Cytotherapy, 2011; 13: 528–538

informa
healthcare

Differential surface antigen expression and 1α,25-dihydroxyvitamin D_3 responsiveness distinguish human dermal fibroblasts with age-dependent osteogenic differentiation potential from marrow-derived stromal cells *in vitro*

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Abstract

Background aims. Recent studies have demonstrated that cells committed to a fibroblastic lineage, including dermal fibroblasts, may undergo osteoblastic differentiation when treated with steroid hormones. However, stem cells have also been isolated from the dermis, making it unclear whether osteoinduction of dermal fibroblasts is the result of transdifferentiation of committed fibroblasts or differentiation of resident multipotent stromal cells, which are morphologically indistinguishable. *Methods.* Flow cytometry was used to characterize the expression of CD26, CD90 and CD105 on neonatal and adult human dermal fibroblasts and adult human bone marrow-derived stromal cells. These cells were then cultured with the steroid hormones 1α,25-dihydroxyvitamin D_3 and dexamethasone, and evaluated for protein expression and mineral deposition typical of an osteoblastic phenotype. *Results.* The surface peptidase, dipeptidyl peptidase IV (CD26), was differentially expressed between human neonatal (98.22 ± 1.47%) and adult (90.73 ± 7.97%) dermal fibroblasts and adult bone marrow-derived stromal cells (6.84 ± 5.07%). In addition, neonatal dermal fibroblasts treated with vitamin D_3 expressed alkaline phosphatase, osteocalcin and bone sialoprotein, and deposited mineral, which is consistent with an osteoblastic phenotype. Such differentiation was not observed in adult dermal fibroblasts. In contrast, marrow-derived stromal cells required dexamethasone in order to undergo osteoblastic differentiation. *Conclusions.* Taken together, the differential surface antigen expression and disparate response to steroid hormones suggest that committed neonatal dermal fibroblasts are distinct from mesenchymal stromal cells and possess osteogenic differentiation potential.

Key Words: *dexamethasone, fibroblast, mineralization, osteoinduction, surface antigen, stromal cell, vitamin D*

Introduction

Primary osteoblasts are difficult to isolate and expand while maintaining the differentiated phenotype *in vitro* (1), creating a need for an alternate source of cells for use in osseous tissue repair strategies. Ideally, this source of cells should be non-immunogenic, easily expandable, readily available through a minimally invasive harvesting procedure, and replicate the biologic function of the native osteoblast. Cells with multilineage differentiation potential have been isolated from the stroma of multiple tissues, including bone marrow (2–13), fat (14–16), muscle (17–19) and skin (19–23). Although stem cells isolated from these various sources have been used for tissue engineering applications, they often involve complex harvesting procedures and result in a low yield of colony-forming cells (3,6,7,11).

Recently, several studies have investigated the ability of cells committed to a fibroblastic lineage to undergo osteoblastic differentiation. For example, gingival (24,25) and dermal fibroblasts (26,27) were able to express an osteoblast phenotype following transduction with vectors overexpressing bone morphogenetic proteins or Runx2, an osteoblast transcription factor. Additionally, dermis-derived fibroblasts have been shown to differentiate into osteoblasts when treated with vitamin D_3 (22,28) or dexamethasone (23,29). Therefore, these findings suggest that dermal fibroblasts may be a prime candidate for use in the regeneration of osseous tissues, as they can be obtained easily from a small skin biopsy, readily expanded up to passage 14 without a decrease in collagen synthesis or growth rate (30), and can be redirected toward the osteoblastic lineage.

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(Received 7 May 2009; accepted 12 November 2010)

ISSN 1465-3249 print/ISSN 1477-2566 online © 2011 Informa Healthcare
DOI: 10.3109/14653249.2010.542454

Although dermis-derived cells have been shown to undergo osteoblastic differentiation, it is unclear whether this is due to transdifferentiation of committed fibroblasts or to differentiation of resident stem cells (22,23,28,29,31). Given that dermal fibroblasts and mesenchymal stromal cells are morphologically indistinguishable, it is necessary to identify markers that are differentially expressed between the cell types. Immunophenotyping using flow cytometry is one method of separating distinct cell populations from a larger pool of cells. In particular, a subset of clusters of differentiation (CD) antigens has been used to identify and isolate mesenchymal stromal cells from bone marrow. These include adhesion molecules, CD63 (HOP26, LIMP) and CD166 (SB10, ALCAM), cell-surface glycoproteins, CD90 (THY-1), and transforming growth factor-β (TGF-β) receptors, CD105 (SH3, endoglin) (8,9,15,19,32–39). Interestingly, dermal fibroblasts also express these surface antigens, possibly because of their common mesodermal origin (23,29,35,39). One class of surface antigens that is typically found on committed dermal fibroblasts is surface peptidases, including common acute lymphocytic leukocyte antigen (CD10, CALLA), aminopeptidase N (CD13) and dipeptidyl peptidase IV (CD26). In addition to being expressed by committed dermal fibroblasts, CD10 and CD13 expression has been reported on mesenchymal stromal cells (34–36,38,40,41). CD26, a surface peptidase with multiple functions that is found in the liver, kidney and intestine, and on T and B cells (42), is expressed by dermal fibroblasts (43–45). However, CD26 expression on mesenchymal stromal cells has not been reported, suggesting that it may serve as a potential marker to distinguish between dermal fibroblasts and resident stem cells. To date, no such marker has been described in the literature.

Regardless of origin, differentiation of progenitor cells toward an osteoblastic lineage is enhanced by the steroid hormones vitamin D_3 and dexamethasone. Vitamin D_3, which undergoes two hydroxylation steps to become the biologically active form 1α, 25-dihydroxyvitamin D_3 (46), is a potent stimulator of the osteoblast phenotype. Treatment of osteoblasts *in vitro* with vitamin D_3 results in increased expression of osteoblast-specific proteins (46–48). For instance, vitamin D_3 is known to increase the expression of osteopontin and osteocalcin in primary osteoblasts and give rise to elevated alkaline phosphatase (ALP) gene expression and enzyme activity (47,48). Dexamethasone is a synthetic glucocorticoid that has also been used to enhance the differentiation and matrix mineralization of osteoblasts and progenitor cells (10,13,49–51). However, the effect of donor age on steroid hormone-mediated osteoinduction of human dermis-derived cells, an important factor for clinical translation, has not been reported.

The objective of the current study was to identify surface markers that are differentially expressed on dermal fibroblasts and marrow-derived stromal cells. In addition, the ability of this population of dermal fibroblasts to undergo osteoblastic differentiation in response to vitamin D_3 and dexamethasone treatment as a function of age was investigated in comparison with marrow-derived stromal cells. We hypothesized that human dermal fibroblasts exhibit a surface marker profile that is distinct from bone marrow-derived stromal cells and are capable of undergoing osteogenic differentiation when supplemented with vitamin D_3 and dexamethasone.

Methods

Cell culture

All cell culture supplies were purchased from Gibco-Invitrogen (Carlsbad, CA, USA) unless otherwise noted. Human neonatal (hDFn-1, -2, newborn; Cascade Biologics, Portland, OR, USA) and adult [hDFa-1 (23 years old), -2 (34 years old), -3 (37 years old); Cascade Biologics] dermal fibroblasts were maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 100 U/mL penicillin, 100 μg/mL streptomycin and 0.075% $NaHCO_3$ and incubated at 37°C in a humidified atmosphere with 5% CO_2. Additional neonatal dermal fibroblasts (hDFn-3, hDFn-4) were isolated from neonatal foreskin specimens in accordance with approved guidelines. The dermis and epidermis were separated manually and the dermis diced into 1 × 1-mm pieces. Dermal explants were maintained in MEM containing 20% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 0.075% $NaHCO_3$, and 1% fungizone, in tissue culture-treated Petri dishes. Dermal fibroblasts were passaged from the cell outgrowth and maintained in the same manner as the commercially available cells. Human adult bone marrow-derived stromal cells [BMSC-1 (24 years old), –2 (27 years old), –3 (32 years old); Tulane University Center for Gene Therapy, New Orleans, LA, USA] (9,10,52) were maintained in minimum essential medium-Alpha (MEM-α) supplemented with 16.5% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine, and incubated at 37°C in a humidified atmosphere with 5% CO_2.

Flow cytometry

Four neonatal dermal fibroblast (passages 2–4), three adult dermal fibroblast (passages 3–4) and three adult bone marrow-derived stromal cell strains