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Immortalization of human adipose-derived stromal cells as possible strategy to employ their paracrine characteristics in regenerative therapies

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Human adipose-derived stromal cells (hASCs) are feasibly isolated and expanded. In addition, it is well established that they secrete large amounts of growth factors. Several findings suggest that rather than trans-differentiation, hASCs could exert a therapeutic potential through a potent paracrine mechanism. All these features make hASCs a real and powerful therapeutic tool for the treatment of several human diseases. However, hASCs limited culture life-span can obstacle full employment of their paracrine characteristics in regenerative therapies. Under this perspective, hASCs immortalization may be a possible strategy to circumvent this problem. hASCs were immortalized by co-transducing human telomerase reverse transcriptase (hTERT) gene with either SV-40 or human papilloma virus (HPV) E6/E7 genes. Flow cytometry was assessed to analyze mesenchymal marker expression. Differentiation potential was evaluated by immunocytochemistry and the levels of two cytokines, HGF and VEGF were assayed by ELISA. Finally, the behavior of immortalized hASCs in basal medium was also investigated. Both hTERT/SV40 (TS) and hTERT/HPV E6/E7 (TE) co-transductions successfully immortalized hASCs. hASCs-TS and hASCs-TE reached elevated Population Doubling Levels (up to 100) and were expanded to obtain elevated cell number. Flow cytometry showed that both hASCs-TS and hASCs-TE retained a mesenchymal phenotypic profile while differentiation potential decreased particularly in hASCs-TS. Of note, the levels of HGF and VEGF secreted by hASCs-TS and hASCs-TE remained elevated after immortalization. Finally, both hASCs-TS and hASCs-TE were successfully cultured in basal medium without FBS and or exogenous recombinant proteins. We have developed a "hybridoma-like model" that by coupling hASCs immortalization and their paracrine characteristics may have potential application in discovering and producing molecules to use in regenerative medicine (process scale-up). Moreover, such model may be very useful to eliminate direct injection of cells thus avoiding potential adverse effects.

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