Factors Derived from Bioactive Kidney Cells Provide Anti-fibrotic Signals in vitro and may Mediate Regenerative Outcomes in vivo

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Introduction

The regenerative potential of a particular tissue or treatment modality is often defined as its inherent capacity to re-establish appropriate function in vivo by direct replacement of lost or damaged cells. Of equal importance, however, is the ability of a cell to stimulate regeneration and attenuate the progression of disease through indirect mechanisms. In previous studies, we have shown that intra-renal delivery of bioactive kidney cells into a Lewis rat model of chronic kidney disease preserves kidney functions, resulting in significant reductions in glomerular and tubulointerstitial fibrosis and attenuation of pro-fibrotic pathways when compared to untreated controls. In the present study, we employed in vitro cell-based assays to investigate potential paracrine mechanism(s) by which bioactive kidney cells could modulate fibrosis through mediators such as Plasminogen Activator Inhibitor-1 (PAI-1).

Materials and Methods

Conditioned media was collected from rat and human cultures of bioactive kidney cells1-2 under serum- and supplement-free conditions and utilized for in vitro assays. Commercially available human-derived renal mesangial cells were used as surrogates for host-response tissues in the in vitro assays because mesangial cells are a source of PAI-1 production in injured or diseased kidneys. PAI-1 gene and protein expression were assayed by quantitative RT-PCR and Western blot, respectively. Vesicular particles shed by cells into the culture media (exosomes) were collected by high-speed centrifugation and total RNA was extracted from the pellet with TRIzol reagent (Invitrogen). RNA content of the vesicles was screened using PCR-based arrays of known microRNA sequences (Qiagen).

Figure 1. Working model for how extracellular matrix might accumulate in chronically-diseased tissues

In a normal kidney there is a balance between ECM synthesis and degradation. Interruption of this balance can occur at the onset of disease and, if balance is not restored, can lead to chronic kidney disease (CKD). Plasminogen activator inhibitor (PAI-1) and Transforming Growth Factor beta 1 (TGFβ1) affect each other in a positive feedback loop and are associated with processes that could result in inappropriate matrix accumulation and glomerulosclerosis. This study looks at the association of TGFβ1 and PAI-1 with CKD tissue pathology in vivo and the in vitro response of these genes to conditioned medium from rat bioactive kidney cells in mesangial cell cultures in the context of this working model.

Figure 2. Intra-renal delivery of bioactive kidney cells reduces the expression of fibrotic markers in vivo

In vitro experiments were designed to mimic the treatment effect of bioactive kidney cells observed in vivo. Human renal mesangial cells (HRMC) express increased levels of PAI-1 in the presence (+) of 5 ng/ml TGFβ1. Co-culture with conditioned media (CM) derived from human bioactive kidney cells attenuates TGFβ1-induced PAI-1 protein expression (A). PAI-1 expression at the mRNA level was unaltered by CM (data not shown). CM from rat bioactive kidney cells had similar effect on cultured HRMC induced (+) and uninduced (-) with TGFβ1. CM supernatant (Deplete Rat CM) collected after centrifugation was less effective at attenuating PAI-1 expression, suggesting that the CM component responsible for the increased attenuation of PAI-1 protein might be associated with vesicles secreted by the rat bioactive kidney cells.

Figure 3. Conditioned media from human and rat bioactive kidney cells attenuates TGFβ1-induced PAI-1 expression in vitro

In vitro findings support the working model of how bioactive kidney cells might improve renal function in chronically-diseased kidneys by modulating fibrotic pathways such as the TGFβ1/PAI-1 feedback loop.

Conclusions

• In vivo PAI-1 protein levels in glomeruli decrease after treatment of CKD induced by 5/6 nephrectomy with bioactive renal cells
• Depleted vesicles from bioactive renal cell cultures contain components that attenuate PAI-1 induced by the TGFβ1/PAI-1 feedback loop
• Depleted vesicles contain miR-449a and upregulate miR-449a into mesangial cells reduces PAI-1 expression
• Taken together, these data support the hypothesis that one mechanism by which intra-renal delivery of bioactive kidney cells improves renal function might be via cell-cell transfer of components that modulate fibrotic pathways in resident kidney cells

References cited: