Differential miRNA expression profiles in human keratinocytes in response to protein kinase C inhibitor

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Abstract. Aberrant expression of microRNAs (miRNAs) is widely accepted to be involved in keratinocyte differentiation and to be dependent on activation of the protein kinase C (PKC) pathway. However, the miRNA profiles and biological characteristics of keratinocytes induced by specific inhibitors of PKC have yet to be elucidated. The present study aimed to explore the differential miRNA expression profiles in keratinocytes treated with the PKC inhibitor GF109203X, by conducting a bioinformatics analysis. Parts of the GF109203X-induced keratinocytes formed distinct clones after 2 days of culture, and the expression of intergrin β1, cytokeratin (CK)19 and CK14 were positive, whereas CK10 expression was negative. A total of 79 miRNAs were differentially expressed in keratinocytes treated with GF109203X, among which 45 miRNAs were upregulated and 34 were downregulated. The significantly upregulated microRNAs includedhsa-miR-1-3p and miR-181c-5p, whereas hsa-miR-31-5p and hsa-let-7c-3p were significantly downregulated. In addition, the results of reverse transcription-quantitative polymerase chain reaction exhibited consistency with the microarray results. An enrichment analysis demonstrated that certain target genes of the differentially expressed miRNAs serve an important role in cell proliferation and differentiation, cell cycle progression and apoptosis, etc. These results revealed that GF109203X induced the differential expression of certain miRNAs when keratinocytes began showing the characteristics of epidermal-like stem cells, which may provide a novel approach for wound healing and regeneration of skin tissues.

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Introduction

MicroRNAs (miRNAs) are short noncoding RNA molecules, usually between 22 and 23 nucleotides in length, which regulate the expression of protein-coding genes at the post-transcriptional level by interfering with the translation of mRNAs or by inducing their degradation (1). In humans, miRNAs have been proposed to regulate ~60% of all protein-coding genes and fulfill regulatory functions, as established by their involvement in numerous processes and diseases (2,3). In addition, previous studies have reported that miRNAs are involved in the self-renewal and cell-fate decisions of stem cells, control of the cell cycle, and maintenance of the balance of keratinocyte proliferation, differentiation and apoptosis, whereas their aberrant expression can lead to disease development (4,5). For example, our previous study (6) observed them iRNA expression profiles of epidermal cells at various stages of differentiation; concluding that the expression of 191 miRNAs was significantly altered, the target genes of which are closely correlated with cell proliferation, differentiation, apoptosis and migration. Furthermore, Liu *et al (*7) detected a significant differential miRNA expression profile in cutaneous wounds between diabetic rats and normal rats, which may be closely associated with the mechanisms underlying diabetic wound healing. Sonkoly *et al (*8) reported that upregulation of miR-203 in human keratinocytes may be required for their differentiation, which is dependent on activation of the protein kinase C (PKC)/activator protein-1 (AP-1) pathway. Conversely, pretreatment with the specific PKC inhibitor, GF109203X, not only suppressed 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced miR-203 expression, but also suppressed it to below the basal level. A downstream target for PKC action in keratinocytes is AP-1, which is a transcription factor that consists of homodimers or heterodimers of the Jun and Fos families of nuclear proteins, and serves essential roles in the regulation of keratinocyte growth and differentiation (8). miRNAs regulate keratinocyte differentiation by activating the PKC signaling pathway; however, to the best of our knowledge, there are currently no reports on the differential miRNA expression profiles of keratinocytes following treatment with the specific PKC inhibitor, GF109203X.

PKC was initially discovered in 1977 as a proteolytically activated protein kinase. Later, it was verified as a

Ca^{2+}-activated, phospholipid-dependent Ser/Thr kinase, firmly associated with signal transduction (9). PKC family isoforms are divided into three subgroups: The calcium- and phorbol ester-dependent 'classical/conventional' subgroup (PKCα, βI, βII, γ), the calcium-independent 'novel' subgroup (PKC σ, δ, ε, η, θ) and the calcium- and phorbol ester-independent 'atypical' subgroup (PKC ζ, ι, λ) (9). The rapid activation of PKC enzymes forms part of the signal transduction pathways elicited by numerous hormones, and their phosphorylation of target proteins leads to various cellular responses, including cell proliferation, differentiation and apoptosis (10). In keratinocytes, several cellular functions are also mediated by signaling via PKC, including translocation of the desmoyokin/AHNAK protein, inhibition of proliferation, and differentiation (11). GF109203X is a specific inhibitor of PKC, which competes at the ATP-binding site and regulates the development of keratinocytes. Le Panse *et al* (12) indicated that GF109203X inhibited c-Fos and c-Jun mRNA expression; in keratinocytes these proto-oncogenes are involved in the cellular differentiation process rather than in cellular proliferation. In addition, it has been verified that GF109203X effectively inhibits granular cell differentiation marker expression when used at 1 and 5 μM concentrations; however, it does not alter keratin (K)1 or K14expression (13). GF109203X has also been reported to block TPA-induced tumor susceptibility gene 101 protein and K10 upregulation during early keratinocyte differentiation (14). Furthermore, keratinocyte differentiation is preceded by a commitment to irreversible cell cycle withdrawal, and GF109203X may induce marked protection from loss of growth potential in human keratinocytes (15). GF109203X may also suppress the ultraviolet B-induced reduction of cell survival, caspase-9 activation, downregulation of human inhibitor of apoptosis protein-1, X-linked inhibitor of apoptosis proteinand PKB (but not myeloid cell leukemia-1), and upregulation of glucose-regulated protein 78 in HaCaT cells (16). Overall, these data indicated that GF109203X may have influence on keratinocyte differentiation. However, the miRNA profiles and biological characteristics of keratinocytes induced by specific PKC inhibitors have yet to be elucidated.

The present study aimed to explore the differential miRNA expression profile and biological characteristics of keratinocytes treated with the specific PKC inhibitor, GF109203X. The findings of the present study may provide a novel approach for wound healing and regeneration of skin tissues.

Materials and methods

Sample collection. Prepuce samples were obtained from 5 male patients (age, 16-30 years) who were healthy patients except their prepuce was too long and underwent circumcision at the Department of Urology Surgery, The First Affiliated Hospital of Nanchang University (Nanchang, China) between March 2014 and April 2014. The present study was conducted in accordance with the Declaration of Helsinki, with approval obtained from the Nanchang University Ethics Committee. Written informed consent was obtained from all participants.

Cell culture and identification. The epidermis was digested with trypsin (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 4˚C in the dark for 8 h. Rapid adhesion to collagen IV (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was used to isolate human differentiated keratinocytes from epidermal stem cells, as previously described (6). The differentiated keratinocytes were cultured *in vitro* in keratinocyte serum-free medium supplemented with 10 μg/l epidermal growth factor and 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in a humidified chamber with 5% CO_2 for 2 days, and were then divided into two groups. In the experimental group (EXP), the primary keratinocytes were treated with GF109203X (Selleck Chemicals, LLC, Houston, TX, USA) for 2 days, at a final concentration of 10 μM. In the control group (CON), the primary keratinocytes were treated with dimethyl sulfoxide (DMSO; Sigma-Aldrich; Merck KGaA) for 2 days, at a final concentration of 10 μM. The cellular morphology of the two groups was observed under an inverted phase contrast microscope (CTR6000; Leica Microsystems GmbH, Wetzlar, Germany). Immunostaining of integrin β1 (catalog no. AW5254), cytokeratin (CK)19 (catalog no. AM8477b), CK1 (catalog no. AP9695c) and CK10 (catalog no. AP6704c; Abgent Inc., San Diego, CA, USA) was used for cell identification, which was performed according to the manufacturer's protocols.

Extraction of total RNA. Total RNA was isolated using TRIzol (Invitrogen; Thermo Fisher Scientific. Inc.) and purified with the RNeasy mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. RNA quality and quantity were measured using a NanoDrop spectrophotometer (ND-1000; NanoDrop; Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and RNA integrity was determined by electrophoresis on a denaturing agarose gel, which was prepared in house. On the denaturing gel, the 28S and 18S ribosomal RNA bands were visible, which suggested that the extracted total RNA was complete, RNA degradation and contamination were low and the extracted total RNA exhibited high levels of purity.

miRNA labeling and array hybridization. After quality control, miRNA was labeled with the miRCURY™ Power Labeling kit (Exiqon A/S, Vedbaek, Denmark) according to the manufacturer's protocol. Briefly, 5 μl calf intestine phosphatase (CIP) reaction solution (1 μl total RNA, 0.5 μl CIP buffer, 0.5 μl CIP and 3 μl ddH_2O) was incubated at 37˚C for 30 min, and then at 95˚C for 5 min to terminate the reaction. Subsequently, 3.0 μl labeling buffer, 1.5 μl fluorescent label (Hy3™), 2.0 μl DMSO and 2.0 μl labeling enzyme were added to the mixture. The system was incubated at 16˚C for 1 h, and subsequently at 65˚C for 15 min to terminate the labeling reaction. The Hy3™-labeled samples were hybridized on the miRCURY™ LNA Array (v.18.0) (Exiqon A/S) according to the manufacturer's protocol. Briefly, 25 μl Hy3™-labeled samples were mixed with 25 μl hybridization buffer and were denatured for 2 min at 95˚C, after which the samples were incubated on ice for 2 min and hybridized to the microarray for 16-20 h at 56˚C in a 12-Bay Hybridization system (Roche Nimblegen, Inc., Madison, WI, USA). Following hybridization, the slides were obtained and washed several times using a wash buffer kit (Exiqon A/S). Finally, the slides were scanned using the Axon GenePix 4000B Microarray Scanner (Axon Instruments; Molecular Devices, LLC, Sunnyvale, CA, USA).

Table I. Primer sequences used for quantitative polymerase chain reaction.

miRNA	Sequences	Annealing temperature (˚C)	Product length (bp)
U6	F:5'GCTTCGGCAGCACATATACTAAAAT3'		
	R:5'CGCTTCACGAATTTGCGTGTCAT3'	60	89
hsa-miR-1-3p	GSP:5'GGGGCTGGAATGTAAAGAAGT3'		
	R:5'GTGCGTGTCGTGGAGTCG3'	60	65
hsa-miR-31-5p	F:5'GGAGGCAAGATGCTGGC3'		
	R:5'CAGTGCGTGTCGTGGAGT3'	60	64

miRNA/miR, microRNA; F, forward; R, reverse.

Data processing and analysis. Scanned images were then imported into GenePix Pro 6.0 software (Axon Instruments; Molecular Devices, LLC) for grid alignment and data extraction. Replicated miRNAs were averaged and miRNAs withintensities ≥30 in all samples were chosen for calculating the normalization factor. Expressed data were normalized using the median normalization. Following normalization, significant differentially expressed miRNAs between the two groups were identified through fold change and P-value (fold change >2 and P<0.05). Differential miRNA expression between the two cell groups was analyzed using a Student's t-test. Finally, hierarchical clustering was performed to detect distinguishable miRNA expression profiling among samples.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and miRNA target prediction. According to the microarray results, the expression levels of hsa-miR-1-3p were upregulated and the expression levels of hsa-miR-31-5p were downregulated in the experimental group compared with the control group, exhibiting strong original signals and clear differences. Therefore, both of these miRNAs were selected for RT-qPCR verification. In RT-qPCR, small nuclear (sn)RNA U6 was used as an endogenous control. Firstly, cDNA was synthesized using a Gene Amp PCR system 9700 (Applied Biosystems, Thermo Fisher Scientific, Inc.). RT was performed in a 20 μl reaction containing 200 ng total RNA, 0.3 μl 1 μM RT primer, 2 μl 2.5 mM dNTP (HyTest Ltd, Turku, Finland), 2 μl 10x RT buffer (Epicentre; Illumina, Inc., San Diego, CA, USA), 1 μl 50 U/μl RT enzyme (Epicentre; Illumina, Inc.), 0.3 μl 40 U/μl RNase inhibitor (Epicentre; Illumina, Inc.), 20 μl nuclease free water and 0.2 μl MMLV High Performance Reverse Transcriptase (Epicentre; Illumina, Inc.). The stem-loop RT reaction was performed at 16˚C for 30 min, followed by 42˚C for 30 min and 85˚C for 5 min. A total of 2 μl RT reaction was then used with 1 μl specific primers for each of the hsa-miR-1-3p and hsa-miR-31-5p in triplicate wells for PCR on an Applied Biosystems ViiA 7 Real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Inc.). The thermal cycling parameters were as follows: An initial predenaturation step at 95˚C for 10 min, followed by 40 cycles of denaturation at 95˚C for 10 sec, annealing at 60˚C for 60 sec, followed by 95˚C for 10 sec, 60˚C for 60 sec and 95˚C for 15 sec. The primers, which were synthesized by Bioligo Life Technology (Shanghai, China) and the sequences are presented in Table I. Expression levels were calculated using the comparative quantitative cycle (Cq) method (17). RT-qPCR was performed in triplicate for each treatment group. To demonstrate the function of differential miRNAs, target gene prediction and functional analysis were conducted. The following websites: http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/ and http://www.targetscan.org/were used to predict target genes of the differentially expressed miRNAs. In addition, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used to identify the roles of these target genes in biological pathways or GO terms, which were accessed from the databases of http://www.geneontology.org/ and http://www.genome.jp/kegg/, respectively.

Results

Biological characteristics of the cells. Non-adherent cells were irregular in shape, size and distribution after culturing for 2 days, and were loosely attached to the plate wells with no clones detected under an inverted microscope. These results suggested that the characteristics of non-adherent cells were in accordance with terminally differentiating epidermal keratinocytes. In the experimental group, parts of the keratinocytes induced by GF109203X attached to the well and formed clones, and the expression of CK19, CK14 and integrin β1 was positive, whereas CK10 expression was negative, which is in agreement with the characteristics of epidermal-like stem cells (data not shown). However, in the control group, the number of cells was significantly decreased with no clones detected, and the expression of CK10 was positive, whereas the expression of CK19, CK14 and integrin β1 was negative, which is in accordance with the characteristics of terminally differentiating epidermal keratinocytes.

Extraction and qualification of total RNA. The A260/A280 ratio of RNA is a method used to detect RNA purity; samples ~2.0 are considered to represent pure RNA. A ratio <1.8 indicates sample contamination. A ratio >2.0 indicates RNA hydrolysis. Therefore, a ratio range between 1.8 and 2.1 is considered acceptable. In addition, the A260/A230 ratio should be >1.8 for pure RNA. As demonstrated in Table II, the extracted RNAs conformed to the quality standards and therefore qualified for the subsequent miRNA experiments. On the denaturing electrophoresis gel (Fig. 1), the 18S and 28S rRNA bands were clearly visible in the RNA samples, suggesting good integrity.

Table II. RNA quantification and quality assurance, as determined by NanoDropND-1000.

Group	OD260/280 ratio	OD260/230 ratio	Concentration (ng/μl)	Quantity (ng)	Result
EXP	1.85	2.12	101.82	1018.2	Pass
CON	1.86	2.06	337.7	3377	Pass

EXP, experimental; CON, control; OD, optical density.

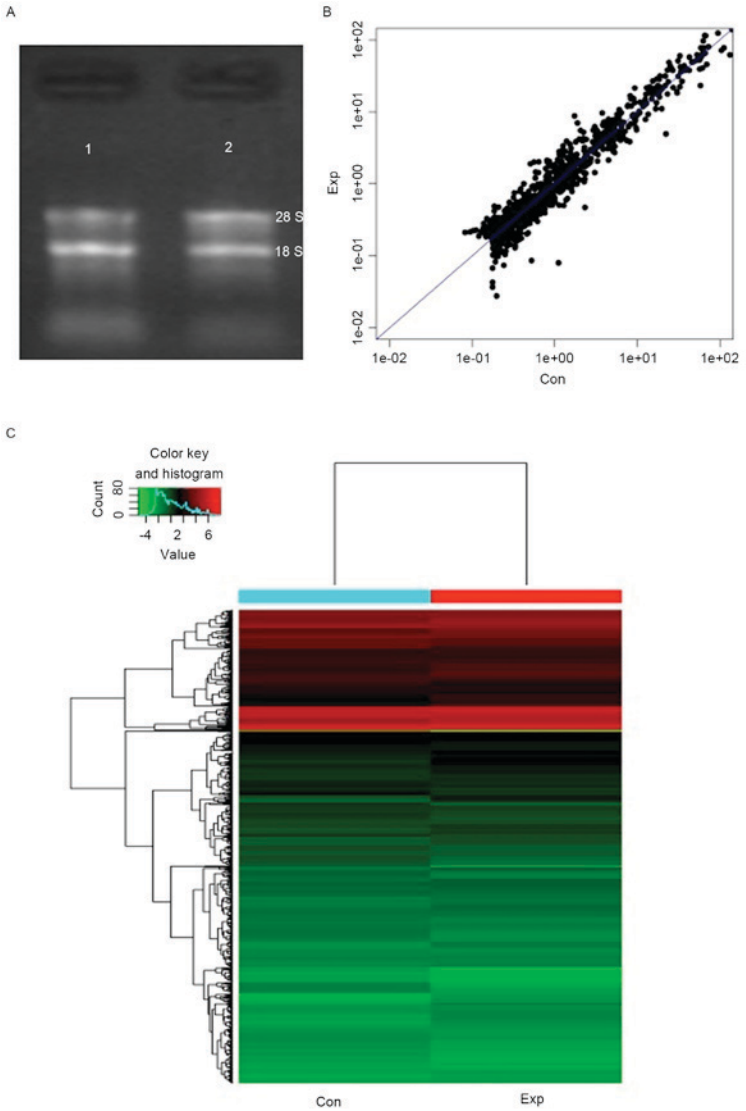

Figure 1. (A) Ribosomal RNA on a denaturing agarose gel. Lane 1, EXP group; lane 2, CON group. (B) Scatter plot for fluorescence signal intensity of the miRNAs in the two groups. Axes represent the fluorescence signal intensity of the miRNAs in chips, and each point represents the fluorescence signal intensity of one probe set (miRNA). (C) Hierarchical clustering in the EXP and CON groups. Red represents up-regulation, green represents down-regulation. EXP, experimental; CON, control; miRNA, microRNA.

Differential miRNA expression. The miRNA expression variations and patterns between the two groups are presented in Fig. 1B and C. According to data processing and analysis, a total of 45 miRNAs were upregulated, whereas 34 miRNAs were downregulated in the experiment group compared with expression in the control group (Table III). The miRNAs with the greatest upregulation and downregulation were hsa-miR-1-3p (5.0265-fold) and hsa-miR-31-5p (13.9011-fold), respectively.

Verification of the microarray data by RT-qPCR. In order to verify the microarray results, RT-qPCR assays were performed on selected miRNAs (hsa-miR-1-3p and hsa-miR-31-5p) in the EXP and CON groups. Amplification and dissociation curve charts for hsa-miR-1-3p, hsa-miR-31-5p and snRNA U6 were generated (Fig. 2). The $2^{-\Delta\Delta Cq}$ value of the miRNAs was calculated according to the relative quantitative method. The $2^{-\Delta\Delta Cq}$ analysis revealed an upregulation of hsa-miR-1-3p (1.724) and downregulation of hsa-miR-31-5p (0.458), which is consistent

Table III. Differential expression of miRNAs.

miRNA probe ID	miRNA	EXP/CON
Upregulated		
10916	hsa-miR-1-3p	5.026537
42660	hsa-miR-144-5p	3.747800
145844	hsa-miR-374a-5p	3.715758
148228	hsa-miR-3656	3.560661
168635	hsa-miR-378e	3.138800
147755	hsa-miR-378c	2.817634
42654	hsa-miR-483-5p	2.683051
46944	hsa-miR-1297	2.674556
147851	hsa-miR-3201	2.632528
147604	hsa-miR-4285	2.624769
29577	hsa-miR-374a-3p	2.570440
168935	hsa-miR-4687-3p	2.562501
146072	hsa-miR-1469	2.484515
168944	hsa-miR-4707-5p	2.460301
17752	hsa-let-7f-5p	2.456365
11053	hsa-miR-32-5p	2.405171
33596	hsa-miR-126-5p	2.382427
147926	hsa-miR-4329	2.377924
27536	hsa-miR-190a-5p	2.370629
42782	hcmv-miR-UL148D	2.338837
42640	hsa-miR-20b-5p	2.337647
11004	hsa-miR-203a-3p	2.295231
169221	hsa-miR-4748	2.294769
169230	hsa-miR-4747-3p	2.281731
17503	hsa-miR-590-5p	2.268278
147840	hsv2-miR-H9-3p	2.262449
4040	hsa-miR-9-5p	2.239972
169395	hsa-miR-4484	2.239785
148620	hsa-miR-454-3p	2.196783
42800	hsa-miR-582-5p	2.188416
17315	kshv-miR-K12-3-3p	2.175560
169399	hsa-miR-4750-5p	2.144243
10923	hsa-miR-107	2.143846
169183	hsa-miR-4644	2.128833
169170	hsa-miR-4472	2.104725
146089	hsv1-miR-H8-5p	2.094303
168696	hsa-miR-4739	2.094056
168893	hsa-miR-4505	2.093298
169272	hsa-miR-4419b	2.080859
42496	hsa-miR-181c-5p	2.041768
169110	hsa-miR-4497	2.040852
168670	hsa-miR-4694-5p	2.031255
146086	hsa-miR-30a-5p	2.030778
148509	hsa-miR-328-5p	2.028205
169375	Has-miR-660-3p	2.005712
Downregulated		
11052	hsa-miR-31-5p	13.901180
42668	hsa-let-7c-3p	7.137004
42959	hsa-miR-514a-3p	6.094606
169159	hsa-miR-4521	4.968959
147809	hsa-miR-514b-3p	4.793510
17848	hsa-miRPlus-A1087	4.497239
42686	hsa-miR-136-3p	4.108723
148402	hsa-miR-3920	3.275565
145689	hsa-miR-543	2.614642
42516	kshv-miR-K12-12-5p	2.564381
147842	hsv2-miR-H11-5p	2.556539
11023	hsa-miR-222-3p	2.505895
145838	hsa-miR-125b-1-3p	2.407407
11140	hsa-miR-508-3p	2.396755
11139	hsa-miR-507	2.307986
29379	hsa-miR-452-5p	2.302862
168958	hsa-miR-2681-5p	2.283967
11037	hsa-miR-299-3p	2.269868
145914	hsa-miR-135b-5p	2.266023
168606	hsa-miR-4633-5p	2.253791
169239	hsa-miR-4732-5p	2.236971
46789	hsa-miR-513b-5p	2.213730
169379	hsa-miR-4694-3p	2.194923
147501	hsa-miR-98-3p	2.165957
46917	hsa-miR-205-5p	2.142624
145751	hsa-miR-23b-5p	2.138643
148278	hsa-miR-138-2-3p	2.126352
168963	hsa-miR-664b-5p	2.123429
146111	hsa-miR-767-5p	2.105775
168953	hsa-miR-4704-5p	2.097161
146165	hsa-miR-1973	2.089585
29190	hsa-miR-708-5p	2.072155
17818	hsa-miR-27a-5p	2.064144
31076	hsa-miR-559	2.018320

miR/miRNA, microRNA; EXP, experimental; CON, control.

with the microarray results, thus suggesting that the microarray data were reliable.

Prediction of target genes. To demonstrate the function of differential miRNAs, target gene prediction and functional analysis were conducted. Databases of identified target genes can be accessed to compile potential targets for differential miRNAs, due to the development of numerous computational algorithms (18). The present study obtained all the target genes of the 79 differentially expressed miRNAs according to three public databases. Subsequently, GO and KEGG analysis were used to identify the biological functions of these target genes.

The most enriched GO terms of the three ontologies are listed in Tables IV-VI. Transcription process, apoptosis process and cell proliferation process were among the most significantly enriched in terms of biological process; the cellular component GO analysis demonstrated that the target genes were associated with the nucleus, cytoplasm and cytosol; and protein binding, DNA binding, ATP binding

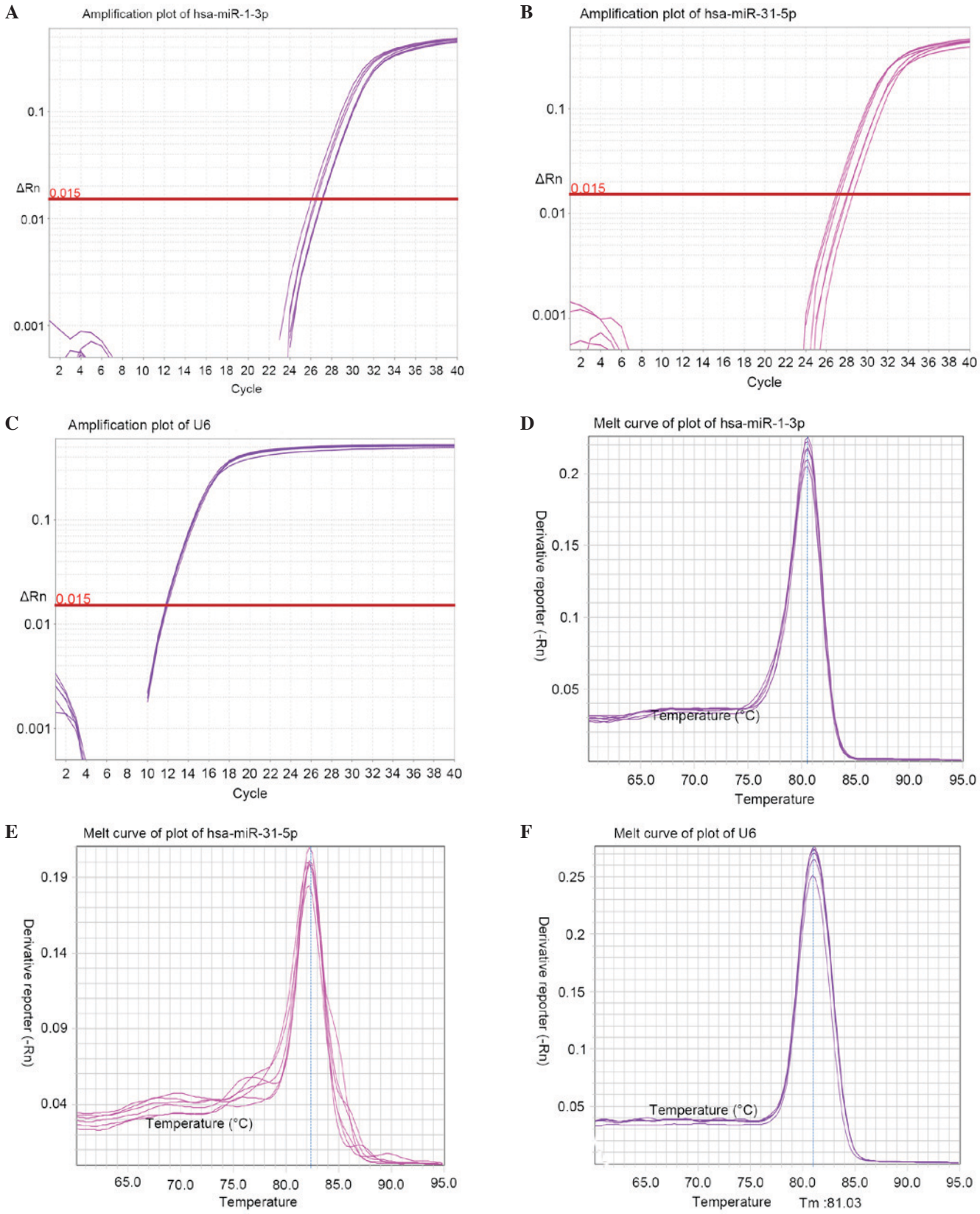

Figure 2. (A-C) Amplification and (D-F) dissociation curve charts for (A and D) hsa-miR-1-3p, (B and E) hsa-miR-31-5p and (C and F) U6. In the amplification curve charts, the x-axis represents cycle number and the y-axis represents the real-time fluorescence signal intensity of the corresponding cycle number. In the dissociation curve charts, the x-axis represents the temperature of the RT-qPCR products and the y-axis represents the real-time fluorescence-signal-intensity change rate with increasing temperature. Differently colored curves correspond to different RT-qPCRs. miR, microRNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

and transcription factor binding were significantly enriched in terms of molecular function. Taken together, these results suggested that the target genes of differentially expressed miRNAs may be involved in cell proliferation, division,

Table IV. Enriched terms in GO biological process.

Term ID	Term name	Hit number
GO:0006351	Transcription, DNA-dependent	514
GO:0006355	Regulation of transcription, DNA-dependent	328
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	259
GO:0007165	Signal transduction	243
GO:0006915	Apoptotic process	216
GO:0045893	Positive regulation of transcription, DNA-dependent	200
GO:0000122	Negative regulation of transcription from RNA polymerase II promoter	198
GO:0010467	Gene expression	181
GO:0045892	Negative regulation of transcription, DNA-dependent	161
GO:0043066	Negative regulation of apoptotic process	143
GO:0008285	Negative regulation of cell proliferation	123
GO:0006366	Transcription from RNA polymerase II promoter	112
GO:0008284	Positive regulation of cell proliferation	105
GO:0008283	Cell proliferation	99
GO:0051301	Cell division	94
GO:0007049	Cell cycle	85
GO:0006357	Regulation of transcription from RNA polymerase II promoter	79
GO:0006367	Transcription initiation from RNA polymerase II promoter	73
GO:0016055	Wnt receptor signaling pathway	65
GO:0001525	Angiogenesis	65
GO:0043065	Positive regulation of apoptotic process	64
GO:0007173	Epidermal growth factor receptor signaling pathway	62
GO:0006917	Induction of apoptosis	60
GO:0007050	Cell cycle arrest	58
GO:0007067	Mitosis	58
GO:0000082	G_1/S transition of mitotic cell cycle	47
GO:0007243	Intracellular protein kinase cascade	43
GO:0007219	Notch signaling pathway	43
GO:0006260	DNA replication	42
GO:0030335	Positive regulation of cell migration	41
GO:0016477	Cell migration	40
GO:0019827	Stem cell maintenance	40

GO, Gene Ontology.

mitosis, apoptosis and differentiation. Finally, KEGG analysis indicated that 77 pathways were associated with the target genes of differentially expressed miRNAs; 15 significantly enriched pathways are presented in Table VII, including the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway, the mitogen-activated protein kinase (MAPK) signaling pathway, protein processing in endoplasmic reticulum, focal adhesion and mammalian target of rapamycin (mTOR) signaling, which are associated with cell growth, differentiation, apoptosis and migration.

Discussion

In the present study, following incubation with GF109203X, some of the surviving keratinocytes reverted from a differentiated to a dedifferentiated state, as evidenced by the high colony-forming efficiency and expression of biological markers of keratinocyte stem cells, including intergrin β1, CK19 and CK14. However, in the CON group these alterations were not detected. These findings suggested that terminally differentiating epidermal keratinocytes may acquire some stem cell characteristics by modulation with GF109203X treatment. Therefore, dedifferentiation of human terminally differentiating keratinocytes may be induced by GF109203X *in vitro*. Mature cell dedifferentiation is a popular phenomenon, in which terminally differentiating epidermal cells can revert to their ancestor cells by dedifferentiation; i.e., epidermal cells can revert from the 'old' differentiated state to the not fully differentiated 'young' state, or even the 'naive' state with the characteristics of epidermal stem cells. Previous studies have confirmed that keratinocytes can be dedifferentiated into their progenitor cells, and have identified that dedifferentiated young epidermal cells may be used to treat severe wounds (19,20). In addition, Sun *et al* (21) demonstrated

Table V. Enriched terms in GO molecular functions.

Term ID	Term name	Hit number
GO:0005515	Protein binding	1,554
GO:0003677	DNA binding	428
GO:0005524	ATP binding	413
GO:0003700	Sequence-specific DNA binding transcription factor activity	295
GO:0043565	Sequence-specific DNA binding	166
GO:0003723	RNA binding	142
GO:0003682	Chromatin binding	126
GO:0008134	Transcription factor binding	111
GO:0042802	Identical protein binding	109
GO:0019901	Protein kinase binding	104
GO:0005525	GTP binding	98
GO:0003713	Transcription coactivator activity	91
GO:0003714	Transcription corepressor activity	70
GO:0004672	Protein kinase activity	70
GO:0019899	Enzyme binding	67
GO:0003924	GTPase activity	67
GO:0044212	Transcription regulatory region DNA binding	63
GO:0019904	Protein domain specific binding	58
GO:0008022	Protein C-terminus binding	51
GO:0003705	RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity	39
GO:0005096	GTPase activator activity	37
GO:0004725	Protein tyrosine phosphatase activity	36
GO:0001077	RNA polymerase II core promoter proximal region Sequence-specific DNA binding transcription factor Activity involved in positive regulation of transcription	32

GO, Gene Ontology.

that dedifferentiation of human terminally differentiating keratinocytes into their precursor cells may be induced by basic fibroblast growth factor. Another study indicated that dedifferentiated epidermal cells are able to form clones and generate a complete epithelium following migration to cutaneous wounds (22). Zhao *et al (*23) demonstrated that LiCl and glycogen synthase kinase-3β inhibitor-induced cells are able to regenerate skin, in a manner equivalent to that of epidermal stem cells. These findings suggested that dedifferentiation is a promising method for the production of abundant epidermal stem cells, which may be used to bioengineer skin equivalents and as stem cell-based therapies in cutaneous repair and regeneration. It is well known that poor wound healing after trauma, surgery, acute illness or chronic disease conditions affects millions of people worldwide each year (24), and the cost of non-healing wounds is a great burden to health care systems (25). The efficacy of conventional approaches to treating cutaneous wounds is limited; dressings, periodic debridement, eliminating causative factors and innovations in surgical autologous grafting techniques are inherently limited to the size of available donor sites and are insufficient for global burn injuries (26). Therefore, the present study offers a potential novel strategy for the treatment of cutaneous wounds. Furthermore, dedifferentiated cells are readily available in large quantities with the use of simple methods, and are considered moral and ethical alternatives for disease therapy, with no risk of genetic incompatibility or tissue rejection.

The present study used microarray hybridization to comparably observe the expression of miRNAs between EXP and CON groups. The results detected 45 upregulated miRNAs and 34 downregulated miRNAs when keratinocytes began exhibiting the characteristics of epidermal-like stem cells. In the present study, hsa-miR-1-3p was the most significantly upregulated miRNA and hsa-miR-31-5p was the most significantly downregulated miRNA. Hsa-miR-1-3p is also known as miR-1, which is significantly positively correlated with expression of the proliferation marker Ki67, and is involved in proliferation (27). A previous study demonstrated that inhibition of PKC prevented the upregulation of miR-1 induced by constitutively active Gαi2, demonstrating a role for PKC in the regulation of muscle-specific miRNA (28). In the present study, miR-1 was upregulated in keratinocytes treated with the PKC inhibitor GF109203X, which may also serve an important role in proliferation. Hsa-miR-31-5p is also known as miR-31, which has been implicated as a key regulator of keratinocyte differentiation and proliferation. Peng *et al (*29)

Table VI. Enriched terms in GO cell component.

Term Id	Term name	Hit number
GO:0005634	Nucleus	1,321
GO:0005737	Cytoplasm	1,104
GO:0005829	Cytosol	668
GO:0005730	Nucleolus	459
GO:0005654	Nucleoplasm	303
GO:0005794	Golgi apparatus	213
GO:0016020	Membrane	198
GO:0005783	Endoplasmic reticulum	168
GO:0005789	Endoplasmic reticulum membrane	167
GO:0048471	Perinuclear region of cytoplasm	150
GO:0000139	Golgi membrane	139
GO:0043231	Intracellular membrane-bounded organelle	110
GO:0005667	Transcription factor complex	83
GO:0005925	Focal adhesion	50
GO:0031965	Nuclear membrane	50
GO:0005765	Lysosomal membrane	43
GO:0005938	Cell cortex	40
GO:0005741	Mitochondrial outer membrane	39
GO:0005911	Cell-cell junction	36
GO:0005819	Spindle	34
GO:0000790	Nuclear chromatin	32
GO:0000151	Ubiquitin ligase complex	25
GO:0017053	Transcriptional repressor complex	22

GO, Gene Ontology.

indicated that miR-31 is an endogenous negative regulator of factor inhibiting hypoxia-inducible factor-1 expression, which results in keratinocyte differentiation by enhancing Notch signaling; this finding is in accordance with the results of the present study. Furthermore, nuclear factor-κB-induced miR-31 promotes keratinocyte proliferation by suppressing protein phosphatase 6 in psoriasis (30). Recently, in human metastatic cutaneous squamous cells, the increased expression of miR-31 was revealed to promote migration, invasion and colony forming ability (31). Taken together, these findings suggested that miR-31 is a multifunctional miRNA that serves important roles in physiological and pathological conditions of epidermal keratinocytes; however, the molecular mechanisms of PKC and miR-31 remain poorly characterized and require further study. In addition, the present study demonstrated that miR-181c-5p and miR-374a were predominantly expressed in GF109203X-induced keratinocytes, which is in accordance with our previous observation that these miRNAs were upregulated in native keratinocyte stem cells (6). Hsa-miR-181c-5p has functional relevance in the maintenance of stemness, which may regulate cell proliferation and cell cycle progression via the Notch signaling pathway and bone morphogenetic protein pathway in cancer stem cells (32). In addition, miR-374a has been reported to promote the proliferation of osteosarcoma cells by targeting Axin2 (33). Overall, these data indicated that these miRNAs may promote proliferation and maintain the undifferentiated state when keratinocytes were induced to re-express the biological characteristics of epidermal-like stem cells by GF109203X.

The enrichment analysis of the differentially expressed miRNAs demonstrated that hsa-miR-181c-5p, hsa-miR-378c and hsa-miR-20b-5p are involved in numerous KEGG pathways that regulate cell proliferation, differentiation and motility, which may serve important roles in dedifferentiation. Hsa-miR-181c-5p, hsa-miR-20b-5p, hsa-miR-374a-5p, hsa-miR-590-5p, hsa-let-7f-5p and hsa-miR-378c are involved in the MAPK signaling pathway, which has been reported to be involved in cell proliferation, differentiation, inflammation and tumor growth (34,35). PKCδ/p38δ MAPK signaling, which is a key controller of keratinocyte proliferation and differentiation, increases p21 (Cip1) expression to suppress keratinocyte proliferation (36). A further study demonstrated that PKCδ/p38δ MAPK signaling suppresses methylosome protein 50 expression, leading to reduced H3/H4 arginine dimethylation at the p21 (Cip1) promoter; this was associated with enhanced p21 (Cip1) expression and reduced cell proliferation (37). Previous research has indicated that the MAPK signaling pathway may increases p21 (Cip1) expression to suppress keratinocyte proliferation, which indicated that hsa-miR-181c-5p, hsa-miR-20b-5p, hsa-miR-374a-5p, hsa-miR-590-5p, hsa-let-7f-5p and hsa-miR-378c may be associated with p21 (Cip1) expression and keratinocyte proliferation. In addition, the PI3K-Akt and mTOR signaling pathways may regulate the growth and differentiation of

Table VII. Pathways associated with the differentially expressed miRNAs.

Pathway	Function	Related differentially expressed miRNAs
PI3K-Akt signaling pathway	Activated by many types of cellular stimuli or toxic insults; regulates fundamental cellular functions, including transcription, translation, proliferation, growth and survival	hsa-miR-181c-5p, hsa-miR-20b-5p, hsa-miR-1297, hsa-miR-378e, hsa-miR-378c
MAPK signaling pathway	Highly conserved module, involved in various cellular functions, including cell proliferation, differentiation and migration	hsa-miR-181c-5p, hsa-miR-20b-5p, hsa-miR-374a-5p, hsa-miR-590-5p, hsa-let-7f-5p, hsa-miR-378c, hsa-miR-299-3p
Protein processing in endoplasmic reticulum	Promotes cell apoptosis	hsa-miR-181c-5p, hsa-miR-1297, hsa-miR-374a-5p, hsa-miR-299-3p, hsa-miR-20b-5p
Focal adhesion	Serves essential roles in important biological processes, including cell motility, cell proliferation, cell differentiation, regulation of gene expression and cell survival	hsa-miR-1-3p, hsa-miR-181c-5p, hsa-miR-20b-5p, hsa-miR-374a-5p, hsa-miR-378e, hsa-miR-4644, hsa-miR-378c
Hippo signaling pathway	Promotes Mats localization in the cytoplasm, leading to cell apoptosis and restricting organ size overgrowth	hsa-miR-20b-5p, hsa-miR-374a-5p, hsa-miR-4644
Wnt signaling pathway	Required for basic developmental processes, including cell-fate specification, progenitor-cell proliferation and control of asymmetric cell division	hsa-miR-20b-5p, hsa-miR-1297, hsa-miR-374a-5p, hsa-miR-222-3p, hsa-miR-135b-5p
Cell cycle	Regulation of cell mitosis	hsa-miR-1-3p, hsa-miR-20b-5p, hsa-miR-1297
TGF-β signaling pathway	TGF-β family members are involved in a wide spectrum of cellular functions, including proliferation, apoptosis, differentiation and migration	hsa-miR-181c-5p,hsa-miR-20b-5p,hsa-miR-374a-5p, hsa-miR-590-5p, hsa-miR-454-3p, hsa-miR-135b-5p
Adherens junction	Important for maintaining tissue architecture and cell polarity, and can limit cell movement and proliferation	hsa-miR-181c-5p, hsa-miR-1-3p, hsa-miR-20b-5p, hsa-miR-378e, hsa-miR-4644, hsa-miR-378c
p53 signaling pathway	p53 activation is induced by a number of stress signals, including DNA damage, oxidative stress and activated oncogenes, thus resulting in three major outputs: Cell cycle arrest, cellular senescence and apoptosis	hsa-miR-1297
Apoptosis	Apoptosis is a genetically controlled mechanism of cell death involved in the regulation of tissue homeostasis	hsa-miR-20b-5p
Hedgehog signaling pathway	Involved in control of stem cell proliferation in adult tissues	hsa-miR-4644
mTOR signaling pathway	Regulates cell growth and cell differentiation	hsa-miR-181c-5p, hsa-miR-20b-5p, hsa-miR-454-3p
ErbB signaling pathway	Regulates diverse biological responses, including proliferation, differentiation, cell motility and survival	hsa-miR-181c-5p, hsa-miR-378e, hsa-miR-4644, hsa-miR-378c
VEGF signaling pathway	Mediates the proliferation and migration of endothelial cells, and promotes their survival and vascular permeability	hsa-miR-1-3p, hsa-miR-4644

miRNA/miR, microRNA; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; TGF, transforming growth factor; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor.

keratinocytes (38,39), whereas the Hedgehog signaling pathway is a critical regulator of lineage-specific stem cells that maintains specialized sensory compartments in the epidermis (40) which may serve a key role in re-expression of biological characteristics in induced keratinocytes.

The present study aimed to determine whether the differentially expressed miRNAs are associated with dedifferentiation of keratinocytes induced by GF109203X. It is well known that Oct-3/4, sex determine region Y-box 2 (Sox2), Nanog, c-Myc and Kruppel-like factor 4 (KLF4) are associated with dedifferentiation (41,42). In cancer cells and mouse embryonic stem cells, zinc finger E-box binding homeobox 1 links epithelial-mesenchymal transition activation and maintenance of stemness by suppressing stemness-inhibiting miRNAs, including miR-200c, miR-203 and miR-183, which cooperate to suppress expression of stem cell factors, such as Sox2 and KlLF4 (43). In addition, miR-134, miR-296 and miR-470, which are upregulated during retinoicacid-induced differentiation of mouse embryonic stem cells, target the amino acid coding sequence of Nanog, Oct4 and Sox2 genes, leading to transcriptional and morphological alterations characteristic of differentiating mouse embryonic stem cells, and resulting in a novel phenotype (44). Lauschke *et al (*45) identified that miRNAs are important drivers of hepatic dedifferentiation. Taken together, dedifferentiation is a process associated with modulation of numerous genes, in which miRNAs may have an important role; this may explain why were so many differentially expressed miRNAs were detected during GF109203X-induced keratinocyte dedifferentiation.

In conclusion, when treated with the PKC inhibitor GF109203X, keratinocytes exhibited a series of alterations, including altered morphology, expression of epidermal cell-specific markers and differentially expressed miRNAs. Bioinformatics analysis of the differentially expressed miRNAs indicated that inhibition of PKC signaling was associated with cell proliferation, differentiation and dedifferentiation. Considering that pre-clinical and clinical studies have demonstrated that modulation of miRNA expression by administration of specific miRNA mimics or inhibitors may be beneficial for treating diseases (46), the present study may offer novel miRNAs for regulation of the PKC pathway. However, the exact mechanisms underlying the differentially expressed miRNAs remain unclear and require further study.

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References

1. Moreno-Moya JM, Vilella F and Simón C: MicroRNA: Key gene expression regulators. Fertil Steril 101: 1516-1523, 2014.
2. Suzuki HI: Dissecting microRNA biogenesis and microRNA-mediated regulation of gene network. Seikagaku 87: 413-421, 2015 (In Japanese).
3. He L and Sedwick C: Lin He: 'Junk' DNA isn't. J Cell Biol 211: 4-5, 2015.
4. Martin EC, Qureshi AT, Dasa V, Freitas MA, Gimble JM and Davis TA: MicroRNA regulation of stem cell differentiation and diseases of the bone and adipose tissue: Perspectives on miRNA biogenesis and cellular transcriptome. Biochimie 124: 98-111, 2016.
5. Yao S: MicroRNA biogenesis and their functions in regulating stem cell potency and differentiation. Biol Proced Online 18: 8, 2016.
6. Song Z, Liu D, Peng Y, Li J, Zhang Z and Ning P: Differential microRNA expression profile comparison between epidermal stem cells and differentiated keratinocytes. Mol Med Rep 11: 2285-2291, 2015.
7. Liu YF, Ding M, Liu DW, Liu Y, Mao YG and Peng Y: MicroRNA profiling in cutaneous wounds of diabetic rats. Genet Mol Res 14: 9614-9625, 2015.
8. Sonkoly E, Wei T, Pavez Loriè E, Suzuki H, Kato M, Törmä H, Ståhle M and Pivarcsi A: Protein kinase C-dependent upregulation of miR-203 induces the differentiation of human keratinocytes. J Invest Dermatol 130: 124-134, 2010.
9. Gutcher I, Webb PR and Anderson NG: The isoform-specific regulation of apoptosis by protein kinase C. Cell Mol Life Sci 60: 1061-1070, 2003.
10. Diaz-Meco MT and Moscat J: The atypical PKCs in inflammation: NF-κB and beyond. Immunol Rev 246: 154-167, 2012.
11. Mitev V and Miteva L: Signal transduction in keratinocytes. Exp Dermatol 8: 96-108, 1999.
12. Le Panse R, Coulomb B, Mitev V, Bouchard B, Lebreton C and Dubertret L: Differential modulation of human fibroblast and keratinocyte growth by the protein kinase C inhibitor GF 109203X. Mol Pharmacol 46: 445-451, 1994.
13. Lee YS, Yuspa SH and Dlugosz AA: Differentiation of cultured human epidermal keratinocytes at high cell densities is mediated by endogenous activation of the protein kinase C signaling pathway. J Invest Dermatol 111: 762-766, 1998.
14. You HL, Eng HL, Hsu SF, Chen CM, Ye TC, Liao WT, Huang MY, Baer R and Cheng JT: A PKC-Sp1 signaling pathway induces early differentiation of human keratinocytes through upregulation of TSG101. Cell Signal 19: 1201-1211, 2007.
15. Tibudan SS, Wang Y and Denning MF: Activation of protein kinase C triggers irreversible cell cycle withdrawal in human keratinocytes. J Invest Dermatol 119: 1282-1289, 2002.
16. Park YK and Jang BC: UVB-induced anti-survival and pro-apoptotic effects on HaCaT human keratinocytes via caspase- and PKC-dependent downregulation of PKB, HIAP-1, Mcl-1, XIAP and ER stress. Int J Mol Med 33: 695-702, 2014.
17. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
18. Dweep H and Gretz N: miRWalk2. 0: A comprehensive atlas of microRNA-target interactions. Nat Methods 12: 697, 2015.
19. Kormos B, Belso N, Bebes A, Szabad G, Bacsa S, Széll M, Kemény L and Bata-Csörgo Z: In vitro dedifferentiation of melanocytes from adult epidermis. PLoS One 6: e17197, 2011.
20. Poloni A, Maurizi G, Anastasi S, Mondini E, Mattiucci D, Discepoli G, Tiberi F, Mancini S, Partelli S, Maurizi A, *et al*: Plasticity of human dedifferentiated adipocytes toward endothelial cells. Exp Hematol 43: 137-146, 2015.
21. Sun X, Fu X, Han W, Zhao Y, Liu H and Sheng Z: Dedifferentiation of human terminally differentiating keratinocytes into their precursor cells induced by basic fibroblast growth factor. Biol Pharm Bull 34: 1037-1045, 2011.
22. Li JF, Duan HF, Wu CT, Zhang DJ, Deng Y, Yin HL, Han B, Gong HC, Wang HW and Wang YL: HGF accelerates wound healing by promoting the dedifferentiation of epidermal cells through beta1-integrin/ILK pathway. Biomed Res Int 2013: 470418, 2013.
23. Zhao Z, Zhang C, Fu X, Yang R, Peng C, Gu T, Sui Z, Wang C and Liu C: Differentiated epidermal cells regain the ability to regenerate a skin equivalent by increasing the level of β-catenin in the cells. Cells Tissues Organs 196: 353-361, 2012.
24. Eming SA, Martin P and Tomic-Canic M: Wound repair and regeneration: Mechanisms, signaling, and translation. Sci Transl Med 6: 265sr6, 2014.
25. Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ, Marks R, Naldi L, Weinstock MA, Wulf SK, *et al*: The global burden of skin disease in 2010: An analysis of the prevalence and impact of skin conditions. J Invest Dermatol 134: 1527-1534, 2014.

26. Sun BK, Siprashvili Z and Khavari PA: Advances in skin grafting and treatment of cutaneous wounds. Science 346: 941-945, 2014.
27. Boštjančič E, Jerše M, Glavač D and Zidar N: miR-1, miR-133a/b and miR-208a in human fetal hearts correlate to the apoptotic and proliferation markers. Exp Biol Med (Maywood) 240: 211-219, 2015.
28. Minetti GC, Feige JN, Bombard F, Heier A, Morvan F, Nürnberg B, Leiss V, Birnbaumer L, Glass DJ and Fornaro M: Gαi2 signaling is required for skeletal muscle growth, regeneration, and satellite cell proliferation and differentiation. Mol Cell Biol 34: 619-630, 2014.
29. Peng H, Kaplan N, Hamanaka RB, Katsnelson J, Blatt H, Yang W, Hao L, Bryar PJ, Johnson RS, Getsios S, *et al*: microRNA-31/factor-inhibiting hypoxia-inducible factor 1 nexus regulates keratinocyte differentiation. Proc Natl Acad Sci USA 109: 14030-14034, 2012.
30. Yan S, Xu Z, Lou F, Zhang L, Ke F, Bai J, Liu Z, Liu J, Wang H, Zhu H, *et al*: NF-κB-induced microRNA-31 promotes epidermal hyperplasia by repressing protein phosphatase 6 in psoriasis. Nat Commun 6: 7652, 2015.
31. Wang A, Landén NX, Meisgen F, Lohcharoenkal W, Ståhle M, Sonkoly E and Pivarcsi A: MicroRNA-31 is overexpressed in cutaneous squamous cell carcinoma and regulates cell motility and colony formation ability of tumor cells. PLoS One 9: e103206, 2014.
32. Sanchez-Diaz PC, Hsiao TH, Chang JC, Yue D, Tan MC, Chen HI, Tomlinson GE, Huang Y, Chen Y and Hung JY: De-regulated microRNAs in pediatric cancer stem cells target pathways involved in cell proliferation, cell cycle and development. PLoS One 8: e61622, 2013.
33. Lu T, Zhang C, Chai MX, An YB and Jia JL: miR-374a promotes the proliferation of osteosarcoma cell proliferation by targeting Axin2. Int J Clin Exp Pathol 8: 10776-10783, 2015.
34. Mishra S, Tripathi A, Chaudhari BP, Dwivedi PD, Pandey HP and Das M: Deoxynivalenol induced mouse skin cell proliferation and inflammation via MAPK pathway. Toxicol Appl Pharmacol 279: 186-197, 2014.
35. Wang X, Pesakhov S, Weng A, Kafka M, Gocek E, Nguyen M, Harrison JS, Danilenko M and Studzinski GP: ERK 5/MAPK pathway has a major role in 1α, 25-(OH)2 vitamin D3-induced terminal differentiation of myeloid leukemia cells. J Steroid Biochem Mol Biol 144: 223-227, 2014.
36. Saha K, Adhikary G, Kanade SR, Rorke EA and Eckert RL: p38δ Regulates p53 to Control p21Cip1 expression in human epidermal keratinocytes. J Biol Chem 289: 11443-11453, 2014.
37. Saha K and Eckert RL: Methylosome protein 50 and PKCδ/p38δ protein signaling control keratinocyte proliferation via opposing effects on p21Cip1 Gene Expression. J Biol Chem 290: 13521-13530, 2015.
38. Xiao X, He Y, Li C, Zhang X, Xu H and Wang B: Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through Notch and phosphoinositide 3-kinase/AKT signaling pathways. Br J Dermatol 174: 522-532, 2016.
39. Patruno A, Pesce M, Grilli A, Speranza L, Franceschelli S, De Lutiis MA, Vianale G, Costantini E, Amerio P, Muraro R, *et al*: mTOR Activation by PI3K/Akt and ERK signaling in short ELF-EMF exposed human keratinocytes. PLoS One 10: e0139644, 2015.
40. Xiao Y, Thoresen DT, Williams JS, Wang C, Perna J, Petrova R and Brownell I: Neural Hedgehog signaling maintains stem cell renewal in the sensory touch dome epithelium. Proc Natl Acad Sci USA 112: 7195-7200, 2015.
41. Nakamura Y, Ishikawa H, Kawai K, Tabata Y and Suzuki S: Enhanced wound healing by topical administration of mesenchymal stem cells transfected with stromal cell-derived factor-1. Biomaterials 34: 9393-9400, 2013.
42. Tao R, Sun TJ, Han YQ, Xu G, Liu J and Han YF: Optimization of in vitro cell labeling methods for human umbilical cord-derived mesenchymal stem cells. Eur Rev Med Pharmacol Sci 18: 1127-1134, 2014.
43. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, Waldvogel B, Vannier C, Darling D, zur Hausen A, *et al*: The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 11: 1487-1495, 2009.
44. Tay Y, Zhang J, Thomson AM, Lim B and Rigoutsos I: MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455: 1124-1128, 2008.
45. Lauschke VM, Vorrink SU, Moro SM, Rezayee F, Nordling Å, Hendriks DF, Bell CC, Sison-Young R, Park BK, Goldring CE, *et al*: Massive rearrangements of cellular miRNA signatures are key drivers of hepatocyte dedifferentiation. Hepatology 64: 1743-1756, 2016.
46. Sethupathy P: The promise and challenge of therapeutic MicroRNA silencing in diabetes and metabolic diseases. Curr Diab Rep 16: 52, 2016.