HEPARANASE: A POTENTIAL NEW FACTOR INVOLVED IN THE PRO-FIBROTIC RENAL BIOLOGICAL MACHINERY ACTIVATED BY THE ISCHEMIA/REPERFUSION INJURY.

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OBJECTIVES

Ischemia-reperfusion (I/R) injury is a relatively frequent clinical condition following several local and systemic events characterized by limited tissue perfusion such as delayed graft function, major vascular surgery complications, trauma and resuscitation, myocardial infarction, sickle cell disease and acute kidney injury. It is an important cause of acute renal failure and delayed graft function, and it may induce chronic renal damage by activating epithelial to mesenchymal transition (EMT) of renal tubular cells [1,2]. Heparanase (HPSE), an endoglycosidase that regulates FGF-2 and TGFβ-induced EMT, may have an important role [3]. Therefore, aim of this study was to evaluate its role in the I/R-induced renal pro-fibrotic machinery by employing in vitro and in vivo models.

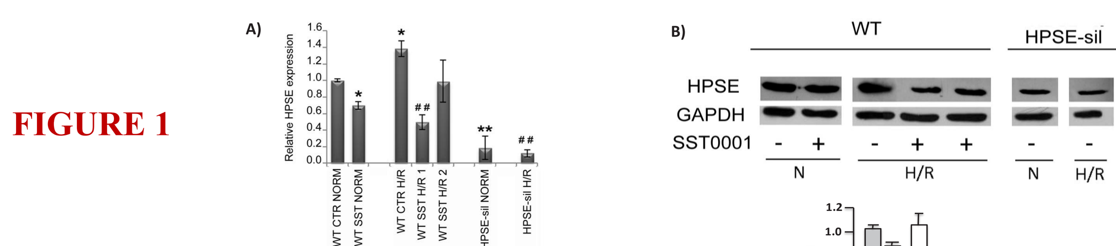
METHODS

In vitro: Human renal proximal tubular cells (HK2) were grown according to our protocol. A stably HPSE-silenced HK2 cell line was obtained by transfection with shRNA plasmid targeting human HPSE (NM_006665) purchased from OriGene. Hypoxic condition was established for 24 h by using an Anaerob Atmosphere Generation Bags and an indicator test for checking the anaerobic conditions. The system reduces oxygen concentration below 1% in 1 h without changing the medium pH. Subsequently, the cells were cultured under normoxic conditions for 6 h (gene expression analysis) or 24 h (protein expression analysis) (reoxygenation phase). WT HK2 cells were also treated with or without 200 ug/ml SST0001 both before hypoxia and prior to reoxygenation. The cells investigated after a complete cycle of hypoxia/reoxygenation (H/R) are hereinafter indicated as H/R cells.

In vivo: Acute ischemia was induced in WT and heparanase-overexpressing (HPA-tg) Balb/c mice. Both right and left renal arteries were exposed and clamped for 30 min during which time the kidney was kept warm and moist. The clamp was then removed, the kidney was observed for return of blood flow, and the abdominal wall incision was sutured. The kidneys were harvested and weighted. One half of the left kidney was snap-frozen in liquid nitrogen and stored at −70°C until further molecular processing; the other half was fixed in formalin, paraffin-embedded, and sectioned (4 μm). Paraffin sections were stained with hematoxylin-eosin and examined histologically. Sham operated mice that underwent an identical procedure except for renal artery clamping, served as controls.

RESULTS

FIGURE 1



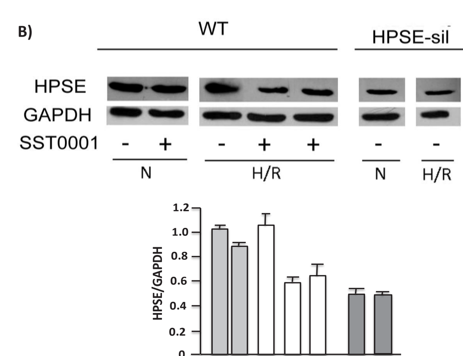


FIGURE 2

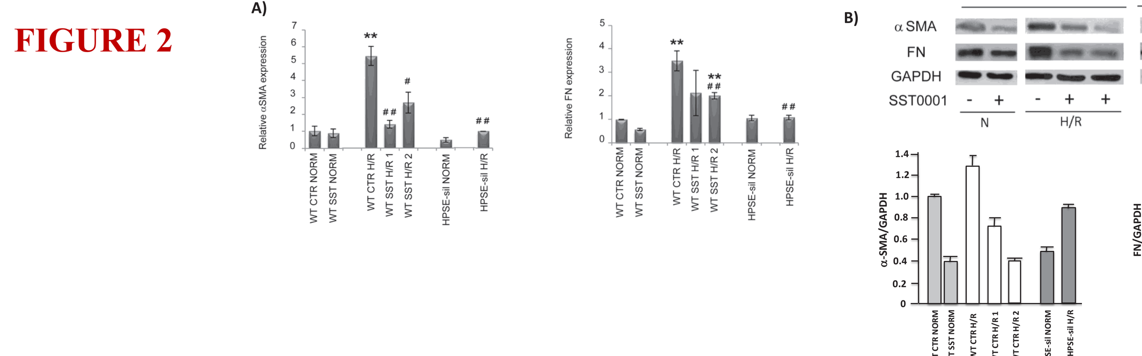


FIGURE 3

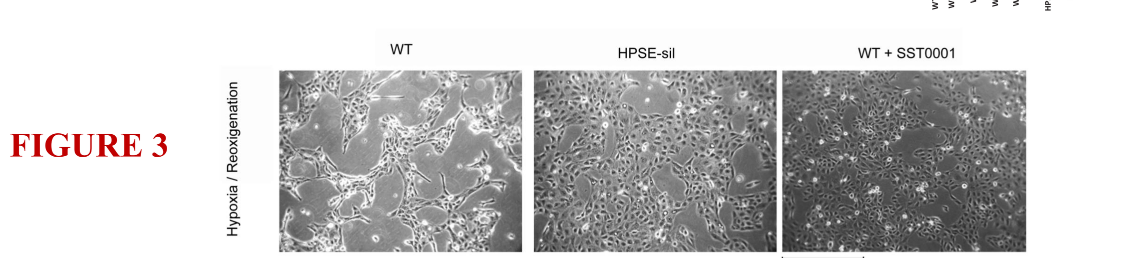
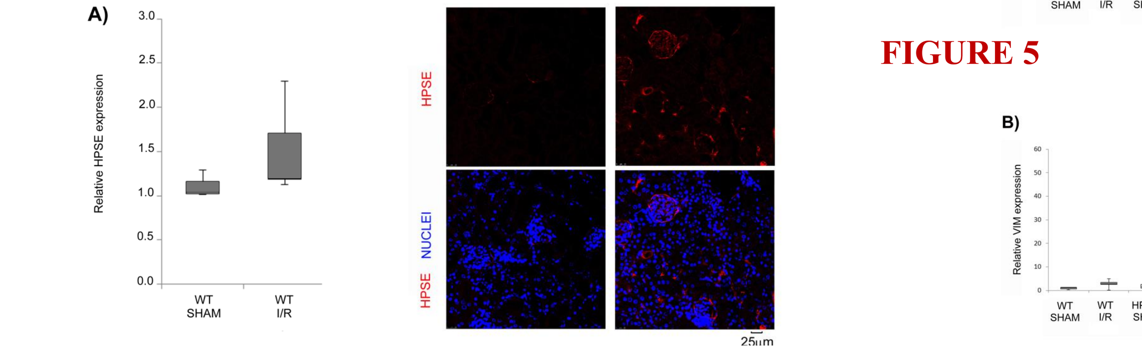


FIGURE 4

FIGURE 5



In vitro: Gene expression **(FIGURE 1A)** and protein analysis (**FIGURE 1B**) revealed that H/R was able to induce a significant HPSE up-regulation in WT cells, but neither in HPSE-silenced cells nor in those treated with the specific inhibitor. Notably, treatment of WT cells with SST0001 reduced HPSE gene expression below basal levels.

Moreover, gene and protein expression analyses revealed that H/R up-regulates the production of both α-SMA and FN in WT tubular cells. Treatment with SST0001 significantly reduced H/R-associated EMT, while HPSE-silenced cells exposed to H/R showed low expression level of both EMT markers (**FIGURE 2**). Additionally, optical microscopy revealed that only wild type cells showed a morphological change after H/R displaying an elongated phenotype (**FIGURE 3**).

In vivo: WT mice after 48 hours of reperfusion showed an increment of HPSE gene expression in renal tissue (**FIGURE 4A**).

Immunofluorescence staining of renal tissue 72 hours post-reperfusion confirmed the up-regulation of HPSE at both glomerular and tubulointerstitial levels (**FIGURE 4B**).

Interestingly, gene expression analysis of total kidney lysates 48 hours after I/R and immunofluorescence staining for α-SMA and VIM 72 hours post-reperfusion showed that HPA-tg mice exhibited remarkable up-regulation of these biological elements (**FIGURE 5**).

Instead, WT mice showed only a slight but not statistically significant increment of the transcriptional level of EMT markers after 48 hours (**FIGURE 5A and B**) and no increment in protein expression 72 hours after I/R **(FIGURE 5C and D**).

CONCLUSIONS

Our results add new insights towards understanding the renal biological mechanisms activated by I/R and they demonstrate, for the first time, that HPSE is a pivotal factor involved in the onset and development of I/R-induced EMT. It is plausible that in future the inhibition of this endoglycosidase may represent a new therapeutic approach to minimize/prevent fibrosis and slow down chronic renal disease progression in native and transplanted kidneys.

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