Molecular analysis of Spheroids from Adipose-derived Stem Cells (S-ASCs) during *in vitro* long-term culture.

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Introduction: Adhesion-based culture conditions have been the standard for *in vitro* expansion of ASCs. However, stem cells from different organs may grow in suspension as spheroids. Spheroids from Adipose Stem Cells (S-ASCs) seem to represent an upstream stage of adherent ASCs (aASCs) that 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. The miRNAs and mRNAs profile were analyzed using Taqman Assay in S-ASCs and aASCs. Statistically significant changes are considered up/down-regulation of miRNA expression higher than 2 folds compared to control ($p<0.001$).

Results: We have previously demonstrated that SASCs displayed significant up-regulation of miR-142-3p and SOX2/OCT4/NANOG, typically expressed in pluripotent stem cells and down-regulation of early and late RNAs correlated with mesenchymal differentiation. We have performed the same molecular analysis in S-ASCs during long-term *in vitro* culture up to 28 days. We demonstrated the maintenance of stemness only in S-ASCs. Furthermore, we studied the molecular levels of miRNAs and mRNAs (such as miR-126 and VEGF) involved in angiogenesis, demonstrating the possibility of using SASCs also in tissue neovascularization.

Conclusion: During long-term *in vitro* culture, stem cells can undergo morphological and genes alternations involved in cell regulation and senescence. Our molecular pattern supports the upstream nature and 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 true stem cell population within adipose tissue.