

CHARACTERIZATION OF CULTURED HUMAN ADIPOSE TISSUE-DERIVED PERICYTES

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Introduction Mesenchymal stem cells (MSCs), also called mesenchymal stromal cells, are multipotent cells initially isolated from bone marrow and, more recently, from almost all tissues of the body. The ample distribution of MSCs could be explained by their association with blood vessels; indeed, evidence from different experimental approaches suggests that perivascular cells may give rise to the cultured cells characterized as MSCs. Here, the surface profile and differentiation ability of pericytes from adipose tissue were evaluated in order to verify if these cells exhibit MSC characteristics.

Methods Four adipose tissue samples obtained from liposuction surgeries were enzymatically disaggregated, and fractions of the resulting cell suspensions were cultured under MSC conditions or stained with the 3G5 antibody and an anti-CD31 antibody. Fresh 3G5+CD31- cells were isolated by FACS and cultured under conditions optimized for the maintenance of pericytes to provide sufficient cell numbers for subsequent experiments. The immunophenotypic profile of these pericyte-derived cells (PDCs) was analyzed by flow cytometry using antibodies that recognize surface molecules characteristic of MSCs and pericytes. The differentiation potential of PDCs toward osteogenic and adipogenic pathways was assessed by culture in media containing dexamethasone, ascorbic acid 2-phosphate and beta-glycerophosphate for osteogenesis, and dexamethasone and rosiglitazone for adipogenesis.

Results PDCs exhibited a typical MSC surface profile. They were positive for CD13, CD29, CD44, CD49a, CD49e, CD54, CD73, CD90, CD105, CD146, CD166 and HLA-ABC, in addition to the nerve/glial antigen-2 (NG2), and negative for CD14, CD31, CD34, CD45, CD106 and HLA-DR. Although fresh pericytes were isolated based on the presence of the 3G5 ganglioside on their surface, the expression of this antigen was lost after a few passages, as did the expression of CD140b. PDCs were proficient at depositing calcium-rich extracellular matrix when cultured in osteogenic medium. In addition, nearly 100% of PDCs accumulated lipid vacuoles when cultured under adipogenic conditions. Conventional adipose tissue-derived MSCs, on the other hand, did not show efficient adipogenic differentiation under the same conditions, although they were able to deposit mineralized extracellular matrix.

Conclusion The immunophenotypic profile and in vitro differentiation ability of PDCs are in agreement with the hypothesis that pericytes and MSCs are closely related cell types. Poor adipogenic differentiation in standard MSC cultures may reflect the presence of other cell types that inhibit differentiation into adipocytes. Finally, the methods used for pericyte isolation and expansion in culture may prove valuable for the provision of cells for therapeutic purposes.