HEPARANASE IS A PLAYER IN RENAL FIBROSIS: IT REGULATES TGF-β EXPRESSION AND ACTIVITY.

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OBJECTIVES

Epithelial-mesenchymal transition (EMT) of tubular cells is one of the mechanisms which contribute to renal fibrosis and transforming growth factor-β (TGF-β) is one of the main triggers. Heparanase (HPSE) is an endo-β-D-glucuronidase that cleaves heparan-sulfate thus regulating the bioavailability of growth factors (FGF-2, TGF-β). HPSE controls FGF-2-induced EMT in tubular cells (1) and is necessary for the development of diabetic nephropathy in mice (2).

The aim of this study was to investigate whether HPSE can modulate the expression and the effects of TGF-β in tubular cells.

METHODS

Two different cell lines were used: 1) HK2 (human kidney 2), a human renal proximal tubular cell line; 2) a stably HPSE-silenced HK2 cell line obtained by transfection with shRNA plasmid targeting human HPSE as recently described elsewhere (3). Cells were treated with TGF-β1 (BD Bioscience), or 100µg/ml BSA (Sigma), or 100µg/ml of glycated albumin (AGE), or 10ng/ml FGF-2 (BD Bioscience) or 50µg/ml low molecular weight heparin (LMWH) for 6, 24 or 48h.

Gene expression levels of α-SMA, FN, VIM, MMP-9, TGF-β and HPSE were quantified by real-time PCR and normalized to GAPDH. Protein expression of α-SMA, FN, VIM was assesed by IF . TGF-β release was measure by ELISA. MMPs activity was measured by zimography.

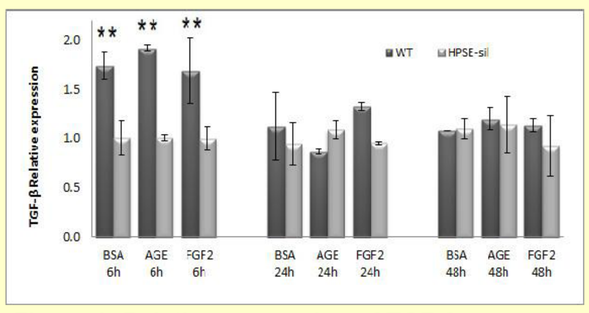

Figure 1: Relative expression of TGF-β.

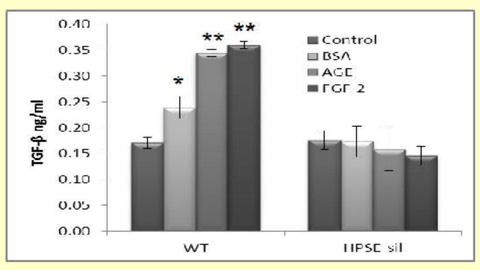

Figure 2: TGF-β release

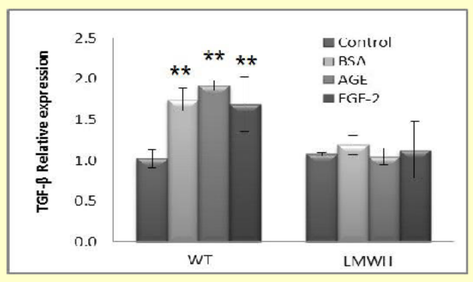

Figure 3: TGF-β expression in LMWH-treated cells

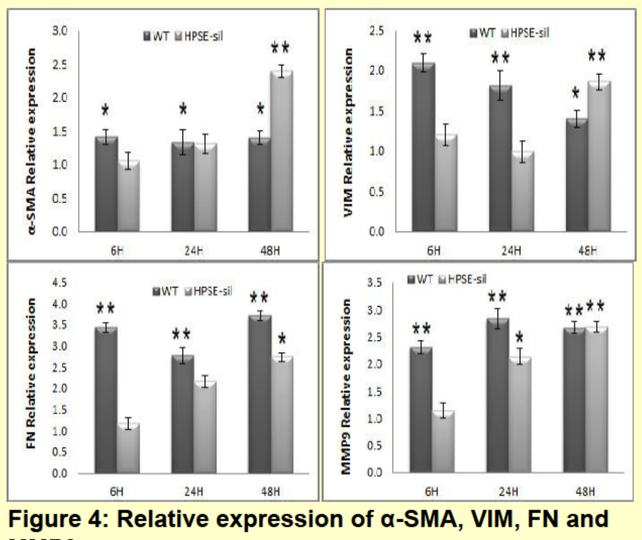

Figure 4: Relative expression of α-SMA, VIM, FN and MMP9

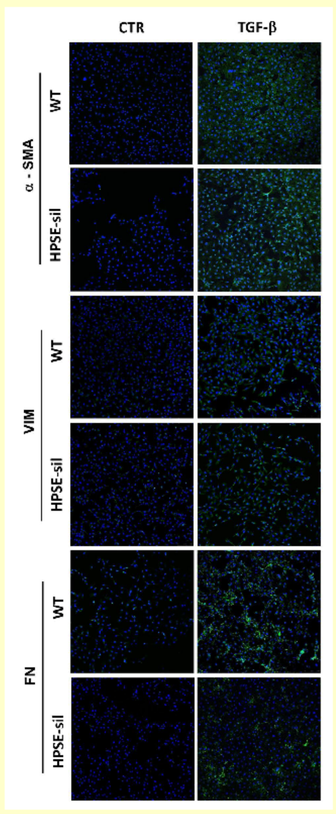

FIGURE 5: α-SMA, VIM and FN protein expression after TGF-b treatment

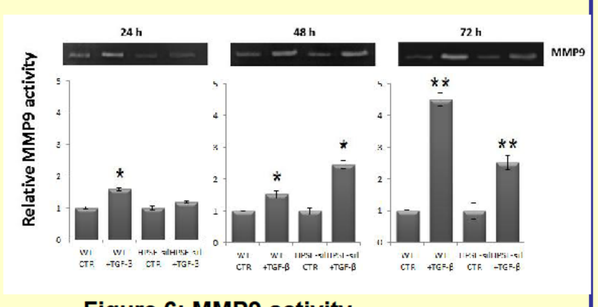

Figure 6: MMP9 activity

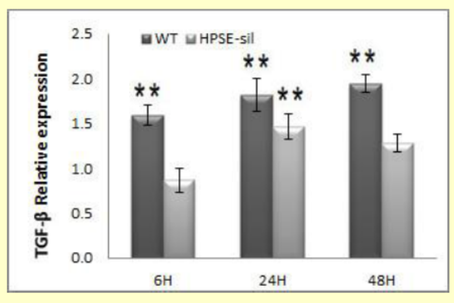

Figure 7: Relative expression of TGF-β

RESULTS

First we proved that the lack of HPSE (Fig 1 and 2) or its inhibition (Fig 3) prevents the increased expression and synthesis of TGF-β by tubular cells in response to pro-fibrotic stimuli such as FGF-2, advanced glycosylation end products (AGE) and albumin overload.

Second, since TGF-β may derive from sources different from tubular cells, we investigated whether HPSE modulate tubular cell response to exogenous TGF-β. HPSE does not prevent EMT induced by TGF-β although it slows its onset; indeed n wt cells TGF-β induced an early up-regulation of all the above genes from the 6-h point, while the effect in HPSE silenced cells was later, starting from the 24th h or even later for VIM (Fig 4). Moreover, after 48 h of incubation with TGF-β, protein amount of α-SMA, FN and VIM were clearly up-regulated both in wt and HPSE-silenced cells. (Fig 5).

In wt cells TGF-β increase the release of active MMP9 progressively, from 24 to 72 h. In HPSE-silenced cells, TGF-β increased the release of active MMP9 after 48 and 72 h of treatment but not at 24 h (Fig 6).

TGF-β produces an autocrine loop to sustain its signal (and EMT); thus we hypothesized that the delayed response of HPSE-silenced cells to TGF-β could be due to alterations in this feedback.

The treatment induced a rapid significant increase of the TGF-β gene expression in wt HK2 cells and this up-regulation lasted over time. In HPSE-silenced cells TGF-β basal gene expression level was comparable to wt cells, and incubation with TGF-β induced only a delayed and transient overexpression of the TGF-β gene (Fig 7).

CONCLUSIONS

Overall, these data confirm that heparanase is an important player in renal fibrosis : A) HPSE modulates TGF-β induced EMT, in particular, the lack of HPSE delays tubular cell transdifferentiation and impairs the TGF-β autocrine loop and B) HPSE is needed for the pathological TGF-β overexpression in response to pro-fibrotic factors such as overload of albumin, AGE and FGF-2.

Given the incidence of chronic renal failure and the need for effective treatments, these findings put HPSE forward as a potential and important drug target in the treatment of kidney disease and renal fibrosis. The understanding of the molecular mechanisms by which HPSE controls the EMT in renal tubular cells is therefore of fundamental importance.

Altogether, these data confirm that strategies aimed at inhibiting heparanase could be a useful tool in controlling several mechanisms leading to organ fibrosis.

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L) Renal pathology. L1) Experimental pathology.

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