Extracellular vesicles (EVs) are the generic term for nano-sized fragments surrounded by a lipid membrane that are routinely released by virtually all cell types into their surroundings. Within the cell, EVs are derived from the plasma membrane or from endosomal multivesicular bodies. They can be classified into microvesicles (microparticles), typically 100–1000 nm in diameter, released by plasma membrane shedding, and exosomes, 40–100 nm in diameter, generated by exocytosis of intracellular vesicles [1]. Exosomes are often identified using the markers CD63, CD9, CD81 and Hsp70. First
described in the early 1980s, EVs are increasingly being recognized as an important mode of paracrine communication facilitating horizontal shuttling of different kinds of RNAs and proteins from one cell to another [1]. To date, no specific receptor for the binding or cellular uptake of EVs has been identified. Specific RNAs and proteins appear to be enriched within EVs, and mRNA translation into protein seems to be one important mode of their action within target cells [1]. However, there is still no consensus on the potential packaging mechanism. The composition of EVs may change from physiological to diseased states [2] and as such EVs have been described as diagnostic urinary biomarkers of kidney injury [3]. In pathophysiology, EVs can exert severely aggravating roles such as promoting the spread of degenerative disease or cancer, but they may also induce the regenerative capacity of their target cells [2]. This has sparked interest in their use as therapeutics, since EV fractions can be prepared in several ways including ultracentrifugation in vitro, e.g. from stem cells [4]. Hence, translational research in nephrology has started to explore the potential use of their diagnostic and therapeutic application in kidney disease.

The utility of stem cell application in renal injury has been reviewed in more depth elsewhere [5]. About a decade ago, the attenuating effect of intravenously injected bone marrow mesenchyme-derived stromal cells (MSCs) on acute ischaemia-reperfusion kidney injury (AKI) was reported in rats [6, 7]. While no transdifferentiation into epithelial cells was observed, the data suggested that the beneficial effects were mediated by paracrine functions exerted by MSCs which had deposited within the kidney. Similarly, in the alternative AKI model of cisplatin toxicity, disease was ameliorated by the infusion of marrow- or adipocyte-derived stromal cells. Importantly, these beneficial effects could be elicited also by administering the stem cells’ conditioned media alone, underpinning the paracrine hypothesis of stem cell action [8]. Infusion of cultured fibroblasts, on the other hand, did not convey such positive effects in the ischaemia-reperfusion model of AKI [7], demonstrating that the ‘stem cell’ characteristic mattered, too. Unfortunately, stem cell applications are still limited by safety issues. Long-term observations in the rat Thy1.1 model revealed that in up to 20% of glomeruli, injected mesenchymal stem cells eventually maldifferentiated into adipocyte-like cells [9, 10]. Recent studies evaluating the effects of injected MSCs in AKI, however, could not confirm beneficial effects in pigs [11] and in humans (late-breaking clinical trials at the ASN annual meeting, 2014). Thus, it is possible that studies on the beneficial effects of stem cell-derived EV injection (see below) may require confirmation also in larger mammals before considering clinical trials.

Injection of EV preparations is a potential approach to overcome some of the safety issues raised by progenitor cell administration while still preserving the paracrine beneficial effects in AKI. In recent years, research conducted in a significant part by the stem cell research group in Torino, Italy, has embarked on the delivery of stem cell-derived EVs to address this question [12]. In experiments analogous to those described above, EVs derived from either MSCs, endothelial progenitor cells or hepatic stem cells of human origin all proved to be beneficial to treat AKI in rodent models of ischaemia-reperfusion [13, 14], cisplatin toxicity [15] or glycerol-induced rhabdomyolysis [16, 17]. Likewise, the approach of EVs derived from endothelial progenitor cells was reported to drive angiogenesis [18–20] which might also contribute to ameliorating renal disease. Other groups have published similar results supporting a beneficial role of exosomes or microvesicles derived from human umbilical cord cells in cisplatin [21] and ischaemia-reperfusion injury [22], or from MSC-derived vesicles in gentamycin-induced AKI [23].

In this issue of NDT, the research group in Torino extends their approach of endothelial progenitor-derived EVs to the model of anti-Thy1.1 nephritis [24]. In anti-Thy1.1 nephritis, a monoclonal antibody (Ox-7) directed against the Thy1.1 antigen serves to induce acute mesangiolysis; therefore it is a model of acute glomerular injury [25]. Acute mesangiolysis is followed by a phase of re-expansion of the mesangial cell population in an overshooting fashion mimicking mesangio-proliferative conditions. The study by Cantaluppi et al. shows that EVs derived from endothelial progenitors, but not from fibroblasts, improve histology and renal function as well as decrease proteinuria, and leukocyte infiltration. Deposition of complement membrane attack complexes in glomeruli was ameliorated and overall complement haemolytic activity (CH50) was restored from a ~33% reduction back to normal [24].

How might injected EVs exert their functions? The renal and/or glomerular uptake of labelled EVs has been shown to be increased upon proximal tubular injury or in anti-Thy 1.1 glomerulonephritis [24, 26]. EVs are taken up by cultured endothelial cells, mesangial cells or podocytes [24]. Three general mechanisms of action of EVs are currently being discussed [2]: (i) direct transmission of messenger RNAs to target cells. This is supported by a number of in vitro studies [2] and by the fact that the bioeffectivity of EVs is abolished by RNase digestion [13], which was also the case in the present study by Cantaluppi et al. [24]. The present study proposes transfer of mRNAs encoding the complement inhibitors factor H, CD55 and CD59 as a potential mechanism to explain their findings. (ii) Transmission of miRNAs inducing gene silencing via EVs has been demonstrated in vitro [2, 27], and actions of miR-126 and miR-296 have been proposed to mediate beneficial effects in renal ischaemia-reperfusion injury [13]. However, it has been noted that the amounts of miRNA conveyed via EVs might not always be sufficient considering the 1:1 stoichiometry required for gene silencing [28]. In addition, EVs contain small noncoding RNA species of yet undefined functions [1]. (iii) Transmission of proteins such as cytokines and other signalling molecules was suggested by in vitro studies characterizing factors secreted into the conditioned media of cultured stem cells [12]. However, most secreted peptides bind to extracellular receptors, while EVs are taken up into the cytoplasm.

For now, stem cell- and especially endothelial progenitor-derived EVs appear to engage a cocktail of RNAs and proteins that seems surprisingly robust in its ability to ameliorate AKI across a variety of animal models. The precise mechanisms and factors remain to be defined. The mechanistic data from the currently available studies provide stimulating findings. Future studies will have to identify individual RNAs and/or proteins which are necessary and sufficient to mediate the
observed therapeutic effects. Ultimately, spiking inactive (e.g. fibroblast-derived EVs) with specific RNAs and proteins may be a potential approach. Finally, standardized EV preparations will have to be established. Cellular preparations and isolation of EVs usually differ from one institution’s protocol to the other’s [29]. ‘Vesiclepedia’, a joint effort by researchers in the field to create a continuously updated research database is an initiative to address this issue [30]. In summary, the field of research on EVs holds a promising outlook to bring this very exiting diagnostic and therapeutic concept to the bedside.

CONFLICT OF INTEREST STATEMENT

No conflicts declared by any author. The results presented in this paper have not been published previously in whole or part, except in abstract format.


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