SMOOTH MUSCLE CELLS DIFFERENTIATED FROM REPROGRAMMED FIBROBLASTS THROUGH DKK3 SIGNALLING ARE POTENT FOR TISSUE ENGINEERING OF VASCULAR GRAFTS

Q Xu, E Karamariti, A Margariti, Y Hu King’s College London
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Background Smooth muscle cells (SMCs) are a key component of tissue-engineered vessels. However, the sources by which they can be isolated are limited. The generation of induced pluripotent stem (iPS) cells is a useful tool for regenerative medicine. Nevertheless, the risk of tumor development of the aforementioned cells should be addressed before they can be used for clinical applications. During the reprogramming process a number of signal pathways are activated, which may lead to direct differentiation of specific cell lineages prior to the cells reaching the pluripotent state.

Methods and Results We hypothesised that a large number of SMCs could be obtained by direct reprogramming of fibroblasts to SMCs. Therefore, we designed a combined protocol of reprogramming and differentiation in an attempt to achieve direct differentiation of fibroblasts to specific cell lineages. Human fibroblasts were shortly reprogrammed by overexpression of four reprogramming factors (OCT4, SOX2, KLF4 and c-MYC) and maintained in reprogramming media on a gelatin substrate for four days. These cells were defined as partially induced pluripotent stem (PiPS) cells. PiPS cells did not form tumors in vivo and differentiated into SMCs when seeded on a collagen IV substrate and maintained in differentiation media. The PiPS-SMCs expressed a panel of SMC markers such as SMA, SM22 and calponin at mRNA and protein levels. In order to elucidate the mechanism of PiPS cell differentiation into SMCs, data from a series of experiments indicated that the gene DKK3 was involved in this differentiation. DKK3 was expressed in
parallel with SMC markers, while its overexpression or stimulation induced SMC marker expression. Furthermore, DKK3 silencing resulted in downregulation of SMC markers on both the mRNA and protein levels. Additional experiments revealed that the upregulation of SMC markers by DKK3 is mediated by interaction of DKK3 with the transmembrane receptor Kremen 1, potentiation of Wnt signalling and ultimately β-catenin translocation. Finally, PiPS-SMCs repopulated decellularised vessel grafts and ultimately gave rise to functional tissue-engineered vessels when combined with the previously established PiPS-endothelial cells, leading to increased survival of SCID mice after transplantation of the vessel as a vascular graft.

**Conclusion** In the present study, we have developed a protocol to generate SMCs from PiPS cells through a DKK3 signalling pathway, which are useful for generating functional tissue-engineered vessels. These findings provide new insight into the mechanisms of SMC differentiation with vast therapeutic potential.