ISAKOS

International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine

10th Biennial ISAKOS Congress • June 7-11, 2015 • Lyon, France

Paper #126

Could Tendon Progenitor Cells Be Useful in Regenerative Medicine Application? Characterization and in Vitro Comparison with Human Adipose-Derived Stem Cells

Deborah Stanco, MD, ITALY
Marco Viganò, MSc, ITALY
Carlotta Perucca Orfei, MSc, ITALY
Alessia Di Giancamillo, MD, ITALY
Gabriele Thiébat, ITALY
Giuseppe Peretti, ITALY
Laura De Girolamo, PhD, ITALY

Galeazzi Orthopaedic Institute
Milan, ITALY

Summary:
Our results demonstrate that TSPCs do not significantly differ from ASCs, both in term of marker expression and differentiation potential, potentially opening up the possibility of using TSPCs for musculoskeletal regenerative medicine.

Abstract:
Adipose-derived stem cells (ASCs) have been deeply characterized for their usefulness in musculoskeletal tissue regeneration; recently, other mesenchymal stem cell (MSC) sources have also been proposed. This study compares for the first time human tendon stem/progenitor cells isolated from hamstring tendons with human ASCs. Human TSPCs and ASCs were isolated from tendon portions and adipose tissue of healthy donors undergoing ACL reconstruction or liposuction, respectively (n=7). At early passages of culture, proliferation and CFU-F ability were observed for both cell populations. The typical stem cells marker expression was evaluated by both RT-PCR and FACS analysis. TSPCs and ASCs multi-differentiation potential was assessed in cells cultured in specific differentiation media: alkaline phosphatase (ALP) activity and extracellular calcified matrix deposition were analyzed after 14 and 21 days of osteogenic induction, respectively; glycosaminoglycans (GAGs) production was quantified in pellet culture (DMMB assay) after 21 days of chondrogenic differentiation; the adipogenic differentiation was evaluated quantifying the lipid vacuoles production stained by Oil Red O after 14 days in adipogenic medium. The expression of osteogenic (RUNX2), chondrogenic (ACAN and SOX9), adipogenic (LEPTIN) and tenogenic (SCX and DCN) genes was assessed by RT-PCR. The amount of discarded tendon tissue was significantly lower with respect to lipoaspirates ($p<0.001$). However, after cell isolation, the number of cells at passage 1 normalized per grams of tissue was 2.2 ± 1.8 X 105 and 0.7 ± 0.5 X 105, respectively for TSPCs and ASCs ($p<0.05$). Both populations showed a homogeneous fibroblast-like morphology typical of MSC and during passages in culture proliferated with a similar doubling time and a similar clonogenic ability (around 10%). Moreover, both populations were highly positive for the typical MSCs surface markers CD90, CD105, CD73 and CD44, and expressed similarly the stemness-specific transcription factors KLF4 and POU5F1. Both osteo-differentiated TSPCs and ASCs showed a significant increase of ALP activity levels (+173%, +177%, respectively) and extracellular calcified matrix production (+46%, +410%, respectively) respect to undifferentiated cells. However, OSTEO-ASCs were able to produce a higher amount of calcified matrix ($p<0.001$) and expressed higher mRNA levels of RUNX2 (+136%, $p<0.01$) than OSTEO-TSPCs. Interestingly, only CHONDRO-TSPCs showed a significant increase of GAGs levels (+83%, $p<0.05$) respect to untreated cells, whereas ASCs were not affected by the chondro-inductive medium. Undifferentiated and differentiated TSPCs also showed higher ACAN gene expression with respect to the respective ASC (+1208% and +7256%, respectively). On the other hand, after adipogenic differentiation, TSPCs showed a significant lower lipid vacuoles production than ASCs ($p<0.05$). Finally, the tendon-related genes SCX and DCN were more expressed in TSPCs compared to the respective ASCs ($p<0.05$).

Paper #126

In conclusion, semitendinosus and gracilis tendons, like adipose tissue, contain a subpopulation of cells with peculiar features of stem cells able to differentiate efficiently toward osteogenic and chondrogenic lineages, and thus they represent a convenient cell source for musculoskeletal regenerative medicine. For this reason, due to the large number of available waste hamstring fragments, it could be noteworthy to ameliorate the knowledge of the potential of these cells to possibly exploit them for future allogeneic applications.