Insights in Stem Cells

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Stem Cell Research 2018 - Adult Stem Cell Subsets from Human Dermis - Agustin Vega-CrespoUniversity of California

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Introduction

Adult human mesenchymal stem cells (hMSC) have the ability to adhere to common plastic treated by tissue culture, to develop in vitro into a clinically relevant number of cells and to differentiate into a variety of cell types, including osteoblasts, chondrocytes and adipocytes. This multi-lineage capacity differs according to the niches, the conditions and the duration of the in vitro culture. The hMSC cell surface signature makes it possible to identify and isolate certain cellular derivatives of stromal type, which can undergo differentiation ex vivo resulting in committed cell lines. The cell surface markers reported in the literature which may accompany an increase in the potential for differentiation in vitro are certainly important, however, this potential varies considerably between tissues and in some cases, the surface signature is not correlated with the degree of phenotypic functionality in vitro. Among these cellphones surface markers, canonical 5'-nucleotidase surface markers, ecto (CD73) or NT5E, thymocyte antigen 1 (CD90) or Thy-1 and Endoglin CD105), were initially described as unique for mesenchymal stromal cells , but have since been identified as distributed among various types of human cells, including fibroblasts with mutipotency in vitro or not. Previous research on adult human fat cells and human neonatal dermal derivatives suggests that the melanoma cell adhesion molecule (CD146) or MCAM and the nerve growth factor receptor (CD271) or NGFR, respectively, as suspected candidate markers to isolate mesenchymal stromal cells from derived adult human dermis, which would increase the ability to form bones, cartilage and fat ex vivo. In theory, adult multipotent dermal fibroblasts can be easily reached using a minimally invasive and relatively painless skin punch biopsy and hMSC derivatives purified via cell surface markers for clinical applications in vitro or in vivo. The aim of our study includes the identification and isolation of subsets of progenitors
from the adult dermis and characterization of the multipotent potential in vitro associated with a specific phenotype

Material and Methods

In vitro culture of primary human skin cells

The LAVIV® adult human skin fibroblasts (azficel-T from Fibrocell Science, DR01) used in this study were obtained from a 4 mm skin biopsy as described on Isolagen Standardized
Manufacturing process EX-GTR-110 version number 00. All the cells derived from human biopsy were cultured in regular cell culture media made up of the medium nutritive mixture Eagle modified by Dulbecco DMEM / F-12 (Life Technologies, 11320 - 082) supplemented with 10% fetal bovine serum (Life Technologies, 26140-079), 1% non-essential amino acids MEM (Life Technologies, 11140-076), 2 mM GlutaMAX (Life Technologies, 35050-061) and Primocin 100 µg / mL (Invivogen, ant-pm -1). The culture medium was changed every 2 days. The cells were allowed to expand to> 90% confluence before passing with 0.05% trypsin-EDTA (Gemini Bio-Products, 400-150) and replacing them at 8400 cells / cm2.

Live cell staining and fluorescence activated cell sortingbased purification

Approximately 1 × 107 cells were trypsinized and washed twice with ice-cold phosphate-buffered saline (PBS) + 2% goat serum (Gemini Bio-Products, 100-109) (PBS-G). The cells were then passed through a 40 µm filter to remove clumps and resuspended as 4-5 × 106 cells in 0.1 mL of ice-cold PBS-G containing 1:100 CD146:FITC antibody (AbD Serotec, MCA2141F) 1:200 CD271:AlexaFluor 647 (BD Bioscience,

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560326), and 1:100 CD73:PE-Cy7 (BD Bioscience, 561258), CD90:PE antibody (BD Bioscience, 555596), and CD105: AlexaFluor 647 antibody (BD Bioscience, 561439). Samples were incubated for 30 min in the dark at 4°C with gentle nutation. The cells were washed thrice with ice-cold PBS-G, resuspended in 1 mL of ice-cold PBS-G, passed through a 40 µm filter, and immediately analyzed and sorted on a FACSAria cell sorter (BD Biosciences). Data were analyzed; DAPI-stained (Life Technologies, D1306) dead-cell exclusion and doublet-exclusion gating were performed; and viable single-cell subpopulations were sorted using BD FACSDiva Software (BD Biosciences). Positive and negative purified fractions alongside a mix-whole sort sample were allowed to recover and expanded for 1-week before a second round of FACS-purification

Discussion

In this study, we isolated skin derivatives with MSC-like competence in vitro, which, to our knowledge, have not been documented in adult dermal cells. The human dermis contains a diverse collection of cells whose ratios depend on the age, site and depth of the biopsy and many other factors. These cells in vivo have a discrete and unique set of capacities and once removed from their niche, the adherent cells adapt quickly to the conditions of culture ex vivo by maintaining or losing these properties. Previous research has revealed that a subset of primary human adherent dermal cells (PHAD) expressing the stage-specific embryonic-3 antigen (SSEA-3) is transcriptionally similar to adipocyte-derived stem cells (AdSc). Human cells expressing CD146 from various sources have been shown to differentiate in vitro not only into osteoblasts, chondrocytes and adipocytes but also into hepatocyte-like cells and smooth muscles. CD271 is an epitope that was originally identified in situ in skin biopsies and characterized as a powerful hMSC marker on adherent dermal cells

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