ARPCS CAN REVERT LPS-INDUCED ENDOTHELIAL-TO-MESENCHYMAL TRANSITION OF ENDOTHELIAL CELLS

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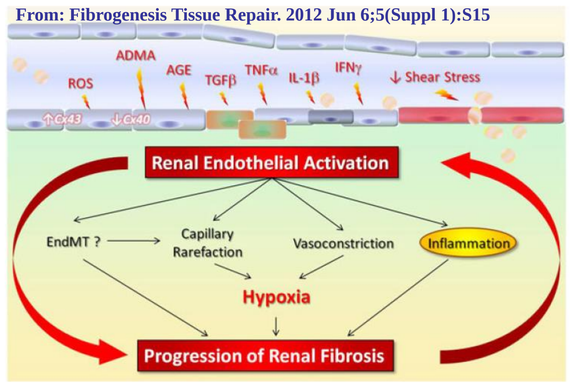
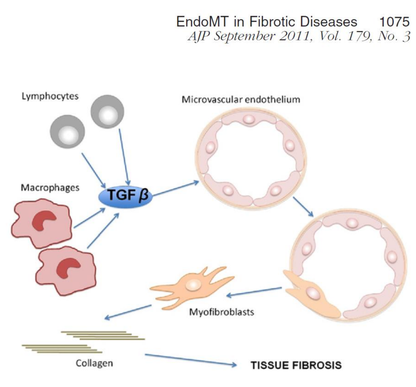
INTRODUCTION AND OBJECTIVES

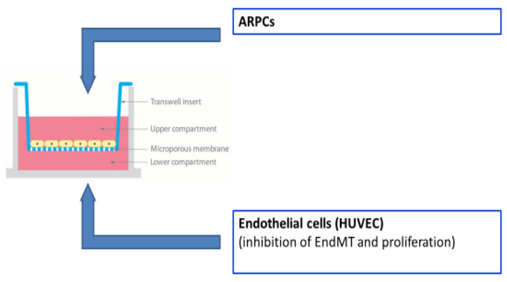




Among the several disorders encountered in sepsis, Acute Kidney Injury (AKI) is the major complication and it is mainly characterized by endothelial cell (EC) dysfunction. EC acquire a myo-fibroblast phenotype, by endothelial-to-mesenchymal transition (EndMT), contributing to the renal fibrosis. Gram-negative bacteria and their cell wall component lipopolysaccharides (LPSs) are frequently involved in the pathogenesis of AKI. The transition of endothelial cells into myofibroblasts can be initiated by the TGF-β secreted by tissue-infiltrating chronic inflammatory cells such as macrophages and lymphocytes. Moreover, EndMT can be induced by the canonical Wnt activation. Since EndMT contributes to the accumulation of activated fibroblasts and myofibroblasts in kidney fibrosis, targeting EndMT might have therapeutic potential. Noteworthy, resident adult renal stem/progenitor cells (ARPCs) enhance tubular regenerative mechanism during AKI, but little is known about their effects on endothelial compartment. The aim of the present study is to investigate the effects of ARPCs on endothelial dysfunction.

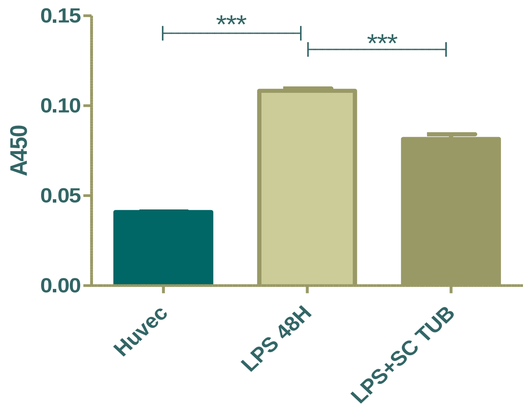
METHODS

Endothelial cells (EC) were stimulated in vitro with LPS (4µg/ml) for 48h and co-cultured with ARPCs for 24h. MTT cell viability assay was used to analyze the EC proliferation rate following LPS stimulation and in co-culture with ARPCs. FACs analysis was used to study the expression of CD31, VE-cadherin, Collagen I and Vimentin. The gene expression profile was obtained from ARPCs and endothelial cells with Agilent SurePrint G3 Human Gene Expression Microarrays. Genespring and R software were used for analysis. RT-PCR was used to study the expression of Wnt-pathway related genes.



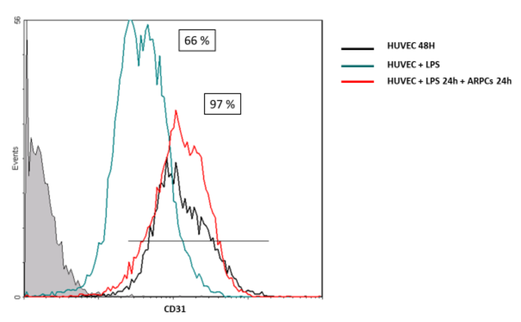
RESULTS

MTT

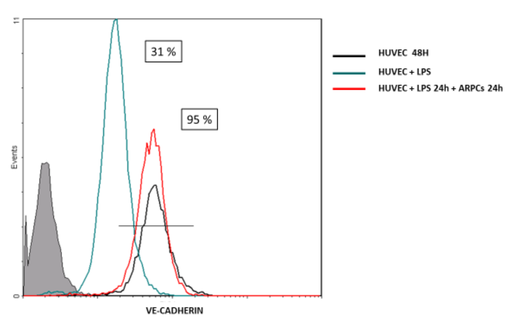


When ECs were stimulated with 4µg/ml LPS, we observed, through MTT, an increased proliferation of ECs (1.7 fold compared to basal 48h h, P =0.05).
Instead, ARPCs in co-culture with EC normalized their proliferation rate and decrease the cell growth rate (P =0.05), even in presence of LPS, after only 24 hours of co-culture.

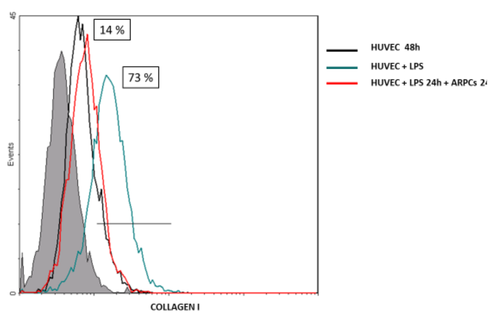
CD31 FACS ANALYSIS



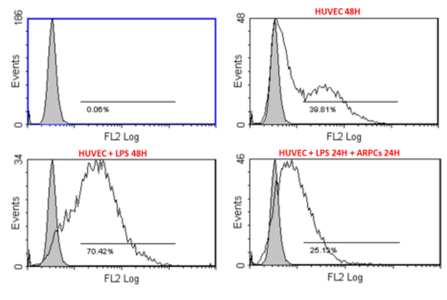
VE-CADHERIN FACS ANALYSIS



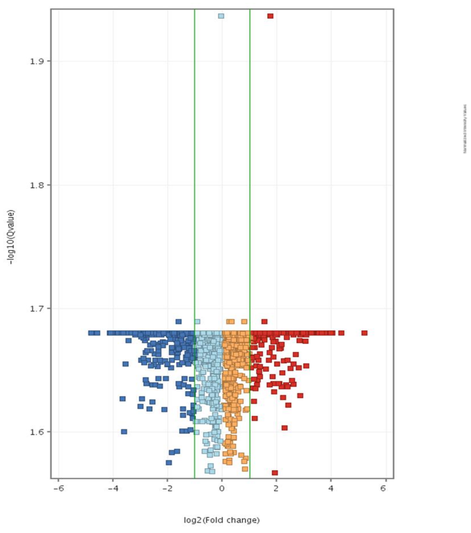
COLLAGEN I FACS ANALYSIS

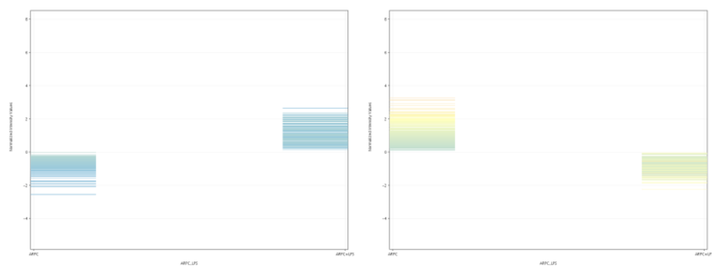


VIMENTIN FACS ANALYSIS

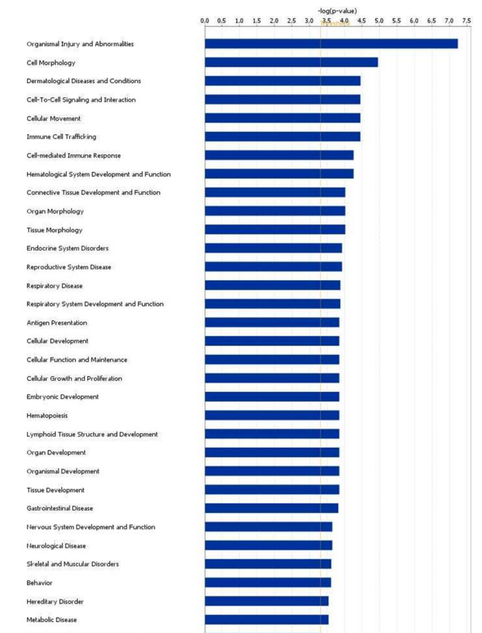


LPS induced EndMT, decreasing significantly specific ECs markers such as CD31 (67% vs 97% basal, p=0.001) and VE-cadherin (31% vs 96% basal, p=0.001). In addition, LPS up-regulated markers of ECs dysfunction such as Collagen I (73% vs 14% basal p=0.001) and Vimentin (50.86% vs basal 30 % p=0.001). Interestingly, ARPCs in co-culture with EC abrogated the LPS-induced EndMT by restoring the high expression of CD31 (95% vs 66% EC without ARPCs, p=0.005) and VE-cadherin (96% vs 31% EC without ARPCs, p=0.001) and limiting Collagen I (18% vs 73% EC without ARPCs, p=0.001) and Vimentin (35 % vs 50.86% EC without ARPCs, p=0.001) expression.





Gene Set	Total Genes	Genes found	q-valu	ES [ARPC+LPS] vs [ARPC]	NES [ARPC+LPS] vs [ARPC]
ZWANG_TRANSIENTLY_UP_BY_2ND_EGF_PULSE_ONLY	1725	35	0	-0.2493158	
GO_DEFENSE_RESPONSE	1231	27	0	-0.2660338	-1.5446887
MEISSNER_BRAIN_HCP_WITH_H3K4ME3_AND_H3K27M	1069	20	0	-0.3162981	-1.0407219
GO_CHEMICAL_HOMEOSTASIS	874	17	0	-0.40806174	-1.398349
GO_G_PROTEIN_COUPLED_RECEPTOR_SIGNALING_PATH	1193	33	0	-0.2840053	-1.1689856
MIKKELSEN_MEF_HCP_WITH_H3K27ME3	590	21	0	-0.45413083	-2.2411408
WWTAAGGC_UNKNOWN	1896	31	0	0.20952082	1.4561726
GO_TISSUE_DEVELOPMENT	1518	29	0	0.15234566	
GO_SYNAPSE	754	21	0	0.24589741	1.043077
GO_IDENTICAL_PROTEIN_BINDING	1209	17	0	0.41901758	1.225483
GO_ENZYME_BINDING	1737	21	0	-0.21149743	
GO_SIGNAL_TRANSDUCER_ACTIVITY	1731	40	0	-0.33517355	-1.3557633
GO_REGULATION_OF_CELLULAR_COMPONENT_MOVEM	771	21	0	-0.22118992	
GO_POSITIVE_REGULATION_OF_CELL_COMMUNICATIOI	1532	35	0	-0.26053512	-1.7251083
GO_RESPONSE_TO_ENDOGENOUS_STIMULUS	1450	29	0	-0.24855068	-1.0152811
GO_CELL_JUNCTION	1151	25	0	0.1944282	
GO_NEURON_DEVELOPMENT	687	17	0	-0.21307984	
WTTGKCTG_UNKNOWN	722	19	0	-0.19657516	
GO_POSITIVE_REGULATION_OF_PHOSPHORUS_METABC	1036	23	0	-0.4013585	
GO_RESPONSE_TO_ORGANIC_CYCLIC_COMPOUND	917	16	0	-0.40535682	-1.1268115
GO_CYTOSKELETAL_PART	1436	15	0	0.30301806	
GO_REGULATION_OF_CELL_DEATH	1472	22	0	0.18184352	
PEREZ_TP53_TARGETS	1174	19	0	0.18700266	1.2030797
GO_SMALL_MOLECULE_METABOLIC_PROCESS	1767	23	0	0.3460766	
GO_BIOLOGICAL_ADHESION	1032	24	0	-0.2942402	-1.2163678



We performed a transcriptome screening of ARPCs to identify LPS-modulated genes. We identified 999 significantly modulated genes (Q value <0.05 and Fold change> 2), 305 over-expressed by LPS and 694 under-expressed.
Gene set enrichment analysis and pathway analysis allowed us to identify a set of 27 genes specifically involved in processes involved specifically in damage limiting biological processes or preventing/recovering from infection caused by external agents.
ARPCs significantly produced inhibin-A and BMP2 that are capable to modulate TGF-B super-pathway as well as Wnt pathway and that could control the EndMT process.

CONCLUSIONS

Our data demonstrate that ARPCs could preserve EC phenotype during LPS-induced endothelial dysfunction by regulating the EndMT process, thereby representing a potential therapeutic cellular target to prevent LPS-induced acute kidney injury.

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