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ORIGINAL ARTICLE

Comparison of Gene Expression Profiles between Keratinocytes, Melanocytes and Fibroblasts

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Background: The skin has many important functions such as protection, preservation, temperature regulation, and vitamin D synthesis. It is composed of a variety of cell types including keratinocytes, melanocytes and fibroblasts. **Objective:** We attempted to compare the gene expression profiles between keratinocytes, melanocytes and fibroblast, using cDNA microarray. **Methods:** Keratinocytes, melanocytes and fibroblasts were primary cultured from five foreskin specimens. Total RNAs were extracted and pooled to reduce the individual variations, and then used for cDNA microarray. **Results:** Total 12,028 genes were selected as the reliable genes whose expression was detected in at least one of the three cell types. By comparing the relative expression levels with cutoff limitation as a fourfold change, we obtained 126 fibroblast-specific, 179 keratinocyte-specific and 173 melanocyte-specific genes, many of which are known to be characteristically expressed in each cell type. In addition, we identified many genes whose skin-specific functions have not yet been determined. **Conclusion:** Our data provide important information on which to base further investigation into the specification of skin cell types. **(Ann Dermatol 25(1) 36～45, 2013)**

-Keywords-
cDNA microarrays, Fibroblasts, Keratinocytes, Melanocytes

INTRODUCTION

The skin is the largest organ of the body, covering an area of approximately 1.4～2.0 m^2 and with a weight that can reach around 4 kg. It plays a variety of functional roles, including protection, preservation, temperature regulation, and Vitamin D synthesis. Skin is composed of many cell types, among which keratinocytes, melanocytes and fibroblasts are regarded as the most abundant and functionally important. Keratinocytes constitute a majority of the epidermis, and form cornified layers that help to contain body fluids and provide barrier protection from the environment[1]. Keratinocytes are ectodermally derived and play an essential role in the formation of hair, nail and sebum. Melanocytes are derived from the neural crest and located in the lower epidermis and hair follicles, where they generate melanin to provide coloration and protection from ultraviolet damage[2]. Fibroblasts, the major cells of the dermal portion, are derived from mesenchyme and synthesize essential extracellular matrix (ECM) components to provide structural support[3].

The characteristics of each skin cell are determined by cell type-specific gene expression patterns. Thus, in order to understand the specification of each cell type, it is necessary to identify the genes whose expressions are confined to specific cells. To profile the gene expression, several noteworthy methods have been developed. DNA microarray technology is a powerful tool to facilitate massive screening of expression for thousands of genes[4]. One major application of this technology is the sequence-specific detection and quantification of mRNA expression.

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Oligonucleotide DNA microarray allows the identification of mRNA whose level is within a wide linear range. Furthermore, owing to the high specificity of DNA sequence detection, oligonucleotide DNA microarray is used for DNA sequencing[5]. In parallel with oligonucleotide DNA microarrays, cDNA microarray has also been developed[6,7]. This technology, in contrast to oligonucleotide microarray, uses cDNA molecules to interrogate mRNA samples. The fabrication of cDNA microarray differs in many ways from that of oligonucleotide microarray. Specifically, cDNA microarray is generated by spotting cDNA molecules of different lengths onto a given matrix of glass, nitrocellulose or nylon[6,7]. By pre-treating the surface of the microarray slide with polylysine or aminosilane, the specific hybridization of DNA strands to the target may be enhanced, while at the same time the enhanced hydrophobicity of the matrix helps to avoid non-specific attachment of target molecules. Using the cDNA microarray technique, in this study we attempted to compare the gene expression profiles between keratinocytes, melanocytes and fibroblasts. Our data provide important insight into the specification of each skin cell.

MATERIALS AND METHODS

Cell culture and RNA isolation

Normal human skin samples were obtained from circumcisions. Keratinocytes, melanocytes and fibroblasts were isolated from the foreskins of five donors. Specimens were briefly sterilized in 70% ethanol, minced, and then treated with dispase overnight at 4°C. The epidermis was separated and placed in a solution containing 0.05% trypsin and 0.02% EDTA for 15 minutes at 37°C. For keratinocytes, cells were resuspended in keratinocyte-serum free medium (K-SFM) supplemented with bovine pituitary extract and recombinant human epidermal growth factor (Invitrogen, Grand Island, NY, USA), as previously reported[8]. Third passaged keratinocytes (60～75% confluency) were used in this experiment. For melanocytes, cells were resuspended in Medium 154 and human melanocyte growth supplement (Cascade Biologics, Portland, OR, USA). During the primary culture of human melanocytes, 200 μg/ml of G418 (geneticin sulfate, Duchefa, Haarlem, The Netherlands) was added to the growth medium to suppress the proliferation of fibroblasts[9]. Primary dermal fibroblasts were obtained by the explant culture method, and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen).

cDNA microarray

Total RNA samples were extracted using RNeasy Mini Kits (Qiagen, Hilden, Germany) according to the recommended protocol. The quality of all RNA samples was evaluated using agarose gel electrophoresis. Keratinocytes, melanocytes and fibroblasts were primary cultured from five foreskin specimens. Total RNAs were extracted, pooled to reduce the individual variations, and then used for cDNA microarray. For cDNA microarray analysis, aliquots of 100 μg of total RNA were labeled with Cy3 or Cy5 monoreactive dyes (Amersham, Buckinghamshire, UK) using a Superscript cDNA system (Gibco BRL, Grand Island, NY, USA). Labeled Cy3 and Cy5 cDNA probes were then cleaned using a Qiaquick nucleotide removal kit (Qiagen). The purified probes were dried and resuspended in 40 μl of hybridization buffer containing 5X SSC, 0.1% SDS, 20 μg of Cot-1 DNA (Gibco BRL), 20 μg of poly(A) RNA, and 20 μg of yeast tRNA (Gibco BRL). The probes were denatured at 95°C for 3 minutes before being applied to the microarray slides. Hybridization was performed at 42°C for 16 hours, and the microarray slides were washed once with 2X SSC, 0.1% SDS at 42°C for 4 minutes, once with 0.1X SSC, 0.1% SDS at room temperature for 10 minutes, and three times with 0.1X SSC at room temperature for 1 minute. The microarray slides were then washed with distilled water and spin-dried.

Microarray scanning and normalization of the data were performed using a GenePix 4000B scanner and GenePix Pro 3.0 software (Axon Instruments, Union City, CA, USA). Poor-quality spots (sum of median <500) were filtered from the raw data before analysis. For each hybridized spot, the background intensity was subtracted and normalized by the global median normalization method[10]. The ratio of Cy5 and Cy3 fluorescence intensity was assigned as the gene expression value (GEV) for determination of the relative gene expression level. The cDNA microarray experiments were repeated twice, and the average of two GEVs for each gene was used for further analysis. Genes with a fourfold or more differential expression were analyzed. To further analyze the gene expression patterns, the self-organizing map (SOM) method was used, and the node number for SOM clustering was set at 3. Gene expression pattern clustering (hierarchical clustering and SOM) was performed using the programs Cluster version 2.12 and TreeView version 1.50[11].

Statistical analysis

The fluorescence intensities from the microarray results were measured as indicators of the gene expression levels. We used foreground intensities after transforming the

values by a logarithm of base two as expression values for the subsequent analysis. The majority of microarray data analysis was conducted using R software (http://www.r-project.org). We normalized the expression values of the 15,704 genes within a slide by the regional Lowess method[12]. To identify the differentially expressed genes among different cell types, we adopted the analysis of variance (ANOVA) model approach. To control for an inflated false positive rate due to multiple testing, we adjusted individual *p*-values using Benjamin-Hochberg's method, which controls the false discovery rate (FDR)[13]. To group genes according to their relative expression patterns among three different cell types, we used the combined approach with K-means clustering and Linear Discriminant Analysis (LDA). At first, we clustered genes using K-means algorithm with as many as 20 centroids for groups to obtain as many homogeneous pattern groups as possible. From the clustering result, we classified genes into three groups as follows: (1) upregulated in fibroblasts, (2) upregulated in keratinocytes, (3) upregulated in melanocytes. To provide a graphical view of the expression patterns of these three groups, we drew a heatmap using R. Finally, gene ontology analysis was performed to find the over-representative functions of genes in each group using GOstat (http://gostat.wehi.edu.au/)[14].

Real-time polymerase chain reaction (PCR)

Total RNA was extracted using an RNeasy kit and cDNA was made using a reverse-transcription kit. PCR was

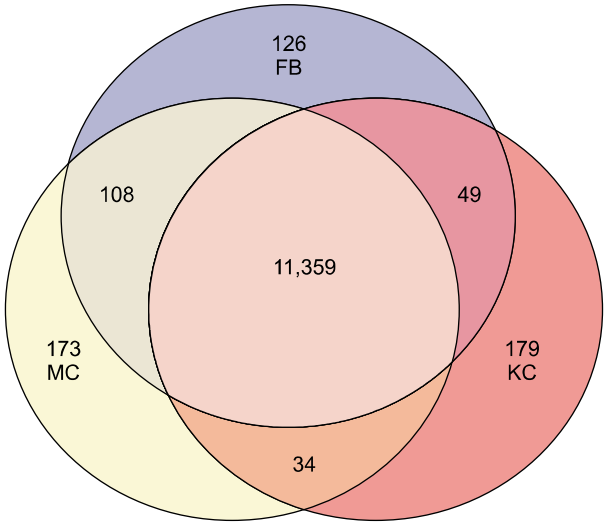

Fig. 1. Venn diagram summarizing the number of specific genes identified by cDNA microarray. A total 12,028 genes were obtained for which expression was detected in at least one of three cell types. KC: keratinocytes, FB: fibroblasts, MC: melanocytes.

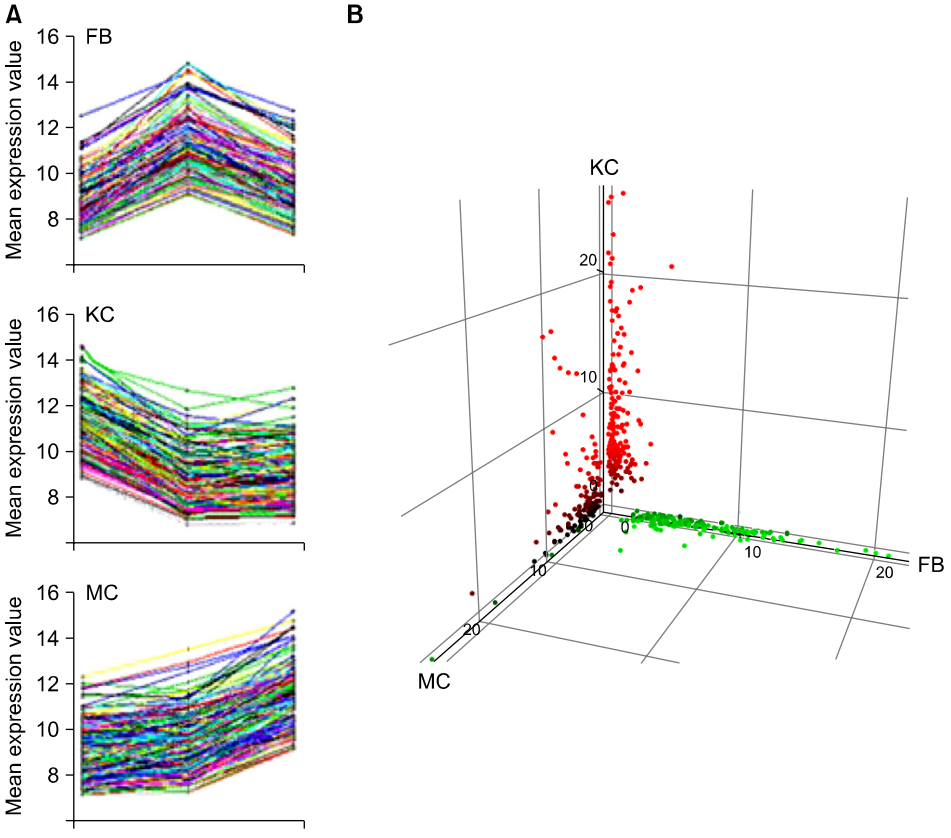

Fig. 2. Self-organizing maps (SOM) analysis of the differentially expressed genes. (A) Four hundred and seventy eight genes were selected as differentially expressed genes, the expression levels of which showed changes greater than fourfold over the other two cell types. Each cluster is represented by its gene expression value. The numbers in each graph show the number of genes belonging to the corresponding cluster. (B) Three-dimensional deposition of differentially expressed genes. KC: keratinocytes, FB: fibroblasts, MC: melanocytes.

performed on a Rotor-Gene 2000 real-time amplification operator (Corbett Research, Mortlake, Australia). Primers were designed using Primer 3 and all primers were verified by BLAST to ensure that each sequence was unique for the specific target gene. The PCRs contained a final concentration of 1×SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), 10 μM specific primers, and 2.5 ng of cDNA. The expression of genes was calculated according to the threshold cycle value, normalized using the value of the sample with the lowest level of each product, and the data were expressed as the ratio of each gene to cyclophilin.

RESULTS AND DISCUSSION

Gene expression profiles

To analyze the gene expression profiles between keratinocytes, melanocytes and fibroblasts, we performed cDNA microarray analysis, in which a loop design was adopted. That is, the gene expression levels were compared between two cells types using Cy5 and Cy3 probes: keratinocytes (Cy5) vs melanocytes (Cy3), melanocytes (Cy5) vs fibroblasts (Cy3), and fibroblasts (Cy5) vs. keratinocytes (Cy3). For accuracy of analysis, dye swap experiments were also performed. We used 17 K cDNA microarray for hybridization, and obtained 12,028 relevant genes whose expression was detected in at least one of three cell types (Fig. 1). By comparing the relative expression levels by ANOVA test with cutoff limitation as a fourfold change, we obtained 478 genes that revealed differential expression in each of the three cell types. We further analyzed the gene expression pattern by SOM and classified the genes into three major gene clusters (Fig. 2). These clusters correspond to groups of coordinately regulated genes that represent expression signatures for fibroblasts, keratinocytes or melanocytes. Cluster 1 includes 126 genes that were expressed at high levels in fibroblasts, but at low levels in keratinocytes and melanocytes (Table 1). Cluster 2 contains 179 genes that were highly and specifically expressed in keratinocytes (Table 2). Cluster 3 includes 173 genes transcripts that were expressed at the highest levels in melanocytes (Table 3).

The expression of randomly selected genes from three SOMs was verified using real-time PCR analysis. There

Table 1. List of top 25 upregulated genes in the fibroblasts

cDNA ID	Gene	Accession NO	Expression ratio		
			KC	FB	MC
AA447781	Lumican	BG114678	1.00	43.55	4.47
AA490172	Collagen, type I, alpha 2	J03464	1.00	40.34	4.99
R98936	Membrane metallo-endopeptidase	NM_007289	1.00	30.31	4.79
H08561	Human insulin-like growth factor binding protein	NM_000599	1.00	28.24	1.10
R62603	Collagen, type VI, alpha 3	0	1.00	25.77	4.67
AA664101	Aldehyde dehydrogenase 1 family	NM_000689	1.00	25.49	1.18
AA464526	Interleukin 1 receptor, type I	M27492	1.00	24.19	4.27
H17615	Fibulin 5	BX537531	1.00	21.67	4.49
AA912115	Steroid-5-alpha-reductase, alpha polypeptide	NM_000348	1.00	19.83	2.07
AI131555	Orphan seven-transmembrane receptor	NM_178445	1.00	18.36	1.58
T98612	Collagen, type III, alpha 1	NM_000090	1.00	17.92	2.59
H99676	Collagen, type VI, alpha 1	NM_001848	1.00	17.59	4.70
H19440	Down syndrome critical region gene 1-like	0	1.00	17.47	1.95
AW084720	Complement component 1, s subcomponent	AK126711	1.00	15.21	3.64
AA703652	Slit (Drosophila) homolog 3	AB011538	3.07	15.03	1.00
AA496283	Thy-1 cell surface antigen	AK090996	1.00	14.81	1.77
H80685	P311 protein	CR749489	1.00	14.75	2.49
AI653116	KIAA1077 protein	AF545571	1.00	14.44	1.42
H10959	Deleted in bladder cancer chromosome reg	NM_014618	1.00	13.16	1.50
AA443302	Ras homolog gene family, member E	X97758	2.06	12.79	1.00
AA670200	Procollagen C-endopeptidase enhancer	BM994449	1.00	12.57	3.19
AI655374	Stromal cell-derived factor 1	BX647204	1.00	12.32	1.23
R43734	Laminin, alpha 4	BC066552	1.00	11.98	3.90
AI888228	Alanyl (membrane) aminopeptidase	AB209918	1.00	11.95	2.97
H22826	LIM domain only 7	NM_005358	1.00	11.76	2.89

KC: keratinocytes, FB: fibroblasts, MC: melanocytes.

Table 2. List of top 25 upregulated genes in the keratinocytes

cDNA ID	Gene	Accession NO	Expression ratio		
			KC	FB	MC
AA455929	Tumor protein 63 kDa with strong homolog	NM_003722	29.74	1.00	1.55
AA857364	Homo sapiens pyruvate dehydrogenase kinase	0	27.39	1.00	1.40
AI969825	Cutaneous T-cell lymphoma-associated tum	AB209182	25.33	1.15	1.00
AW085631	Keratin 5	M21389	24.87	1.00	1.72
AI949576	Annexin A3	AB209868	24.65	1.02	1.00
AA677534	Laminin, gamma 2 (nicein (100 kD)	Z15008	23.53	1.00	1.05
AA235002	Annexin A8	BM675395	21.57	1.06	1.00
H66158	Plakophilin 2	NM_004572	19.91	1.00	1.09
T97710	Ladinin 1	NM_005558	19.00	1.00	1.05
R43483	Integrin, alpha 6	X53586	17.96	1.00	1.41
T49159	Serine (or cysteine) proteinase inhibitor	BC012609	17.24	2.24	1.00
AI971049	Myocilin, trabecular meshwork inducible	BC029261	16.91	1.00	1.14
AA458849	Serine protease inhibitor, Kunitz type	AK127479	15.29	1.01	1.00
AA035637	Junction plakoglobin	BX648177	14.81	2.37	1.00
AA459123	Dual adaptor of phosphotyrosine	0	14.43	1.00	1.16
R45640	Diphtheria toxin receptor	BC033097	14.42	1.00	1.24
AI308916	Protease, serine, 4 (trypsin 4, brain)	BQ687514	14.16	1.88	1.00
AI222585	Cytochrome P450, subfamily XXVIIB	BC020267	14.09	1.00	1.12
AA933616	Jagged 1 (Alagille syndrome)	AF003837	13.96	1.00	1.61
AA931758	Putative lymphocyte G0/G1 switch gene	CD511787	13.83	1.15	1.00
AI369378	Creatine kinase, mitochondrial 1	AK094322	13.69	1.00	1.37
H44785	Bullous pemphigoid antigen 1 (230/240 kD)	NM_183380	13.62	1.18	1.00
AA001432	Laminin, alpha 3 (nicein (150 kD)	NM_198129	13.40	1.20	1.00
AI017342	Interferon regulatory factor 6	NM_006147	13.16	1.00	1.22
AA284669	Plasminogen activator, urokinase	NM_002658	13.03	1.32	1.00

KC: keratinocytes, FB: fibroblasts, MC: melanocytes.

were good correlations between the average SOM values and the real-time PCR results for each gene. The expression of insulin-like growth factor binding protein 3 (IGFBP3) and WNT 5A, which were classified into cluster 1, was highly expressed in fibroblasts, but expressed at very low levels in keratinocytes and melanocytes (Fig. 3A). G protein-coupled receptor 110 (GPR110) and Guanine nucleotide binding protein (G protein), alpha 15 (Gq class) (GNA15), which were classified into cluster 2, were verified as a highly expressed in keratinocytes by real-time PCR analysis (Fig. 3B). Real-time PCR analysis convincingly confirmed that two genes belonged to cluster 3 and were overexpressed in melanocytes: Pirin (iron-binding nuclear protein) (PIR), and Basic helix-loop-helix domain containing, class B, 3 (BHLHB3) (Fig. 3C).

Cluster 1

Cluster 1 includes those genes that were highly expressed in fibroblasts and had relatively low expression levels in keratinocytes and melanocytes. Fibroblasts are derived from mesechyme and are responsible for the tensile strength of skin tissue by synthesizing various connective tissue matrix proteins and a number of soluble factors[15]. They are also implicated in skin photoaging[16]. Relative to young or normal skin, the dermis of photoaged skin shows both qualitative and quantitative differences in dermal collagen, elastins, and other structural components produced by fibroblasts[17]. In our cDNA microarray, the genes that were highly expressed in fibroblasts contained many ECM components such as collagen 1A2, 6A3, and 3A1 (Table 1), indicating that the experiments were methodologically appropriate. As noted, the physiologic role of collagen fibers in the skin is to provide the tensile properties that allow the skin to serve as a protective organ against external trauma. Collagen 1A2 is a fibrillar forming collagen that is found in skin, bone tendon and ligament[18]. Defects in this gene have been linked with defects in skin ranging from hyperextendability to poor wound healing[19]. Collagen 6A3 assembles into a relatively thin microfibrillar network, which may perform an anchoring function by stabilizing the assembly of the broad collagen fibers as well as the basement membranes[20]. Mutations in type VI collagen genes can lead to congenital muscular dystrophy, with no apparent phenotype in the

Table 3. List of top 25 upregulated genes in the melanocytes

cDNA ID	Gene	Accession NO	Expression ratio		
			KC	FB	MC
N31933	Tyrosinase (oculocutaneous albinism IA)	N31933	1.00	1.35	34.54
N66177	Microphthalmia-associated transcription factor	NM_198159	1.00	4.91	31.14
AA775447	Alpha-2-macroglobulin	CR749334	1.00	1.72	29.13
AA424996	Tyrosinase-related protein 1	X51420	1.00	1.40	23.59
N21654	Solute carrier family 16	NM_004694	1.00	1.33	23.21
AA458653	GS3955 protein	NM_021643	1.00	3.45	22.51
N24824	v-kit Hardy-Zuckerman 4 feline sarcoma	BC071593	1.00	2.44	22.01
AA973938	Ocular albinism 1 (Nettleship-Falls)	BG288765	1.00	1.28	19.18
N67770	Silver (mouse homolog) like	AK092881	1.00	1.34	18.55
AI095082	Rag D protein	AL137502	1.00	1.82	17.65
H04789	Glycogenin 2	NM_003918	1.00	2.07	17.62
AA989072	G protein-coupled receptor, family C	AF520570	1.00	2.42	16.15
AI097486	Oculocutaneous albinism II	NM_000275	1.00	1.60	15.40
AI365209	Deleted in esophageal cancer 1	AK056153	1.00	1.80	14.56
N32199	Melan-A	BF573723	1.00	1.99	13.56
AA976578	SRY (sex determining region Y)-box 10	BC018808	1.00	1.10	13.14
N35112	ATPase, Class V, type 10C	AB051358	1.00	2.37	13.08
AA143331	Matrix metalloproteinase 1 (interstitial	BC013875	1.00	1.66	12.83
AI364029	Homo sapiens mRNA; cDNA DKFZp434N1728	NM_018602	1.16	1.00	12.17
AI431804	Hypothetical protein	CR936670	1.00	1.08	11.94
AW025698	Latent transforming growth factor beta b	NM_206943	1.00	3.01	11.68
AI522182	ATP-binding cassette, sub-family C	U49248	1.13	1.00	11.51
AA775223	Hydroxyprostaglandin dehydrogenase 15	NM_000860	1.11	1.00	11.34
AA520979	Transmembrane 7 superfamily member 1	AL832142	1.00	2.29	11.16
AA282134	Glutaminyl-peptide cyclotransferase	BC047756	1.00	1.09	10.66

KC: keratinocytes, FB: fibroblasts, MC: melanocytes.

skin but with limited joint hypermobility[21]. Collagen 3A1 predominates in human skin during embryonic life[22]. Mutations in Collagen 3A1 cause the vascular type of Ehlers-Danlos syndrome.

Other examples in this cluster include procollagen C-endopeptidase enhancer and lumican. The former stimulates the C-proteinase activity that is required for conversion of procollagen to collagen[23]. A lack of this enzyme results in impaired tensile strength of collagen fibers in the skin. Lumican belongs to the corneal leucine-rich repeat proteoglycan family, which play a critical role in generating and maintaining a transparent matrix within the corneal stroma[24]. So far, it is unknown whether lumican is expressed in dermal fibroblasts and further investigation of this topic is required.

Cluster 2

This cluster contains genes that were highly expressed in keratinocytes as compared with other two cell types. The keratinocytes are the ectodermally derived cells that constitute more than 80% of the epidermal cells. All keratinocytes contain cytoplasmic keratin intermediate filaments in their cytoplasm and form desmosomes or modified desmosomal junctions with adjacent cells. In accordance with the fact that keratin filaments are the hallmark of the keratinocytes, in our cDNA microarray results keratin 5 was highly ranked in this cluster (Table 2). Keratin 5 is a well-established biomarker for basal keratinocytes, and it has been demonstrated that mutations in this gene cause a blistering disorder of human skin, epidermolysis bullosa simplex[25-27]. It has been known that many of the keratinocyte-specific genes are located at the region of epidermal differentiation complex (EDC) in chromosome 1q21, and that EDC contains a large number of genes related to cornified envelope formation such as loricrin, involucrin, filaggrin, and the S100 family (or annexins)[28]. In our cDNA microarray, however, expression of those cornified envelope-related genes was not detected and/or they were ranked as low expressed genes. Because we used keratinocytes cultured in low calcium medium, which does not permit keratinocyte differentiation and thereby represents the basal layer characteristics, the low level expression of those genes was somewhat plausible.

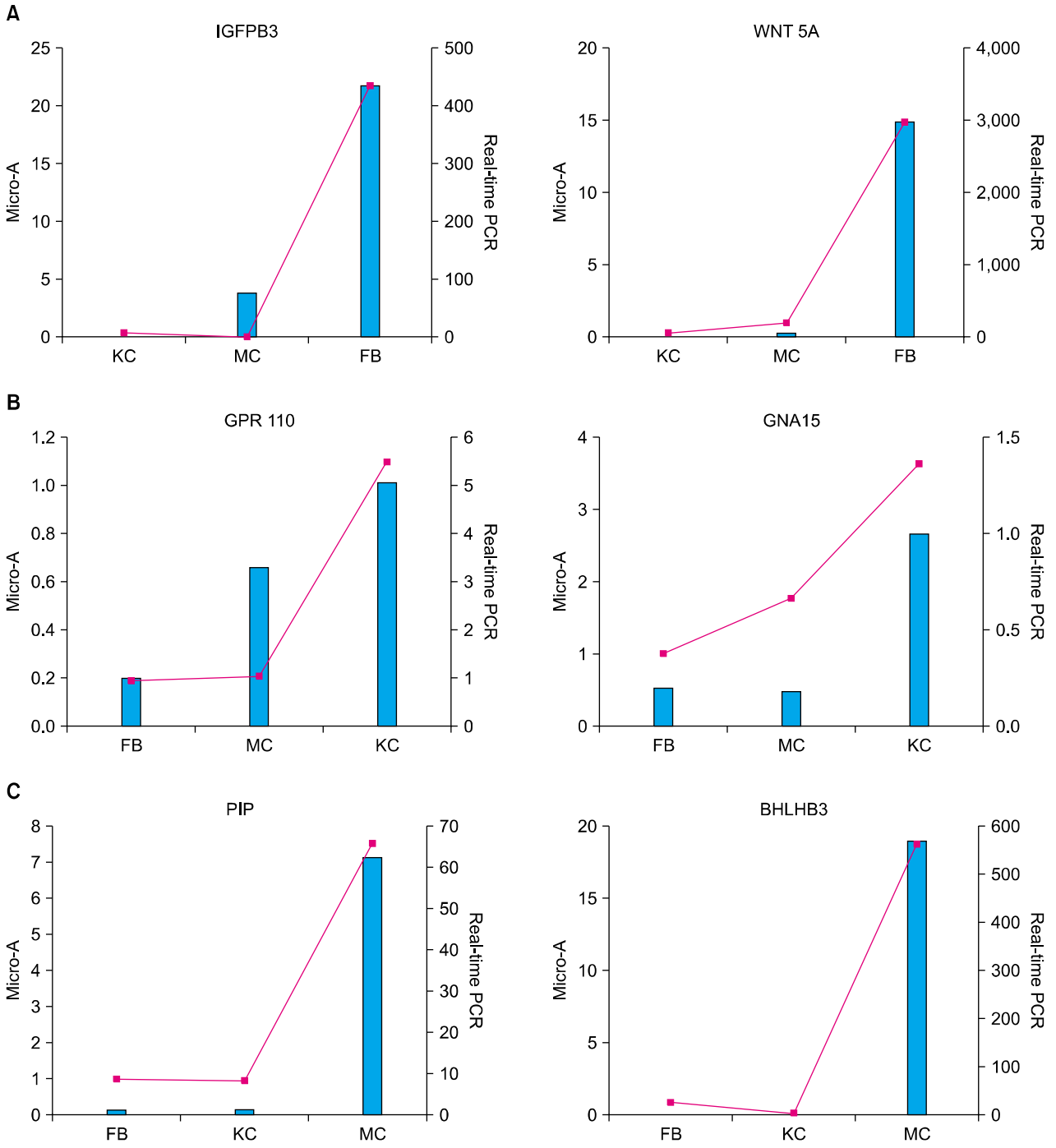

Fig. 3. Real-time PCR analysis of selected genes from SOMs. The data are logarithmically transformed and represented as expression relative to cyclophilin (left scale, line graph). Microarray results of gene expression value (GEV) for selected genes are depicted as bar graphs (right scale). (A) Insulin-like growth factor binding protein 3 (IGFBP3) and WNT 5A were overexpressed in fibroblasts. (B) G protein-coupled receptor 110 (GPR110) and Guanine nucleotide binding protein (G protein), alpha 15 (Gq class) (GNA15) were overexpressed in keratinocytes. (C) Pirin (iron-binding nuclear protein) (PIR), and Basic helix-loop-helix domain containing, class B, 3 (BHLHB3) were overexpressed in melanocytes. KC: keratinocytes, FB: fibroblasts, MC: melanocytes, PCR: polymerase chain reaction, SOM: self-organizing maps.

In our cDNA microarray, three annexins (A2, A3, and A8) were ranked as being highly expressed genes in keratinocytes. Annexin A3, also called "lipocortin 3" or "placental anticoagulant protein 3" (PAP-III), is a member of the lipocortin/Anx family that binds to phospholipids and membranes in a calcium-dependent manner[29]. Annexin

A3 has been shown to have anticoagulant and anti-phospholipase A2 properties *in vitro*[30] and to promote the calcium-dependent aggregation of isolated specific granules from human neutrophils[31]. Annexin A8 proteins are involved in injury response, inflammation, and in tumor suppression[32]. Although the functional roles of Annexin A3 and A8 have not yet been identified, it is interesting that an earlier report indicates that Annexin 1 and 2 bind to calcium, then exert their effects as the pro-differentiating factors in keratinocytes *in vitro*[33]. From this, we speculate that Annexin A3 and A8 may have similar effects on keratinocytes. Elucidation of the putative roles of those gene products will be an interesting topic for further study.

In our cDNA microarray, many of the genes related to the cell-cell junction were identified as being especially highly expressed in keratinocytes. Compared to the other two cell types, keratinocytes are packed in the epidermis in a condensed way, reflecting the importance of cell to cell contacts. For example, keratinocytes have numerous juctional structures, including desmosome, hemidesmosome and adherens junctions and tight junctions. The desmosomal plaque is composed of several intracellular plakin proteins, including phlakophilin and plakoglobin. Desmosome mechanically couples adjacent keratinocytes. Moreover it plays a significant role in epidermal differentiation and is part of the cornified cell envelope. Mutations in phlakophilin and plakoglobin cause ectodermal dysplasia/skin fragility syndrome and Naxos syndrome, respectively[34,35]. Hemidesmosome, anchoring filaments and anchoring fibrils are components of the dermal-epidermal junction basement membrane. Bullous pemphigoid antigen-1 (BPAg-1) is the major component of the hemidesmosomal inner plaque. Deletions in BPAg-1 cause epidermolysis bullosa simplex. Integrin is a large class of transmembrane ECM binding proteins that provide cell attachment and subsequent signal transduction. Integrin $\alpha 6\beta 4$ has a high affinity for laminin 5, and is therefore essential to integration of hemidesmosome with the underlying basement membrane and stroma. Mutations in $\alpha 6$ or $\beta 4$ chains result in dermal-epidermal instability[36,37]. Laminin 5 has been localized to the anchoring filaments and is essential in keratinocyte adhesion. Mutations in $\alpha 3$ or $\gamma 2$ chains of laminin 5 result in severe Herlitz' junctional epidermolysis bullosa[38]. In this experiment, $\alpha 3$ and $\gamma 2$ chains of laminin 5 and integrin $\alpha 6$ chains showed signature expression in the keratinocytes.

Cluster 3

This cluster contains genes that were highly expressed in melanocytes and expressed at very low levels in fibroblasts and keratinocytes. Melanocytes are dendritic, pigment-synthesizing cells derived from neural crust, for which pigmentation-related genes are excellent signature biomarkers[39,40]. In accordance with this fact, in our cDNA microarray results, pigmentation-related genes such as tyrosinase, microphthalmia associated transcription factor (MITF), tyrosinase-related protein 1, ocular albinism I, silver, oculocutaneous albinism II, and melan-A were ranked as the highly expressed genes in melanocytes. Besides this, we found several genes that have not been previously known in melanocytes such as glutaminyl-peptide cyclotransferase (QPCT), which has been well-studied in bovine pituitary[41]. The putative role of this gene remains to be determined.

In our results, Disheveled (Dvl) (the 65th gene in the upregulated genes in the melanocytes) was identified as a signature gene in melanocytes. Dvl is required for Wnt signals, which enhance the induction of melanoblasts from neural crust cells[42]. Wnts are secreted glycoproteins that play a critical role in melanocyte fate decision by activating the Frizzled family. Activation of Frizzled receptor by Wnt triggers the activation of Dvl, which in turn inactivates glycogen synthase kinase 3β (GSK3β). As a consequence, the level of β-catenin increases, allowing its translocation to the nucleus and facilitates the activation of genes by Tcf/Lef transcription factors. A key target of Tcf/Lef/ β-catenin in early melanocyte precursors is MITF[43,44]. Therefore, we speculate that a high expression of Dvl is required for proper specification of melanocytes.

In summary, we compared the gene expression profiles between keratinocytes, melanocytes and fibroblasts. We found many novel signature genes for each cell type, the functions of which have not yet been reported. Although the precise functional roles of such genes remain to be determined, our data add new information on which to base further investigation into the regulatory mechanism underlying the specification of skin cells.

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