CMLS, Cell. Mol. Life Sci. 61 (2004) 2774–2781
1420-682X/04/212774-08
DOI 10.1007/s00018-004-4288-4

CMLS Cellular and Molecular Life Sciences

Research Article

Isolation of human epidermal stem cells by adherence and the reconstruction of skin equivalents

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Received 2 July 2004; received after revision 20 August 2004; accepted 10 September 2004

Abstract. The isolation of human epidermal stem cells is critical for their clinical applications. In the present study, we isolated three populations of epidermal keratinocytes according to their ability to adhere to collagen type IV: i.e., rapidly adhering (RA), slowly adhering (SA), and non-adhering (NA) cells. The aim of this study was to characterize RA cells and to investigate the possibility of using these cells for epidermis reconstruction. To identify RA cells, flow cytometric analysis was performed using anti-α_6 integrin and anti-CD71 antibodies. RA cells express high levels of α_6 integrin and low levels of CD71, which are considered as markers of an epidermal stem cell nature. Furthermore, electron microscopy showed that RA cells are small and have a high nuclear to cytoplasmic ratio, whereas SA and NA cells have well-developed cellular organelles and abundant tonofilaments. Western blot analysis showed that RA cells are slow cycling and express p63, a putative epidermal stem cell marker, whereas SA and NA cells express c-Myc, which is known to regulate stem cell fate. To compare epidermal regenerative abilities, skin equivalents (SEs) were made using RA, SA, and NA cells. The epidermis constructed from RA cells was well formed compared to those formed from SA or NA cells. In addition, only SEs with RA cells expressed α_6 integrin and β_1 integrin at the basal layer. These results indicate that RA cells represent epidermal stem cells and are predominately comprised of stem cells. Therefore, the isolation of RA cells using a simple technique offers a potential route to their clinical application, because they are easily isolated and provide a high yield of epidermal stem cells.

Key words. Skin equivalents; epidermal stem cell; FACS; p63; β_1 integrin.

Introduction

The epidermis is constantly renewed, and consists of keratinocytes showing variable degrees of differentiation. Cell kinetic analyses of epidermal turnover can be used to divide keratinocytes into stem cells, transit amplifying (TA) cells, and postmitotic differentiating cells [1, 2]. Moreover, epidermal stem cells can contribute to the maintenance of the epidermis via their self-renewing ability. In addition, stem cells are deeply involved in cellular regeneration, wound healing, and neoplasm formation [3].

Although many researchers have tried to identify a universal epidermal stem cell marker, no specific markers have been commonly accepted. In general, slow-cycling cells having a high proliferative potential are widely viewed as stem cells [4]. A homologue of the p53 tumor suppressor gene, p63, is regarded as another potential stem cell marker based on the phenomenon that $p63^{-/-}$ mice failed to develop stratified squamous epithelia [5].

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Moreover, clonal analysis revealed that p63 is abundantly expressed in epidermal and limbal holoclones, which are considered to be a stem cell population [6]. Additionally, the proto-oncogene c-Myc is thought to regulate stem cell fate. Thus, c-Myc is important in the conversion of a stem cell to a TA cell [2]. These studies indicate that both p63 and c-Myc are essential regulators of the fate of keratinocytes. Recently, candidate mouse keratinocyte stem cells were isolated by fluorescence-activated cell sorting (FACS) based on high levels of α_6 integrin and low levels of the transferrin receptor, CD71 (α_6 integrin^{bri}CD71^{dim}) [7]. However, α_6 integrin^{bri}CD71^{dim} cells require further characterization as stem cell markers.

Isolating α_6 integrin^{bri}CD71^{dim} cells by FACS is both time-consuming and laborious. In addition, stem cell yields obtained by FACS are relatively low, and stem cell viability after FACS should also be considered. Murine epidermal stem cells, however, can be selected using rapid substrate attachment [8]. Thus, a suitable extracellular matrix could provide a simple and excellent method for isolating human epidermal stem cells. Collagen type IV is the ligand of β_1 integrin, and it was reported that human interfollicular epidermal stem cells express high levels of β_1 integrin [9]. Thus, collagen type IV may be a possible candidate for the selection of epidermal stem cells [10].

In this study, we performed FACS using anti-α_6 integrin and anti-CD71 antibodies with the aim to classify all human epidermal cells according to their cell surface markers. To collect putative stem cells, we isolated three populations of keratinocytes according to their ability to adhere to collagen type IV, namely, rapidly adhering (RA), slowly adhering (SA), and non-adhering (NA) cells. These RA, SA, and NA cells were then characterized using various methods, i.e., FACS analysis, Giemsa staining, and electron microscopy. In addition, the expression levels of p63 and c-Myc were investigated to compare epidermal stem cell markers among RA, SA, and NA cells. Furthermore, we prepared three-dimensional skin equivalents (SEs) to examine the epidermal regenerative abilities of RA, SA, and NA cells. In addition, immunohistochemical studies were performed using some putative stem cell markers to determine the distributions of stem cells in the SEs.

Materials and methods

Isolation of primary epidermal cells

Human keratinocytes were isolated from human foreskins obtained during child circumcision. Skin specimens were processed according to the method of Rheinwald and Green [11], as modified in our laboratory using thermolysin (Sigma, St. Louis, Mo.). Isolated keratinocytes were maintained in keratinocyte growth medium (KGM; Clonetics, San Diego, Calif.). Collagen-coated IV dishes were prepared by coating 100-mm dishes with type IV collagen (20 µg/ml) overnight at 4 °C, and cells were separated according to their abilities to adhere to type IV collagen. The primary epidermal cells from foreskins were plated onto type IV collagen-coated dishes. A portion of these cells was selected according to their ability to adhere within 10 min at 37 °C (RA cells), and non-adherent cells were rinsed off and transferred to another culture dish. These non-adherent cells were then maintained for a further 24 h and adherent cells were again selected (SA cells). Non-adherent cells were rinsed off and collected (NA cells). Both types of adherent cells were trypsinized and subjected to the following experiments.

Measurement of cell size and the nuclear cytoplasmic ratio

About 40,000 cells per slide were cytospun at 100 × *g* for 5 min. Slides were stained with Giemsa, and the cells were observed under a phase contrast microscope (Olympus, Tokyo, Japan) and then photographed with a DP50 digital video camera system (Olympus) supported by OLYSIA-BioReport software (Olympus).

Transmission electron microscopy

Separated cells were gently centrifuged at 250×g for 5 min, and cell pellets were fixed in freshly prepared fixative solution (4% paraformaldehyde and 2% glutaraldehyde in 0.1 M pH 7.2 phosphate buffer) for 1 h. After washing with PBS, the cells were postfixed in 2% OsO_4 in PBS for 1 h, dehydrated in graded ethanol, and embedded in Epon. Sections were cut using an ultramicrotome (Leica, Wetzlar, Germany) and stained with a saturated solution of uranyl acetate in acetone, and then with a solution of 0.2% lead citrate. Samples were observed under a JEM-100CX (JEOL, Tokyo, Japan) transmission electron microscope.

Immunofluorescence staining and flow cytometric analysis

Separated cells were used for flow cytometric analysis. Dual staining was performed using FITC-conjugated CD71 monoclonal antibody (No. sc-7327 FITC) from Santa Cruz Biotechnology (Santa Cruz, Calif.) and PE-conjugated α_6 integrin monoclonal antibody (No. MCA 1457PE) from Serotec (Raleigh, N. C.). Mouse IgG_1: FITC/mouse IgG_1:RPE antibody (No. DC012) from Serotec was used as an isotype-matched negative control. Labeling reactions were performed in a light-shielded state for 1 h at 4 °C. The cells were then resuspended in PBS containing 2% FBS at $2 - 3 \times 10^6$/ml. FACS analyses were performed on a FACS Calibur (Becton-Dickinson, Franklin Lakes, N. J.). Experiments were repeated at least twice using the same conditions and settings.

Western blot analysis

Cells were lysed in cell lysis buffer [62.5 mM Tris-HCl (pH 6.8), 2% SDS, 5% β-mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride, protease inhibitors (Complete, Roche, Mannheim, Germany), 1 mM Na_3VO_4, 50 mM NaF, and 10 mM EDTA]. Ten micrograms of protein per lane was separated by SDS-polyacrylamide gel electrophoresis and blotted onto PVDF membranes, which were saturated with 5% dried milk in Tris-buffered saline containing 0.4% Tween 20. Blots were then incubated with the appropriate primary antibodies at a dilution of 1:1000, and then further incubated with horseradish peroxidase-conjugated secondary antibody. Antibodies against p63 (No. sc-8431), c-Myc (No. sc-40), α_6 integrin (No. sc-6597), $p21^{WAF1/CIP1}$ (No. sc-397), cyclin D1 (No. sc-6281), and actin (I-19) were obtained from Santa Cruz Biotechnology, and against cyclin D2 from Pharmingen (San Diego, Calif.). Bound antibodies were detected using an enhanced chemiluminescence plus kit (Amersham, Little Chalfont, U.K.).

Colony forming assay

Keratinocytes were isolated as described above. Twenty thousand cells per population were seeded in 100-mm dishes, and grown at 37 °C in 5% CO_2 in serum-free KGM prepared from keratinocyte basal medium (KBM) by adding 0.1 ng/ml of recombinant epidermal growth factor (EGF), 5.0 µg/ml insulin, 0.5 µg/ml hydrocortisone, 0.15 mM calcium, 30 µg/ml bovine pituitary extract, 50 µg/ml gentamicin sulfate, and 50 ng/ml amphotericin B. Cultures were grown for 10 days and then observed under a phase contrast microscope (Olympus) and photographed using a $CoolSNAP_{cf}$ digital video camera system (Roper Scientific, Tucson, Ariz.) supported by RS Image software (Roper). To visualize colony growth, culture medium was removed, and the cells were stained with 0.1% crystal violet in 10% ethanol for 5 min at room temperature and rinsed four times.

Regenerative efficacy of separated cells

Dermal substitutes were prepared according to the method of Bell et al. [12], with some modifications [13]. Briefly, type I collagen from rat tail tendons was dissolved by stirring in 1/1000 glacial acetic acid at 4 °C for 48 h. Dermal substitutes were made by mixing eight volumes of type I collagen with one volume of 10 × concentrated DMEM and one volume of neutralization buffer (0.05 N NaOH, 0.26 mM $NaHCO_3$, and 200 mM HEPES) and adding 5×10^5 fibroblasts. Three milliliters of this mixture was then poured into a 30 mm polycarbonate filter chamber (3.0 µm Millicell; Millipore, Bedford, Mass.), and culture medium was added after gelation at 37 °C. Human keratinocytes were seeded at a density of 1×10^6 cells onto the dermal substitute, and cultured in a submerged state for 1 day and then for 12 days at the air-liquid interface. The growth medium consisted of DMEM and Ham's nutrient mixture F12 at a ratio of 3:1 supplemented with 5% FBS, 0.4 µg/ml hydrocortisone, 1 µM isoproterenol, 25 µg/ml ascorbic acid, and 5 µg/ml insulin. A low concentration of EGF (1 ng/ml; Invitrogen, Carlsbad, Calif.) was also added during submerged culture and a higher concentration of EGF (10 ng/ml) during the air-liquid interface culture. The medium was changed three times per week. Experiments were repeated at least twice using the same conditions and settings.

Histology and immunohistochemistry

After 13 days, SEs were fixed in Carnoy's solution (ethanol/chloroform/acetic acid 6:3:1) for 30 min and processed for conventional paraffin embedment. Four- to six-micrometer-thick sections were then prepared. For morphological observations, sections were stained with hematoxylin and eosin (H&E), and for immunohistochemical analysis, sections were processed using the avidin-biotin-peroxidase complex technique (DAKO, Glostrup, Denmark). Antibodies against p63 (No. sc-8431), α_6 integrin (No. sc-6597), and β_1 integrin (No. sc-9970) were obtained from Santa Cruz Biotechnology; against PCNA (No. M0879) from DAKO, and against involucrin from Sigma.

Results

Epidermal cell populations as determined by cell surface marker analysis

To determine the distribution of human epidermal keratinocytes according to their cell surface markers, primary keratinocytes were isolated from human foreskins and analyzed by FACS, as reported previously [1]. Because stem cells may not maintain their characteristics in culture in the absence of their neighboring niche cells [1, 14, 15], we analyzed only freshly isolated cells in these experiments. Figure 1A shows a representative dot plot in which cells are represented by the expressions of α_6 integrin (x-axis) and CD71 (y-axis). The same cells are also plotted in figure 1B according to size (FSC, forward light scatter) and granularity (SSC, side light scatter). From these results, we grouped cells into three populations. R1 represents a high level of α_6 integrin and a low level of CD71 expression, i.e., potential stem cell candidates (fig. 1A). Dot plots of FSC and SSC indicated that R1-gated cells are composed of relatively small cells (blue dots in fig. 1B). In figure 1B, the R2 population is represented by larger more granulated cells. These cells showed low levels of α_6 integrin and high levels of CD71 (red dots in fig. 1A). The remainders of the cells were grouped as another population and are indicated by black dots.

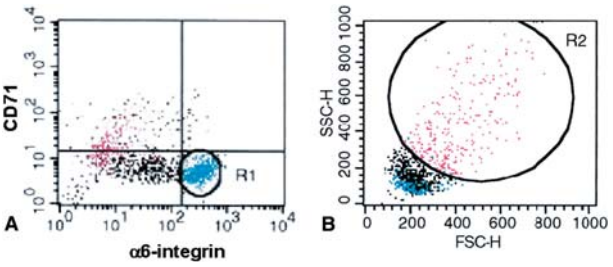

Figure 1. FACS analysis of epidermal cells according to cell surface markers. (*A*) Two-color flow cytometric analysis of α_6 integrin and CD71 expression on epidermal cells: R1-gated cells (blue dots) represent α_6 integrin-positive and CD71-negative cells. (*B*) Forward scatter (FSC) and side scatter (SSC) dot plots of epidermal cells: R2-gated cells (red dots) represent larger more granulated cells. These results are representative of three independent experiments.

Separation of epidermal cells according to their ability to adhere

There have been reports that epidermal stem cells can be selected using a rapid attachment method [8, 16]. Accordingly, primary foreskin epidermal cells were plated onto type IV collagen-coated culture dishes, and a small proportion of the cells were observed to adhere quickly to dish surfaces. Cells adhering within 10 min (RA cells) were selected, and non-adherent cells were transferred to another culture dish. The non-adherent cells were then maintained for a further 24 h and adherent cells were again separated (SA cells) from non-adherent cells (NA cells).

To characterize adhering human epidermal cells, the separated RA, SA, and NA cells were analyzed by FACS. The three cell types were examined using two-color fluorescence dot plots for α_6 integrin (x-axis) and CD71 (y-axis) (fig. 2A–C, respectively), and for FSC and SSC (fig. 2D–F). We found that RA cells expressed high levels of α_6 integrin and low levels of CD71 (fig. 2A), and that they are smaller and less granulated than SA or NA cells (fig. 2D). In contrast, NA cells expressed low levels of α_6 integrin but high levels of CD71 (fig. 2C). They were also larger and more granulated than RA or SA cells (fig. 2F). On the other hand, SA cells expressed low levels of α_6 integrin and CD71 and were intermediate in size and granularity. These results indicate that cell adherence can easily be used to isolate α_6 integrin-positive and CD71-negative cells.

Cell size and ultrastructure

Giemsa staining of cytospin preparations of each of the three cell fractions showed that they had different cell sizes and nuclear to cytoplasmic (N/C) ratios. In agreement with the FACS analysis, the RA cell population was found to contain smaller cells with a higher N/C ratio than the other two populations (fig. 3A), whereas most of the NA cells were larger with a lower N/C ratio, and thus an increased cytoplasmic area (fig. 3C). SA cells showed intermediate characteristics (fig. 3B). These differences in the morphological appearance of the three populations were further verified by transmission electron microscopy (fig. 3D–F), which demonstrated that RA cells are smaller and have less cytoplasmic space than the other two cell types. In contrast, more tonofila-

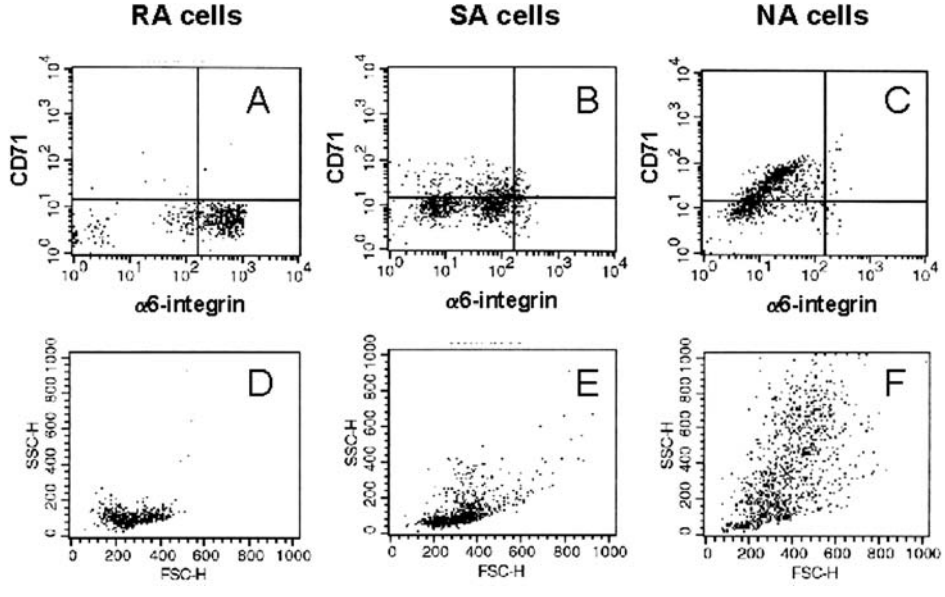

Figure 2. Separation of epidermal cells according to their ability to adhere. Epidermal cells were separated into rapidly adhering (RA), slowly adhering (SA), and non-adhering (NA) cells, as described in Materials and methods. Dot plots show the results of flow cytometric analyses of RA (*A*), SA (*B*), and NA (*C*) cells double-labeled with anti-α6 integrin and anti-CD71 antibodies. RA (*D*), SA (*E*), and NA (*F*) cell distributions were plotted by forward scatter (FSC) and side scatter (SSC). The figures shown are representative of three independent experiments.

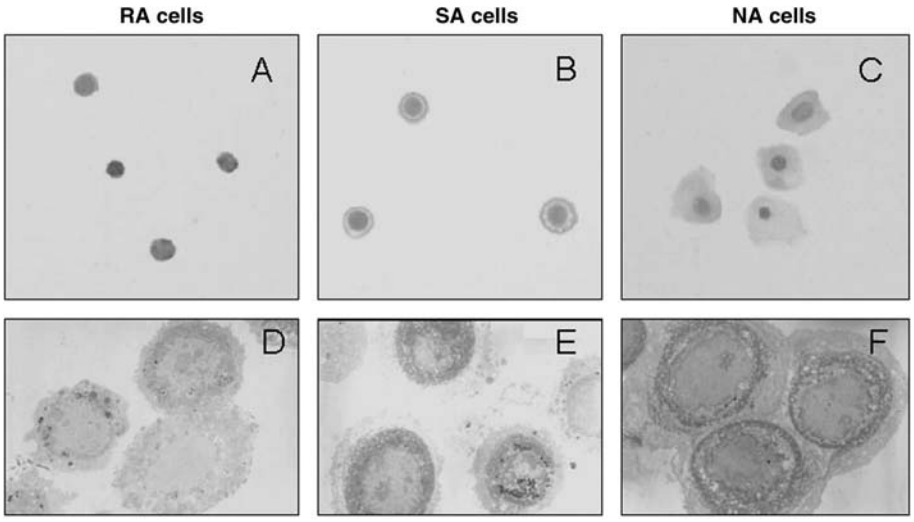

Figure 3. Cell size, N/C ratio, and the ultrastructures of separated cells. Giemsa-stained cytospin preparations of RA (*A*), SA (*B*), and NA (*C*) cells. Phase contrast pictures were taken using a color video camera (×400). Transmission electron microscopy of RA (*C*), SA (*E*), and NF (*F*) cells was performed as described in Materials and methods (×2700). RA cells were found to have higher N/C ratios.

ments and well-developed organelles were observed in SA and NA cells.

Expressions of putative stem cell markers

Several reports have indicated that p63 expression is inherently associated with keratinocyte stem cells [5, 6]. On the other hand, c-Myc plays a crucial role in the conversion of stem cells to TA cells. Western blot analysis showed that RA cells express p63 strongly, whereas SA and NA cells strongly expressed c-Myc (fig. 4). In agreement with the FACS analysis, α_6 integrin was detected only in RA cells. From these results, we concluded that p63 positivity is indicative of α_6 integrin positivity and CD71 negativity.

Stem cells cycle slowly, and thus we examined the cell cycle-related proteins, cyclin D1, cyclin D2, and $p21^{WAF1/CIP1}$, to determine whether RA cells also have this characteristic. As shown in figure 4, RA cells expressed low levels of cyclin D1 and cyclin D2 protein, but contained elevated levels of $p21^{WAF1/CIP1}$, which suggests that RA cells have stem cell properties.

RA, SA, and NA cells and SE reconstruction

After separating RA and SA cells, the cell populations were seeded on 100-mm dishes. As shown in figure 5A and C, RA cells produced a larger number of large colonies after 10 days of culture, indicating that they have a higher proliferative potential. We next determined the epidermal regenerative ability of the different cell populations. SEs reconstructed using RA, SA, and NA cells revealed various morphological features. The SE produced using RA cells showed a regular stratification of thick epidermis, whereas SA cells produced a rela-

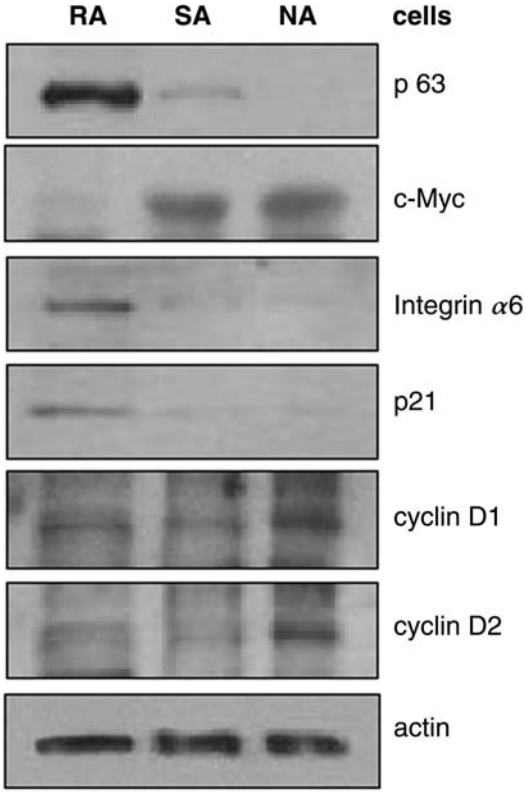

Figure 4. Expressions of putative stem cell markers. RA, SA, and NA cells were subjected to Western blot analysis with antibodies against p63, c-Myc, α_6 integrin, $p21^{WAF1/CIP1}$, cyclin D_1, and cyclin D_2. Equal protein loadings were confirmed using anti-actin antibody. p63 and α_6 integrin expression were markedly upregulated in RA cells.

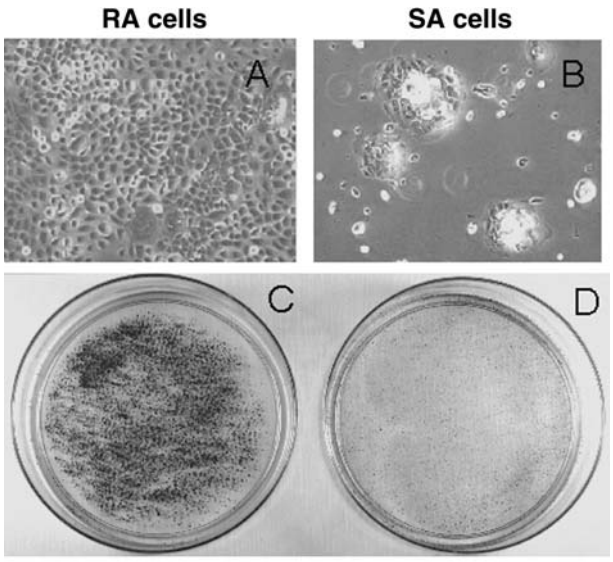

Figure 5. Colony forming activities of RA and SA cells. RA and SA cells were grown in serum-free KGM for 10 days, and phase contrast pictures were taken using a color video camera (×100) (*A*, *B*). To visualize colony growth, the cells were stained with 0.1% crystal violet (*C*, *D*).

tively thin epidermis, and the horny layer was not well differentiated (fig. 6A–C). In addition, the RA cells produced a more compact basal layer and had a more cubic appearance than the other cell types. However, NA cells formed a poor epidermal structure (fig. 6A–C). We also analyzed by immunohistochemistry, the phenotypes of the SEs produced using each cell population. The expression of involucrin was more evident in RA-constructed SEs, indicating that the cells were well differentiated (fig. 6D). PCNA, a nuclear marker of proliferating cells, was mainly observed in the basal keratinocytes of SEs (fig. 6G–I), and p63, a putative intracellular keratinocyte stem cell marker, was also detected in this location (fig. 6J–L). However, p63 expression was attenuated in the SEs of SA or NA versus RA. In the literature, β_1 and α_6 integrin are considered epidermal stem cell markers. Interestingly, β_1 and α_6 integrin expressions were detected in the basement membrane zone only of RA SEs (fig. 6M, P). These findings concur with our flow cytometric findings, i.e., that RA cells are α_6 integrin-positive cells (fig. 2A).

Discussion

Epidermal stem cells are promising clinical candidates for the treatment of burns, chronic wounds, and ulcers. To isolate stem cells, a knowledge of their specific markers would be useful. In the skin, however, no definitive molecular stem cell markers are known, although several molecules such as β_1 integrin, α_6 integrin, p63, K19, and β-catenin have been reported as putative epidermal stem cell markers [17]. Among these, β_1 integrin is expressed on basal keratinocytes and has been used to isolate epidermal stem cells [9, 10]. However, there are also reports that not all cells expressing β_1 integrin are epidermal stem cells [1, 18].

Stem cells have also been separated based on the relative expression levels of another integrin molecule, α_6 integrin, and of a proliferation-associated cell surface marker, CD71 [1, 7, 18]. These workers categorized basal epidermal cells into three groups by FACS: stem cells with a high α_6 integrin expression and a low CD71 expression (α_6 integrin^{bri}CD71^{dim} cells), TA cells with a high α_6 integrin expression and a high CD71 expression (α_6 integrin^{bri}CD71^{bri} cells), and differentiating cells with a low α_6 integrin expression (α_6 integrin^{dim} cells). Even in the absence of specific stem cell markers, this method was thought to be useful for isolating the most well-characterized epidermal stem cell population described to date [19, 20]. Therefore, α_6 integrin^{bri}CD71^{dim} could be used as a parameter to separate the epidermal stem cell proportion. However, no definite cut-off was suggested for α_6 integrin^{bri}CD71^{dim} cells, and the α_6 integrin^{bri}CD71^{dim} population was defined only as a arbitrarily boxed region. Thus, the boundaries between stem cells, TA cells, and differentiating cells are somewhat ambiguous. Recently, α_6 integrin^{bri} CD71^{bri} cells and α_6 integrin^{dim} cells were also reported to be capable of prolonged tissue regeneration [21], and that the term 'epidermal stem cell' could be extended to the committed progeny of stem cells.

In the present study, we performed FACS analysis not only on basal epidermal cells, but also on whole epidermal cells. Figure 1 shows that the α_6 integrin-positive and CD71-negative region produces a distinct cell population, which appears to be composed of putative stem cells, because these cells are small and homogenous by FSC and SSC analysis. It was reported that keratinocytes that rapidly adhere to several extracellular matrix proteins show high colony-forming efficiencies [9, 22]. The suggestion has further been made that keratinocytes can be purified on the basis of rapid adhesion to type IV collagen, fibronectin, or to extracellular matrix deposited by keratinocytes. In view of the possibility that β_1 integrin is a potent stem cell marker [10], we used type IV collagen as a β_1 integrin ligand to separate RA cells. Interestingly, the RA cells obtained also had an α_6 integrin-positive/CD71-negative phenotype, indicating that β_1 integrin-positive RA cells resemble epidermal stem cells.

Epidermal reconstruction is arguably the best way to examine the usefulness of stem cells in clinical application. To build a stratified squamous epidermis, the ability to self-renew and to terminally differentiate are important [23]. SEs produced using RA cells showed a well-organized, regular stratification, whereas SEs produced using SA or NA cells showed incomplete stratification. Moreover, PCNA, a proliferation marker, was found to be ex-

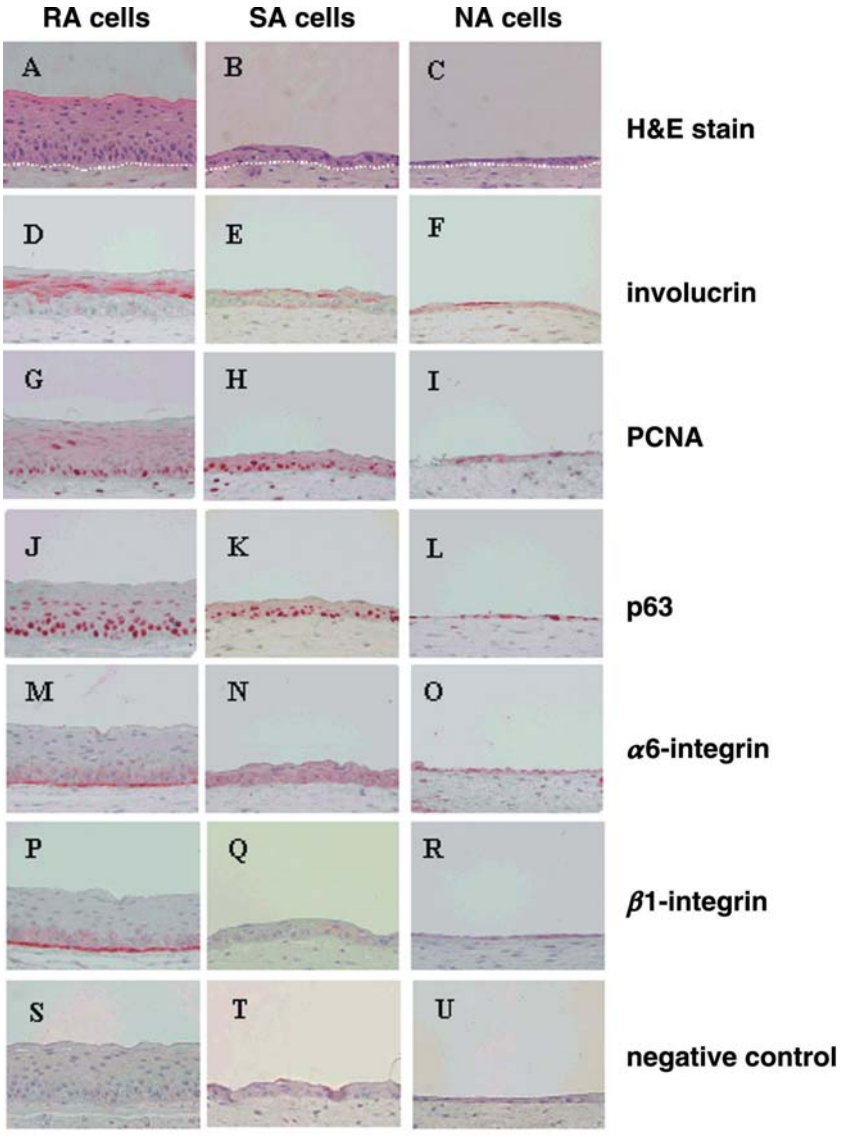

Figure 6. The effects of RA, SA, and NA cells on SE reconstruction SEs were produced from RA (*A*, *D*, *G*, *J*, *M*, *P*, *S*), SA (*B*, *E*, *H*, *K*, *N*, *Q*, *T*), and NA (*C*, *F*, *I*, *L*, *O*, *R*, *U*), as described in Materials and methods, and sections of SEs were stained with H&E (*A*, *B*, *C*), and for involucrin (*D*–*F*), PCNA (*G*–*I*), p63 (*J*–*L*), α_6 integrin (*M*–*O*), or β_1 integrin (*P*–*R*); negative control: (*S*–*U*). The dotted line indicates the epidermal-dermal junction. Original magnification: ×400.

pressed in the basal layers of SEs from RA, SA, and NA cells. These results indicate that SA and NA cells contain cells with proliferative capacity, but that they lack differentiating potential. Interestingly, TA and differentiating cells have also been reported to be capable of tissue regeneration [21]. In addition, we recently reported that dermal compartment-related epidermis modifications are important in the processes of SE reconstruction [24]. Thus, RA cells may be ideal for skin reconstruction, since they contain pure stem cells and a small portion of their committed progeny. The expression patterns of p63, which is currently regarded as a stem cell marker, were found to be similar to those of PCNA [5, 6, 25]. Moreover, we recently suggested that p63 may have a role in maintaining the proliferative potential of keratinocytes [26]. As shown by our Western blot analysis, p63 is more strongly expressed by RA cells than by SA or NA cells. Thus, the intensity of p63 expression could critically determine stem cell fate and be useful for stem cell isolation. However, the possibility of using p63 as an epidermal stem cell marker requires further examination. In general, c-Myc is known to be involved in the stimulation of proliferation and in the suppression of differentiation [27]. Unexpectedly, however, the overexpression of c-Myc in human keratinocytes was found to stimulate terminal differentiation by driving stem cells into the TA cell compartment [16]. In the present study, we found that RA cells did not express c-Myc, whereas SA and NA cells

did. These results suggest that c-Myc expression is important in keratinocyte differentiation and that it stimulates cells to escape from the stem cell compartment. On the other hand, α_6 integrin and β_1 integrin were found in the lower surface of basal cells in SEs reconstructed from RA cells. Hence, α_6 integrin and β_1 integrin could play a crucial role in maintaining the epidermal stem cell phenotype.

Cell isolation based on FACS provides a reliable method for separating epidermal keratinocyte stem cells. However, the method is time-consuming and laborious. Thus, the isolation of RA cells could be used as a simpler alternative, although at the expense of final purity. Because of their strong tissue-regenerative capacity, RA cells could prove to be helpful in clinical applications where epidermal stem cells are needed.

Acknowledgements. This research was supported by a grant (SC13111) from the Stem Cell Research Center of the 21st Century Frontier Program funded by the Ministry of Science and Technology, Republic of Korea.

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