Hypoxia enhances proliferation and stemness of human adipose-derived mesenchymal stem cells

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Abstract The aim of the study was to obtain the highest number of multipotent adipose-derived mesenchymal stem cells (ADMSCs) by using culture conditions which favour cell expansion without loss of mesenchymal stem cells (MSC)-like properties. Based on the assumption that stem cells reside in niches characterized by hypoxic condition, we investigated if the low oxygen tension may improve the proliferation and stemness of ADMSCs. Intact adipose tissue was resected from eight subjects, and the stromal vascular fraction was obtained by using type II collagenase. The heterogeneity of cellular lineages was confirmed by immunophenotypic analysis that showed the presence of leukocytes (CD45+), endothelial cells (CD34+), and pericytes (CD140+). The immunophenotype of confluent ADMSCs was similar to that of bone marrow-derived MSCs, except for the expression of CD34, which was variable (donor-dependent) and inversely correlated to the CD36 expression. ADMSCs showed a high clonal efficiency (94.5 $\pm$ 1 %) and were able to generate osteoblastic, chondrocytic and adipocytic lineages. ADMSCs were cultured under normoxic (21 % O_2) and hypoxic (1 % O_2) conditions, and we found that hypoxia significantly favoured ADMSC proliferation and preserved the expression of stemness genes, i.e. *Nanog* and *Sox2*. Since hypoxia reflects the microenvironment in which ADMSCs must proliferate and differentiate, the culture in hypoxic condition allows to better understand the biology of these cells and their regenerative potential. Low oxygen concentrations promote cell proliferation and stemness, thus enriching the pool of cells potentially able to differentiate into multilineages, and extending the possibility of a long-term expansion.