RENAL INJURY

Early apoptotic extracellular vesicles in injury and repair

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During injury, mitogenic signals from apoptotic cells may compensate for cell loss by promoting organ homeostasis and regeneration. A distinct type of early apoptotic extracellular vesicle with specific mitogenic activity has been identified. The detection of these vesicles in damaged mouse glomeruli highlights their possible role in response to renal injury.

Refers to Gupta, K. H. *et al.* Apoptosis and compensatory proliferation signalling are coupled by CrkI-containing microvesicles. *Dev. Cell* **41**, 674–684 (2017)

The production of vesicles and their uptake by distant target cells is a fundamental aspect of the intercellular exchange of information. The ancestral nature of vesicle-mediated communication can be inferred from its presence in all three domains of life. Cells release different types of extracellular vesicles depending on the mechanism of vesicle generation. Exosomes (50–100 nm in diameter) are generated from intracellular multivesicular bodies, whereas microvesicles bud from the cell surface and include vesicles released by healthy cells (100–500 nm in diameter), and pre-apoptotic microvesicles (500–1,000 nm in diameter). Apoptotic bodies are large vesicles that are released by apoptotic cells and rapidly cleared by phagocytes. Gupta *et al.* now identify a distinct type of early apoptotic extracellular vesicle with specific mitogenic activity, called CRK-containing microvesicles (CRK-MVs), which are found in damaged mouse glomeruli and thus might have regenerative effects in the kidney[1].

Extracellular vesicles are important in the context of tissue damage. For example, the uptake of apoptotic bodies by macrophages might induce a reparative response and limit inflammation and tissue damage[2]. In addition, several studies highlighted a role of apoptotic bodies in stimulating the proliferation and differentiation of organ-resident stem progenitor cells. In particular, apoptotic bodies released from endothelial cells or cardiomyocytes may induce proliferation and differentiation of endothelial progenitor cells[3] or cardiac precursors[4], respectively. Nanosized exosomes or microvesicles are also released by injured cells and can induce epigenetic modifications in organ-resident stem cells through the transfer of genetic information, thus modulating the regenerative potential of stem cells[5]. For example, extracellular vesicles released by damaged proximal tubular epithelial cells induce an epithelial commitment in mesenchymal stem cells through the transfer of microRNAs of the miR-200 family[6].

> “extracellular vesicles within the urinary lumen may represent an intranephron communication signal”

Gupta *et al.* characterized a new type of early apoptotic extracellular vesicle with a specific mitogenic activity[1]. They showed that vesicles of ~1 μm in diameter, which are distinct from both apoptotic bodies and nanosized extracellular vesicles, were released by HeLa cells in the early phases of apoptosis, and promoted compensatory proliferation signalling in surviving neighbouring cells. Interestingly, early apoptotic extracellular vesicles rapidly induced proliferation in almost 100% of the analysed target cells[1]. The researchers further characterized the essential role of CRK, an adaptor protein that promotes apoptosis through the caspase 3 cascade, in the generation of CRK-MVs, and definitively linked the intracellular apoptotic process with the release of CRK-MVs.

The importance of the compensatory proliferation signalling mediated by early apoptotic vesicles for tissue repair is underlined by its prevalence in metazoan species across the evolutionary spectrum (from *Drosophila melanogaster* to mammals) and by the nonspecificity of the apoptotic stimulus. Moreover, the process of CRK-MV release itself is tightly regulated — only a limited fraction of cells (5%) produce vesicles and only a small number of vesicles (3 to 5) are released per cell[1], compared with the more extensive release of exosomes (about 2,200) by cells[7]. An interesting hypothesis proposed by Gupta *et al.*[1] is that the CRK-MVs are released by a stem and progenitor cell population, although it cannot be excluded that their release is related to cell cycle transition or to other functional physiopathological aspects.

The compensatory proliferation mechanism was first described in *D. melanogaster* but is also of particular relevance for mammalian parenchymal organs, including the liver, intestines and kidney[8]. The study by Gupta *et al.*[1] supports this idea, showing that the CRK-MVs can be isolated *ex vivo* from the glomeruli of mice after treatment with nephrotoxic serum. *In vitro*, CRK-MVs stimulate the proliferation of parietal epithelial cells.

Different functional roles could be envisaged for these mitogenic vesicles in glomerular pathophysiology. Parietal epithelial cell proliferation is a detrimental compensatory signal, but CRK-MV-induced proliferation of other cell types, such as those along the tubules, might invariably be of benefit in the repair of acute kidney injury. Along these lines, evidence suggests that extracellular vesicles within the urinary lumen may represent an intranephron communication signal, possibly also comprising glomerular–tubular communication[5]. In addition, the analysis of vesicles released by cells facing the urinary lumen, including podocytes, might represent a useful diagnostic tool for kidney injury[5]. For example, an increased number of podocyte-derived microparticles (0.1–1 μm in diameter) was reported in the urine of diabetic mice, before the onset of albuminuria[9]. As these particles were released by damaged

podocytes, it cannot be excluded that they were early apoptotic extracellular vesicles. Indeed, a specific search for pre-apoptotic CRK-MVs might be informative about podocyte health.

Extracellular vesicles might also modulate tissue integrity and repair. In fact, administration of extracellular vesicles released from healthy cells — in particular, from stem cells — is viewed as a potential therapeutic strategy for tissue regeneration. Specifically, mesenchymal stem-cell-derived extracellular vesicles exert multiple effects during renal tissue regeneration, including stimulating proliferation of tubular cells, and they promote cell survival upon induction of apoptosis, stimulate angiogenesis and limit fibrosis and inflammation[5]. Furthermore, studies aimed at correlating the functional properties and molecular content of different extracellular vesicle populations have identified exosomes as the main vesicle type responsible for regenerative effects in the kidney[10].

A number of bioactive factors, including DNA, RNA and proteins, have been detected within extracellular vesicles, and these factors might also be present in CRK-MVs. It is therefore important to determine the relationship between the biological activities of CRK-MVs and the nature of their cargo. The proteins within CRK-MVs have been analysed, and the effects of CRK-MVs mainly ascribed to TGFβ-mediated and WNT-mediated activation of JUN N-terminal kinase (JNK) signalling in target cells[1]. This finding differs from the mechanism ascribed to the regenerative effects of mesenchymal stem cell extracellular vesicles. In fact, a number of studies, including those using knock down of Drosha and Dicer to limit microRNA generation, show a relationship between transfer of non-coding RNA and biological activity related to target cell reprogramming[7].

In conclusion, extracellular vesicles seem to be a fundamental aspect of cell-to-cell communication, and the identification of early apoptotic CRK-MVs adds a new player to this field. Knowledge about the activity of these extracellular vesicles could inform strategies to modulate unwanted detrimental proliferation and to generate new tools for regenerative medicine, including in kidney repair (FIG. 1).

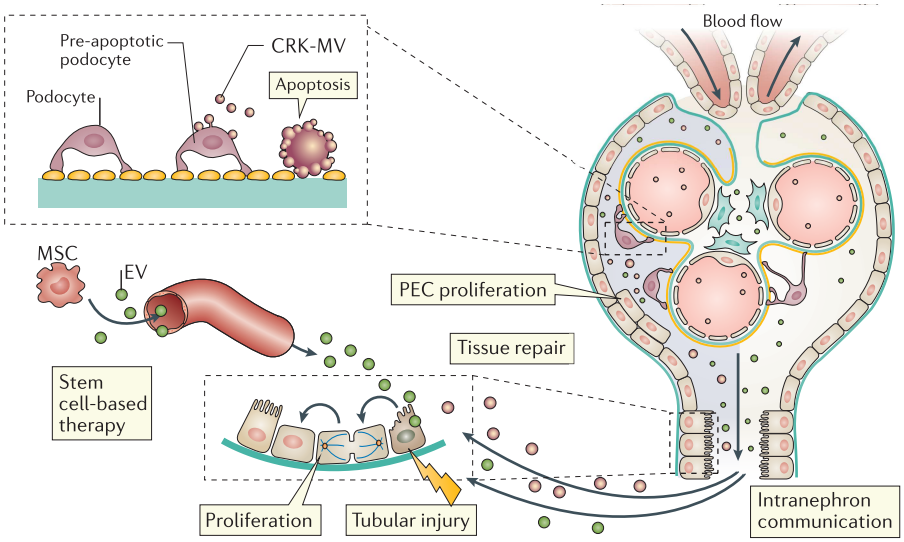

Figure 1 | **Intranephron vesicular communication.** CRK-containing microvesicles (CRK-MVs) released from pre-apoptotic podocytes might induce proliferation of parietal epithelial cells (PECs) and of injured tubular epithelial cells. Extracellular vesicles (EVs) released from different nephron districts may have a role in intranephron communication in physiological and pathological conditions. Mesenchymal stem cell (MSC)-derived EVs might act as paracrine mediators and contribute to nephron repair after injury.

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