Mesenchymal Stromal Cells downregulate Renin-Angiotensin System in Unilateral Ureteral Obstruction model in rats suppressing HuR.

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OBJECTIVES

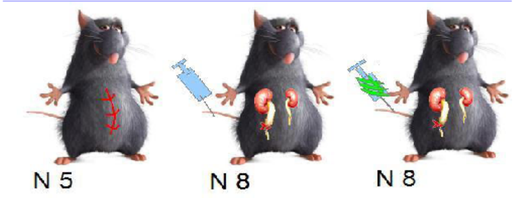
Renin-angiotensin system (RAS) plays a pivotal role in renal fibrosis(1) and renin production is critically dependent on renin mRNA (REN mRNA) stability. We have proved that Mesenchymal Stromal Cells (MSC) prevent inflammation and renal scarring in Unilateral Ureteral Obstruction model in rats (UUO), but at present the mechanism by which MSC protect the kidney from fibrosis is unknown (2-3). HuR is a protein that target a cis-element 3'UTR of REN mRNA and regulate renin production (4). IL-10 inhibits HuR activity, moreover serum IL-10 is higher in UUO rats infused with MSC(5).

Aim: investigate whether the mechanisms by which MSC exert anti-fibrotic effect in UUO involved RENmRNA stabilizing protein HuR .

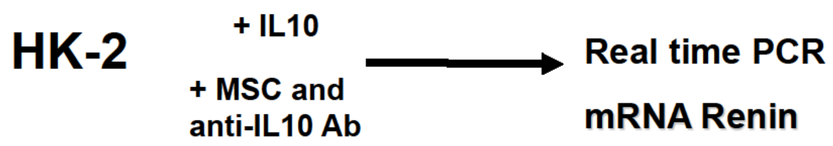
METHODS

In vivo experiments: We studied 3 groups of SD rats. MSC were isolated by bone marrow. Group A: 5 rats sham operated. Group B: 8 rats UUO received saline solution at day 0 . Group C: 8 rats UUO received MSC (3 millions) at day 0 via tail vein. Rats were sacrified at days 1 and 7. Serum ANG II levels were evaluated by ELISA, RENmRNA expression, HuR mRNA expression were evaluated on renal tissue by RT PCR. ***In vitro experiments*** : HK-2 (Homo Sapiens Kidney, Cortex/Proximal tubule) cell line derived from normal kidney were cultured in high glucose (HG) medium to induce upregulation of RENmRNA and incubated for 30 min, 1, 2, 4 hours in presence in presence and absence of IL10 (10 ng), a potent anti-inflammatory cytokine released by MSC, and in presence of neutralizing antibody anti IL-10 able to suppress mRNA stabilizing protein HuR. REN mRNA was evaluated on cell lysate by RT PCR

In vivo experiments:



in vitro experiments:



RESULTS

IN VIVO EXPERIMENTS

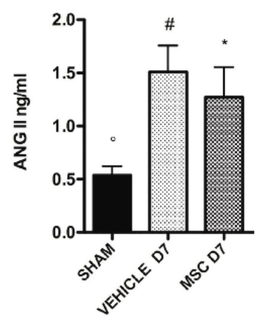


Fig. 1

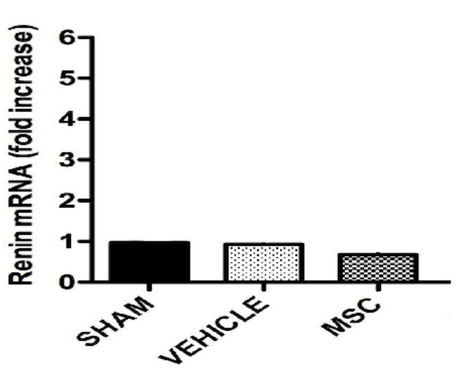


Fig. 2

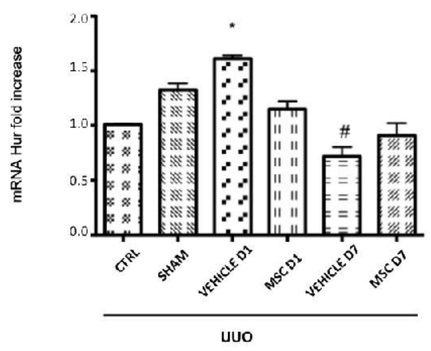


Fig. 3

IN VITRO EXPERIMENTS

HK + IL10

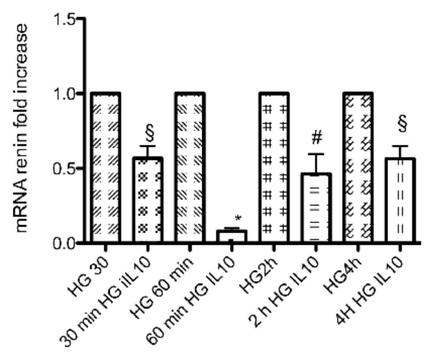


Fig. 4

HK+MSC IN HG medium + neutralizing IL10 Ab

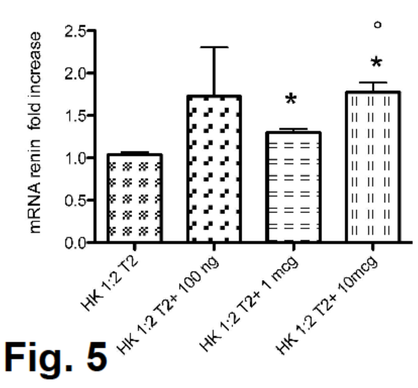


Fig. 5

After 7 days of ureter ligation serum ANG II levels increased in rats of group B compared with group A (p<0.001), MSC infusion reduced significantly serum ANG II levels in rats of group C (p<0.001) compared with group B **(Fig.1)**. REN mRNA expression did not increase in rats of group B compared with group A due to ANGII feedback (**Fig.2**). MSC reduced, but not significantly, REN mRNA in rats of group C, compared with groups A and B. One day from ureter ligation HuR mRNA expression was significantly higher in rats MSC untreated compared to sham operated rats (p<=0.05) . MSC treatment in rats of group C suppressed HuR mRNA expression compared with rats of group B (p<0.005). After 7 days from ligation there were not difference between groups**(Fig. 3)**. *In vitro experiments* showed that REN mRNA expression was suppressed significantly in presence of IL10 (p<0.001,**Fig.4**). Blocking IL10, secreted by MSC, REN mRNA increased significantly in medium additioned with neutralizing IL10 Ab (p< 0,005) **(Fig.5)**.

CONCLUSIONS

Our results show that MSC in UUO model suppress HuR transcription via IL-10. The suppression of HuR trascription may induce decreased REN mRNA stability and inhibition of RAS in UUO.

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L) Renal pathology. L1) Experimental pathology.

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