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SHAKING THE GROUND YOU WALK ON: ARE ADIPOSE-DERIVED STEM CELLS (ASCS) TRUE STEM CELLS?

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Introduction: Adhesion-based culture conditions have been the standard technique for in vitro expansion of ASCs. However, stem cells from different organs grow in suspension and display a more primordial phenotype characterized aggregation in clusters as spheroids. We hypothesized that S-ASCs could represent an upstream stage of the traditional adherent ASCs (aASCs) before they enter an early differentiation pathway leading to their adhesion. Molecular profiles of miRNAs and mRNAs were used between aASCs and S-ASCs to investigate our hypothesis.

Methods: Lipoaspirate samples were processed for the extraction of S-ASCs as previously described by our lab. The miRNAs profile was analyzed using Taqman Array Human MiRNA A Cards in S-ASCs and aASCs cells. Statistically significant changes are considered up- or down-regulation of miRNA expression higher than 2 folds compared to control ($p<0.001$).

Results: After a screening analysis of several miRNAs, we compared molecular patterns between S-ASCs with aASCs and the principal miRNAs and mRNAs involved with the stemness and mesenchymal differentiation. S-ASCs displayed significant up-regulation of miR-142-3p and SOX2, OCT4, NANOG, (5-fold increase) typically expressed in the pluripotent stem cells. Consequently, the early (SOX-9, RUNX-2, PPARg, miR-495, miR-221, miR-30c) and late (LPL, ALP, COL10A, miR-140, miR-143 and miR-100) RNAs correlated with mesenchymal differentiation was up-regulated in aASCs. Furthermore, we have assessed the same molecular analysis in S-ASCs and aASCs during different time in vitro culture up to 28 days. The results have demonstrated the maintenance of stemness only in S-ASCs, expressing high level of pluripotent stem cells markers and low level of differentiation markers.

Conclusion: S-ASCs overexpress important pluripotent stem cells markers typical of iPS cells that are not present in aASCs. Furthermore, miRNAs and mRNAs typical of differentiated cells in multiple lineages were significantly under-expressed in S-ASCs while being over-expressed in aASCs. This molecular pattern supports the upstream nature and the stemness maintenance of S-ASCs and the down-stream and more differentiated precursor nature of aASCs. This data represents the first step in the recognition of S-ASCs as the true stem cell population within adipose tissue.

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THE SECRETOMES OF ADIPOSE DERIVED STEM CELLS AND COMPLETE STROMAL VASCULAR FRACTION ENHANCE MYOBLAST PROLIFERATION VIA DIVERSE SIGNALLING FACTORS

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Introduction: Functional muscle recovery after peripheral nerve injury is far from optimal, mainly because of atrophy of the muscle due to prolonged denervation. Previously, using an experimental animal model, we showed that injections of Schwann cell-like differentiated adipose derived stem cells (dASCs) into denervated muscle reduced the atrophy and enhanced hind limb functionality. In this current study, we have investigated the in vitro interactions between the stem cells and myoblasts.

Methods: Adipose derived stem cells were stimulated with a mix of factors (bFGF, PDGF-AA, neuregulin-1 and forskolin) to induce the Schwann-cell like phenotype. The dASCs, undifferentiated stem cells (uASCs) or complete stromal vascular fraction (SVF) were indirectly co-cultured with primary myoblasts or the L6 cell line. Proliferation of the myoblasts was assessed by MTS, BrdU and qRT-PCR assays. The signalling mechanisms between the cells were investigated using a range of biochemical, molecular and pharmacological assays.

Results: Both dASCs and SVF enhanced proliferation of myoblasts whereas uASCs had no effect. The dASCs and SVF activated ERK1/2 signalling in the myoblasts and inhibition of this pathway with a MEK inhibitor abolished the proliferative effects on the cells. However, the dASCs and SVF appear to act via diverse upstream mechanisms. The dASCs expressed the protein machinery necessary for acetylcholine production but this was not detected in the SVF or uASCs. Treatment of dASCs/myoblast co-cultures with the muscarinic acetylcholine receptor blocker atropine attenuated the proliferation of the myoblasts. In contrast, atropine had no effect on the SVF-evoked myoblast proliferation. Comparison of dASCs and SVF using PCR growth factor arrays showed a number of genes with greater than 20 fold higher expression in the SVF. These included Hgf, Vegfd and Fgf10 and the relative importance of these molecules in SVF-mediated proliferation of myoblasts is under investigation.

Conclusions: These studies provide new insights into how adipose tissue derived cells mediate effects on myoblasts via their secretomes. The knowledge gained could be used to improve regenerative cell therapy strategies for treatment of the injured neuromuscular system.