MESENCHYMAL STROMAL CELLS SUPPRESS *in vitro* RENIN mRNA TRASCRIPTION IN MACROPHAGES AND EPITHELIAL CELLS

V. Corradetti 1, 3, S. Milanesi 1, C. Rocca 1, M.A. Avanzini 2, E.F. Pattonieri 1,3, F. Bosio 1,3, M. Cannone 1,3, N. Maggi 1,3, M. Gregorini 1,3, P. Esposito 1, T. Rampino 1, A. Dal Canton 1,3.

1.S.C. Nefrologia, dialisi e trapianto IRCCS Fondazione Policlinico San Matteo, Pavia

2. Laboratorio di Immunologia e Trapianti, Cell Factory, Oncoematologia Pediatrica IRCCS Fondazione Policlinico San Matteo, Pavia

3. Università degli Studi di Pavia

OBJECTIVES

In animal models of fibrogenic renal and cardiac disease Mesenchymal Stromal Cells (MSC) attenuate the inflammatory injury and the progression of fibrosis by causing a downregulation of the renin-angiotensin-aldosterone system (RAS) [1, 2].

Actually, intrarenal activation of RAS has been proved to be a major culprit for renal fibrosis in several renal disorders, including the leading cause of advanced renal failure, i.e. diabetic nephropathy [3]. In the present shortage of any information on the mechanism by which MSC suppress RAS we believed that the first step to understand was investigate the interaction of MSC with two cell type(s) that are nominal renin source in diabetic nephropathy [4, 5].

METHODS

HK-2 (Homo Sapiens Kidney, Cortex/Proximal tubule) cell line derived from normal kidney were cultured in low (LG) and high glucose (HG) medium. Macrophages (Mϕ) derived from circulating monocytes (MC) were isolated by Ficoll density gradient centrifugation and adhesion for 24h and coltured for 4 days at 37° C with 5% di CO2.

Monocyte to macrophage shifting phenotype was assessed by FACS flow cytometer using CD14FITC antibody. MSC were isolated from bone marrow by healthy donor and expanded in vitro with LG-DMEM, 10% FCS, 1% gentamicin until passage 2/3. HK2 and MSC were co-coltured in well at 1:2, 1:20 and 1:200 concentrations in HG-medium for 1h (T1) and 4h (T2). Mϕ and MSC were co-cultured at 1:2, 1:20 and 1:200 concentration per well for 4 days. Renin mRNA expression was evaluated by RT-PCR.

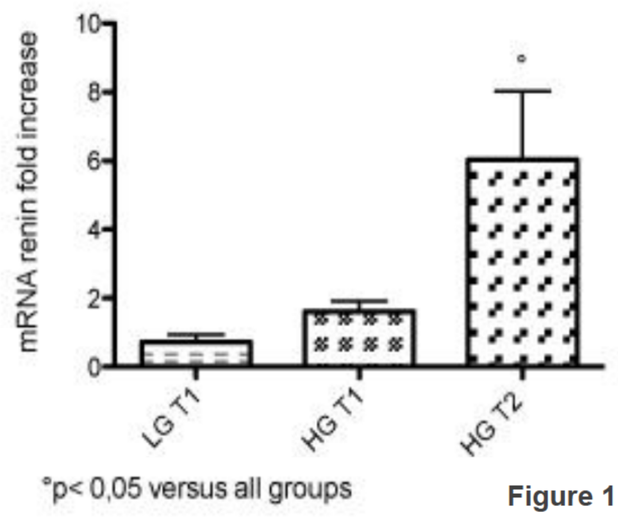

Figure 1

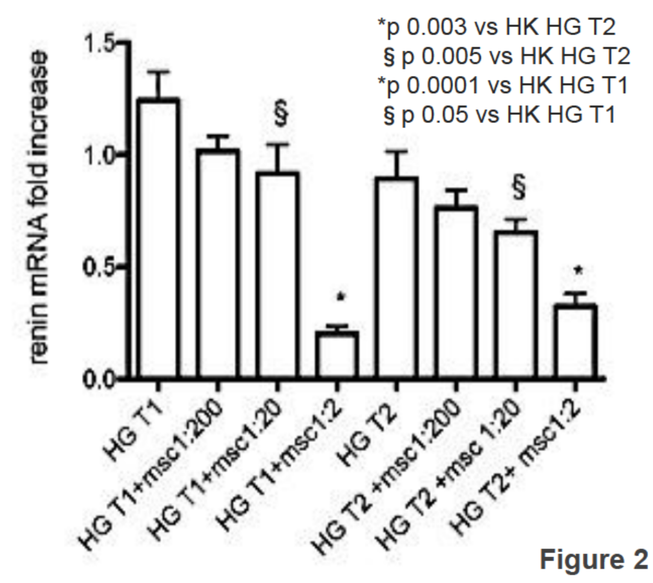

Figure 2

RESULTS

Renin mRNA expression increased significantly in HK2 cultured in HG compared LG medium ($p<0.005$) Figure 1. MSC addition in HK2 HG coltures at concentration 1:2, 1:20 for 1h and 4h suppressed significantly HK2 renin mRNA expression compared with HK2 HG alone ($p<0.005$) Figure 2. Renin mRNA expression increased significantly in macrophages compared with circulating monocytes ($p<0.005$) Figure 3. Renin mRNA expression was suppressed in macrophages co-cultured with MSC at concentration 1:2, 1:20, 1:200 compared with macrophages cultured alone ($p<0.005$) Figure 3.

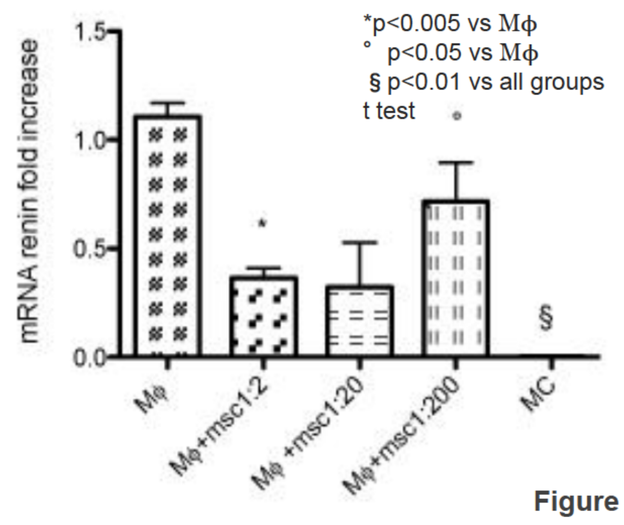

Figure 3

CONCLUSIONS

In conclusion our results show that MSC in vitro downregulate renin mRNA trascription in macrophages and activated epithelial cells, principal actors in many renal diseases. Further studies are needed to understand the mechanisms by which MSC suppress RAS.

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