A CLUSTER OF PROTEINS SECRETED BY HUMAN RENAL STEM/PROGENITOR CELLS (ARPCs) PROVIDE A NOVEL STRATEGY TO REVERT ENDOTHELIAL DYSFUNCTION AND RENAL INJURY IN SEPSIS-INDUCED ACUTE KIDNEY INJURY (AKI)

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INTRODUCTION AND AIMS: Endothelial dysfunction is crucial in the pathophysiology of LPS-induced AKI. Endothelial cells (EC) acquired a fibroblast-like phenotype and contributed to myofibroblasts generation through EndMT process. Noteworthy, ARPCs enhance tubular regenerative mechanism during AKI, but little is known about their effects on EC. The aim of this study was to understand whether ARPCs could prevent sepsis-induced EndMT and to identify the related mechanisms.

METHODS: EC were stimulated in vitro with LPS (4 μg/ml) for 48h, co-cultured with ARPCs for 24h, and analyzed by MTT assay and FACS. Gene expression profile was obtained from ARPCs and LPS-stimulated ARPCs by Agilent SurePrint G3 Human Gene Expression Microarrays. Genespring and R software were used for the analysis. Gene expression was validated by Real-time PCR and ELISA. AKI was induced by i.v. LPS infusion (300 μg/kg) in swine model. Renal biopsies, were performed before and at 9h after LPS infusion, and were analyzed by IF.

RESULTS: We observed a significant proliferation of EC when activated by LPS (vs basal 48h, p<0.05). Moreover, LPS significantly decreased specific EC markers such as CD31 (67% vs 97% basal, p<0.001) and VE-cadherin (31% vs 96% basal, p<0.001). On the contrary, LPS up-regulated myofibroblast markers such as Collagen I (73% vs 14% basal p=0.001) and Vimentin (50.86% vs basal 30% p=0.001). The co-culture with ARPCs normalized EC proliferation rate and downregulated the LPS-induced EndMT by restoring the high expression of EC markers (p=0.003) and the low expression of myofibroblast markers (p=0.0001). Then, we compared gene expression of LPS-stimulated ARPCs with that of basal ARPCs to find genes activated by LPS (Fold change ≥1.5,FDR<0.05). Gene set enrichment analysis showed that most of genes modulated in LPS-stimulated ARPCs belongs to cell activation and defense response pathways. In particular, among most up-regulated genes we found BPIFA2, SAA2, SAA4 and CXCL6. BPIFA2 is recently described as an early biomarker of AKI but little is known about its function in the kidney. The other genes are frequently involved in the response to bacterial infection and kidney injury. RT-PCR analysis showed that these genes were highly expressed in LPS-stimulated ARPCs (vs basal ARPCs, BPIFA2 p=0.001; SAA2 p=0.001; SAA4 p=0.005; CXCL6 p=0.001) and in ARPCs co-cultured with EC in presence of LPS (vs basal ARPCs BPIFA2 p=0.005; SAA2 p=0.01; SAA4 p=0.01, CXCL6 p=0.005). In accordance, ELISA analysis confirmed these data. Finally, in endotoxemic renal biopsies, we observed an increase of CD133ARPCs that expressed BPIFA2 respect to healthy pigs (p=0.0001).

CONCLUSIONS: Taken together these results suggest an underestimate role of ARPCs in preventing endothelial dysfunction by the production of several proteins. The identification of these molecules may offer novel strategies to protect endothelial compartment and promote kidney repair.