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THE IMPORTANCE OF CELL SOURCE FOR SOMATIC TISSUE REPROGRAMMING: ENDOTHELIAL CELL-DERIVED IPS CELLS HAVE ENHANCED CAPACITY TO DIFFERENTIATE INTO FUNCTIONAL ENDOTHELIAL CELLS

E Skinner,[1] M Brittan,[1] O Tura,[1] J Fletcher,[1] P Burton,[2] A Baker,[2] J Mountford,[2] P Hadoke,[1] I Wilmut,[1] N Mills[1] [1]*University of Edinburgh;* [2]*University of Glasgow*

doi:10.1136/heartjnl-2013-304019.193

Background Induced pluripotent stem (iPS) cell technology has primarily focused on the reprogramming of fibroblasts to an embryonic stem (ES)-like state. However, as the field has developed, the importance of somatic cell source has been studied to enhance reprogramming and differentiation efficiencies.

Objectives To generate iPS cells from human fibroblasts and endothelial outgrowth cells from blood. To compare reprogramming efficiency between both cell types, and compare their potential for endothelial differentiation.

Methods and Results Reprogramming: Episomal vectors containing Sox2, Klf4, Oct4 and c-Myc were electroporated into fibroblasts and endothelial cells using the Amaxa system. Successfully reprogrammed fibroblast-derived iPS ('fiPS') and endothelial cell-derived iPS ('eiPS') arose as colonies, and were picked and expanded. Reprogrammed cells expressed pluripotency markers SSEA3, SSEA4, TRA-1-60, Oct4 and NANOG, and developed into all three germ layers following embryoid body (EB) formation. Optimisation of endothelial differentiation protocol: iPS and ES cell lines were aggregated into EBs for three days in stem cell growth media containing mesoderm-inducing cytokines. EBs were then disaggregated and cultured in Endothelial Growth Medium supplemented with VEGF. After seven days, a population of CD31+ cells was isolated by FACS sorting, cultured and mature endothelial cell antigen expression determined using flow cytometry. CD31 + cells were selected for functional assessment in vitro using established assays of angiogenesis, migration and adhesion. Human endothelial cells derived from iPS cells were implanted subcutaneously in a NOD-SCID mouse model of angiogenesis and neovasculogenesis quantified at day 21. Comparison of fiPS and eiPS: eiPS differentiate into endothelial cells with greater efficiency than fiPS (CD31+ cells at day seven is 15.2% and 4.1%, respectively). fiPS-endothelial cells have been characterized phenotypically and shown to express endothelial markers CD146 (86.2%±10.1%), CD31 (92.9%±3.3%), VEFGR2 (44.8%±2.8%), Tie-2 (30.7%±19%) and VE-Cadherin (65.0%±7.1%). When grown on Matrigel %, they form tubule-like structures with a similar number of vessel connections per field to control endothelial cells (54.5±5.5 versus 57.5±4.0). Characterisation of eiPS-endothelial cells is ongoing. In vivo, implantation of endothelial cells derived from fiPS and eiPS increase vessel formation by 78.2%±16.4% and 67.2% ±7.6% respectively, compared to Matrigel - control. By comparison control endothelial cells increased vessel formation by 6.9%±0.8%.

Conclusion Endothelial cells can be isolated from blood and reprogrammed effectively to form eiPS cell lines with greater capacity to differentiate into endothelial cells than iPS cells derived from dermal fibroblasts suggesting reprogrammed cells retain epigenetic memory. Endothelial cells derived from both eiPS and fiPS cells increase angiogenesis compared to mature endothelial cells and have potential therapeutic applications for vascular regeneration.