Editorial Comments

The emerging role of microvesicles in cellular therapies for organ/tissue regeneration

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The field of regenerative medicine is searching for a source of stem and progenitor cells that can be safely and efficiently employed for regeneration of damaged organs (e.g. heart, liver or kidney) [1–3]. In experimental animal models of organ damage (e.g. heart infarct), different types of stem cells isolated from adult tissues have been employed, including bone marrow-derived mononuclear cells, hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), skeletal muscle myoblasts and so-called cardiac stem cells (CSCs). Interestingly, while some beneficial effects have been reported using cellular therapies, there is no solid evidence that cells employed to regenerate damaged tissues truly give rise to organ-specific cell populations (e.g. functional cardiomyocytes in heart, hepatocytes in liver or tubular epithelium in kidney). Furthermore, much of the previously published data purporting to show transdifferentiation of transplanted cells into cells that comprise damaged organs has been subsequently explained by a rare cell fusion phenomenon [4]. As a result of fusing transplanted cells with cells residing in the damaged tissues, new cells with double the number of chromosomes (heterokaryons) are created. Such heterokaryons may express some markers of the transplanted cells employed for therapy and thus may give the false impression that the transplanted cells have replaced the damaged ones. This phenomenon, however, is extremely rare and it is now widely accepted that it was unduly emphasized in the past as a major mechanism contributing to tissue and organ chimerism following cellular therapy [4,5].

The issue remains that in all of the tissue-damage models reported so far, no solid evidence has been presented that transplanted cells truly trans-differentiate into cell populations specific to the injured organ. Moreover, since similar beneficial effects in improvement of organ function (e.g. for the left ventricular ejection fraction) after cell therapy were often observed regardless of which cells were employed, it became obvious that some other cell-mediated effects must be responsible for the observed effects that ameliorate organ damage [6,7]. To begin with, those effects might be explained by paracrine signals from the transplanted cells. In fact, it is very well known that MSCs, and even highly purified HSCs, secrete several growth factors, cytokines or chemokines that prevent apoptosis of cells residing in damaged tissues [8]. In addition, some of the secreted factors could also stimulate angiogenesis and thus, by improving local circulation, improve the function of damaged tissues. However, on the assumption that every cell type possesses a unique repertoire of secreted factors, other common cell-dependent mechanisms may play an important role as well.

The paper published by Dr Camussi’s group in this issue of Nephrology Dialysis and Transplantation provides elegant evidence that some crucial protective and pro-regenerative mechanisms are mediated by cell-derived microvesicles (MVs) [9]. These small, circular membrane structures (100 nM–1 μm in diameter) are also referred to as ‘microparticles’ or ‘exosomes’ in the literature [10–12]. Furthermore, MVs secreted in the developing embryo are potential vehicles for the spread of morphogens through epithelia (e.g. Hedgehog proteins) and are known as argosomes [13]. For simplicity, we will use the traditional name (MVs) in this editorial.

Overall, MVs, as small circular membrane fragments, are shed from the cell surface or released from the endosomal cell membrane compartment and play an important and underappreciated role in cell-cell communication [10–12]. This intriguing MV-mediated cell–cell communication system emerged very early during evolution and served as a template for the further development of intercellular interaction mechanisms involving soluble bioactive mediators and fine-tuned ligand–receptor interactions. The first unicellular organisms communicated with each other by sending and receiving MVs. In bacteria, for example, MVs may transfer antibiotic resistance genes. On the other hand, in amoeba MVs play an important role in marking trails for migrating cells: MV trails left by migrating amoebae provide guidance for other cells that follow ‘in their footsteps’. Furthermore, as mentioned above, in tissues of the develop-
ing embryo, argosome MVs provide a gradient of trophic factors involved in tissue and organ specification [13]. MVs are detectable in all biological fluids. For example, MVs derived from activated blood platelets, leucocytes and endothelial cells continuously circulate in peripheral blood under steady-state conditions, with their number increasing during stress situations (e.g. infection, organ tissue damage or neoplasia) [10–12]. This phenomenon could be exploited from the diagnostic point of view and a new biotechnological platform is currently being developed to employ the molecular signatures [e.g. messenger RNA (mRNA), micro RNA (miRNA) and protein compositions] of MVs circulating in peripheral blood in the diagnosis of different disorders.

There is no doubt that the biological significance of MVs has for many years been largely overlooked, regarded like apoptotic bodies as merely cellular fragments or debris. However, compelling evidence has already accumulated that these tiny circular membrane fragments orchestrate several biological processes [10–12]. On the one hand, it is already acknowledged that MVs are secreted or shed by normal and not dying cells, are much smaller in size than apoptotic bodies and do not contain fragments of nuclei loaded with nuclear DNA. On the other hand, MVs contain numerous structural proteins (e.g. receptors and adhesion molecules), as well as lipids similar to those present in the membranes of cells from which they originate, that they transfer from one cell to another. Furthermore, because they engulf some cytoplasm during membrane blebbing, they may also be enriched in cell-of-origin-derived proteins, enzymes, mRNA and miRNA that are then delivered to target cells [14]. In this transfer of mRNA or proteins, MVs act as a kind of ‘naturally-engineered liposomes’. Interestingly, accumulating evidence suggests that all these molecules are somehow preferentially enriched in MVs by mechanisms that involve docking proteins [10–12].

Figure 1 summarizes the currently acknowledged biological effects mediated by MVs. First, MVs may stimulate target cells directly by surface-expressed ligands, acting as signaling complexes. Second, MVs may transfer surface receptors from one cell to another, deliver proteins, mRNA, bioactive lipids and even whole organelles (e.g. mitochondria) into target cells [15]. Finally, as already mentioned, they may also serve as a vehicle for transferring infectious particles, such as prions or Human Immunodeficiency Virus (HIV), between cells (i.e. a Trojan horse mechanism of infection) [16]. MVs exert several pleiotropic effects that modulate the biology of target cell populations. For example, as previously shown, MVs secreted from embryonic stem cells may stimulate expansion of HSCs, and MVs secreted from damaged lungs may transiently change the phenotype of HSCs [17]. It is likely that several experiments in which transient ‘trans-differentiation’ of cultured cells was observed after coculturing with supernatants or

![Fig. 1. Alternative mechanisms by which MV may interact with target cells. MV may (i) stimulate target cells directly by surface-expressed ligands acting as a kind of ‘signaling complex’; (ii) transfer surface receptors from one cell to another; (iii) deliver proteins, mRNA, bioactive lipids and even whole organelles (e.g. mitochondria) into target cells and finally (not shown in this picture), MV may also (iv) serve as a vehicle (i.e. a Trojan horse mechanism) to transfer infectious particles between cells (e.g. HIV or prions). In this issue of Nephrology Dialysis and Transplantation, Camussi et al. describe how MSC-derived MV protects damaged kidneys against ischemia–reperfusion-induced acute and chronic kidney injury, and MV-transferred mRNA plays a crucial role in this phenomenon.](image-url)
cell extracts were in fact mediated by MVs that transferred surface receptors, proteins or mRNA into the target cells [10–12].

Overall, because all cell types secrete MVs, some of the biologically active components of MVs depend on the cell of origin (e.g. membrane receptors hijacked from parental cells, adhesion molecules, peptides, mRNA and miRNA). Some others that are more commonly shared between cells, for example, bioactive lipids that are components of cell membranes, including sphingosine-1-phosphate (S1P), ceramide-1-phosphate and lysophosphatidic acid, are highly enriched in all types of MVs. It is well known that S1P, for example, inhibits cell apoptosis and stimulates angiogenesis [18].

Interestingly, as shown in Dr Camussi’s recent paper, MSC-derived MVs may protect the kidney against ischemia–reperfusion-induced acute and chronic kidney injury. In this study, MVs isolated from MSCs were injected intravenously into rats immediately after monolateral nephrectomy and transient occlusion of the renal artery and vein to the remaining kidney. The authors observed that a single administration of MVs immediately after ischemia–reperfusion injury protected experimental animals from acute kidney injury by inhibiting apoptosis and stimulating tubular epithelial cell proliferation. This phenomenon was strongly dependent on mRNA present in these MVs, as pretreatment of MVs with RNase abrogated this protective effect. In addition to mRNA, other components of MVs, such as S1P, could also play a pivotal role as well [18]. Based on previous work where a similar protective effect was observed after infusion of MSCs, this paper suggests that MSCs and MSC-derived MVs show similar biological effects. This implies that MVs and their mRNA cargo are crucial players in cellular therapies involving MSCs. In the most likely scenario, transplanted MSCs in a damaged microenvironment release MVs in response to organ injury, which together with secreted paracrine factors, protect kidney cells from damage. Further work should identify the mRNA species that are playing a crucial role. Because MVs contain miRNA in addition to mRNA, it is also important to investigate the composition of miRNA species in these small vesicular structures [19,20].

This interesting phenomenon described by Camussi et al. has several other implications. It suggests that MVs could be successfully employed instead of intact cells to avoid the possible long-term maldifferentiation of engrafted MSCs and eliminate the risk of their malignant transformation. Second, it is possible that new strategies could be developed, where MVs are harvested from MSCs engineered to express high levels of growth factors, surface molecules, mRNA and miRNA that inhibit apoptosis of target cells and promoting neovascularization of damaged tissues. Such custom-engineered ‘super MVs’ could become a new class of cell-derived therapeutics.

Based on this and other recently published papers, MV should no longer be envisioned as mere cell debris or biologically irrelevant cell dust. Many lines of evidence demonstrate that they are important mediators of intercellular communication and exert important biological effects on target cells. Thus, e.g. as shown in the elegant paper from Dr Camussi’s group increase in survival of kidney tubular cells from ischemia–reperfusion-induced injury indicates that the time for application of MVs to regenerative medicine is approaching.

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(See related article by Gatti et al. Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia–reperfusion-induced acute and chronic kidney injury. Nephrol Dial Transplant 2011; 26: 1474–1483)

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Acute kidney injury (AKI) is a frequent complication after orthotopic liver transplantation (OLT). The incidence of post-operative AKI according to acute kidney injury network criteria can be estimated to be as high as 60% of all patients after liver transplantation [1–3]. Besides increasing morbidity and length of hospitalization, graft survival is significantly reduced, even with only modest increase of serum creatinine (> 0.5 mg/dL, AKIN Stage 1) [3,4]. Post-operative AKI is also an independent risk factor for mortality during the first year after transplantation [1].

The development of AKI following liver transplantation is multifactorial and influenced by numerous pre-, intra- and post-operative factors. During the preoperative period, conditions predisposing for post-operative AKI can be present. The most commonly observed preoperative renal dysfunction is due to the hepato-renal syndrome characterized by arterial vasodilatation mainly in the splanchnic vessel area and severe renal vasoconstriction. Intraoperative factors include long periods of vascular crossclamping, hypotension, high doses of vasopressors and large volume load. Post-operative hypotension and calcineurin inhibitors such as cyclosporine and tacrolimus also support conditions potentially culminating in AKI [4].

Currently, there are no effective measures or treatment strategies available for the prevention or treatment of AKI. The development of effective interventions is hampered by the limited ability of early detection of AKI [5, 6]. In order to develop and evaluate strategies for the prevention and treatment of AKI, there is a great need for early biomarkers.

In this issue of Nephrology Dialysis Transplantation, Wagener et al. [7] propose increased urinary neutrophil gelatinase-associated lipocalin (NGAL)/creatinine ratio as an early predictor of AKI following OLT. The data source is a prospective cohort study of 92 patients undergoing OLT at a single centre between 2008 and 2010 (18 living related, 74 deceased). Patients underwent OLT for different reasons (hepatitis C, hepatitis B, nutritive toxic liver cirrhosis, primary sclerosing cholangitis) and showed a modified end stage liver disease score of 21.9 ± 7.4 prior to surgery. Patients did not require renal replacement therapy preoperatively and apparently had intact kidney function according to the serum creatinine (0.99 ± 0.64 mg/dL). Urine samples were collected after induction of anaesthesia prior to incision, immediately after portal reperfusion of the liver graft and then 3, 18 and 24 h later. To compensate for possible urinary dilution or concentration, the results of the NGAL measurements are given as urinary NGAL/creatinine ratio.

NGAL was increased significantly at Day 2 after transplantation, whereas urinary NGAL/creatinine ratio already showed a significant increase after 3 h. According to the study, there is a diagnostic benefit of urinary NGAL compared to serum creatinine for the diagnosis of AKI after OLT. Interestingly, in patients with AKI, NGAL...