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Variable expression of lineage regulators in differentiated stromal cells indicates distinct mechanisms of differentiation towards common cell fate

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ABSTRACT

Mesenchymal stem cells (MSCs) possess a multi-lineage differentiation capacity that makes them important players in the field of regenerative medicine. MSC populations derived from different tissues or donors have been shown to exhibit variable gene expression patterns. Further, it is widely acknowledged that MSC isolates are heterogeneous mixtures of cells at different developmental stages. However, the heterogeneity of expression of lineage regulators has not been linked to differentiation potential of different MSC populations towards mesenchymal lineages. Here, we analyzed variation of expression of differentiation markers across whole population and between single differentiating cells of multipotent stromal cell populations derived from adipose tissue (AdMSCs) and skin (FBs) of seven donors. The results of the analyses show that all cell populations exhibit similar differentiation potential towards adipocyte, osteoblast and chondrocyte lineages despite tissue type- and donor-specific variations of expression of differentiation-associated genes. Further, we detected variable expression of lineage regulators in individual differentiating cells. Together, our data indicate that single cells of stromal cell populations could use distinct molecular mechanisms to reach a common cell fate.

1. Introduction

Mesenchymal stem cells (MSCs) have been isolated from numerous tissues including blood, skin, liver and fat (Da Silva Meirelles et al., 2006; Kassis et al., 2006; Toma et al., 2001; Zuk et al., 2001). MSCs can be differentiated into a variety of cell types including adipocytes, osteoblasts, chondrocytes and myocytes (Gimble and Guilak, 2003; Lee and Kemp, 2006; Zuk et al., 2002). Surface molecules Ecto-5′-nucleotidase/CD73, Thy-1/CD90 and Endoglin/CD105 have been used to discriminate MSCs from other cell types (Dominici et al., 2006). However, different studies (Gronthos et al., 2001; Mitchell et al., 2006; Zuk et al., 2002) report different expression of these surface proteins on MSCs. Stromal fibroblasts (FBs) have been described as cells that are distinct from MSCs (Bae et al., 2009; Ishii et al., 2005; Wagner et al., 2005). However, in other reports it has become evident that both MSCs and FBs express surface antigens CD105, CD90, CD73 and CD166, and differentiate into adipocytes, osteoblasts and chondrocytes under similar culture conditions (Blasi et al., 2011; Haniffa et al., 2007; Hanson et al., 2010; Jääger and Neuman, 2011; Jääger et al., 2012; Lorenz et al., 2008). Hence, while MSC and FB populations are subject to donor-, tissue source and culture-specific variations of gene expression, they are functionally equivalent cells.

The multipotency of MSCs is typically demonstrated by their ability to differentiate into three mesodermal cell types including adipocytes, osteoblasts and chondrocytes in *in vitro* culture conditions. Each differentiation pathway is initiated and controlled by a specific set of sequentially induced genes. Peroxisome proliferator-activated receptor γ (PPARγ) regulates development of adipocytes together with members of the CCAAT/enhancer-binding protein (C/EBP) family, whose coordinated actions result in the expression of adipocyte genes including fatty acid binding protein 4 (FABP4, also known as aP2)(Rosen, 2005; Tontonoz et al., 1994). Development of osteoblasts is governed by a number of transcription factors including runt-related transcription factor 2 (Runx2), Osterix, Twist and Msx/Dlx proteins (Franceschi et al., 2009; Frith and Genever, 2008), whereas Runx2 has been identified as the central regulator of cell commitment and differentiation into osteoblasts (Banerjee et al., 1997). The transcription factor Sox9 is one of the

Abbreviations: AB, Alcian Blue; ACAN, aggrecan; AdMSC, adipose-derived mesenchymal stem cell; ALP, alkaline phosphatase; APN, adiponectin; ARS, Alizarin Red S; C/EBP, CCAAT/enhancer-binding protein; Col II, collagen type II; FABP4, fatty acid-binding protein 4; FB, fibroblast; ORO, Oil Red O; PPARγ, peroxisome proliferator-activated receptor gamma.

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earliest genes expressed in cells undergoing chondrogenic differentiation (Zhao et al., 1997), and it is required for cartilage development (Bi et al., 1999), including expression of cartilage-specific matrix proteins (Sekiya et al., 2000). These differentiation pathways have been well studied using biochemical methods as well as genome-wide gene expression and regulation analyses of murine 3T3-L1 and C3H10T1/2 cell lines, whereas the differentiation mechanisms of human primary stromal cells are not fully described, probably due to cellular heterogeneity of stromal cell populations.

In this study, we asked whether two stromal cell populations — adipose-derived MSCs (AdMSCs) and dermal fibroblasts (FBs) isolated from the same seven donors exhibit variations in expression patterns of well-known lineage-specific genes along differentiation into adipocytes, osteoblasts and chondrocytes. Our results revealed both tissue source- and donor-specific differences in gene expression patterns in cell populations of similar differentiation potential. Importantly, we evidenced heterogenous expression of lineage markers in individual differentiating cells that suggests different alternative molecular mechanisms of stromal cells to reach a common cell fate.

2. Materials and methods

2.1. Cell culture

Human AdMSCs were isolated from subcutaneous adipose tissue and FBs from dermis of the same seven individuals as previously described (Jääger et al., 2012). Briefly, adipose tissue was digested using 0.1% collagenase (Gibco) and stromal cell pellet was plated and cultivated in αMEM (Gibco) supplemented with 10% FBS (PAA) and 1% penicillin/streptomycin (PAA). FBs were derived from skin explants following cell migration, and cultivated in high-glucose DMEM (Gibco) supplemented with 10% FBS and 1% penicillin/streptomycin. All cell populations were cultured under standard conditions and passaged 3–5 times until experimental analysis. All human subjects were females

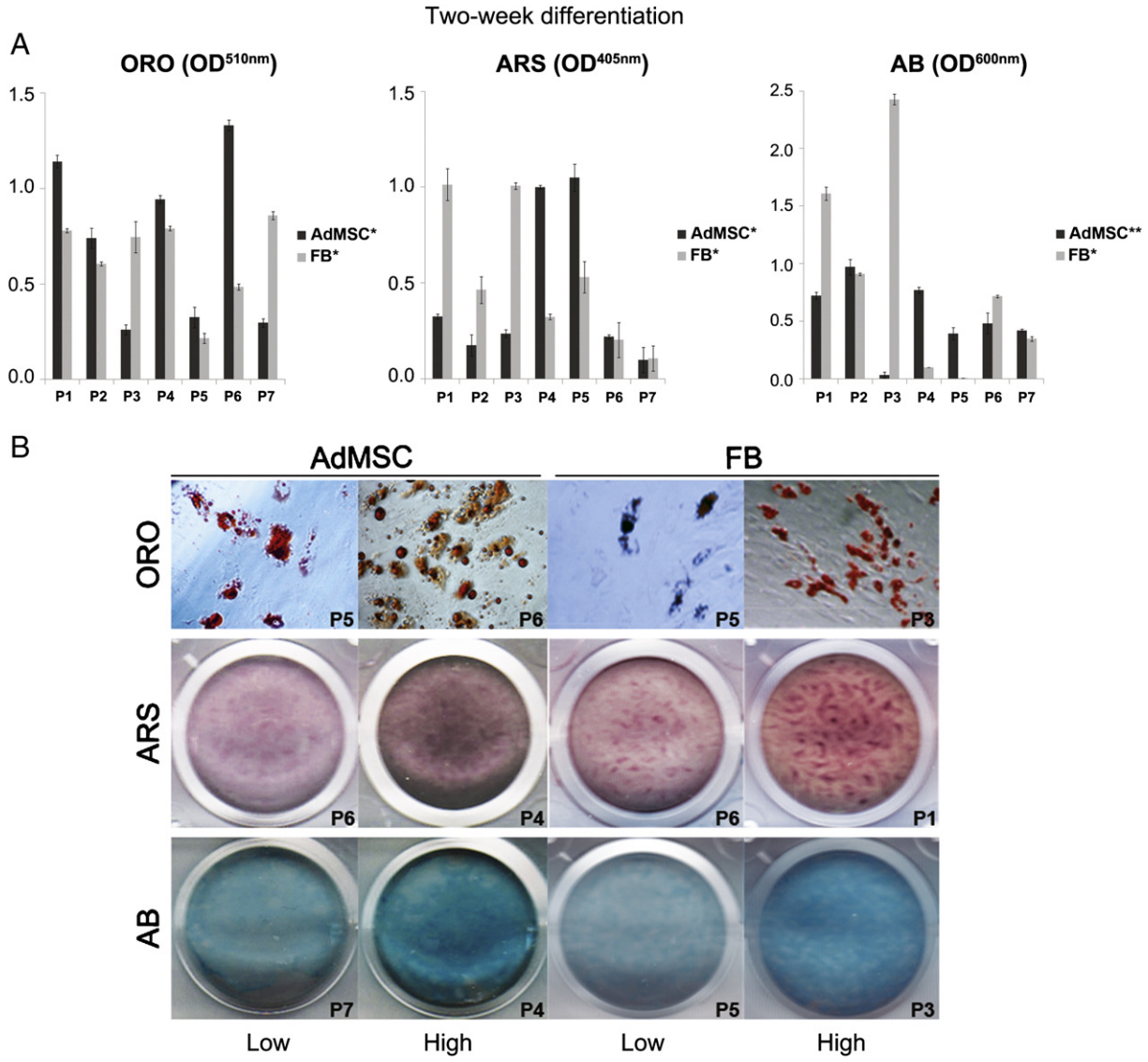

Fig. 1. Tissue-specific staining shows multipotency of all AdMSC and FB populations. Differentiated AdMSC and FB cultures derived from the same donors were stained with ORO for detection of lipid droplets in adipocytes, with ARS for detection of calcified matrix of osteoblasts, and with AB for detection of proteoglycan-rich matrix of chondrocytes on day 14 upon differentiation. (A) Staining intensities were quantified based on optical density (OD) at indicated wavelengths. Error bars represent mean +/− SD of triplicate measurements. ANOVA resulted in statistically significant differences across different cell populations (*$P < 0.05$), except for AB-stained AdMSC populations ($0.05 < **P < 0.25$), denoted next to legends. (B) Cell cultures with 'low' or 'high' staining intensities. Abbreviations: AdMSC — Adipose-derived mesenchymal stem cell; FB — fibroblast; P — population; ORO — Oil Red O; ARS — Alizarin Red S; AB — Alcian Blue.