Transcriptional Regulators of Steroidogenesis, DAX-1 and SF-1, are Expressed in Human Skin

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DAX-1 and SF-1 are members of the orphan nuclear receptor superfamily that are critical regulatory components of the hypothalamic-pituitary-adrenal-gonadal axis. In adrenal and gonadal tissues they regulate the expression of the cytochrome P450 steroid hydroxylase genes, key mediators of steroidogenesis. The identification of a number of steroid hydroxylases in human skin prompted us to investigate the presence of DAX-1 and SF-1. Immunohistochemical analysis of human skin revealed a distinctive staining pattern for DAX-1 and SF-1 in skin and its appendages. Prominent staining for DAX-1 was confined to the epidermis, sebaceous glands, sweat glands, and outer root sheath of the hair follicle with weaker expression in the inner root sheath, matrix cells, and dermal papilla cells. Similarly, SF-1 was also detected in the epidermis but displayed a scattered nuclear pattern across all layers. SF-1 immunoreactivity was also detected in the exocrine glands and was stronger than DAX-1 in the inner root sheath, matrix cells, and dermal papilla cells. Co-localization of DAX-1 and SF-1 was demonstrated by immunocytochemistry in the HaCaT keratinocyte cell line, primary keratinocytes, preadipocytes, and dermal papilla cells. Reverse transcriptase-polymerase chain reaction analysis demonstrated the expression of DAX-1 and SF-1 mRNA in whole human skin and Western analysis also confirmed the presence of DAX-1 protein in skin-derived cells. Our investigations demonstrate that two important regulators of steroidogenesis are present in human skin and its appendages. These transcription factors may have a role in cutaneous steroidogenesis and thus be involved in hair follicle cycling or pathologies associated with steroids. Further studies are needed to determine the functional roles of DAX-1 and SF-1 in human skin.

Keywords: adrenal/epidermis/hair/nuclear receptor/steroids.

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The cytochrome P450 steroid hydroxylases are a group of enzymes predominantly located in the adrenal cortex and gonads, where they act in a sequential cascade converting cholesterol, the common precursor of steroid hormones, into biologically active steroids. The steroid hormones synthesized by these enzymes are involved in a plethora of physiologic and developmental processes, including ion balance, metabolism, sexual development, and reproductive function.

Studies of several mouse steroid hydroxylase promoters led to the identification and cloning of an orphan nuclear receptor, steroidogenic factor-1 (SF-1) (Lala et al, 1992), also known as adrenal 4 binding protein (Ad4BP) (Honda et al, 1993). It was found to induce the expression of all the steroid hydroxylases by binding as a monomer to consensus estrogen receptor half site (AGGTCA) regulatory elements in their gene promoters. Disruption of Ftzf1, the gene that encodes murine SF-1, causes adrenal gland and gonadal deficiency accompanied by prominent XY sex reversal (Luo et al, 1994), which is consistent with the phenotype recently detected in a female XY human who had a heterozygous mutation in the FTZ-F1 gene (Achermann et al, 1999). These studies demonstrated the critical role that SF-1 plays in the development and function of these endocrine organs.

SF-1 has also been found to regulate the expression of other components of the hypothalamic-pituitary-gonadal-adrenal axis such as the adrenocorticotrophic hormone receptor (ACTH-R), the gonadotrophin-releasing hormone receptor (GnRH-R), the β-subunit of luteinizing hormone (LHβ) and follicle stimulating hormone β-subunit (FSHβ), the cholesterol transporter sterol regulatory element binding protein (SREBP), and Mullerian inhibiting substance, a member of the transforming growth factor β family (reviewed in Morohashi, 1999). In addition, SF-1 is important for the regulation of the transcriptional repressor, dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1; SF-1, steroidogenic factor-1.
directly with SF-1 to repress its transcriptional activity (Ito et al., 1997; Zazopoulos et al., 1997). DAX-1 is also able to block the transcriptional activity of other proteins involved in steroidogenesis and thus impede the steroidogenic process. One such regulated gene includes StAR, a protein that mediates the first step of steroidogenesis by transporting extramitochondrial cholesterol into the inner mitochondrial membrane for conversion into pregnenolone by CYP11A1 (Stocco and Sodeman, 1991; Zazopoulos et al., 1997; Reinhart et al., 1999).

In addition to steroidogenic tissues, Asa et al. (1996) showed preferential SF-1 expression in human anterior pituitary gonadotropes, and Ramayya et al. (1997) reported the presence of SF-1 mRNA transcripts by northern blot analysis in the spleen and widespread expression throughout many components of the human brain and central nervous system. Similarly, DAX-1 has also been detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in nonsteroidogenic tissues, including human adult and fetal brain, pituitary, and hypothalamus, in addition to its prevalent expression in steroidogenic tissues (Guo et al., 1995).

Human skin is a target tissue for the steroid hormones released from and tightly controlled by the hypothalamic-pituitary-adrenal-gonadal axis. The capacity for skin to intracellularly metabolize weak circulating androgens such as dehydroepiandrosterone (DHEA) and androstenedione into the more potent forms, testosterone and dihydrotestosterone (DHT), has been well characterized and these hormones are known to be involved in the regulation of human hair growth. In contrast, it has not yet been proved that human skin is an organ that actively participates in de novo steroid biosynthesis from cholesterol, even though most of the essential components required for androgen and estrogen synthesis, such as cholesterol and the steroidogenic enzymes, namely CYP11A1, CYP17, CYP19, 3β-HSD, 17β-HSD, and StAR, have been identified in human skin (Crouchay et al., 1996; Sloominski et al., 1996; Venencie et al., 1999).

The identification and localization of all the cytochrome P450 steroid hydroxylases in human skin prompted us to investigate whether their expression in skin is controlled in a similar manner as found in the adrenal cortex. In this study, we have shown for the first time using immunodetection, reverse transcription and polymerase chain reaction (RT-PCR) techniques that DAX-1 and SF-1 appear to be expressed in human skin and its appendages. Our findings also show some differences in expression, in particular, DAX-1 immunoactivity is predominantly localized to basal cells in the epidermis, whereas SF-1 immunoactivity is found in all layers except the stratum corneum.

MATERIALS AND METHODS

Cell culture and tissues
Primary human skin keratinocytes were cultured on an irradiated mouse 3T3 feeder layer as previously described (Navarra et al., 1994) in medium consisting of a mixture of Dulbecco's modified Eagle's medium and Ham's F12 in a ratio of 3:1 (vol/vol), supplemented with 10% (vol/vol) fetal calf serum, 0.4 μg hydrocortisone per ml, 10 μg/mL choler toxin, 10 ng epidermal growth factor per ml, 100 μg streptomycin per ml, and 5 μg insulin per ml. HaCaT cells, a spontaneously immortalized keratinocyte cell line (Boukamp et al., 1988), were cultured using the same medium as described above but in the absence of a 3T3 feeder layer. The primary keratinocytes and HaCaT cells were cultured in a same medium as described above but in the absence of a 3T3 feeder layer. 1% v/v glutamine.

Cell culture and tissues

Facelift skin was obtained from male patients undergoing surgery and was immediately snap-frozen in liquid nitrogen and stored at −80°C until required for hair follicle isolation, sectioning, and protein and RNA extraction. Approval by the East London and City Health Authority Research Ethics Committee (number: T/98/008) for the use of redundant human skin was given. Hair follicles were isolated from facelift skin and transiently maintained in culture as previously described (Philpott et al., 1993). Isolation of dermal papilla cells from the isolated hair follicles was carried out according to the method of Jahoda et al. (1984). Briefly, the follicle bulb was dissected away from the isolated hair follicles by means of a scalpel. Using a sterile syringe needle the dermal papilla cells were microdissected out and seeded into T25 tissue culture flasks containing Williams E media (Sigma, Poole, U.K.), supplemented with 15% (vol/vol) fetal calf serum, 100 μg hydrocortisone per ml, 100 units penicillin G per ml (Sigma), 100 μg streptomycin per ml, and 2 mM glutamine. The cells were cultured in a humidified atmosphere at 37°C with 5% CO2 until they reached 70% confluence, after which they were split one in five. Passage two cells were harvested and used for immunocytochemistry and protein extraction.

RNA isolation
Total RNA was isolated from snap-frozen facelift skin using TRIzol LS Reagent (Gibco BRL, Life Technologies) and from cell cultures using the Qiagen RNeasy Mini kit (Qiagen, Crawley, U.K.). Removal of genomic DNA from RNA extracted using either method was treated with DNase I (Gibco BRL, Life Technologies), followed by ethanol precipitation to obtain concentrated RNA. After resuspension in RNase-free water the DNase I was removed using the cleanup protocol from the RNeasy mini kit. The concentration of RNA was estimated by measuring the absorbance at 260 nm and stored at −70°C until required.

RT-PCR
First-strand DNA synthesis was carried out using a First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). Briefly, 5 μg of total RNA in 20 μl of RNase-free water was heated to 65°C for 10 min and chilled on ice, after which it was added to 11 μl of Bulk First-Strand Reaction Mix, 1 μl of a 5 μg per ml Not I-d(T)11 bifunctional primer solution and 1 μl of a 200 mM dithiothreitol solution. The total reaction mix was incubated at 37°C for 1 h and stored at −20°C until use. For PCR, 5 μl of First-strand cDNA were directly pipetted into a mastermix composed of 5 μl of 10 × PCR buffer (Amersham Pharmacia Biotech), 2.5 units of Tag DNA polymerase (Amersham Pharmacia Biotech), 20 nM nucleotide mix (Amersham Pharmacia Biotech), and 40 pmol of primers made up to a total volume of 50 μl using water. PCR of the SF-1 product also required the addition of 5% (vol/vol) dimethyl sulfoxide to the mastermix to remove any secondary structures present in the cDNA causing low GC-rich regions, of the primers used were designed to have an intron spanning and included SF-1 forward 5'-CCTCATCCGGTG-TGAGACG-3' (nucleotide 987, GenBank accession: D84206), and SF-1 reverse 5’-GGTGCACGTGTAGTCTGTTG-3' (nucleotide 371, GenBank accession: D84207), which amplify a product of 198 bp, DAX-1 forward 5’-AAGGATACGGCTACCTCCA-3', and DAX-1 reverse 5’-TCCATGCTGACTGTGCCGAT-3' (nucleotides 1359–1592, GenBank accession: S74720), which amplify a product of 251 bp. Prior to amplification, the PCR reactions were heated for 3 min at 94°C to remove any secondary structures present in the cDNA-RNA heteroduplex. Amplification was carried out for 35 cycles, with denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s in a Perkin-Elmer Thermocycler. PCR products were separated by electrophoresis on a 1.6% agarose gel accompanied by a φX174 DNA Hae III digest molecular weight marker (Promega, Hants, U.K.) to determine the size of products, and visualized with ethidium bromide. The PCR products were purified using the QiAquick gel extraction kit (Qiagen, Crawley, U.K.) and sequenced on both strands using the ABI Prism BigDye Terminator cycle sequencing ready reaction kit (PE Applied Biosystems, Warrington, U.K.). The human adrenocortical H295R cell line was used as a positive control for RT-PCR, as they have previously been shown to express DAX-1 by immunofluorescence (Lalli et al., 2000) and SF-1 by northern analysis (Leers-Sucheta et al., 1997). DNA sequencing of products amplified from skin and H295R cDNA confirmed their identity with SF-1 and DAX-1 on the GenBank database.

Immunohistochemistry
Six-micrometer thick longitudinal sections were cut from hair-bearing human male scalp skin and mounted on superfrost microscope slides. The slides were stored at −80°C until...
needed for immunohistochemistry. The slides were left to air-dry at room temperature for 20 min followed by treatment in 3% (vol/vol) H2O2 in methanol for 15 min to block endogenous peroxidase activity. The sections were then treated with 0.1% (vol/vol) Triton X-100 in PBS for 15 min. The sections were subsequently washed in TBS, followed by incubation with 0.3% (wt/vol) hydrogen peroxide in methanol for 15 min. The sections were washed in TBS twice before incubation with 10% (wt/vol) normal goat serum for 30 min. The sections were then incubated with the primary antibody for 1 h at room temperature. The sections were then washed in TBS/0.1% (vol/vol) Tween-20 for 5 min each, and subsequently incubated with goat anti-rabbit horseradish peroxidase (DAKO, Cambridge, U.K.) at 0.03 μg per ml in blocking buffer for 15 min. The sections were washed three times with TBS/0.1% (vol/vol) Tween-20, and the coverslips were washed three times with TBS. Cell nuclei were stained with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) in TBS, and mounted in DePex mounting medium (BDH Laboratory Supplies, Poole, U.K.). Omission of the primary antibody for negative controls resulted in no detection demonstrating specificity of the primary antibodies (data not shown).

Immunocytochemistry

Dermal papilla cells, HaCaT cells, normal human keratinocytes, preadipocytes, and αT3-1 cells were trypsinized and seeded at a density of 1 × 106 cells on to flame-sterilized glass coverslips that had been placed in 6-well tissue culture plates (Vector Laboratories) for 45 min at room temperature. The sections were subsequently washed in TBS followed by incubation with an avidin-biotin-peroxidase complex (Vector Laboratories) for 45 min at room temperature. Immunoreactivity was visualized using diaminobenzidine (DAB) (BioGenex, San Ramon, CA) as a chromogenic substrate in H2O2. Sections were dehydrated and then mounted in Permount mounting medium (Biomax, Inc.,字

RESULTS

Immunolocalization of DAX-1 and SF-1 in human skin

Immunohistochemistry on human facelift skin sections for DAX-1 and SF-1 revealed distinctive differential nuclear staining for both transcription factors in specific regions of the human hair follicle and skin (Fig 1). Within the epidermis, prominent nuclear and cytoplasmic DAX-1 expression was detected in the basal layer, with decreasing expression towards the differentiated suprabasal keratinocytes (Fig 1a), whereas nuclear SF-1 immunoreactivity was diffuse across all strata of the epidermis, namely the stratum basale, stratum spinosum, and stratum granulosum (Fig 1b).

Immunolocalization for both transcription factors was also apparent in the dermal fibroblasts (Fig 1a, b). In the human hair follicle, there was a distinguishable difference in DAX-1 and SF-1 staining (Fig 1c, d). Strong nuclear staining for DAX-1 was confined to the outer root sheath with weaker expression in the inner root sheath, matrix cells (Fig 1c) and significantly lower immunoreactivity in the dermal papilla cells (Fig 1c, e). There was no difference in distribution for SF-1 throughout the hair follicle in comparison with DAX-1 expression, but the levels of immunoreactivity for SF-1 in the outer root sheath, inner root sheath, matrix cells (Fig 1d), and dermal papilla cells of the hair bulb (Fig 1d, f) were stronger than that for DAX-1. A similar pattern of expression for both orphan nuclear receptors was detected in the exocrine glands of skin, including the basal layer and sebocytes of the sebaceous gland (Fig 1g, h) and in the sweat glands (Fig 1i, j). Omission of the primary antibody as a negative control for immunohistochemistry resulted in no staining (data not shown).

Immunolocalization studies have also been carried out on facelift skin from female subjects (data not shown) but no apparent differences were observed in the expression pattern for SF-1 and DAX-1 between male and female skin.

Immunocytochemistry confirms co-localization of DAX-1 and SF-1 in cells derived from human skin

Co-localization of DAX-1 with SF-1 in the cells of human skin was examined by immunocytochemistry as SF-1 has previously been reported to regulate DAX-1 gene transcription (Burris et al., 1995; Kawabe et al., 1999). Nuclear staining for DAX-1 and SF-1 was observed in every cell type studied, including primary cultures of epidermal keratinocytes (Fig 2a, b), the immortalized keratinocyte cell line HaCaT (Fig 2d, e), primary cultures of preadipocytes (Fig 2g, h), primary cultures of dermal papilla cells (Fig 2j, k), and in the positive control murine αT3-1 gonadotrope cells (Fig 2m, n). The presence and co-localization of DAX-1 in the dermal papilla cells with SF-1 was confirmed by immunocytochemistry; however, a longer incubation in the substrate was required for visual detection of DAX-1, confirming earlier immunohistochemical observations in whole human skin that less DAX-1 immunoreactivity appeared to be present in the dermal papilla cells in comparison with SF-1. Immunocytochemical analysis on primary dermal fibroblasts also resulted in nuclear staining for both transcription factors (data not shown), suggesting that nuclear staining observed earlier in the dermis of whole human skin is due to fibroblasts expressing DAX-1 and SF-1. Specific staining was not observed in any of the negative controls for each cell type in which the primary antibodies were omitted (Fig 2c, f, i, l, o).
DAX-1 and SF-1 mRNA transcripts are expressed in human skin. RT-PCR analysis revealed the presence of DAX-1 and SF-1 mRNA in whole human skin (Fig 3). The gene-specific and intron spanning primers for SF-1 were designed to flank the coding sequence of the DNA-binding domain of SF-1 to produce a 198 bp amplicon. Amplification of the expected 198 bp product from

Figure 1. Immunohistochemical analysis reveals nuclear staining for both DAX-1 and SF-1 in specific regions of the human hair follicle and skin. Snap-frozen human facelift skin was processed for immunohistochemistry using anti-DAX-1 antibody and anti-SF-1 antibody with a DAB detection system. DAX-1 and SF-1 are detected in the epidermis and the dermis of human skin (a) and (b), respectively. There is differential DAX-1 and SF-1 immunoreactivity in the human hair follicle (c) and (d), respectively, and in the hair bulb shown at a higher magnification (e) and (f). DAX-1 and SF-1 are localized in the sebaceous gland (g) and (h), respectively, and in the sweat glands (i) and (j). Abbreviations: BL, basal layer; DER, dermis; DP, dermal papilla; E, epidermis; HS, hair shaft; IRS, inner root sheath; MC, matrix cells; ORS, outer root sheath; SG, sebaceous gland; SW, sweat gland. Scale bars: (a, b, g, h) 64 μm; (c, d) 100 μm; (e, f) 34 μm; (i, j) 72 μm.
whole human skin and from the positive control H295R adrenocortical cell line only occurred in the presence of 5% dimethylsulfoxide in the PCR mastermix (Fig 3), but the expected product was also accompanied by a smaller band of 161 bp in size (data not shown). Further investigations of the 161 bp product revealed that the SF-1 primers were mispriming and annealing to genomic DNA contaminants present in the PCR reaction to produce a smaller 161 bp amplicon, which contained most of intron 2 and part of exon 3 of the SF-1 gene. DNase I treatment of the RNA extracted from human skin and adrenocortical cells eliminated the appearance of the 161 bp product with the expected 198 bp product being amplified alone. RT-PCR of whole human skin and H295R cell RNA yielded the expected 251 bp DAX-1 product (Fig 3). DNA sequencing of the 198 bp SF-1 band and the 251 bp DAX-1 band amplified from whole human skin and the H295R cell line by their respective primers, confirmed their identity with human DAX-1 and SF-1 sequences in the human GenBank database.

**Western analysis confirms the expression of DAX-1 protein in human skin cells** Using the same DAX-1 antibody as that

![Figure 2](image2.png) Figure 2. Nuclear localization of DAX-1 and SF-1 by immunocytochemistry in cultured cells derived from human skin. Methanol fixed cells cultured on glass coverslips were permeabilized with 0.1% (v/v) Triton X-100 and incubated in primary antibodies against DAX-1 and SF-1. Nuclear immunoreactivity for DAX-1 and SF-1 is apparent in primary cultures of human keratinocytes (a) and (b), respectively; immortalized human keratinocytes (d) and (e); primary cultures of human preadipocytes (g) and (h) and primary cultures of human dermal papilla cells (i) and (k). DAX-1 and SF-1 were detected in the positive control cells, the αT3-1 cells (n) and (o), respectively. The negative controls for each cell type in which the primary antibodies were omitted (c, f, i, l, o) do not show any nuclear staining. Scale bars: (a–f) 25 μm; (g–l) 50 μm; (n–o) 12.5 μm.

![Figure 3](image3.png) Figure 3. DAX-1 and SF-1 mRNA is detected in whole human skin by RT-PCR. Lane 1, the 198 bp SF-1 mRNA amplicon was detected in whole human skin; lane 2, and in the positive control human adrenocortical H295R cells. Lane 3, the 251 bp DAX-1 mRNA amplicon was detected in whole human skin; lane 4, and in the positive control human adrenocortical H295R cells. Lanes 5 and 6, SF-1 and DAX-1 negative controls without cDNA, respectively; lane 7, φX174 DNA Hae III digest molecular weight markers.
used for immunocytochemistry, a correct sized band corresponding to human DAX-1 protein was observed by Western blotting confirming the expression of DAX-1 in primary cultures of dermal papilla cells, HaCaT cells, primary cultures of normal human keratinocytes, primary cultures of preadipocytes, and in the positive control αT3-1 gonadotrope cell line (Fig 4). Quantitation and normalization of nuclear protein in each cell extract was performed prior to loading and Coomassie blue staining (data not shown) confirmed equal protein loading to each lane. Despite demonstrating the presence of SF-1 protein in skin by immunohistochemistry we have as yet been unable to show SF-1 protein by immunoblotting. This may be because the commercially available SF-1 antibody lacks the required sensitivity for use in this technique.

DISCUSSION

Since the initial studies when SF-1 was identified and named, several human tissues other than steroidogenic tissues have also been shown to express this orphan nuclear receptor. Our studies using immunohistochemistry, mRNA expression, and western blotting would suggest that these orphan nuclear receptors are also present in human skin. Immunohistochemistry of DAX-1 and SF-1 revealed a nuclear staining pattern in human skin, which is in agreement with their localization in steroidogenic tissues such as the adrenal cortex. Although we found predominant nuclear DAX-1 staining in skin, cytoplasmic staining was also observed in the basal layer of the epidermis, a finding which is in agreement with that recently made by Lalli et al (2000), who reported that DAX-1 is able to shuttle between the nucleus and cytoplasm. Western analysis confirmed that the nuclear protein detected by immunohistochemistry and immunocytochemistry was indeed DAX-1.

The expression of SF-1 and DAX-1 mRNA transcripts was confirmed in whole human skin; however, we experienced difficulties in amplifying the SF-1 transcript from skin by PCR and found that this was only feasible from skin that was immediately snap-frozen upon removal.

Our immunocytochemical studies show that both transcription factors co-localize with each other in all skin–derived cultured cells, including human keratinocytes, dermal papilla cells, and preadipocytes. A distinct staining pattern was also observed for DAX-1 in the epidermis of human skin with prominent staining in the basal layer compared with the scattered pattern for SF-1 in all layers, except the cornified layer. By immunohistochemistry DAX-1 immunoreactivity also appeared to be lower than that found for SF-1 in dermal papilla cells; however, factors such as the affinity of the antibodies against their antigens and the concentrations of the antibodies used must be taken into consideration.

The expression of the steroidogenic enzymes CYP11A1, CYP17, and CYP21 in the adrenal cortex and CYP19 in the gonads is under the transcriptional control of DAX-1 and SF-1 and many of these enzymes have previously been reported to be expressed throughout human skin (Table I).

The distribution of all the steroidogenic enzymes in different regions of human skin is concordant with our immunohistochemical findings for DAX-1 and SF-1 expression, suggesting that both transcription factors may also be involved in regulating their gene expression in human skin. Furthermore, the cutaneous expression of DAX-1 and SF-1 supports the hypothesis that de novo steroid synthesis from cholesterol could take place in human skin.

It was recently shown that oxysterols such as 25-hydroxy cholesterol are able to induce human keratinocyte differentiation by increasing activator protein 1 (AP-1)-dependent transcription of the involucrin gene, which encodes a protein involved in the formation of the cornified envelope of differentiated keratinocytes (Hanley et al, 2000). 25-Hydroxycholesterol has also been shown to stimulate SF-1–dependent transcription in non-steroidogenic CV-1 cells and was believed to be a ligand for the orphan receptor (Lala et al, 1997). Mellon and Bair (1998) demonstrated that SF-1 transcriptional activity remained unaffected in the presence of oxysterols in steroidogenic mouse MA-10 Leydig cells and showed that oxysterols were not ligands for SF-1 in known steroidogenic cells. It is conceivable, however, that oxysterols may act as ligands

![Figure 4](image-url)  
**Figure 4.** DAX-1 protein is detected in skin derived cells by Western blotting. Nuclear protein extracts from skin derived cells blotted onto a nitrocellulose membrane were analyzed for the presence of DAX-1 protein using 0.08 μg per ml anti-DAX-1 antibody. Lane 1, primary culture of normal human keratinocytes (NHK); lane 2, HaCaT cells; lane 3, primary culture of preadipocytes (P-A); lane 4, primary culture of dermal papilla cells (DP); lane 5, positive control αT3-1 gonadotrope cells.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function in adrenal/gonads</th>
<th>Location in human skin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP11A1 (cholesterol side-chain cleavage enzyme or desmolase)</td>
<td>20α-hydroxylation, 22-hydroxylation and scission of the cholesterol side-chain to yield pregnenolone</td>
<td>Basal layer of epidermis and eccrine gland</td>
<td>Venencie et al, 1999</td>
</tr>
<tr>
<td>CYP17 (17α-hydroxylase)</td>
<td>17α-hydroxylation of pregnenolone and progesterone to 17α-hydroxyprogrenolone and 17α-hydroxyprogesterone, respectively, followed by scission of the C17, 20 carbon bond to yield dehydroepiandrosterone and androstenedione, respectively</td>
<td>Epidermis, inner root sheath, outer root sheath, sebaceous gland, and sweat gland</td>
<td>Venencie et al, 1999</td>
</tr>
<tr>
<td>CYP21 (21-hydroxylase)</td>
<td>21-hydroxylates progesterone and 17α-hydroxyprogesterone to yield deoxycorticosterone and 11-deoxycorticisol, respectively</td>
<td>Whole skin</td>
<td>Slominski et al, 1996</td>
</tr>
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</table>
for SF-1 in human keratinocytes. Moreover, SF-1 is a likely candidate through which oxysterols may induce keratinocyte differentiation as the activation function 2 (AF2) subdomain that resides in the ligand binding domain of SF-1 is able to interact with AP-1 to modulate gene transcription (Li et al, 1998).

Our immunohistochemical data of DAX-1 and SF-1 localization in the human epidermis suggests that SF-1 may facilitate cellular mechanisms in skin such as keratinocyte differentiation in a positive manner, whilst DAX-1 could act in a repressive manner. The persistent expression of DAX-1 and SF-1 in suprabasal cells, however, might imply additional functions for these transcription regulators.

The well-described function of these transcription factors during development and their widespread expression in most components of human skin and the pilosebaceous unit, provokes speculation that they may be involved in some regulatory aspects of the human hair cycle by controlling cutaneous steroidogenesis. Furthermore, if DAX-1 and SF-1 are indeed regulating local steroidogenesis in human skin, they may influence further effects in the steroidogenic cascade by controlling the availability of steroids for their conversion into more potent forms such as DHT, and thus be involved in initiating pathologies associated with androgens, for instance acne or hirsutism. Further studies must be performed to elucidate the intriguing roles that they may play in human skin.

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REFERENCES


Barnhart KM, Mellon FP: The orphan nuclear receptor, steroidogenic factor-1, regulates the glycoprotein ecto-subunit gene in pituitary gonadotropes. Mol Endocrinol 8:878±885, 1994


Lala DS, Rice DA, Parker KL: Steroidogenic factor 1, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fusha tarzu-factor 1. Mol Endocrinol 6:1249±1258, 1992

Lala DS, Syka PM, Lazarchil SB, Mangelsdorfr DJ, Parker KL, Heyman RA: Activation of the orphan nuclear receptor steroidogenic factor 1 by oxysterols. Proc Natl Acad Sci 94:4985±4980, 1997

Lalli E, Ohe K, Hindelang C, Sassone-Corsi P: Orphan receptor DAX-1 is a shuttling RNA binding protein associated with polyribosomes via mRNA. Mol Cell Biol 20:4910±4921, 2000


Stocco DM, Soderman TC: The 30 kDa mitochondrial proteins induced by hormone stimulation in MA-10 mouse Leydig tumour cells are processed from larger precursors. J Biol Chem 266:19731±19738, 1991

