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NEURAL POTENTIAL OF THE STEM CELL POPULATION IN THE ADIPOSE AND CUTANEOUS TISSUES

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Objective: Adult human stem cells have gained progressive interest as a promising source of autologous cells to be used as therapeutic vehicles. Multipotent stem cells isolated from skin (skin-derived precursors-SKPs) and adipose tissue (adipose-derived stem cells-ADSc) demonstrated an exciting potential for neural regeneration, which has yet to be fully explored.

Methods: Adult stem cells isolated from skin and from adipose tissue derived from the same adult donor were treated with epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2). Neurospheres obtained were first expanded and evaluated in term of proliferative ability, and then seeded onto two tridimensional scaffold types: hyaluronan based membrane and fibrin-glue meshes. Neurospheres were then induced to acquire a glial and neuronal-like phenotype. Gene expression, morphological feature and chromosomal imbalance (kariotype) were analyzed and compared.

Results: Adipose- and skin-derived neurospheres grew in suspension as spheres in the presence of the mitogens FGF2 and EGF. Adipose and skin derived neurospheres are able to grow well and to differentiate into glial/neuron cells without any chromosomal imbalance in both scaffolds. Adult cells are able to express typical cell surface markers such as S100; GFAP; nestin; Œ≤III tubulin; CNPase.

Conclusion: We have demonstrated that neurospheres isolated from skin and adipose tissues are able to differentiate in glial/neuron-like cells, without any chromosomal imbalance in two scaffold types, useful for tissue engineering application: hyaluronan based membrane and fibrin-glue meshes. The work described in this paper provides the framework for our attempts to develop a co-culture of Schwann cells and neurons obtained by differentiation of skin-derived and adipose-derived stem cells. The co-culture system will be conceived as an in vitro model to study the re-myelination of the axons, the structure and permeability of the junctional channels through the layers of non-compact myelin, for further dissection of the sequence of events that end in demyelination followed by axonal loss.

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BONE MORPHOGENETIC PROTEIN RECEPTOR IB AS A MARKER FOR ENRICHMENT OF OSTEOGENIC PRECURSORS

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Background: Cell sorting has identified subpopulations with enhanced osteogenic potential. However, these markers (CD90, CD105) undergo a phenotypic drift in vitro. Type I BMP receptors play critical roles in the specification of osteoblasts and adipocytes, and may offer a more reliable cell surface marker for prospective sorting. The present study evaluated the potential of a combinatorial approach, employing both FACS and manipulation of the BMP pathway, to enhance bone formation.

Methods: BMPR-IB+, BMPR-IB-, and unsorted ASCs were sorted using FACS. Phenotype was analyzed at Days 0, 7, and 14 using anti-CD90, -CD105, and -BMPR-IB antibodies. Each group was then treated with or without Noggin suppression, and cultured in ODM with or without BMP-2. Alkaline phosphatase and quantification were performed on Day 7, alizarin red staining and quantification on Day 14. Osteogenic gene expression was examined by qRT-PCR. In vivo, critical-sized calvarial defects were created in nude mice, and repair was performed using the abovementioned subpopulations delivered on a HA-PLGA scaffold with or without BMP-2. Healing was monitored using micro-CT scans for eight weeks. Calvaria were harvested at Week 8, and sections were stained with Movat's Pentachrome.

Results: Staining assays and qRT-PCR revealed that BMPR-IB+ ASCs were more osteogenic than BMPR-IB- or unsorted. Suppression of Noggin in ASCs resulted in increased osteogenic gene expression and in vitro osteogenic differentiation, and the addition of BMP-2 to Noggin knockdown even further promoted osteogenesis. In vivo, similar results were found, with Noggin-suppressed BMPR-IB+ ASCs seeded on BMP-2 scaffolds experiencing the greatest skeletal healing compared to the other groups over eight weeks.

Conclusions: Our findings demonstrate that subpopulations of ASCs may be identified with enhanced osteogenic capacity and that ASC-mediated bone formation can be promoted through manipulation of the BMP pathway. Use of BMPR-IB+ as a cell surface marker effectively identified an enriched group of cells which could facilitate more rapid regeneration of skeletal defects.