Mesenchymal stem cell–derived extracellular vesicles attenuate kidney inflammation

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Mesenchymal stem/stromal cells (MSCs) have distinct capability for renal repair, but may have safety concerns. MSC-derived extracellular vesicles emerged as a novel noncellular alternative. Using a porcine model of metabolic syndrome and renal artery stenosis we tested whether extracellular vesicles attenuate renal inflammation, and if this capacity is mediated by their cargo of the anti-inflammatory cytokine interleukin (IL) 10. Pigs with metabolic syndrome were studied after 16 weeks of renal artery stenosis untreated or treated four weeks earlier with a single intrarenal delivery of extracellular vesicles harvested from adipose tissue–derived autologous MSCs. Lean and sham metabolic syndrome animals served as controls (seven each). Five additional pigs with metabolic syndrome and renal artery stenosis received extracellular vesicles with pre-silenced IL10 (IL10 knock-down). Single-kidney renal blood flow, glomerular filtration rate, and oxygenation were studied *in vivo* and renal injury pathways *ex vivo*. Retention of extracellular vesicles in the stenotic kidney peaked two days after delivery and decreased thereafter. Four weeks after injection, extracellular vesicle fragments colocalized with stenotic-kidney tubular cells and macrophages, indicating internalization or fusion. Extracellular vesicle delivery attenuated renal inflammation, and improved medullary oxygenation and fibrosis. Renal blood flow and glomerular filtration rate fell in metabolic syndrome and renal artery stenosis compared to metabolic syndrome, but was restored in pigs treated with extracellular vesicles. These renoprotective effects were blunted in pigs treated with IL10-depleted extracellular vesicles. Thus, extracellular vesicle–based regenerative strategies might be useful for patients with metabolic syndrome and renal artery stenosis.

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Advances in regenerative medicine have uncovered a distinct potential of mesenchymal stem cells (MSCs) for kidney repair,[1] which resides in their remarkable anti-inflammatory and immunomodulatory properties. We have previously shown that intrarenal delivery of autologous adipose tissue–derived MSCs decreased inflammation and improved function in the stenotic kidney of pigs with non-atherosclerotic renal artery stenosis (RAS)[2,3] or with atherosclerotic RAS undergoing revascularization.[4,5] However, concerns regarding safety and transplantation of viable replicating cells, such as induction of tumors, malformations, or microinfarctions, may limit their translational capacity.[6]

MSCs are avid producers of extracellular vesicles (EVs), including microvesicles (50–1000 nm in size), formed by outward budding and fission of the plasma membrane, and exosomes (40–100 nm), formed in multivesicular bodies and released upon fusion with the membrane.[7] MSC-derived EVs shuttle functional components capable of reducing tissue injury and/or enhancing repair in recipient cells, thereby mediating MSC paracrine action.[8] We have recently shown that cultured porcine adipose tissue–derived MSCs release EVs that contain genes and proteins capable of modulating inflammation, angiogenesis, adipogenesis, and other pathways in recipient cells.[9,10] Indeed, delivery of EVs derived from MSCs has been shown to restore renal structure and function in experimental rodent models of acute renal failure.[11–14] However, their ability to rescue kidney function in chronic renal injury in a large preclinical animal model is unknown. Moreover, the mechanisms associated with the MSC-derived EV renoprotective effect remain to be defined.

Interleukin (IL)-10 is an anti-inflammatory cytokine that regulates the functions of immune cells, and a key determinant of alternatively activated (M2) macrophage phenotype. Macrophages can change their expression profile in response to insults, and their effector phenotype determines the nature and severity of renal injury.[15] Classically activated (M1) macrophages express pro-inflammatory cytokines, whereas M2 macrophages attenuate inflammation and promote tissue repair. We have previously shown that IL-10 expression is blunted in both porcine[2] and human[16] stenotic kidneys, and correlates inversely with tubular injury score and renal fibrosis.[17] Furthermore, MSC delivery into the stenotic kidney favors a phenotypic switch

from M1 to M2 macrophages and improves IL-10 expression, as well as renal function and structure,[2,3] suggesting that this cytokine might be implicated in the MSC-induced reparative process. However, whether IL-10 mediates the paracrine actions of the MSC progeny EVs to preserve kidneys subjected to chronic ischemia has not been directly explored.

The current study took advantage of a novel porcine model of coexisting metabolic syndrome (MetS) and RAS (MetS + RAS), comorbidities that accentuate renal inflammation, associated with prominent glomerular and tubular alterations.[18] We studied the temporal pattern of EV uptake and distribution, and tested the hypothesis that intrarenal delivery of autologous MSC-derived EVs would attenuate inflammation, ameliorating structural and functional decline in the MetS + RAS kidney. Furthermore, we tested whether the renoprotective properties of EVs are partly determined by their cargo of IL-10.

RESULTS

MetS + RAS

MetS + RAS was achieved by inducing unilateral RAS in domestic pigs 6 weeks after initiating a high-cholesterol and -carbohydrate diet. MetS pigs were studied *in vivo* and *ex vivo* after 10 weeks of RAS untreated or treated 4 weeks earlier with a single intrarenal delivery of EVs harvested from autologous adipose tissue–derived MSCs or saline vehicle (Figure 1a). The systemic characteristics in all pigs 4 weeks after EV delivery or sham are summarized in Table 1. Body weight and blood pressure were similarly higher in all MetS groups compared with a lean group. All RAS groups developed a moderate but significant stenosis of a similar degree ($P > 0.05$ analysis of variance). Common to the chronic phase of untreated RAS, plasma renin activity (PRA) levels were similar among the groups. Lipid fraction levels were comparably elevated in all MetS groups compared with lean, and their fasting insulin and homeostasis model assessment of

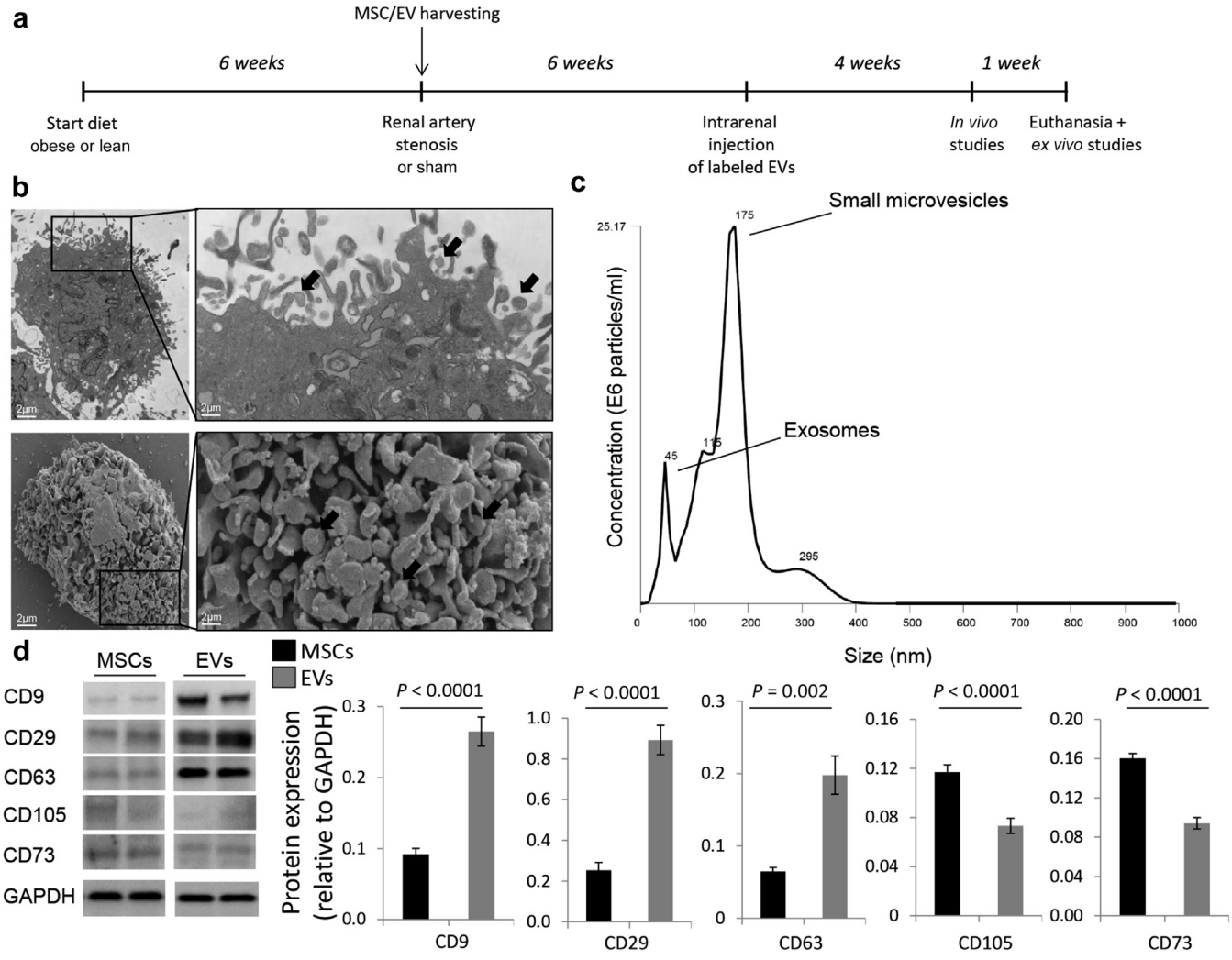

Figure 1 | Cultured porcine mesenchymal stem cells (MSCs) release extracellular vesicles (EVs). (**a**) Schematic of the experimental protocol. (**b**) Transmission (top) and scanning (bottom) electron microscopy showing that cultured MSCs release substantial amounts of EVs (black arrows). (**c**) Size distribution of isolated EVs. (**d**) EVs expressed common EV and MSC markers. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.