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Pangenomic changes induced by DHEA in the skin of postmenopausal women

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ABSTRACT

The objective of this study was to explore, for the first time, the changes in the pangenomic profile induced in human skin in women treated with dehydroepiandrosterone (DHEA) applied locally.

Sixty postmenopausal women participated in this phase II prospective, randomized, double-blind and placebo-controlled study. Women were randomized to the twice daily local application of 0% (placebo), 0.3%, 1% or 2% DHEA cream. Changes in the pangenomic expression profile were studied using Affymetrix Genechips.

Significant changes ($p < 0.05$) in sixty-six DHEA-responsive probe sets corresponding to 52 well-characterized genes and 9 unknown gene sequences were identified. A dose-dependent increase in the expression of several members of the collagen family was observed, namely COL1, COL3 and COL5 as well as the concomitant modulation of SPARC, a gene required for the normal deposition and maturation of collagen fibrils in the dermis. Several genes involved in the proliferation and differentiation of keratinocytes were also modulated. In addition, topical DHEA reduced the expression of genes associated with the terminal differentiation and cornification of keratinocytes.

Our results strongly suggest the possibility that DHEA could exert an anti-aging effect in the skin through stimulation of collagen biosynthesis, improved structural organization of the dermis while modulating keratinocyte metabolism.

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1. Introduction

The overall changes observed in the skin during aging, including thinning of the dermis, are likely related to a combination of intrinsic factors, especially changes in the hormonal status due to aging as well as extrinsic factors, especially photodamage by ultraviolet and infrared radiation from the sun and the influence of cigarette smoking [1].

Humans, along with the other primates, are unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids dehydroepiandrosterone (DHEA) and especially DHEA-sulfate (DHEA-S), which are converted into potent androgens and/or estrogens in peripheral tissues [2–4]. It is important to mention that recent data have shown that women have at least 50% as much androgens as men, the main source being DHEA of adrenal origin [5]. However, serum DHEA as well as total androgens start to decline by the age of 30 years and they have already decreased by 60% at time of menopause [5], thus potentially providing an explanation for part of the mechanisms of skin aging. Tissue-specific treatment with DHEA appears a valuable replacement for traditional systemic hormonal replacement therapy (HRT) or estrogen replacement therapy (ERT) and thus avoid the reported side-effects, especially the increased risk of breast cancer [6,7].

All the steroidogenic enzymes that transform DHEA into dihydrotestosterone (DHT) or 17β-estradiol (E2) are present in human skin, thus providing the enzymatic machinery necessary for the local synthesis of sex steroids from the inactive adrenal steroid precursor DHEA [8–10]. In fact, after cessation of estrogen secretion by the ovaries at menopause, practically all sex steroids acting in the skin are synthesized locally from DHEA [11]. DHEA has already been shown to have beneficial effects on the skin of postmenopausal women, especially correction of the hyposeborrheic condition of the aged skin [12], increase of skin hydration and thickness, and reduction of skin pigmentation [13]. Some data have already reported an effect of DHEA on collagen synthesis and degradation [14–16].

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Little is known, however, about the gene expression changes induced by DHEA in the skin. This is the first global analysis of the DHEA-induced changes of the skin transcriptome. This study defines the effect of DHEA in postmenopausal women using Affymetrix microarrays to scan the whole human genome [17,18].

2. Materials and methods

2.1. Patients and sample preparation

Sixty healthy postmenopausal Caucasian women aged 60–65 years participated in this study after IRB approval and having given written informed consent. No subject had taken hormone replacement therapy during the previous 6 months. No subject was suffering from an endocrine disorder, and none was under treatment with lipid- or glucose-lowering agents. There was no migraine and no diabetes mellitus not controlled by conventional therapy. There was no corticosteroid treatment within 6 weeks of study entry as well as treatment with β-carotenoid, retinoic acid, hydroquinone, α-hydroxyacid (including inhaled, topical or oral).

There was no administration of any investigational drug within 30 days of screening visit or previous treatment with androgens or anabolic steroids within 6 months prior to the screening visit. There was no exposure to or use of antidepressants, antipsychotics, or analgesics, within 30 days prior to enrollment.

The body weight ranged between 18.5 and 29.9 of ideal body weight according to Body Mass Index (BMI). There was no hepatic or renal impairment or condition known to affect drug or steroid metabolism. All subjects had a medical history, complete physical examination, serum biochemistry as well as complete blood and urine analysis.

Each subject was randomly assigned to one of the four treatment groups, namely placebo, 0.3% DHEA, 1% DHEA and 2.0% DHEA. Daily, before breakfast, and after supper, for 13 weeks, subjects received 6.0 ml of one of the four emulsions (i.e. Water-in-oil cream containing 50% propylene glycol and 5% ethyl alcohol plus emollient, emulsifying and antioxidant agents for the placebo group and with the addition of the respective concentrations of DHEA for the treated groups). All subjects were instructed to apply the study treatment twice daily during 13 weeks, as previously described [5].

Two biopsies were obtained from the thigh of each patient, one before and the second one after treatment. The biopsies were done on the external face of the thighs 8 cm below the trochanter on a vertical line using the right or left side for pretreatment sample and the opposite side following 13 weeks of treatment. To collect the punch biopsies, local anesthesia was used, and a 2-mm diameter cylindrical core of skin was removed. Biopsies were immediately submerged and stored in liquid nitrogen until RNA extraction.

With 15 subjects in each group on day 1, there were 15, 15, 10 and 3 subjects per group at week 13 for the placebo, 0.3%, 1% and 2% DHEA creams, respectively. In the 1% and 2% DHEA groups, 5 and 10 women, respectively, were excluded at week 8 of treatment for serum levels of DHEA above 11 ng/ml. From the remaining women at the end of the study, samples from 13, 14 and 7 subjects, from placebo, 0.3% and 1% DHEA, respectively were analyzed. Since the women having the highest serum DHEA values were those stopped at week 8, the samples obtained at week 13 for the remaining women were not retained for the microarray study. In addition, 4 of the 10 women stopped after week 8 of 2% DHEA treatment were also analyzed.

2.2. High density oligonucleotide array hybridization

Total RNA was isolated by Trizol (Invitrogen) from individual biopsies. The samples were processed following the Small Sample Labeling Protocol version II from Affymetrix. This protocol was based on the principle of performing two cycles of cDNA synthesis and in vitro transcription (IVT) reactions for target amplification. Labelled cRNA was isolated using the RNeasy Mini Kit column (QIAGEN). Purified cRNA was fragmented to 200–30 mer using a fragmentation buffer. The quality of total RNA, cDNA synthesis, cRNA amplification and cRNA fragmentation was monitored by capillary electrophoresis (Bioanalizer 2100, Agilent Technologies). Fifteen micrograms of fragmented cRNA was hybridized in duplicate for 16 h at 45 °C with constant rotation, using a human oligonucleotide array U133 Plus 2.0 (Genechip, Affymetrix, Santa Clara, CA). After hybridization, chips were processed by using the Affymetrix GeneChip Fluidic Station 450 (protocol EukGE-WS2v5_450). Staining was made with streptavidin-conjugated phycoerythrin (SAPE) (Molecular Probes), followed by

Table 1
RT-PCR primers.

Gene symbol	GenBank	Region	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Size (bp)
CIRBP	NM_001280	430–592	GAGGAGGGGACCGAGGCTATG	TTGTGTGTAGCGTAACTGTCGTAACTG	163
CLDN8	NM_199328	571–759	TACTTAGGATGGACCACGGCA	ACACAACTACACATACTGACTTCTGGA	189
COL1A1	NM_000088	4698–4876	AACCGAACATGACCAAAAACCAAAA	GGGCAGCATTGGGGTTTCATAA	173
COL5A2	NM_000393	4630–4890	AGCGGAATGGAAATGTGGGCA	TGCAGGATCAGCCATTACTTCAAGAG	261
DNASE1L3	NM_004944	950–1158	CACCACCCCAGAGACATCCGTTA	CCGTGGTGTCCTCTTGGTCCC	209
EEF2KL	NMJ13302	2792–3020	TGCACACCACCACACTCAGCJAAT	AGGGTACACACTGAACTTTGGAACTAA	229
EFNA1	NM_004428	1100–1343	GAGAGAGCCAGGATGCCCAGAT	GCTACACTCTAAGAACAGGTTGGCACA	244
ERBP	NM_014597	1848–2093	CTGCAGAAAGCCGTCATTACACCT	GGGGAAGCCATCTCTATCATTTTT	246
GBA/GBAP	NM_000157	1953–2199	AAAAGATCAGTAAGCCCCAGTGT	CCTGATGCTGACACTGCTGCT	247
JAG1	AK023793	3339–3510	AGGTTGGGGTGCTGATTTAGTG	AATAAACACCTCGAGCTCAAGCA	172
KNTC2L	NM_006101	1549–1760	TGGAGGATACTTTAGAACAATTGAATG	TTCACTGAGCCCCTGGTTAACA	212
KRT1B	BC033366	864–1096	CCTAAGCTCTGCATCATAACCACTCT	CACAGCTACAGAGAACAGAAGGCA	233
LEP7	AF005081	26–305	TCCTTGCCAGAGCTATTATGTTCA	AATTCCCAAACAGCAGCACCC	280
MT1X	NM_005952	12–232	GCGTGTTTTCCTCTTGATCGGG	GCTGCACTTGTCTGACGTCCCT	221
NOL3	NM_003946	753–1001	GGATAGGACCTGGGATGCTGCT	GGGTGCAGCCTGGACTCCTAA	249
PLLP	NM_015993	928–1152	ACGGGGGATCTGAGGCTGTGT	ACGGTGTTTAAAGGAAGGAGAGTGGTT	225
RORCL	NM_005060	2771–3031	GTGGAGAAGGAAGCAGATGTGAT	GTTGGGGTTTATGTTTACTCAGATGAA	261
RRM2	NM_001034	1435–1678	TTACCAACTAGCCACACCATGAAT	TGGCTGTGCTGGTTAAAGGACT	244
S100A8/MRP8	NM_002964	82–335	GAACTCTATCATCGACGTCTACCACAA	ACTCTTTGTGGCTTTCTTCATGGCT	254
S100A9/MRP14	NM_002965	56–334	AAATGTCGCAGCTGGAACGCA	CCTCGTGCATCTTCTCGTGGGA	279
SERPIN B3	BC005224	1036–1154	GCGGTCTCGTGCTATCTGGA	ATTAGTTGAAGTAGGTGATGATCCGAA	119
SPARC	NM_003118	1804–2056	AGGGACTGCCAGGCTGTTTCA	TGAAATGCTTGGAGGTGAACGAGT	253
SPRR2G	NM_001014291	278–511	TGAAACAACAAGATCCAGTGGCT	AACACACTTGCTCTCAGGATAGGA	234
ZNF677	AK026366	233–463	GCCCACACAATATGACCTCGG	TCCCTGTACAAGGCCCTCTGG	231

Table 2
Genes up or down-regulated by 1% or 2% DHEA ($p < 0.05$) in human skin of postmenopausal women.

Gene title	Gene symbol	Up or down		Molecular function
		DHEA 1%	DHEA 2%	
Dermis ECM				
Collagen, type V, alpha 2	COL5A2	▲	▲	Extracellular matrix structural constituent /// protein binding
Collagen, type V, alpha 1	COL5A1	n.s.	▲	Extracellular matrix structural constituent /// protein binding
Collagen, type I, alpha 1	COL1A1	▲	▲	Extracellular matrix structural constituent /// protein binding
Collagen, type I, alpha 2	COL1A2	▲	▲	Extracellular matrix structural constituent /// protein binding
Collagen, type III, alpha 1	COL3A1	▲	▲	Extracellular matrix structural constituent /// protein binding
Secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	n.s.	▲	Collagen binding /// metal ion binding
Actin, alpha 1, skeletal muscle	ACTA1	▲	▲	Structural constituent of cytoskeleton /// protein binding /// ATP–ADP binding
Actin, alpha, cardiac muscle	ACTC	▲	▲	Nucleotide binding /// protein binding /// ATP binding /// structural molecule activity
Myosin, heavy polypeptide 3	MYH3	▲	▲	Microfilament motor activity /// nucleotide binding /// actin binding
Epidermal differentiation				
Serine (or cysteine) proteinase inhibitor, clade B, member 3	SERPINB3	▲	▲	Serine-type endopeptidase inhibitor activity
Serine (or cysteine) proteinase inhibitor, clade B, member 4	SERPINB4	▲	▲	Serine-type endopeptidase inhibitor activity
S100 calcium binding protein A9 (calgranulin B)	S100A9	▲	▲	Signal transducer activity /// calcium ion binding
S100 calcium binding protein A8 (calgranulin A)	S100A8	n.s.	▲	Calcium ion binding /// calcium ion binding
Corneodesmosin	CDSN	n.s.	▼	Protein homodimerization activity
Claudin 8	CLDN8	n.s.	▼	Structural molecule activity /// protein binding
WAP four-disulfide core domain 3	WFDC3	n.s.	▼	Serine-type endopeptidase inhibitor activity /// endopeptidase inhibitor activity
Small proline-rich protein 2G	SPRR2G	▼	n.s.	Structural molecule activity
Keratin 1B	KRT1B	n.s.	▼	Structural molecule activity
Keratin 7	KRT7	▼	▼	Structural molecule activity /// protein binding
Late envelope protein 7	LEP7	▼	▼	Molecular function unknown
AHNAK nucleoprotein (desmoyokin)	AHNAK	▼	▼	Protein binding
Proliferation				
Nucleobindin 2	NUCB2	▲	▲	DNA binding /// calcium ion binding /// calcium ion binding
Sjogren syndrome antigen B (autoantigen La)	SSB	▲	▲	Nucleic acid binding /// RNA binding
Deoxyribonuclease I-like 3	DNASE1L3	n.s.	▲	DNA binding /// endonuclease activity /// calcium ion binding
Topoisomerase (DNA) II alpha 170 kDa	TOP2A	▲	▲	DNA topoisomerase (ATP-hydrolyzing) activity /// protein kinase C binding
Synaptotagmin binding, cytoplasmic RNA interacting protein	SYNCRIP	▲	n.s.	Protein binding/// nucleic acid binding
Kinetochore associated 2	KNTC2	n.s.	▲	Protein binding
Ribonucleotide reductase M2 polypeptide	RRM2	n.s.	▲	Ribonucleoside-diphosphate reductase activity /// iron ion binding /// protein binding
Transcriptional machinery				
Cold inducible RNA binding protein	CIRBP	n.s.	▼	Nucleic acid binding
RAR-related orphan receptor C	RORC	n.s.	▼	Transcription factor activity /// steroid hormone receptor activity /// zinc ion binding
Transformer-2 alpha	TRA2A	n.s.	▼	Nucleotide binding /// RNA binding /// nucleic acid binding
Eukaryotic elongation factor-2 kinase	EEF2K	n.s.	▼	Calcium ion binding /// translation factor activity, nucleic acid binding
Zinc finger protein 447	ZNF447	▼	n.s.	Transcription factor activity /// metal ion binding /// nucleic acid binding
Zinc finger protein 677	ZNF677	n.s.	▼	DNA binding /// metal ion binding /// nucleic acid binding
Nucleolar protein 3 (apoptosis repressor with CARD domain)	NOL3	▼	▼	RNA binding /// identical protein binding /// protein binding
Odd-skipped related 1 (Drosophila)	OSR1	▼	▼	Nucleic acid binding /// zinc ion binding /// metal ion binding
MutS homolog S (*E. coli*)	MSHS	▼	▼	Nucleotide binding /// damaged DNA binding /// ATP binding
Estrogen receptor binding protein	ERBP	▼	▼	Receptor activity
Miscellaneous				
Decay accelerating factor for complement (CD55)	DAF	n.s.	▼	GPI anchor binding
Carcinoembryonic antigen-related cell adhesion molecule 6	CEACAM6	n.s.	▼	GPI anchor binding
Ephrln-A1	EFNA1	n.s.	▼	Ephrin receptor binding /// GPI anchor binding
Metallothionein 1X	MT1X	n.s.	▼	Metal ion binding /// electron carrier activity
S100 calcium binding protein P	S100P	▼	▼	Metal ion binding /// calcium-dependent protein binding
Interleukin 1 family, member 7 (zeta)	IL1F7	n.s.	▼	lnterleukin-1 receptor antagonist activity /// cytokine activity
Interleukin 1 family, member 10 (theta)	IL1F10	▼	▼	lnterleukin-1 receptor antagonist activity /// cytokine activity
Glucosidase, beta; acid	GBA/GBAP	n.s.	▼	Glucosylceramidase activity /// hydrolase activity, acting on glycosyl bonds
N-acetylgalactosaminyltransferase-like 2	GALNTL2	▼	▼	Transferase activity /// metal ion binding /// sugar binding
Betacellulin	BTC	n.s.	▼	Growth factor activity
Jagged 1 (Alagille syndrome)	JAG1	▼	▼	Notch binding /// structural molecule activity /// growth factor activity
Plasma membrane proteolipid (plasmolipin)	PLLP	n.s.	▼	Ion channel activity
Inositol 1,4,5-triphosphate receptor, type 2	ITPR2	▼	▼	Ion channel activity
Unknown				
Purkinje cell protein 4	PCP4	▲	▲	Molecular function unknown

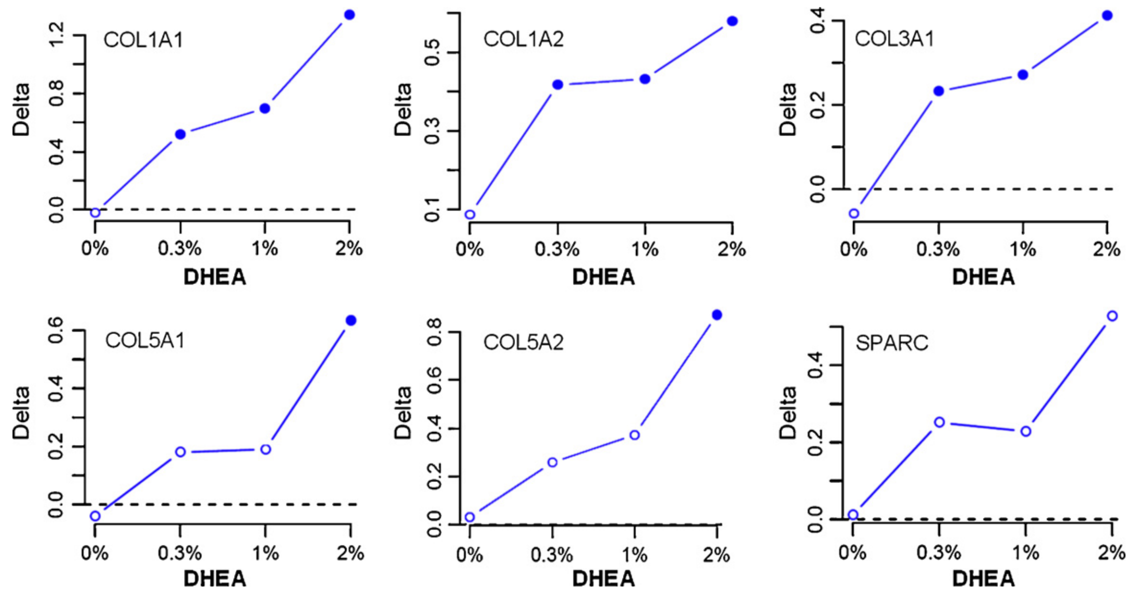

Fig. 1. DHEA stimulation of the expression profile of collagen and SPARC genes after twice daily placebo (0% DHEA), 0.3% DHEA, 1% DHEA or 2% DHEA treatment. Curves represent fold-change values (pre- and post-treatment) expressed as log2 (Delta). Data are expressed as raw signal intensity after normalization by the RMA method. Filled dots correspond to a statistically significant difference ($p < 0.05$).

amplification with a biotinylated anti-streptavidin antibody (Vector Laboratories), and by a second round of SAPE. Chips were scanned using a GeneChip Scanner 3000 G7 (Affymetrix) enabled for High-Resolution Scanning.

The arrays included 1,300,000 unique oligonucleotide features covering over 47,000 transcripts and variants, which, in turn, represent approximately 38,500 of the best characterized human genes.

Chip images were extracted with the GeneChip Operating Software and analyzed with Limma package. Quality control of microarray chips was performed using the AffyQCReport software [19]. A comparable quality between microarrays was required for all microarrays within each experiment.

The background subtraction and normalization of probe set intensities was performed using the method of Robust Multiarray Analysis (RMA) described by Irizarry et al. [20]. The analysis was performed using the affylmGUI Graphical User Interface for the Limma microarray package [21], and GeneSpring v7.2 software (Agilent).

2.3. Real-time PCR (RT-PCR)

The cDNA was reverse-transcribed from total RNA using 30 ng of Oligo(d)T primers, 300 U of Superscript III Rnase H-reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA), 500 μM dNTPs, 5 mM dithiothreitol, and 34 U of human Rnase inhibitor

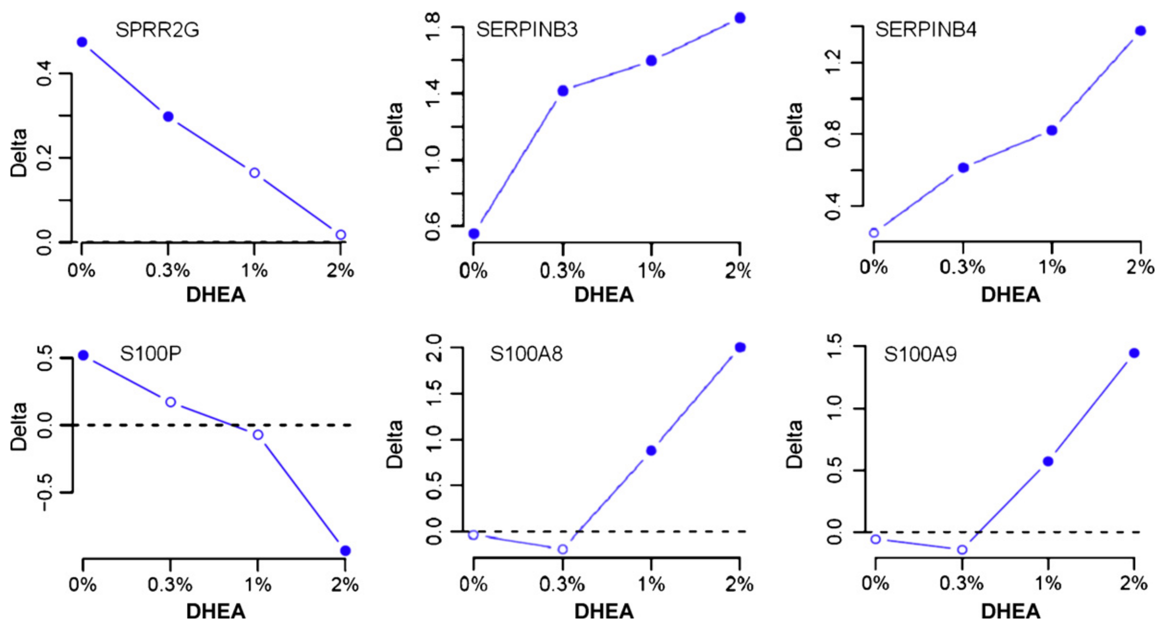

Fig. 2. DHEA modulation of keratinocyte-related genes. Data are expressed as raw signal intensity after normalization by the RMA method. Fold-change values (pre- and post-treatment) are expressed as log2 (Delta). Filled dots correspond to a statistically significant difference ($p < 0.05$).

Table 3
Validation by RT-PCR or genes modulates by 1% or 2% DHEA.

Gene title	Gene symbol	DHEA 1%			DHEA 2%		
		Arrays	RT-PC		Arrays	RT-PCR	
			Fold-change[a]	p-Value		Fold-change[a]	p-Value
S100 calcium binding protein A8 (calgranulin A)	S100A8	n.s.	20.21	<0.0001	▲	44.97	<0.0001
S100 calcium binding protein A9 (calgranulin B)	S100A9	▲	10.74	<0.0001	▲	44.95	<0.0001
Serine (or cysteine) proteinase inhibitor B3	SERPINB3	▲	6.77	<0.0001	▲	8.39	<0.0001
Kinetochore associated 2	KNTC2L	n.s.	2.38	<0.3	▲	6.44	<0.2000
Estrogen receptor binding protein	ERBP	n.s.	4.69	<0.0001	▲	4.69	<0.0001
Collagen, type I, alpha 1	COL1A1	▲	3.69	<0.0001:	▲	3.72	<0.0001
Secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	n.s.	2.68	<0.0001	▲	2.55	<0.0001
Ribonucleotide reductase M2 polypeptide	RRM2	▲	2.03	<0.0009	▲	2.5	<0.0001
Deoxyribonuclease l-like 3	DNASE1L3	n.s.	1.86	<0.0001	▲	1.57	<0.0002
Collagen, type V, alpha 2	COL5A2	▲	1.51	<0.002	▲	1.44	<0.0004
Jagged 1 (Alagille syndrome)	JAG1	▼	−0.79	<0.0024	▼	−0.69	<0.0001
Claudin8	CLDN8	n.s.	−1.44	<0.0001	▼	−1.00	<0.92
RAR-related orphan receptor C	RORC	n.s.	−0.87	<0.7	▼	−1.09	<0.64
Cold inducible RNA binding protein	CIRBP	n.s.	−1.73	<0.005	▼	−1.27	<0.045
Zinc finger protein 677	ZNF677	n.s.	−1.06	<0.05	▼	−1.43	<0.0001
Eukaryotic elongation factor-2 kinase	EEF2K	n.s	−4.94	<0.0002	▼	−1.72	<0.02
Nucleolar protein 3 (apoptosis repressor with CARD domain)	NOL3	▼	−3.31	<0.0001	▼	−1.90	<0.0001
Metallothionein 1X	MT1X	▼	−1.44	<0.0001	▼	−1.98	<0.0001
Glucosidase, beta; acid	GBA/GBAP	n.s.	−3.03	<0.0001	▼	−2.1	<0.0002
Ephrin-A1	EFNA1	n.s.	−2.09	<0.0004	▼	−2.74	<0.0001
Late envelope protein 7	LEP7	▼	−4.00	<0.0001	▼	−3.37	<0.0001
Plasma membrane proteolipid (plasmolipin)	PLLP	▼	−1.43	<0.0001	▼	−3.42	<0.0001
Keratin 1B	KRT1B	n.s.	−3.00	<0.0001	▼	−3.69	<0.0001
Small proline-rich protein 2G	SPRR2G	▼	−4.68	<0.0001	n.s.	−5.05	<0.0001

[a] Fold-change above or below control: value of fold-change between the start and end of treatment with placebo vs. fold-change between the start-end of treatment in the indicated DHEA group.

(Amersham Pharmacia, Piscataway, NJ) in 50 μl final volume as described [22]. Gene-specific primers were designed by GeneTools software (Biotools Inc.). The sequences of primers used are shown in Table 1.

The RT reaction was performed at 50 °C for 2 h, followed by a 30 min at 37 °C incubation with RNAseA and a final purification by column (QIAGEN, Valencia, CA).

RT-PCR quantitative assays was run in triplicates using the LightCycler FastStart DNA Master Plus SYBRGreen I kit (Roche Diagnostics) following the manufacturer's recommendations. PCR was performed using LightCycler (Roche Applied Science, Mannheim, Germany) for 50 cycles of 95 °C for 10 s (denaturation), 59–65 °C for 5 s (annealing), 72 °C for 10–12 s (elongation), and at 78°C for 3 s (lecture). The housekeeping gene Atp5o was used as a reference gene. Amplification specificity was checked using a melting curve following the manufacturer's instructions. Quantification was obtained using the second derivative calculation and double correction [22], and the results were analyzed with LightCycler Software v.3.5 (Roche).

3. Results

3.1. Microarray data analysis of DHEA effect on human skin transcriptome

Sixty-six probe sets corresponding to 52 well-characterized genes (represented by one or more probe sets) (Table 2) and 9 unknown sequences (EST) (data not shown) were significantly modulated ($p < 0.05$) by DHEA. Global analysis of the increased gene expression levels under the influence of DHEA showed a clear over-representation of genes associated with transcription regulation, cell proliferation and keratinocyte differentiation. Of note, the expression of extracellular matrix structural molecules, including several members of the collagen family, was stimulated by DHEA. Clearly, DHEA treatment affected both compartments of human skin present in the biopsies, i.e. epidermis and dermis.

3.2. DHEA increases the expression of several members of the collagen gene family

Among the genes up-regulated by DHEA, a dose-dependent increase in the expression of probe sets corresponding to several members of the collagen family was observed (Fig. 1). The highest level of modulation was observed for COL1A1 and A2, COL3A1 as well as the COL5A1 and A2 genes after 2% DHEA treatment (Fig. 1). Interestingly, an up-regulation of a key gene involved in the synthesis of the extracellular matrix (ECM), namely secreted protein acidic and rich in cysteine (SPARC) was also observed (Fig. 1).

3.3. DHEA modulates the expression of several genes involved in keratinocyte proliferation and differentiation

Among the genes up-regulated by DHEA, we found several genes encoding proteins involved in host defense and generally detected in the upper layers of the epidermis, including Serpins B3 (SCCA-1) and B4 (SCCA-2), S100A8 and A9 (Table 2 and Fig. 2). A whole set of genes linked to DNA remodeling and cell proliferation was also up-regulated (Table 2). On the other hand, the Small Proline-Rich Protein 2G (SPRR2G) and S100 calcium binding protein P (S100P) (Fig. 2) and Keratin 1B genes (Table 2) were down-regulated by DHEA. Additionally, desmosome-associated corneodesmosin (Cdsn), a late marker of terminal differentiation of keratinocytes [23,24] as well as Claudin-8 (Tables 2 and 3) were also down-regulated in the group of DHEA 2% measured after 8 weeks of treatment. Finally, the expression of two other genes involved in terminal differentiation of keratinocytes, namely the Late envelope protein 7 (LEP7) and the Jagged 1 (JAG1) genes, were also down-regulated by DHEA (Fig. 3).

Due to the small amount of RNA obtained from 2 mm biopsies, only 24 genes were selected for RT-PCR validation. The RT-PCR data show that the level of stimulation/repression of the selected genes agrees with the microarray data for at least one of the two highest concentrations of DHEA tested (1% or 2% DHEA) (Table 3). For

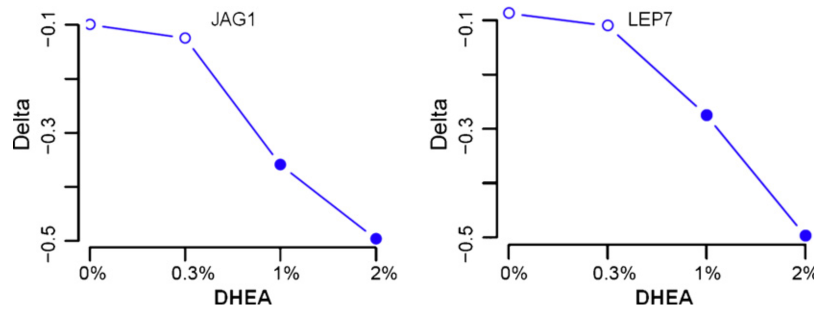

Fig. 3. DHEA down-regulation of JAG1 and LEP7 gene expression. Data are expressed as raw signal intensity after normalization by the RMA method. Fold-change values (pre- and post-treatment) are expressed as log2 (Delta). Filled dots correspond to a statistically significant difference ($p < 0.05$).

example, we have indeed found a progressive increase of SPARC mRNA, starting at 0.3% DHEA (Fig. 1), and confirmed by RT-PCR, for the groups 1% and 2% DHEA (Table 3). In addition, down-regulation of keratinocyte differentiation was also confirmed as LEP-7 in the 1% DHEA group as well as SPRR2G, in the 2% DHEA group measured after 8 weeks of treatment. On the whole, only 2 out of 25 gene changes were not validated by RT-PCR (i.e. KNTCL2 and RORc).

Altogether, these results show that, in response to DHEA treatment, the balance between epidermal proliferation and differentiation is modified in favor of proliferation, while structural dermal genes are activated.

4. Discussion

The present study is the first comprehensive analysis and characterization of the changes induced by DHEA in the transcription profile of the whole genome in human skin. Such an approach is likely to provide significant insights into the potential role of DHEA in skin aging.

The thinning and wrinkling of the skin [25] and the loss of skin elasticity and firmness [26] are believed to be caused by decreased collagen synthesis and increased collagen degradation [27]. Considering that the dermis predominantly contains type I and III collagens (85–90% and 10–15% of total proteins, respectively), the alterations of collagen are thus believed to be the causes of clinical changes during aging, especially skin wrinkles and loss of elasticity [28,29].

With increasing age, a decrease in collagen biosynthesis is observed, concomitant with increased collagen degradation by matrix metalloproteinases (MMP) [30]. In fact, collagen content has been shown to decrease 2% per postmenopausal year [31]. In agreement with these observations, collagen types I and III are believed to have decreased by as much as 30% 5 years after menopause [32,33]. In addition, a reduction in the type III/type I ratio is observed in the dermis at menopause [33].

Some effects of DHEA on collagen gene expression and protein synthesis have already been described. For example, DHEA increased human α1 (s) procollagen and reduced collagenase (MMP-2) gene expression in cultured skin fibroblasts [34], while in human skin, DHEA treatment up-regulated procollagen type 1 and TIMP-1 expression and down-regulated MMP-1 [16]. Here we show that in whole human skin, DHEA indeed stimulates the expression of not only COL1A1, COL1A2 but also other members of the family of collagen genes, namely COL3A1 and COL5A2. Interestingly, the co-stimulation of both COL1A1 and COL1A2 genes suggest that common cis-acting elements are present on both genes [35] and modulated in parallel in human skin by DHEA.

In fact, in most tissues, type III collagen is co-expressed with type I collagen and, under physiological conditions, the amount of collagen III regulates the diameter of type I collagen fibrils, thus controlling the mechanical characteristics of the tissue [36,37]. In addition, collagen V is a quantitatively minor component of most connective tissues that are rich in collagen I fibrils. On the other hand, both collagens I and V co-polymerize to form heterotypic fibrils. In analogy with collagen III, it has been suggested that collagen V could serve as a negative regulator of collagen I fibril diameter (reviewed by Fichard et al. [38]). Finally, together with COL1A1 and A2, COL3A1, COL5A1 and A2, SPARC expression was also stimulated by DHEA treatment. Accordingly, a remarkable parallelism (see Fig. 1) was observed between the expression of SPARC and almost all mRNAs encoding collagens which bind this glycoprotein, namely COL1A1, COL1A2, COL3A1, COL5A1 and COL5A2.

Since SPARC is known to be required for collagen deposition and collagen fiber maturation in the dermal ECM, the synthesis of the ECM particularly during matrix remodeling [39] and the maintenance of skin mechanical strength [40], the co-stimulatory effect of DHEA on COL1, COL3, COL5 and SPARC expression could result into a remodeling of dermis architecture and increased firmness of the skin. Interestingly, these up-regulated genes are also detected during the repair process of wound healing [41]. Their DHEA-induced up-regulation thus suggests that DHEA-treated skin could share some features with wound healing skin, linked to keratinocyte proliferation and delayed differentiation.

For example, S100A8 and S100A9, also known as calgranulin A or MRP8 and calgranulin B or MRP14, respectively, are two additional genes that are up-regulated by topical treatment with DHEA. These components are the most abundant naturally occurring homo- and heterodimers of the S100 family [42]. Interestingly, their expression is increased during epidermal repair [43] and the hyperproliferative phase of wound healing [44]. In line with this, DHEA treatment also decreases significantly the levels of Keratin 1, a well known marker of early differentiation of keratinocytes [45,46], as well as SPRR2G, a member of the SPRRs family preferentially expressed in the suprabasal cells of keratinized epithelia, and localized essentially in the spinous cells [47,48].

In addition to the above-mentioned early differentiation markers, we have found that desmosome-associated corneodesmosin (Cdsn), another late marker of terminal differentiation of keratinocytes [23,24], is also down-regulated in the group of 2% DHEA examined after 8 weeks of treatment. Claudins, like Cdsn, are located in the cells of the granular layer of the epidermis, and colocalize into tight junctions (TJs) with TJs plaque proteins and occludines [49]. The present study shows that claudin-8 expression is also down-regulated by DHEA treatment, together with two other genes involved in keratinocyte terminal differentiation, namely Jagged 1 (JAG1) and LEP7. JAG1 is one of the mediators of the Notch signaling pathway [50], sufficient to trigger terminal differentiation and corneogenesis in a living human epidermal equivalent model system [51]. LEP7, also identified as Chromosome 1 open-reading frame 68 (C1orf68), or Human skin-specific protein (xp32

gene) [52] is induced during keratinocyte terminal differentiation and relocates to the envelope very late in terminal differentiation. The concomitant down-regulation of JAG1, LEP7, Claudin-8, Cdsn, SPRR2G, all genes associated with the late keratinocyte differentiation and cornification process, may indicate that the effect of DHEA on epidermis could be mediated by a delay in keratinocyte differentiation, reducing the transition rate from spinous to granular cell and ultimately the production of the cornified component of the skin.

Interestingly, several genes modulated by DHEA like S100A8, S100A9, SPRR2G and LEP7 are co-localized in the Epidermal Differentiation Cluster (EDC) on human chromosome 1q21 [53], which unites a remarkable number of functionally related genes that play an important role in terminal differentiation of the human epidermis [54]. Further studies will however be necessary to assess the real impact of DHEA in keratinocyte differentiation, and ultimately, in skin barrier properties.

Finally, almost all genes modulated by 1% DHEA after 12 weeks were also modulated in the same way by 2% DHEA after 8 weeks of treatment. Additionally, all the genes significantly modulated only by 2% DHEA but non-significantly changed by 1% DHEA, were found significantly modulated when a more sensitive method, namely RT-PCR, was used with the same samples (Tables 2 and 3). Moreover, certain genes, especially the collagen genes and members of the SERPIN family appear very sensitive at the lowest dose of DHEA used (Figs. 1 and 2, respectively). These observations could suggest that topical DHEA may trigger genomic changes in the skin even at low doses (0.3% DHEA) and that the effect of high doses (2% DHEA) may be reached by lower doses after longer exposure time.

Taken together, the present data suggest that DHEA could counteract the effect of aging by acting simultaneously on both skin compartments namely the dermis, by stimulating collagen biosynthesis and deposition, and in the epidermis by modulating keratinocyte proliferation and differentiation. Some of the effects observed are reminiscent of wound healing, thus suggesting that DHEA treatment could potentially lead to apparent skin rejuvenation. It is recognized that the skin undergoes regressive changes after menopause and that these changes are mainly related to a loss of skin collagen content. The present data suggest that DHEA could exert anti-aging effects in the skin, secondary to DHEA-induced changes in the structural organization of the dermis.

Conflicts of interest

None declared.

References

[1] M. Samuel, R.C.C. Brooke, S. Hollis, C.E.M. Griffiths, Interventions for photodamaged skin, Cochrane Database System Rev (2005), CD001782. doi:10.1002/14651858.CD001782.pub2.

[2] C. Labrie, A. Bélanger, F. Labrie, Androgenic activity of dehydroepiandrosterone and androstenedione in the rat ventral prostate, Endocrinology 123 (1988) 1412–1417.

[3] F. Labrie, Intracrinology, Mol. Cell. Endocrinol. 78 (1991) C113–C118.

[4] F. Labrie, V. Luu-The, A. Bélanger, S.-X. Lin, J. Simard, C. Labrie, Is DHEA a hormone? Starling review, J. Endocrinol. 187 (2005) 169–196.

[5] F. Labrie, A. Bélanger, P. Bélanger, R. Bérubé, C. Martel, L. Cusan, J.L. Gomez, B. Candas, I. Castiel, V. Chaussade, C. Deloche, J. Leclaire, Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women, J. Steroid Biochem. Mol. Biol. 99 (2006) 182–188.

[6] Women's Health Initiative, Risks and benefits of estrogen plus progestin in healthy postmenopausal women, JAMA 288 (2002) 321–333.

[7] V. Beral, D. Bull, G. Reeves, Endometrial cancer and hormone-replacement therapy in the Million Women Study, Lancet 365 (2005) 1543–1551.

[8] F. Labrie, V. Luu-The, C. Labrie, G. Pelletier, M. El-Alfy, Intracrinology and the skin, Horm. Res. 54 (2000) 218–229.

[9] R. Hoffman, S. Niiyama, A. Huth, S. Kissling, R. Happle, 17alpha-estradiol induces aromatase activity in intact human anagen hari follicles ex vivo, Exp. Dermatol. 4 (2002) 376–380.

[10] S. Gingras, C. Turgeon, N. Brochu, F. Labrie, J. Simard, Characterization and modulation of sex steroid metabolizing activity in normal human keratinocytes in primary culture and hacat cells, J. Steroid Biochem. Mol. Biol. 87 (2003) 167–179.

[11] F. Labrie, V. Luu-The, C. Labrie, A. Bélanger, J. Simard, S.-X. Lin, G. Pelletier, Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone, Endocr. Rev. 24 (2003) 152–182.

[12] F. Labrie, P. Diamond, L. Cusan, J.L. Gomez, A. Belanger, B. Candas, Effect of 12-month dehydroepiandrosterone replacement therapy on bone, vagina, and endometrium in postmenopausal women, J. Clin. Endocrinol. Metab. 82 (1997) 3498–3505.

[13] E.E. Baulieu, G. Thomas, S. Legrain, N. Lahlou, M. Roger, B. Debuire, V. Faucounau, L. Girard, M.P. Hervy, F. Latour, M.C. Leaud, A. Mokrane, H. Pitti-Ferrandi, C. Trivalle, O. de Lacharriere, S. Nouveau, B. Rakoto-Arison, J.C. Souberbielle, J. Raison, Y. Le Bouc, A. Raynaud, X. Girerd, F. Forette, Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 4279–4284.

[14] K. Sakyo, A. Ito, Y. Mori, Effects of dehydroepiandrosterone sulphate on the production of collagenase and gelatinolytic metalloproteinase by rabbit uterine cervical cells in primary cultures, J. Pharmacobiodyn. 9 (1986) 276–286.

[15] K. Yoshida, R. Tahara, T. Nakayama, T. Yanaihara, Effect of dehydroepiandrosterone sulphate, oestrogens and prostaglandins on collagen metabolism in human cervical tissue in relation to cervical ripening, J. Int. Med. Res. 21 (1993) 26–35.

[16] M.H. Shin, G.E. Rhie, C.H. Park, K.H. Kim, K.H. Cho, H.C. Eun, J.H. Chung, Modulation of collagen metabolism by the topical application of dehydroepiandrosterone to human skin, J. Invest. Dermatol. 124 (2005) 315–323.

[17] F. Labrie, V. Luu-The, E. Calvo, C. Martel, J. Cloutier, S. Gauthier, P. Belleau, H. Boucher, J. Morissette, M.H. Lévesque, C. Labrie, Tetrahydrogestrione (THG) induces a genomic signature typical of a potent anabolic steroid, J. Endocrinol. 184 (2) (2005) 427–433.

[18] F. Labrie, V. Luu-The, C. Martel, A. Chernomoretz, E. Calvo, J. Morissette, C. Labrie, DHEA is an anabolic steroid like testosterone and THG, J. Steroid Biochem. Mol. Biol. 100 (2006) 52–58.

[19] L. Gautier, L. Cope, B.M. Bolstad, R.A. Irizarry, Affy—analysis of Affymetrix GeneChip data at the probe level, Bioinformatics 20 (2004) 307–315.

[20] R.A. Irizarry, B. Hobbs, F. Collin, Y.D. Beazer-Barclay, K.J. Antonellis, U. Scherf, T.P. Speed, Exploration, normalization, and summaries of high density oligonucleotide array probe level data, Biostatistics 4 (2003) 249–264.

[21] J.M. Wettenhall, K.M. Simpson, K. Satterley, G.K. Smyth, AffylmGUI: a graphical user interface for linear modeling of single channel microarray data, Bioinformatics 22 (2006) 897–899.

[22] V. Luu-The, G. Pelletier, F. Labrie, Quantitative appreciation of steroidogenic gene expression in mouse tissues: new roles for type 2 5alpha-reductase, 20alpha-hydroxysteroid dehydrogenase and estrogen sulfotransferase, J. Steroid Biochem. Mol. Biol. 93 (2005) 269–276.

[23] M. Simon, N. Jonca, M. Guerrin, M. Haftek, D. Bernard, C. Caubet, T. Egelrud, R. Schmidt, G. Serre, Refined characterization of corneodesmosin proteolysis during terminal differentiation of human epidermis and its relationship to desquamation, J. Biol. Chem. 276 (2001) 20292–20299.

[24] N. Jonca, M. Guerrin, K. Hadjiolova, C. Caubet, H. Gallinaro, M. Simon, G. Serre, Corneodesmosin, a component of epidermal corneocyte desmosomes, displays homophilic adhesive properties, J. Biol. Chem. 277 (2002) 5024–5029.

[25] C.Y. Tan, B. Statham, R. Marks, P.A. Payne, Skin thickness measurement by pulsed ultrasound: its reproducibility, validation and variability, Br. J. Dermatol. 106 (1982) 657–667.

[26] F. Henry, C. Pierard-Franchimont, G. Cauwenbergh, G.E. Pierard, Age-related changes in facial skin contours and rheology, J. Am. Geriatr. Soc. 45 (1997) 220–222.

[27] G.J. Fisher, S. Kang, J. Varani, Z. Bata-Csorgo, Y. Wan, S. Datta, J.J. Voorhees, Mechanisms of photoaging and chronological skin aging, Arch. Dermatol. 138 (2002) 1462–1470.

[28] G.J. Fisher, Z.Q. Wang, S.C. Datta, J. Varani, S. Kang, J.J. Voorhees, Pathophysiology of premature skin aging induced by ultraviolet light, N. Engl. J. Med. 337 (1997) 1419–1428.

[29] J. Varani, R.L. Warner, M. Gharaee-Kermani, S.H. Phan, S. Kang, J.H. Chung, Z.Q. Wang, S.C. Datta, G.J. Fisher, J.J. Voorhees, Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin, J. Invest. Dermatol. 114 (2000) 480–486.

[30] G.J. Fisher, S.C. Datta, H.S. Talwar, Z.Q. Wang, J. Varani, S. Kang, J.J. Voorhees, Molecular basis of sun-induced premature skin ageing and retinoid antagonism, Nature 379 (1996) 335–339.

[31] M. Brincat, E. Versi, C.F. Moniz, A. Magos, J. de Trafford, J.W. Studd, Skin collagen changes in postmenopausal women receiving different regimens of estrogen therapy, Obstet. Gynecol. 70 (1987) 123–127.

[32] M. Brincat, C.J. Moniz, J.W. Studd, A. Darby, A. Magos, G. Emburey, E. Versi, Long-term effects of the menopause and sex hormones on skin thickness, Br. J. Obstet. Gynaecol. 92 (1985) 256–259.

[33] P. Affinito, S. Palomba, C. Sorrentino, C. Di Carlo, G. Bifulco, M.P. Arienzo, C. Nappi, Effects of postmenopausal hypoestrogenism on skin collagen, Maturitas 33 (1999) 239–247.

[34] K.S. Lee, K.Y. Oh, B.C. Kim, Effects of dehydroepiandrosterone on collagen and collagenase gene expression by skin fibroblasts in culture, J. Dermatol. Sci. 23 (2000) 103–110.
[35] G. Karsenty, R.W. Park, Regulation of type I collagen genes expression, Int. Rev. Immunol. 12 (1995) 177–185.
[36] X. Liu, H. Wu, M. Byrne, S. Krane, R. Jaenisch, Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 1852–1856.
[37] K. Stevenson, U. Kucich, C. Whitbeck, R.M. Levin, P.S. Howard, Functional changes in bladder tissue from type III collagen-deficient mice, Mol. Cell. Biochem. 283 (2006) 107–114.
[38] A. Fichard, J.P. Kleman, F. Ruggiero, Another look at collagen V and XI molecules, Matrix Biol. 14 (1995) 515–531.
[39] J.P. Alexander, J.R. Samples, E.M. Van Buskirk, T.S. Acott, Expression of matrix metalloproteinases and inhibitor by human trabecular meshwork, Invest. Ophthalmol. Vis. Sci. 32 (1991) 172–180.
[40] A.D. Bradshaw, P. Puolakkainen, J. Dasgupta, J.M. Davidson, T.N. Wight, E. Helene Sage, SPARC-null mice display abnormalities in the dermis characterized by decreased collagen fibril diameter and reduced tensile strength, J. Invest. Dermatol. 120 (2003) 949–955.
[41] I. Hopkinson, W. Evans, D. Chant, S. Hiscox, D. Berry, K. Harding, Reverse transcription-polymerase chain reaction detection of collagen transcripts in healing human wounds, Eur. J. Clin. Invest. 25 (1995) 539–542.
[42] R.L. Eckert, A.M. Broome, M. Ruse, N. Robinson, D. Ryan, K. Lee, S100 proteins in the epidermis, J. Invest. Dermatol. 123 (2004) 23–33.
[43] C. Marionnet, F. Bernerd, A. Dumas, F. Verrecchia, K. Mollier, D. Compan, B. Bernard, M. Lahfa, J. Leclaire, C. Medaisko, B. Mehul, S. Seite, A. Mauviel, L. Dubertret, Modulation of gene expression induced in human epidermis by environmental stress in vivo, J. Invest. Dermatol. 121 (2003) 1447–1458.
[44] I.S. Thorey, J. Roth, J. Regenbogen, J.P. Halle, M. Bittner, T. Vogl, S. Kaesler, P. Bugnon, B. Reitmaier, S. Durka, A. Graf, M. Wockner, N. Rieger, A. Konstantinow, E. Wolf, A. Goppelt, S. Werner, The Ca^{2+}-binding proteins S100A8 and S100A9 are encoded by novel injury-regulated genes, J. Biol. Chem. 276 (2001) 35818–35825.
[45] H. Green, E. Fuchs, F. Watt, Differentiated structural components of the keratinocyte, Cold Spring Harb. Symp. Quant. Biol. 46 (Pt 1) (1982) 293–301.
[46] S.H. Yuspa, A.E. Kilkenny, P.M. Steinert, D.R. Roop, Expression of murine epidermal differentiation markers is tightly regulated by restricted extracellular calcium concentrations in vitro, J. Cell. Biol. 109 (1989) 1207–1217.
[47] M. Hwang, A. Kalinin, M.I. Morasso, The temporal and spatial expression of the novel Ca^{++}-binding proteins, Scarf and Scarf2, during development and epidermal differentiation, Gene Expr. Patterns 5 (2005) 801–808.
[48] E.B. Lane, Keratins, in: P.M. Royce, B.U. Steinmann (Eds.), Connective Tissue and its Heritable Disorders: Molecular, Genetic, and Medical Aspects, Wiley-Liss, New York, 1993, pp. 237–247.
[49] J.M. Brandner, S. Kief, C. Grund, M. Rendl, P. Houdek, C. Kuhn, E. Tschachler, W.W. Franke, I. Moll, Organization and formation of the tight junction system in human epidermis and cultured keratinocytes, Eur. J. Cell. Biol. 81 (2002) 253–263.
[50] C.E. Lindsell, C.J. Shawber, J. Boulter, G. Weinmaster, Jagged: a mammalian ligand that activates Notch1, Cell 80 (1995) 909–917.
[51] B.J. Nickoloff, J.Z. Qin, V. Chaturvedi, M.F. Denning, B. Bonish, L. Miele, Jagged-1 mediated activation of notch signaling induces complete maturation of human keratinocytes through NF-kappaB and PPARgamma, Cell. Death Differ. 9 (2002) 842–855.
[52] X.P. Zhao, J.T. Elder, Positional cloning of novel skin-specific genes from the human epidermal differentiation complex, Genomics 45 (1997) 250–258.
[53] A.P. South, A. Cabral, J.H. Ives, C.H. James, G. Mirza, I. Marenholz, D. Mischke, C. Backendorf, J. Ragoussis, D. Nizetic, Human epidermal differentiation complex in a single 2.5 Mbp long continuum of overlapping DNA cloned in bacteria integrating physical and transcript maps, J. Invest. Dermatol. 112 (1999) 910–918.
[54] D. Mischke, B.P. Korge, I. Marenholz, A. Volz, A. Ziegler, Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ("epidermal differentiation complex") on human chromosome 1q21, J. Invest. Dermatol. 106 (1996) 989–992.