TS045
Tenogenic potential of human stem cells from the amniotic fluid and adipose tissue

MT Rodrigues[1,2], AI Gonçalves[1,2], SJ Lee[3], A Atala[3], JJ Yoo[3], RL Reis[1,2] and ME Gomes[1,2]

[1]*3B's Research Group, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Portugal;* [2]*ICVS/3B's - PT Government Associate Laboratory, Portugal;* [3]*Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, USA*

Introduction: Tendons are highly prone to injury and the intrinsic hypocellularity and hypovascularity make their natural healing extremely slow and inefficient when severed damaged. Surgical repair with grafts is common but unsuccessful in a long term basis. The development of tissue engineering strategies based on stem cells explores a natural endogenous system of regeneration with potential for tendon application. We propose to establish biochemical culturing conditions to assess the tenogenic potential of human adipose stem cells (hASCs) and amniotic fluid-derived stem cells (hAFSCs), known for their proliferative and differentiation capacities. Since several growth factors (GFs) participate in tendon formation and ECM synthesis, these GFs were added to the culture medium to stimulate tenogenic differentiation of these cells(1, 2). This study also envisions the application of hASCs and/or hAFSCs in cell-based strategies for tendon repair.
Materials and Methods: hASCs were enzymatically isolated from lipoaspirates and expanded in basic medium(α-MEM, 10% FBS, 1% antibiotic) before being cultured in (i) basic medium, (ii) basic medium with glutamine(2 mM) and ascorbic acid(0.2 mM) plus (iii) EGF(10 ng/mL), (iv) FGF(10 ng/mL), (v) PDGF(10 ng/mL) or (vi) TGF-β(10 ng/mL). hAFSCs were obtained from amniocentesis procedures and expanded in α-MEM medium plus 15% embryonic screened FBS, 1% glutamine, 1% antibiotic, 18% Chang B and 2% Chang C. Similarly to hASCs, hAFSCs were cultured in media (i), (iii), (iv), (v) and (vi) but (ii) corresponded to hAFSCs expansion medium. Tenogenic differentiation was weekly evaluated up to 28 days based on cell morphology and Tenascin-C and Collagen3 protein expression, as well as on PCR analysis for tendon related markers (scleraxis, tenascin C, decorin and collagen 1 and 3. Primary tenoblasts obtained from surgery surplus were used as cellular control of the experiment.
Results: hAFSCs and hASCs showed a tenocyte-like aligned distribution in the different culture media by 14 and 21 days, respectively. But hAFSCs tend to lose the typical alignment by growing confluent. Also, hASCs expressed higher levels of Tenascin-C protein than hAFSCs.
Conclusions: Preliminary data on the aligned morphology and tenascin-C expression shows that both cell sources can be biochemically induced towards tenogenic features. Overall, and despite variations found, selected GFs do not actively participate in the tenogenic process of these cells. Ongoing studies on molecular biology and other tendon matrix related markers will clarify which stem cell source has more potential for tendon regeneration strategies.
References:

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TS046
Facs-purified human motor neurons from amplified human stem cell-derived cultures generate robust survival assays

NJ Lamas[1,2,3,4,5,6], B Johnson-Kerner[3,4,5,6], L Roybon[3,4,5,6,7], H Wichterle[3,4,5,6] and CE Henderson[3,4,5,6]

[1]*Life and Health Science Research Institute, School of Health Sciences, University of Minho, Braga, Portugal;* [2]*ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal;* [3]*Project A.L.S./Jenifer Estess Laboratory for Stem Cell Research, New York, NY, USA;* [4]*Center for Motor Neuron Biology and Disease, Columbia University Center, New York, NY, USA;* [5]*Departments of Pathology, Neurology, and Neuroscience, Center for Motor Neuron Biology and Disease and Columbia Stem Cell Initiative, Columbia University Medical Center, New York, NY, USA;* [6]*Columbia Stem Cell Initiative, Columbia University Medical Center, New York, NY, USA;* [7]*Department of Experimental Medical Science Wallenberg Neuroscience Center, Lund University, Lund, Sweden*

Human Embryonic Stem Cells (hESCs) and Induced Pluripotent Stem Cells (hiPSC) can virtually give rise to all cell types in the body, constituting an inexhaustible source of relevant cell types. Motor neurons derived from hESCs and hiPSCs are a potentially important tool to model and to unravel mechanisms of pathological motor neuron cell death. The culturing conditions to specify motor neurons (MNs) from human pluripotent stem cells have been extensively studied and in general work robustly. However, the production of human motor neurons on a scale compatible with high-throughput studies remains impractical due to the relatively low yield of current *in vitro* differentiation protocols. On the other hand, robust survival assays based on purified human motor neurons have not been developed. Here, we demonstrate for the first time the presence of ongoing neurogenesis in human stem cell-derived cultures of mixed spinal cord identity, which leads to the continuous generation of new-born motor neurons for several weeks. We initially exploited this as a means to increase motor neuron yields and screened a collection of bioactive molecules. The Rho-associated kinase (ROCK) inhibitor Y-27632 was identified as a compound that amplifies motor neuron yields up to 4-fold in differentiated hESC and hiPSC cultures. This likely occurs in a ROCK-independent manner, through the promotion of proliferation of Olig2-expressing motor neuron progenitors. Since ongoing neurogenesis constitutes a major potential confound for survival assays, amplified motor neurons expressing the HB9::GFP reporter were therefore FACS-purified and employed to develop a reproducible and robust survival assay for human motor neurons. Using this assay, we were capable to demonstrate for the first time in human motor neurons a significant survival-promoting activity of an array of known neurotrophic factors and also showed that Y-27632 itself supports motor neuron survival. Together, our results highlight Y-27632 as a useful tool to increase yields of human motor neurons from pluripotent stem cells for cell-based screening and biochemical applications.