FOXO1A modifies arterial and venous endothelial development from human pluripotent stem cells; they establish 3D vascular structures in vitro and quantifiable vascular networks in vivo

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Background and purpose: Endothelial derivatives of human pluripotent stem cells may offer regenerative treatments in ischemic cardiovascular diseases. We aimed to investigate the regulatory role of PI3K/FOXO1A signalling pathway on arterial and venous identity of endothelial subpopulations as well as the fate of generated cells in 3D cultures in vitro and via transplantation into small animals in vivo.

Methods and results: Human embryonic stem cells (hESC) were differentiated via either embryoid body (EB) or monolayer method under normoxic and hypoxic conditions. CD31-positive endothelial cells (EC) were sorted by FACS and compared with human induced pluripotent stem cell-derived endothelial cells (hiPSC-EC). Both hESC-EC and hiPSC-EC showed mature endothelial phenotype in vitro, including cobblestone pattern, ac-LDL uptake and tube formation. Proteome profiling revealed high abundance of angiogenesis-related proteins both in cell lysates and supernatant. Expressions of arterial (EphrinB2, Notch1–2) and venous (EphB4) endothelial markers were increased during differentiation, suggesting the presence of mixed endothelial population in culture. Transfection of hESC-EC/hiPSC-EC with plasmids encoding FOXO1A-eGFP or pmaxGFP was carried out by electroporation. Human ESC-EC and hiPSC-EC with high FOXO1A showed downregulated expressions of universal (CD31, angiopoietin-2 and ve-cadherin) as well as arterial and venous markers. Indeed, arterial index (EphrinB2/EphB4 mRNA ratio) decreased in response to FOXO1A overexpression (hESC-EC 8.16±3.22 vs. 2.24±0.49, $p<0.01$; hiPSC-EC 6.46±2.75 vs. 1.67±0.72, $p<0.05$; n=3 biological replicates). This suggests a key role of PI3K/FOXO1A signalling pathway in the modulation of arterial and venous phenotype. For engineering 3D vascular constructs decellularised human aortic slices (300μm) were repopulated with hESC-EC and hiPSC-EC in small bioreactor systems. Cells remained viable on engineered matrices. Imaging with Calcein AM live staining and 3DHistech

analysis proved recellularisation with CD31-positive, viable endothelial cells. Human ESC-EC and hiPSC-EC were transplanted subcutaneously into athymic nude rats in Matrigel containing endothelial growth factors. 3DHistech analyses of transplantation sites proved development of capillary networks from CD31 positive human EC. Network area and number of CD31 positive cells in hESC-EC and hiPSC-EC structures were comparable with those in HUVEC control endothelial cells.

Conclusions: We found that PI3K/FOXO1A pathway has strong effects on arterial and venous endothelial identity. Human ESC-EC and hiPSC-EC remained viable on 3D vascular matrices and they formed capillary networks via transplantation in vivo. Human ESC-EC and hiPSC-EC behave comparable with mature control endothelial cells in vitro and in vivo. In-depth analyses of phenotype and functional characteristics of hESC-EC and hiPSC-EC may enhance their therapeutic application for vascular tissue engineering.