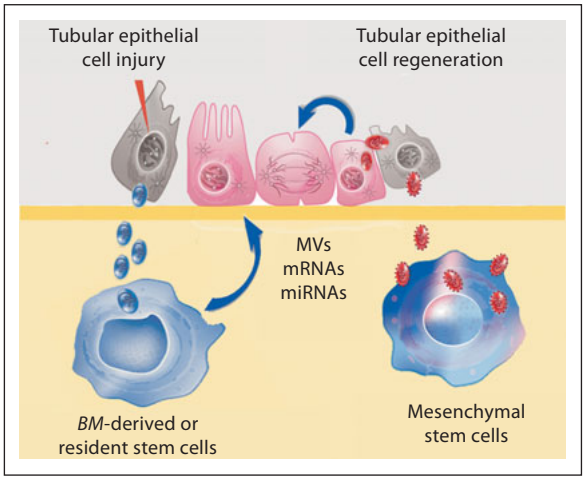

Fig. 1. Schematic representation of MV-mediated bidirectional exchange of genetic information between stem cells and tissue-injured cells. MVs released from injured tubular cells may reprogram the phenotype of bone morrow (BM)-derived or resident stem cells to acquire tissue specific phenotype by delivering the mRNAs and/or miRNA specifics of tubular cells to stem cells. On the other hand, MVs produced by stem cells, either resident or recruited from the circulation, or MVs produced ex vivo by exogenous stem cells and administered to experimental animals with AKI, may reprogram tubular cells which survived injury by delivering mRNA and/or miRNA that induce dedifferentiation, production of soluble paracrine mediators and cell cycle re-entry allowing tissue regeneration.

considered rare events and several studies suggest the involvement of a paracrine/endocrine mechanism rather than cell transdifferentiation. As an alternative to transdifferentiation and fusion, Quesenberry et al. [22] suggested that stem cell differentiation depends on epigenetic cell changes mediated by signals received from injured cells and delivered by MVs. They demonstrated that bone marrow cells co-cultured with injured lung cells induced the expression of lung-specific genes and proteins mediated by transfer of lung-specific mRNA delivered by MVs released from injured cells to bone marrow cells. In preliminary experiments, we found that MVs released from injured renal tubular epithelial cells may induce expression of tubular cell markers in human MSCs (fig. 1). Therefore, MVs released from injured tissue could reprogram bone marrow-derived stem cells as well as resident stem cells. MV-mediated transfer of genetic information 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 [28].

On the other hand, MVs derived from stem cells may reprogram cells which survived injury and favor tissue regeneration. It has been shown that MSC administration in AKI favors functional and morphological recovery

[28]. These beneficial effects are associated only with a transient recruitment of MSCs within the renal vasculature with a minimal engraftment within tubules [28]. Several studies on tubular repopulation after AKI indicate a prominent contribution of renal tubular cells which survived injury. Therefore, it has been suggested that MSC may provide paracrine support to the repair of injured tissue by releasing factors that limit injury and favor regeneration [23, 29].

We recently demonstrated that MVs released from stem cells recruited at the site of tissue injury may account for such paracrine/endocrine effects by inducing dedifferentiation of resident cells which survived injury and a tissue regenerative program (fig. 1) [23, 29]. MVs released from MSCs are able to deliver their mRNA cargo to the epithelial cells, thus stimulating proliferation and apoptosis resistance and accelerating functional and morphological recovery [18]. Given that the efficacy of MVs is comparable to that of MSC administration, we suggest that the beneficial effect of MSCs is mostly due to the release of MVs. The mechanism of repair is mainly dependent on RNA delivery as suggested by the inhibitory effect of RNA inactivation and by the presence in MVs of a defined subset of transcripts with multiple differentiative and functional properties of MSCs. Moreover, MSC-derived MVs may deliver miRNAs that activate translational control mechanisms or specific checkpoints for the transcripts after entry in target cells [21]. Therefore, administration of MVs produced by stem cells may provide a potential therapeutic strategy that would avoid the possible maldifferentiation of stem cells once engrafted in the kidney in the long term.

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 for 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, tumors, and sepsis. Many points require further investigation: (1) the stimuli and the molecular pathways that regulate the assembly within MVs of the biological 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 remove potentially harmful MVs from circulation, 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 MVs has opened a new era, with new perspectives of investigation on the horizon.

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Giovanni Camussi
Dipartimento di Medicina Interna
Ospedale Maggiore S. Giovanni Battista
Corso Dogliotti 14, IT–10126 Torino (Italy)
Tel. +39 011 633 6708, E-Mail giovanni.camussi@unito.it