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Comparative analysis of stemness between dermal papilla cells and human dermal stem/progenitor cells

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Abstract

Adult stem cells (ASCs) have great applicative potential in tissue regeneration. Comparative analyses of ASCs derived from various niches are essential for comprehending the unique traits of each population and evaluating their potential for therapeutic use. In this study, the proliferation ability, stem cell marker expressions, and differentiation potential of skin-derived ASCs were compared between hair follicle dermal papilla cells (HFDPCs) and human dermal stem/progenitor cells (hDSPCs). The cell division capacity of hDSPCs was significantly increased compared with HFDPCs, and the differentiation capacity into adipocytes, chondrocytes, and osteoblasts was significantly increased in hDSPCs. On the contrary, HFDPCs showed significantly increased expression of dermal papilla–related markers (*SOX2, S100β, CORIN* and *Snai2*) compared with hDSPCs. To analyze why these two types of ASCs have different properties, I analyzed intracellular signaling by protein kinase assay. Protein kinase assays showed that the phosphorylation of ERK1/2, c-JUN, CREB, YES, and GSK3α/β is significantly changed in HFDPCs and hDSPCs compared with dermal fibroblasts. HFDPCs have increased expression of markers related to hair regeneration compared with hDSPCs, on the other hand, hDSPCs are more multipotent than HFDPCs. The five above-mentioned phosphorylated signaling proteins (ERK1/2, c-JUN, CREB, YES, and GSK3α/β) are responsible for the characterization of HFDPCs and hDSPCs. The different characteristics of each skin-derived ASC might be a major factor influencing their effective use for tissue regeneration and therapeutics.

Keywords: Adult stem cells, Hair follicle dermal papilla cells, Human dermal stem/progenitor cells, Multipotency, Hair regeneration

1. Introduction

Stem cells, such as embryonic stem cells (ESCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs), are valuable sources of tissue regeneration because of self-renewal and potential to differentiate into various somatic cells [1]. Although ESCs and iPSCs have shown significant potential for tissue regeneration, they still have challenges, such as risk of teratoma formation, ethical concerns, immune concerns, and risks in genetic manipulation of iPSCs [1, 2]. By contrast, multipotent ASCs, also known as mesenchymal stromal cells (MSCs), are immunocompatible, and such cells have no ethical problems. Moreover, these ASCs have a great therapeutic benefit because of their paracrine effects on the restoration of damaged tissues by activating endogenous tissue regeneration upon transplantation [3].

Androgenetic alopecia (AGA) is a non-scarring condition marked by the progressive miniaturization of hair follicles, following a characteristic pattern in genetically predisposed men and women. It is a common cause of hair consultation. Epidemiologic studies show that 80% of Caucasian men and 40%–50% of Caucasian women are affected by AGA, with its prevalence rising with age [4-6]. The prevalence of AGA in the Asian population is low: AGA was observed in 14.1% of Korean men across all ages [7]. AGA significantly affects patients' quality of life and self-esteem, and their expectations regarding therapy outcomes often exceed actual results.

Treatment options for men and women suffering from hair loss include hair transplant surgery, low-level laser therapy, micropigmentation of the scalp, and topical concealer fibers [8-10]. However, these treatment options have limitations; thus, researchers are always looking for new and alternative therapies [11, 12]. A growing area of clinical and scientific interest involves investigating the role of adipose tissue, particularly autologous adipose transplantation, in promoting hair growth. [13].

Skin-derived stem cells also hold promise as a cell source to improve AGA. Skin precursor cells (SKPs), a type of ASC, are present in the dermis. In addition, previous studies have reported that SKPs have similar properties to MSCs [14–18]. SKPs have been studied in rodents and humans for their role in repairing damaged dermis and maintaining skin homeostasis. Toma *et al*. demonstrated that SKPs have neural stem cell–like properties and can differentiate into mesoderm-derived cells such as chon-

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drocytes, adipocytes, and osteoblasts. These cells can be differentiated into ectodermal and endodermal origin, including neurons and hepatocytes [14]. Another study has confirmed the presence of ASCs in the human dermis using single-cell cloning analysis [15]. Shim *et al*. reported the isolation of human dermal stem/progenitor cells (hDSPCs) from human dermis–derived fibroblasts based on their ability to adhere to type IV collagen, the binding partner of integrin beta 1 (CD29) [17, 18]. Compared with non-hDSPCs, hDSPCs have a colony-forming ability and characteristics of ASCs, and hDSPCs can be differentiated into ectoderm- and mesoderm-derived cells. In addition, the hDSPC-conditioned medium (hDSPCs-CM) improved intrinsic and extrinsic aged dermal fibroblasts [19, 20].

Another skin-derived stem cell is hair follicle dermal papilla cells (HFDPCs). HFDPCs are essential not only in hair growth, formation, and cycling but also in the hair inductive capacity (trichogenecity). HFDPCs stimulate the proliferation and differentiation of the follicular epithelium [21, 22]. In addition, HFDPCs are an important class of ASCs involved in the regeneration of the adult body, particularly the hair follicle.

The two types of ASCs mentioned above, hDSPCs and HFDPCs, have been shown as cell sources for treating AGA. Previously, comparative studies between fibroblasts and HFDPCs have been conducted, such as proteomic analysis and secretome analysis [23, 24]; however, no studies have been conducted on the comparative analysis between hDSPCs and HFDPCs. Here, I investigated the proliferative capacity, differentiation potential (adipocyte, osteoblasts, chondrocytes) and intracellular signaling between HFDPCs and hDSPCs. These characteristics were compared to provide a platform for the use of hDSPCs and HFDPCs.

2. Materials and Methods

2.1. Enrichment of hDSPCs and cell culture

Human hair follicle dermal papilla cells (HFDPCs; Cell Applications, USA) isolated from normal human scalp hair follicles were cultured in HFDPC growth medium (Cell Applications) containing a growth supplement at 37°C. HFDPCs were used within three passages.

Human adult dermal fibroblasts (HDFs; Cell Applications) were cultured in DMEM (Welgene, Korea) containing 10% FBS (Equitech-Bio, USA) and 1X antibiotics at 37°C. The HDFs were used within three passages.

For the enrichment of human dermal stem/progenitor cells (hDSPCs), tissue culture dishes were coated with type IV collagen (20 μg/mL; Sigma Aldrich, USA) overnight at 4°C. HDFs were seeded onto type IV collagen-coated dishes and enriched based on their ability to adhere to type IV collagen within 5 min at 37°C [17, 19, 20].

2.2. In vitro cell proliferation assay

The cells (HDFs, HFDPCs, and hDSPCs) were plated at a density of 1.2×10^5 cells/well in a 35-mm tissue culture dish, and the proliferative capacity was measured using the cell counting kit-8 (CCK-8; DoGenBio, Korea) in accordance with the manufacturer's instruction. In brief, cells were treated with 100 μL of the CCK-8 solution in 900 μL of DMEM (phenol red-free, Welgene) and incubated for 1.5 h at 37°C. The absorbance was measured at 450 nm using a spectrophotometer (Epoch; BioTek, USA).

2.3. RNA extraction and real-time RT-PCR

Total RNA was isolated using a TRIzol® Reagent (Life Technologies, USA), and the RNA concentration was assessed using the Epoch Take3 micro-volume spectrophotometer. One microgram of RNA was reverse transcribed into cDNA using the SuperiorScript III RT Master Mix (Enzynomix, Korea). Reverse transcription was stopped by adding TE buffer to 100 μL of the cDNA solution. Real-time RT-PCR was performed using a LightCycler® 96 Real-Time PCR System (Roche, Germany) in accordance with the manufacturer's instruction. In brief, 20 μL of the PCR mixture contained 10 μL of 2× TaqMan® universal PCR Master Mix (Thermo Fisher Scientific, USA), 1 μL of 20× of TaqMan® Gene Expression assay (Thermo Fisher Scientific), and 50 ng of cDNA. Taqman® gene expression assays used for real-time RT-PCR are listed in Table 1. Human GAPDH was used for normalizing variation in cDNA quantities from different samples.

Table 1. Gene Symbol, name and Assay ID Number in qRT-PCR Analysis.

Symbol	Gene name	Assay ID
SOX2	Sex determining region Y-box 2	Hs01053049_s1
S100β	S100 calcium-binding protein B	Hs00389217_m1
Oct3/4	POU class 5 homeobox 1	Hs04260367_gH
Nanog	Nanog homeobox	Hs04399610_g1
Klf4	Kruppel-like factor 4	Hs00358836_m1
CORIN	Corin, serine peptidase	Hs00198141_m1
VCAN	Versican	Hs00171642_m1
Snai2	Snail family zinc finger 2	Hs00950344_m1
Twist1	Twist basic helix-loop-helix transcription factor 1	Hs01675818_S1
FABP4	Fatty acid binding protein 4	Hs00609791_m1
ADIPOQ	Adiponectin	Hs00605917_m1
COL2A1	Collagen type 2 alpha 1	Hs00264051_m1
ACAN	Aggrecan	Hs00153936_m1
OGN	Osteoglycin	Hs00247901_m1
OCN	Osteocalcin	Hs01587814_g1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	43333764F

2.4. In vitro differentiation

Cells were analyzed for their capacity to differentiate into adipogenic, chondrogenic, and osteogenic lineages. The differentiation medium was changed every 2–3 days.

Adipogenic differentiation was induced with an adipogenic differentiation medium (DMEM supplemented with 10% FBS, 100 U/mL of penicillin, 100 µg/mL of streptomycin, 0.5 mM IBMX, 1 µM dexamethasone, 10 µg/mL of insulin, and 2 µM troglitazone) for 10 days [17, 18].

In inducing chondrogenic differentiation, cells were treated with the hMSC Chondrogenic differentiation medium Bulletkit (Lonza, Swiss) for 10 days [17, 18].

For osteogenic differentiation, the cells were cultured with the hMSC Osteogenic differentiation medium Bullet-Kit (Lonza) for 2 weeks [17, 18].

2.5. Histochemical analysis

Cell morphology was analyzed at 40× or 100× of magnification on a phase-contrast microscope (CKX53, Olympus, Japan). Images were captured using an eXcope T500 digital camera system (DIXI Science, Korea).

In examining hair growth promotion ability, an alkaline phosphatase (ALP) staining kit (Vector Laboratories, USA) was used. In brief, cells were fixed with paraformaldehyde for 5 min at room temperature (RT) and washed with PBS. Then, the cells were treated with ALP substrate for 20 min at 37°C and rinsed three times with tap water.

2.6. Protein kinase array analysis

Analysis of phosphorylated proteins by human phospho-kinase array was performed in accordance with the manufacturer's instruction (R&D systems, USA). In brief, cell lysates (300 µg) were incubated with each array sheet at 4°C overnight on a rocking shaker. The cell lysate was removed, and human phospho-kinase arrays were washed three times with washing buffer. Arrays were incubated with the primary antibody for 2 h at RT and washed three times with washing buffer. The secondary antibody was added and incubated for 1 h on a rocking shaker. The array was washed three times with washing buffer, and phosphorylated protein spots were detected using a chemiluminescence solution. The density of each phosphorylated protein spot was assessed using the Multi Gauge V3.0 program, and the density was calculated by subtracting the background and PBS-negative control.

2.7. Statistical analysis

Statistical analysis comparing three groups was conducted using one-way analysis of variance (ANOVA). For experiments comparing two groups, the Student`s t-test was conducted (Supplementary Fig. 1). The results are presented as the mean ± standard deviation (SD) from at least three independent experiments, with $p < 0.05$ considered statistically significant.

3. Results

3.1. Comparison of the proliferation potential between HFDPCs and hDSPCs

In comparing the proliferation ability, 1.2×10^5 cells were cultured on a tissue culture dish, and CCK-8 analysis was performed. After 1-day culture, no morphological and cell-size differences were observed among the three types of cells in cultures (Fig. 1A–F). However, CCK-8 analysis showed that hDSPCs were 47% highly proliferative compared with HDFs and HFDPCs. This result indicates that hDSPCs are more proliferative than HFDPCs; it also suggests that hDSPCs are potential sources for obtaining highly proliferative cells.

3.2. Dermal papilla cell marker expressions in skin-derived ASCs

In this study, the expression of dermal papilla (DP) cell markers was compared by real-time RT-PCR in cultured HDF, HFDPCs, and hDSPCs. DP cell markers (*SOX2*, *S100ß*, *Corin*, *VCAN*, and *Twist1*) were significantly increased in HFDPCs and hDSPCs compared with dermal fibroblasts (Fig. 2A–E). *SOX2*, *S100ß*, and *Corin* are more highly expressed in HFDPCs compared to hDSPCs, while *VCAN* and *Twist1* are more highly expressed in hDSPCs. Another DP cell marker (*Snai2)* expression was only significantly increased in HFDPCs (Fig. 2F).

Alkaline phosphatase (ALP) is a key enzyme expressed in DP cells [25, 26]. To analyze hair growth potential by ALP staining, each cell type was inoculated at 1×10^5 cells in a 35 mm tissue culture dish, cultured for 2 days, and then stained using the ALP staining kit. ALP was not expressed in HDFs but was expressed in 27.6% and 14.0% of the population in HFDPCs and hDSPCs, respectively (Fig. 3).

3.3. Comparison of multipotency between HFDSCs and hDSPCs

The multipotency was tested by differentiation into mesodermal cell types (adipogenic, chondrogenic, and osteogenic), as described in the materials and methods.

The adipogenic differentiation of each cell type was analyzed for mature adipocyte markers (*FABP4* and *ADIPOQ*). These markers were highly expressed in hDSPC-derived adipocytes compared with HDFs- and HFDPC-derived adipocytes ($p < 0.05$; Fig. 4A and B).

Chondrogenic differentiation was also evaluated after chondrogenic differentiation condition. Real-time RT-PCR revealed increased gene expression levels of chondrocyte-

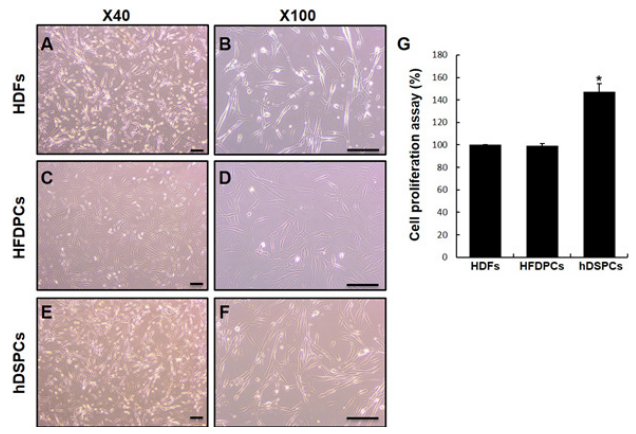

Fig. 1. Characterization of each cell type and comparison of proliferative capacity. Representative phase-contrast images of human dermal fibroblasts (HDFs) (A and B). Hair follicle dermal papilla cells (HFDPCs) (C and D) and human dermal stem/progenitor cells (hDSPCs) (E and F). Quantification of the proliferation capacity of HDFs, HFDPCs, and hDSPCs by cell counting kit-8 (CCK-8) analysis (G). The data represent the mean ± SD of independent experiments run in triplicate. Scale bars; 20 µm, * $p < 0.05$.

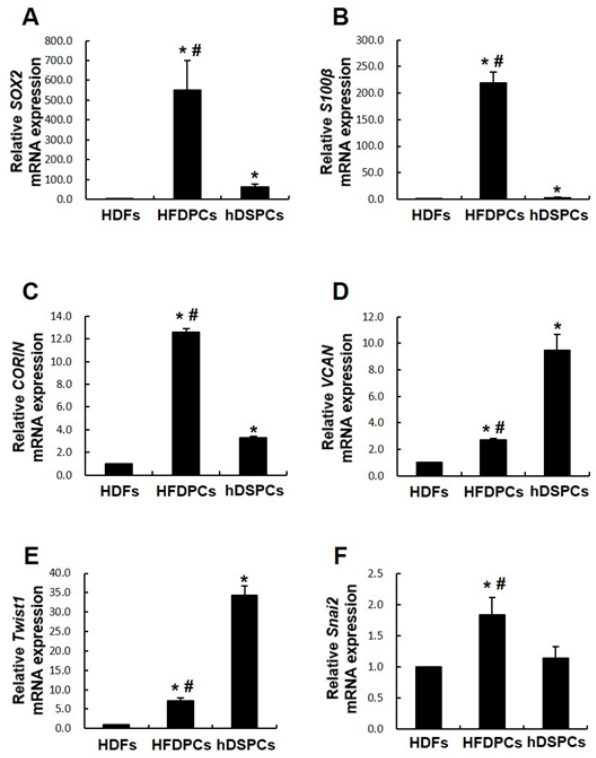

Fig. 2. Comparative analysis of the dermal papilla-related mRNA expression from different cell sources. Real-time RT-PCR analysis showed the relative expression of dermal papilla markers. Values represent the mean ± SD of independent experiments run in triplicate. * compared with HDFs, # compared with hDSPCs. *, # $p < 0.05$.

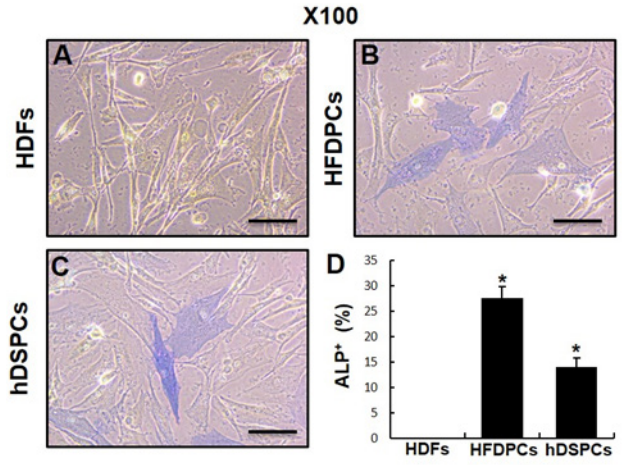

Fig. 3. Comparative analysis of the ALP expression. Representative ALP-positive phase-contrast images of HDFs (A), HFDPCs (B), and hDSPCs (C). Quantification of the ALP expression of HDFs, HFDPC, and hDSPCs by ALP staining analysis (D). The data represent the mean ± SD of independent experiments run in triplicate. Scale bars; 20 μm, * $p < 0.05$.

related gene markers, *COL2A1* and *ACAN*, in hDSPC-derived cells compared with HDFs and HFDPCs ($p < 0.05$; Fig. 4C and D).

Furthermore, 2 weeks after cell differentiation in an osteogenic medium, the mRNA expression levels of *OGN* and *OCN* were increased significantly in hDSPCs after osteogenic induction compared with HDFs. On the contrary, the differentiation of HFDPCs into osteogenic differentiation was significantly decreased compared with HDFs ($p < 0.05$; Fig. 4E and F).

The expression of undifferentiated markers, *Oct3/4*, *Nanog*, and *Klf4* was analyzed with real-time RT-PCR. The expressions of *Oct3/4*, *Nanog*, and *Klf4* were increased by 5.07, 8.89, and 3.81-fold, respectively, in hDSPCs compared with HFDPCs (Supplementary Fig. 1). Fig. 4 and Supplementary Fig. 1 showed that hDSPCs have more multipotency and stemness compared with HFDPCs.

3.4. Analysis of the intracellular signaling of HFDPCs and hDSPCs

The differences among HDFs, HFDPCs, and hDSPCs were indicated by the mentioned experiments. To determine which intracellular signals drive the properties of each cell type, a phospho-kinase array was performed. From the phospho-kinase array experiment, five phosphorylated proteins were identified (Fig. 5A, Table 2). Proteins that were significantly changed in both HFDPCs and hDSPCs compared with HDFs are marked with red squares, and proteins that were significantly changed in only HFDPCs compared with HDFs are marked with green squares. In addition, phosphorylated proteins that were significantly decreased in hDSPCs compared with HDFs are marked with blue squares (Fig. 5). In this experiment, the p-ERK1/2 protein was increased by 60% in HFDPCs and increased by 21% in hDSPCs compared with the phosphorylated protein level of HDFs (Fig. 5B). The phosphorylation of c-Jun was decreased by about 56% in HFDPCs and decreased by 51% in hDSPCs compared

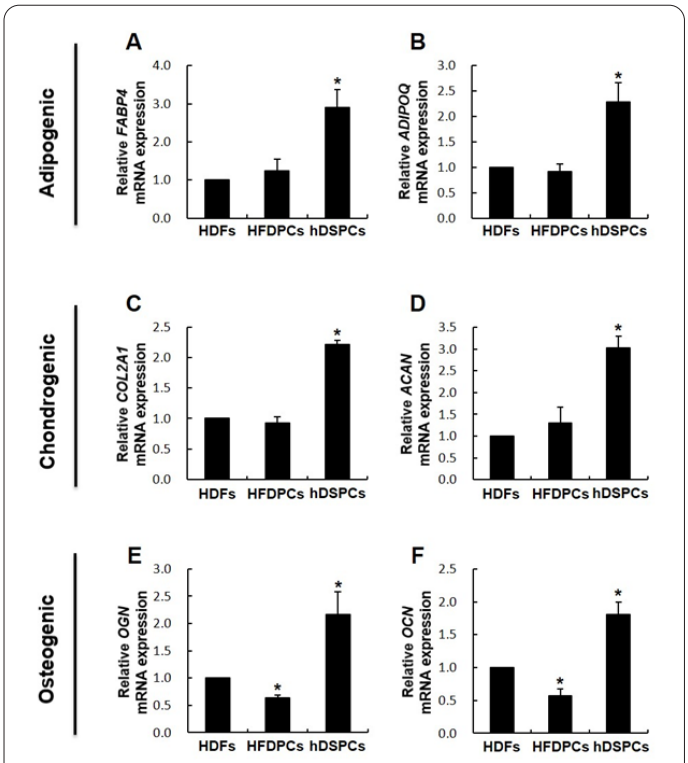

Fig. 4. Comparing the ability of three cell types to differentiate into mesodermal lineage differentiation. Quantitative RT-PCR analysis of mRNA expression involved in adipogenic differentiation (A and B): FABP4 (A) and ADIPOQ (B). Quantitative RT-PCR analysis revealed the expressions of COL2A1 (C) and ACAN (D) upon chondrogenic differentiation. Quantitative RT-PCR analysis showed the expressions of OGN (E) and OCN (F) upon osteogenic differentiation. The data represent the mean ± SD of independent experiments in triplicate. * $p < 0.05$.

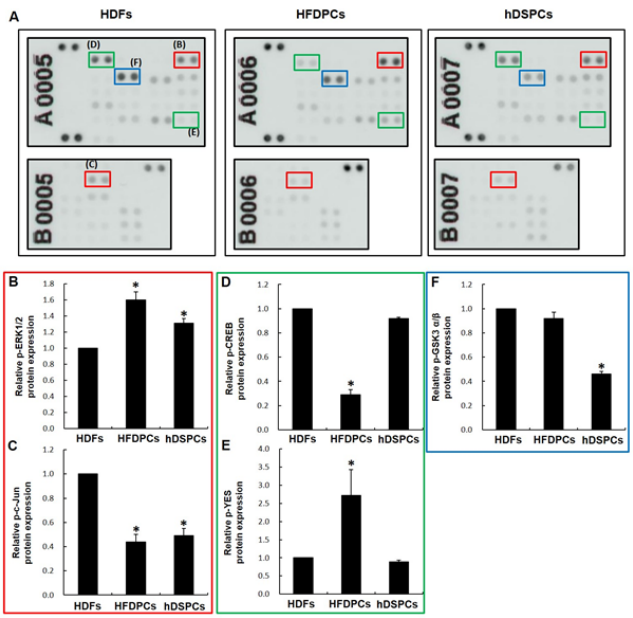

Fig. 5. Phosphorylation analysis of intracellular signaling. After collecting whole-cell lysates, such lysates were applied to the human phospho-kinase array kit. (A) indicates membranes A and B of the phospho-kinase array kit. The density of each array spot was measured. Relative p-ERK1/2 (B), p-c-Jun (C), p-CREB (D), p-YES (E), and p-GSK3α/β (F) protein expression. The graphs are shown as the mean ± SD of three independent experiments. * compared with HDFs, $p < 0.05$.

with the HDFs (Fig. 5C). The expression of p-CREB was reduced by 71% in HFDPCs compared with HDFs, and p-YES was increased by 2.72-fold in HFDPCs, but such difference was not significant in hDSPCs. On the contrary, p-GSK3α/β was reduced by 54% in hDSPCs compared with HDFs, but this difference was not significant in HFDPCs (Fig. 5D–F). These five phospho-proteins are responsible for the different characteristics of HFDPCs and hDSPCs from HDFs.

4. Discussion

Bone marrow-derived MSCs are a great source of cells for therapeutic purposes, but they are difficult to isolate and obtain in large quantities. In comparison to the bone marrow, the skin is the largest organ in the body and can be easily accessed for sample biopsy [14, 17]. Therefore, a substantial amount of ASCs could be easily harvested from the skin, and the skin might be more efficient than other organs for obtaining a large number of relevant MSCs.

The proliferative property and differentiation ability of skin-derived ASCs were investigated to understand the characteristics of each cell population. In particular, hDSPCs were more proliferative than HFDPCs (Fig. 1). *SOX2*, *S100b*, *CORIN*, *VCAN*, *Twist1*, *Snai2*, and ALP are usually used as markers of DP cells [25–29]. In this experiment, the expressions of *SOX2*, *S100β*, *CORIN*, and *Snai2* were investigated, and the result showed that HFDPCs had significantly increased expression of *SOX2*, *S100b*, *CORIN*, and *Snai2* markers compared with HDFs and hDSPCs (Fig. 2A–C and F). ALP staining showed an increased number of ALP-positive cells in HFDPCs and hDSPCs compared with HDFs (Fig. 3). On the other hand, the expressions of *VCAN* and *Twist1* were significantly increased in hDSPCs compared with HFDPCs (Fig. 2D and E). VCAN is a large extracellular matrix proteoglycan that is present in a variety of human tissues such as the skin, blood vessels, and developing heart [30–32]. VCAN has a crucial role in development, guiding embryonic cell migration during heart formation and outlining the pathway for neural crest cell migration. In particular, the expression of VCAN is important to the development of the skin, and higher expression of VCAN has been reported in fetal skin tissue than in adult skin. [32]. The increased expression of *VCAN* in hDSPCs (Fig. 2D) and the expression of ESC markers (Supplementary Fig. 1) suggest that hDSPCs may be a more undifferentiated cell population compared to HFDPCs.

Twist1 plays an important role in some physiological processes involved in angiogenesis, metastasis, extravasation, invadopodia, and chromosomal instability. The underlying mechanism of tumor metastasis is not fully understood, but VCAN has been implicated in the upregulation of matrix metalloproteinases (MMP) and the inhibition of tissue inhibitors of metalloproteinase (TIMP) [33–35]. Based on previous reports, the MMP1 gene is more highly expressed in the dermis than in the dermal sheath where HFDPCs reside [36]. hDSPCs are a stem cell-like population isolated from the dermis, and HFDPCs are cells present in the dermal sheath. The expression of MMP1 in HFDPCs is down-regulated compared with that in hDSPCs (Supplementary Fig. 2). This result suggests that the increased effect of Twist1 in hDSPCs is responsible for the increase of MMP1 in hDSPCs (Fig. 2E).

The differences in multipotency between HFDPCs and hDSPCs led to preferential differentiation into adipose, chondrocytes, and osteoblasts (Fig. 4). Although HFDPCs were not differentiated into various mesodermal lineages, hDSPCs showed a significant increase in differentiation into adipocytes, chondrocytes, and osteoblasts. Although HFDPCs are stem cells present in the skin, they are thought to be biased toward hair lineage compared with hDSPCs. In addition, Supplementary Figure 1 shows that the expression of ESC markers such as Oct3/4, Nanog, and Klf4 is increased in hDSPCs compared to HFDPCs, indicating that hDSPCs are relatively more multipotent than HFDPCs.

To understand what might be causing the differences between hDSPCs and HFDPCs, the intracellular signaling of each cell type was analyzed. A phospho-kinase array was used to screen for intracellular signaling proteins that were increased or decreased in HFDPCs and hDSPCs compared with HDFs. This technique is commonly used to analyze phosphorylated intracellular signaling in many cell types as demonstrated in previous studies [37–39]. The phosphorylation of ERK1/2 was significantly increased in HFDPCs and hDSPCs compared with HDFs (Fig. 5B). In undifferentiated ESCs, the increased phosphorylation of ERK1/2 by the activation of PKC delta is a signaling pathway required to maintain stemness and induce the cell division of ESCs [40]. In addition, the inhibition of FGF4 by FGF4 splice isoform (FGF4si), an antagonist of FGF4 in human ESCs, induces the phosphorylation of ERK1/2, which leads to the self-renewal of human ESCs [41]. In chronic myelogenous leukemia patient-derived K562 cells, the inhibitory mechanism of the erythroid differentiation of K562 cells by signaling activity through ERK/MAPK phosphorylation was reported [42]. These studies have shown that the phosphorylation of ERK1/2 is an im-

Table 2. Relative phosphorylated protein analysis by fold change ratio.

Gene Name(phosphorylation site)	Fold change (mean ± S.D) ratio	
	HFDPCs/Fb	hDSPCs/Fb
CREB(S133)	0.29±0.04	0.92±0.01
EGF R(Y1086)	0.91±0.1	0.93±0.2
eNOS(S1177)	0.95±0.1	0.95±0.1
ERK1/2(T202/Y204, T185/Y187)	1.6±0.1	1.31±0.1
Fgr(Y412)	0.91±0.2	0.96±0.2
GSK-3α/β(S21/S9)	0.92±0.1	0.46±0.02
GSK-3β(S9)	0.91±0.2	0.93±0.2
HSP27(S78/S82)	0.97±0.1	1.08±0.1
JNK1/2/3(T183/Y185, T221/Y223)	0.94±0.2	0.91±0.2
Lck(Y394)	0.99±0.1	0.97±0.1
Lyn(Y397)	0.93±0.1	0.99±0.1
MSK1/2(S376/S360)	0.91±0.2	0.95±0.1
p38a(T180/Y182)	0.95±0.1	0.99±0.1
PDGF Rβ(Y751)	0.92±0.2	0.92±0.2
PLCγ-1(Y783)	0.92±0.2	0.92±0.2
Src(Y416)	1.08±0.2	1.09±0.3
STAT2(Y689)	0.92±0.1	0.97±0.1
STAT5a/b(Y699)	0.94±0.2	0.96±0.2
WNK1(T60)	0.91±0.3	0.90±0.2
Yes(Y426)	2.72±0.7	0.89±0.1
β-catenin	0.91±0.2	1.09±0.3
Akt1/2/3(S473)	0.96±0.1	0.92±0.2
Akt1/2/3(T308)	0.97±0.1	0.91±0.1
c-Jun(S63)	0.44±0.1	0.49±0.1
p53(S392)	0.91±0.1	0.96±0.1
p53(S46)	0.92±0.2	0.91±0.1
p70 S6 kinase(T389)	1.02±0.2	0.94±0.1
p70 S6 kinase(T421/S424)	0.93±0.1	0.92±0.1
PRAS40(T246)	0.91±0.2	0.94±0.2
Pyk2(Y402)	0.9±0.1	0.96±0.2
RSK1/2(S221/S227)	0.91±0.2	1.09±0.2
RSK1/2/3(S380/S386/S377)	0.92±0.2	0.9±0.1
STAT1(Y701)	0.94±0.1	0.92±0.1
STAT3(S727)	0.91±0.1	0.91±0.2
STAT3(Y705)	0.91±0.1	1.09±0.2
STAT6(Y641)	0.94±0.2	0.98±0.3

portant signaling system for inhibiting cell differentiation and maintaining the undifferentiated state. Our results also show that p-ERK1/2 is increased in HFDPCs and hDSPCs compared with HDFs, suggesting that HFDPCs and hDSPCs have stem cell-like properties compared with HDFs (Fig. 5B).

On the contrary, c-Jun phosphorylation increases as cells differentiate. The increased protein expression of JNK1/3 and increased p-JNK have been reported during differentiation from ESCs through neural progenitors to terminally differentiated pyramidal glutamatergic neuron cells [43]. In monocytes, the phosphorylation of c-Jun induces monocytic differentiation through monocyte-specific gene expression [44]. Similar to previous studies showing that phospho-c-Jun triggers cell differentiation, phosphorylated c-Jun protein expression was reduced in HFDPCs and hDSPCs compared with HDFs (Fig. 5C).

Previous studies showed that in MSCs, BMP9 induces differentiation into osteoblasts rather than adipocytes via signaling that triggers the phosphorylation of COX2 and CREB [45]. As shown in Figures 4e and f, the differentiation capacity of HFDPC cells into osteoblasts was relatively less compared with HDFs and hDSPCs. Based on this result and the results shown in Figure 5d, the significantly decreased expression of p-CREB in HFDPCs could inhibit the differentiation capacity of HFDPCs into osteoblasts.

At present, the role of the phosphorylated YES and GSK3α/β in stem cells or HFDPCs has not yet been reported. The phosphorylation of the YES protein, which belongs to the Src kinase family, has been poorly studied and is known to act as an activator of CDC42 in pancreatic islet β cells and to stimulate glucose-induced insulin secre-

tion [46]. In addition, YES is known to regulate endothelial cell plasticity and barrier strength by regulating the phosphorylation of VE-cadherin on endothelial cells. [47]. Adiponectin induces the proliferation of hippocampal neural stem/progenitor cells through the p38 MAPK and GSK3α/β signaling pathways [48]. Some reports indicated that GSK3α/β phosphorylation is involved in neuronal function, neurite growth, synapse formation, and neurogenesis [49]. However, no studies have been conducted on whether YES and GSK3 α/β are involved in hair production or in maintaining the undifferentiated state of cells in HFDPCs or hDSPCs. Thus, investigating the function of these signaling systems in hair production or skin-derived ASCs is necessary in the future.

The skin might be an efficient niche for obtaining ASCs for regenerative medicine because of its easy accessibility and high proliferation ability. HFDPCs and hDSPCs in the skin have the potential of ASCs. HFDPCs are more specialized with regard to hair production, whereas hDSPCs have more potential as ASCs than HFDPCs based on their differentiation potentials into adipocytes, chondrocytes, and osteoblasts. This study confirmed that these two ASCs differ in their differentiation capabilities and hair growth potential and that they express different intracellular signaling systems.

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Author Disclosures
The author states no conflict of interest.

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