

Sebocytes are the Key Regulators of Androgen Homeostasis in Human Skin

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The mRNA expression patterns of the androgen receptor and the androgen metabolizing enzymes 3 β -hydroxysteroid dehydrogenase/ Δ^{5-4} -isomerase, 17 β -hydroxysteroid dehydrogenase, 5 α -reductase, and 3 α -hydroxysteroid dehydrogenase were investigated in three different cell populations originating from human skin, SZ95 sebocytes, HaCaT keratinocytes, and MeWo melanoma cells, by means of reverse transcription polymerase chain reaction. Restriction analysis of cDNA fragments was performed to identify isozymes of 3 β -hydroxysteroid dehydrogenase/ Δ^{5-4} -isomerase and 3 α -hydroxysteroid dehydrogenase. In addition, ³H-dihydroepiandrosterone and ³H-testosterone were used as substrates to determine the metabolic activity of these enzymes in SZ95 sebocytes, primary sebocyte cultures, and HaCaT keratinocytes. Furthermore, the effects of the selective 5 α -reductase type 1 and 2 inhibitors, 4,7 β -dimethyl-4-aza-5 α -cholestan-3-one and dihydrofinasteride, respectively, and of the 3 β -hydroxysteroid dehydrogenase/ Δ^{5-4} -isomerase inhibitor cyproterone acetate on androgen metabolism were investigated. Androgen receptor mRNA was detected in SZ95 sebocytes and HaCaT keratinocytes but not in MeWo melanoma cells, whereas 3 β -hydroxysteroid dehydrogenase/ Δ^{5-4} -isomerase isotype 1 mRNA and metabolic activity were only found in SZ95 sebocytes. The enzyme activity could be inhibited by cyproterone acetate. Type 2 17 β -hydroxysteroid dehydrogenase, type 1 5 α -reductase, and 3 α -hydroxysteroid dehydrogenase mRNA were expres-

sed in all three cell populations tested, whereas type 3 17 β -hydroxysteroid dehydrogenase mRNA could only be detected in SZ95 sebocytes. The major metabolic steps of testosterone in SZ95 sebocytes, primary sebocyte cultures, and HaCaT keratinocytes were its conversion to androstenedione by 17 β -hydroxysteroid dehydrogenase and further to 5 α -androstenedione by 5 α -reductase. The type 1 5 α -reductase selective inhibitor 4,7 β -dimethyl-4-aza-5 α -cholestan-3-one, but not the type 2 selective inhibitor dihydrofinasteride, inhibited 5 α -reductase at low concentrations in SZ95 sebocytes and HaCaT keratinocytes. 5 α -androstenedione was degraded to androsterone by 3 α -hydroxysteroid dehydrogenase, which exhibited a stronger activity in HaCaT keratinocytes than in SZ95 sebocytes and in primary sebocyte cultures. Lower levels of 5 α -dihydrotestosterone and 5 α -androstenediol were also detected in all cells tested. Our investigations show that specific enzyme expression and activity in cultured sebocytes and keratinocytes seem to allocate different duties to these cells *in vitro*. Sebocytes are able to synthesize testosterone from adrenal precursors and to inactivate it in order to maintain androgen homeostasis, whereas keratinocytes are responsible for androgen degradation. **Key words:** androgen metabolism/androgen receptor/3 α -hydroxysteroid dehydrogenase/3 β -hydroxysteroid dehydrogenase/ Δ^{5-4} -isomerase/17 β -hydroxysteroid dehydrogenase/keratinocytes/melanocytes/5 α -reductase/sebaceous glands. *J Invest Dermatol* 116:793-800, 2001

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Abbreviations: 5 α -A-diol, 5 α -androstenediol; A-dione, androstenedione; 5 α -A-dione, 5 α -androstenedione; CPA, cyproterone acetate; DHEA, dehydroepiandrosterone; 5 α -DHT, 5 α -dihydrotestosterone; FP, forward primer; HPTLC, high performance thin layer chromatography; 3 α -HSD, 3 α -hydroxysteroid dehydrogenase; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; Δ^5 -3 β -HSD, 3 β -hydroxysteroid dehydrogenase/ Δ^{5-4} -isomerase; IC₅₀, 50% inhibitory concentration; MK386, 4,7 β -dimethyl-4-aza-5 α -cholestan-3-one; RP, reverse primer; T_a, annealing temperature.

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The function of sebaceous glands and hair follicles appears strongly dependent on biologically active androgens. Dehydroepiandrosterone (DHEA), androstenedione (A-dione), and testosterone have been shown to stimulate sebum secretion in humans (Pochi and Strauss, 1969; Diamond *et al*, 1996). 5 α -Dihydrotestosterone (5 α -DHT) seems to be necessary for male beard growth and scalp hair recession, whereas testosterone alone is sufficient for stimulation of axillary and pubic hair growth (for review see Messenger, 1993). The effect of active androgens is mediated by their binding to nuclear androgen receptors. Lack of functional androgen receptors, e.g., in the total androgen insensitivity syndrome, prevents the action of androgens on skin appendages (Imperato-McGinley *et al*, 1993).

Having recognized the key effects of biologically active androgens on skin, their local synthesis and degradation have

gained special interest. It is known that the precursors of the active androgens in tissue, such as DHEA sulfate, DHEA, and A-dione, are mostly derived from glandular secretion, whereas testosterone in women and 5 α -DHT are mainly synthesized in the periphery. Therefore, an association of a possible local overproduction of active androgens and skin disorders such as acne and androgenetic alopecia in males has been suggested by several studies. Skin in both acne (Sansone and Reisner, 1971) and androgenetic alopecia (Puerto and Mallol, 1990) produced higher rates of testosterone and 5 α -DHT from blood precursors than in healthy individuals. In addition, elevated plasma levels of 5 α -DHT and 3 α -androstenediol glucuronide have been found in female patients with acne, whereas DHEA sulfate, A-dione, and testosterone were normal (Lookingbill *et al*, 1985). As a consequence, elucidation of the peripheral androgen metabolism is crucial for understanding androgen effects on skin.

Five major enzymes are involved in the activation and deactivation of androgens (Hay and Hodgins, 1978; Sawaya and Penneys, 1992). In a first step, 3 β -hydroxysteroid dehydrogenase/ Δ^5 -isomerase (Δ^5 -3 β -HSD) converts DHEA to A-dione. Two isoforms of the enzyme have been described (Labrie *et al*, 1992). Human skin seems to express exclusively type 1 isoform with approximately 5 to 8-fold higher substrate affinity than type 2 (Dumont *et al*, 1992). Experiments using specific substrates in combination with formazan formation (Milne, 1969), immunohistochemical methods (Dumont *et al*, 1992), and semiquantitative mRNA analysis (Courchay *et al*, 1996) led to the conclusion that cutaneous Δ^5 -3 β -HSD is practically located in the sebaceous glands only. In a second step, A-dione is further activated to testosterone through the 17 β -hydroxysteroid dehydrogenase (17 β -HSD). Up to date, seven isoforms of this enzyme have been identified, whereas isoforms 2 and 3 catalyze the conversion of testosterone to A-dione and vice versa, respectively (Wu *et al*, 1993; Geissler *et al*, 1994; Labrie *et al*, 1997; Peltoketo *et al*, 1999). Although little is known about the distribution of these isozymes, skin seems to be one of the organs in which reduction (i.e., synthesis of testosterone from A-dione) is preferred over oxidation (i.e., A-dione synthesis from testosterone) in *in vitro* experiments (Labrie *et al*, 1997). In a third step, 5 α -reductase irreversibly converts testosterone to 5 α -DHT, the most potent naturally occurring androgen (Grino *et al*, 1990). For this enzyme, two isoforms have been described (Russell and Wilson, 1994), and type 1 is dominating in the skin *in vivo* and *in vitro* (Harris *et al*, 1992; Chen *et al*, 1996). Immunohistochemical and enzyme activity studies suggested predominant expression of the enzyme in sebaceous glands, but also in sweat glands, epidermal cells, and hair follicles (Luu-The *et al*, 1994; Eicheler *et al*, 1995; Thiboutot *et al*, 1995; Patel *et al*, 1996; Chen *et al*, 1998). Finally, 3 α -hydroxysteroid dehydrogenase (3 α -HSD), an enzyme existing in three isoforms (Khanna *et al*, 1995; Dufort *et al*, 1996), catabolizes active androgens to compounds that do not bind the androgen receptor. After glucuronidation water-soluble compounds are eliminated through the kidney (Sperling and Heimer, 1993). Alternatively, aromatase can convert testosterone and A-dione to estrogens in certain cell types (Berkovitz *et al*, 1987; Hughes *et al*, 1997).

Androgen metabolism in sebaceous glands has been investigated using isolated nonviable glands, sebaceous-gland-rich skin tissue, and freshly isolated whole sebaceous glands (Baillie *et al*, 1966; Milne, 1969; Hay and Hodgins, 1978; Thiboutot *et al*, 1998). 5 α -Reductase and 17 β -HSD have been studied in human epidermal and follicular keratinocytes (Thiboutot *et al*, 1997, 1998). Human genital melanocytes were also shown to be an androgen target (Tadokoro *et al*, 1997). Androgen activation and deactivation are mainly intracellular events, however, and can differ from cell type to cell type and among cells of different locations (Labrie, 1991). Therefore, we investigated the expression patterns of androgen-metabolizing enzymes in a recently established SZ95 human sebaceous gland cell line, which presents characteristics of primary human sebocytes (Zouboulis *et al*, 1999), in HaCaT keratinocytes, which have many characteristics of primary human keratinocytes

(Schürer *et al*, 1993; Wraight *et al*, 1998), and in MeWo melanoma cells to further clarify androgen metabolism at the cellular level in the skin. Furthermore, we analyzed the metabolism of DHEA and testosterone in SZ95 sebocytes, primary human sebocytes, and HaCaT keratinocytes. The results were compared and correlated to the mRNA expression data. In addition, the effects of selective enzyme inhibitors on androgen metabolism were evaluated.

MATERIALS AND METHODS

Materials Cell culture media and chemicals were from Biochrom (Berlin, Germany) unless otherwise stated. Sterile cell culture plastic ware was from Becton-Dickinson (Heidelberg, Germany). Androgen standards were from Sigma (Munich, Germany). Solvents were purchased from Merck (Darmstadt, Germany) and other fine chemicals were from Merck or Sigma. 3 H-DHEA and 3 H-testosterone were from NEN-Dupont (Dreieich, Germany). Selective inhibitors of the 5 α -reductase type 1 isozyme, 4,7 β -dimethyl-4-aza-5 α -cholestan-3-one (MK386) (Ellsworth *et al*, 1996), and type 2 isozyme, dihydrofinasteride (Azzolina *et al*, 1997), were provided by Merck, Sharp & Dohme, Rahway, NJ. CPA, shown to inhibit the activity of Δ^5 -3 β -HSD (Dumont *et al*, 1992; Tóth *et al*, 1997), was purchased from Sigma.

Cell cultures The spontaneously immortalized human keratinocyte line HaCaT (Boukamp *et al*, 1988) (gift of Prof. Fusenig and Dr. Breitkreutz, Heidelberg, Germany) and the melanoma cell line MeWo were grown in a modified Dulbecco's modified Eagle's medium (DMEM)/Ham's F₁₂ 1:1 with 10% fetal bovine serum (FBS), 100 U per ml penicillin, and 100 μ g per ml streptomycin. The recently described SZ95 human sebaceous gland cell line (Zouboulis *et al*, 1999), derived from facial sebaceous glands, was cultured in the same medium supplemented with 10 ng per ml epidermal growth factor and 5 ng per ml keratinocyte growth factor (both from Boehringer, Mannheim, Germany). Primary human sebocyte cultures were obtained from a 53-year-old female who underwent operation at the fronto-parietal facial area for another reason as described previously (Xia *et al*, 1989; Zouboulis *et al*, 1998) and were maintained without the use of a feeder layer in the culture medium of SZ95 cells supplemented with 10⁻⁹ M cholera toxin (Calbiochem, Bad Soden, Germany) and 1.1 \times 10⁻⁶ M hydrocortisone (Sigma). Cells were maintained at 37°C in 5% CO₂ and were regularly subcultured at a split ratio of 1:3 using trypsin ethylenediamine tetraacetic acid 0.05/0.02% (wt/vol) in phosphate-buffered saline without Ca²⁺ and Mg²⁺ (PBS).

Oligonucleotide sequences for polymerase chain reaction (PCR)

The following oligonucleotides were selected from different gene exons in order to avoid amplification of genomic DNA and were used for mRNA detection by reverse transcription PCR (RT-PCR). The appropriate sequences of mRNA, cDNA, or exons of the genomic DNA were found in the GenBank data bank via the Internet. The accession number of the sequences, the annealing temperature (T_a) used, and the length of the expected products are given in parentheses. All oligonucleotides were synthesized by Eurogentec (Seraing, Belgium): androgen receptor (#M34233), forward primer (FP) 5'-GAA GAC CTG CCT GAT CTG TG, reverse primer (RP) 5'-AAG CCT CTC CTT CCT CCT GT (T_a = 63°C, 269 bp); Δ^5 -3 β -HSD (#M38180 type 1 and #M77144 type 2), FP 5'-GAA GGA GAC ATT CTG GAT GA, RP 5'-TGA GTC TTG GAC TTC AGG TT (T_a = 62°C, 941 bp); 3 α -HSD (#L43822-L43830 exons 1-9 for type 1 and #L43831-L43839 exons 1-9 for type 2), FP 5'-GCA CCT ATG CAC CTC CAG AG, RP 5'-ATT GTA GCT CTT GGC CAG GA (T_a = 63°C, 755 bp); 5 α -reductase type 1 (#M68882-M68886 exons 1-5), FP 5'-TGG CGC TTC TCT ATG GAC TT, RP 5'-GGA AGC AAC ACT GCA GTT GA (T_a = 60°C, 369 bp); 5 α -reductase type 2 (#M74047), FP 5'-TCT GA CTG GAA ATG GAG TC, RP 5'-AAG CCA CCT TGT GGA ATC CT (T_a = 60°C, 202 bp). The primer sequences for 5 α -reductase types 1 and 2 were communicated by Dr. Hoffmann, Marburg, Germany, and the primer sequences used for 17 β -HSD types 1, 2, and 3 had been published previously (Zhang *et al*, 1996). Primers for β -actin, which was used as control, were purchased from Clontech (Bad Soden, Germany).

RT-PCR Cell mRNA was isolated using the RNeasy Midi kit (Qiagen, Hilden, Germany). RNA concentrations were determined using a Pharmacia GeneQuant II spectrophotometer. Three micrograms of RNA were transcribed to cDNA with 30 U M-MLV reverse transcriptase (Biozym, Hess. Oldendorf, Germany) with 0.01 μ M dithiothreitol, 1.25 mM dNTPs (Perkin Elmer, Berlingen, Germany),

30 U RNase inhibitor (Clontech), and 3.7 μ M random hexamers (Pharmacia, Freiburg, Germany) in a total of 30 μ l in the appropriate buffer at 37°C for 1 h. PCR was performed in an MJ Research PTC 100 cycler at 35 cycles with 1.25 U Taq polymerase, 0.2 mM dNTP (both from Perkin Elmer), 3 μ l cDNA solution, and 0.5 μ M of FP and RP, respectively. β -Actin was amplified as internal standard to verify success of the mRNA reverse transcription and cDNA synthesis. RNA samples without reverse transcriptase were run in parallel and were negative for β -actin, ensuring that genomic DNA was not present. Products were separated using a glycerol gel loading buffer (Biometra, Göttingen, Germany) in a 2% agarose gel in TAE buffer with 2 μ g per μ l ethidium bromide for visualization under ultraviolet light. A 123 bp standard for comparison of molecular weights was purchased from Gibco (Karlsruhe, Germany). Documentation of gels was done with a UVP ImageStore 5000 system. Figures in this publication were from TIFF files from the gels.

Restriction analysis of PCR fragments Sequences of Δ^5 -3 β -HSD and 3 α -HSD PCR products were analyzed by an appropriate software for single cutting sites of specific endonucleases (<http://www.medkem.gu.se/cgi-bin/cutter>). The RT-PCR products of Δ^5 -3 β -HSD or 3 α -HSD were purified from buffer salts and the surplus of primers by size exclusion chromatography on Pharmacia HR 300 microspin columns. Five microliters of buffer were added to 45 μ l eluate and the cDNA was digested for 1 h with 5 U of the endonucleases, as indicated in the figures. The resulting fragments were separated on an agarose gel as described above.

Evaluation of DHEA and testosterone metabolism by high performance thin layer chromatography (HPTLC) Single cell solutions of SZ95 sebocytes, primary sebocytes, and HaCaT keratinocytes were seeded in 24-well culture dishes at a density of 5×10^4 cells per well to form subconfluent cultures. All cells were incubated in modified DMEM/Ham's F_{12} medium supplemented with 10% FBS because an influence of the culture conditions on testosterone metabolism to A-dione was detected in preliminary experiments. Twenty-four hours later the medium was removed and fresh medium containing 0.5 μ Ci at 20 nmol per l DHEA or testosterone in 0.5 ml with or without enzyme inhibitor was added. At the time points indicated the medium was collected, the cells were washed once with 0.5 ml PBS, and medium and PBS were added to 2.5 ml chloroform/tetrahydrofuran (3:2, vol/vol) in glass vials. The vials were vortexed and phase separation was performed by centrifugation at $1600 \times g$ for 10 min. The upper aqueous phase was discarded and the lower phase was dried in a Savant Speed-Vac SC 110 A vacuum centrifuge. Probes were then resuspended in 100 μ l chloroform/methanol 2:1 (vol/vol) and the resulting solutions as well as steroid standards were applied to HPTLC plates (Merck) using a Camag Linomat IV lipid applicator. Plates were developed in dichloromethane/ethyl acetate/methanol

85:10:1.5 (vol/vol/vol). The lane containing the standard was cut off, dipped for 10 s in an aqueous 0.6 M copper(II) sulfate solution with 8.5% phosphoric acid, and charred at 150°C. The distance of migration values for steroid standards were as follows: 5 α -androstanediol (5 α -A-dione) 0.85, A-dione 0.59, 5 α -DHT 0.51, DHEA 0.48, androsterone 0.44, testosterone 0.35, and 5 α -androstanedione (5 α -A-diol) 0.29. The metabolism of DHEA and testosterone to different androgens was evaluated after exposure to imaging plates (BAS-TR2040S, Fuji, Osaka, Japan) for 24 h and was read in a Fuji BAS 1000 bio-imaging analyzer. The androgens were identified according to their distance of migration values and the amount of metabolites was determined as a percentage of the total steroid radioactivity. Images of HPTLC plates were obtained either directly from TIFF files received by the bio-imaging analyzer or by autoradiography using Kodak BioMax films. Prior to autoradiography, HPTLC plates were covered with an enhancer spray (En³hance, NEN-Dupont). All metabolism experiments were performed in triplicate and standard deviation was below 5%.

RESULTS

Messenger RNA expression of Δ^5 -3 β -HSD, 17 β -HSD, 5 α -reductase, 3 α -HSD, and androgen receptor Messenger RNA was isolated from subconfluent SZ95 sebocytes, HaCaT keratinocytes, and MeWo melanoma cells. RT-PCR was performed as described above and the products obtained are shown in **Fig 1**. A Δ^5 -3 β -HSD-specific RT-PCR signal was seen in SZ95 sebocytes only. 17 β -HSD type 2 mRNA was found in SZ95 sebocytes and in HaCaT keratinocytes, but not in MeWo melanoma cells. A weak but reproducible signal for 17 β -HSD type 3 could only be detected in the SZ95 sebocytes. No expression of 17 β -HSD type 1 mRNA was determined in the cells tested (data not shown). Strong mRNA expression of the enzymes 5 α -reductase type 1, but not of type 2 (data not shown), and of 3 α -HSD was found in all three cell populations. In addition, expression of androgen receptor mRNA was detected in SZ95 sebocytes and HaCaT keratinocytes only. Taken together, all cell populations would be expected to metabolize testosterone to 5 α -DHT and 5 α -A-diol, but they may have different capabilities for DHEA and A-dione metabolism.

Restriction analysis of Δ^5 -3 β -HSD and 3 α -HSD RT-PCR products The primers for Δ^5 -3 β -HSD and 3 α -HSD PCR were designed to amplify the cDNA of both types 1 and 2 of these enzymes. This was feasible because of a 93% homology for Δ^5 -3 β -HSD type 1 and 2 isozymes and an 80% homology for 3 α -HSD types 1 and 2. Using a computer program as described in *Materials*

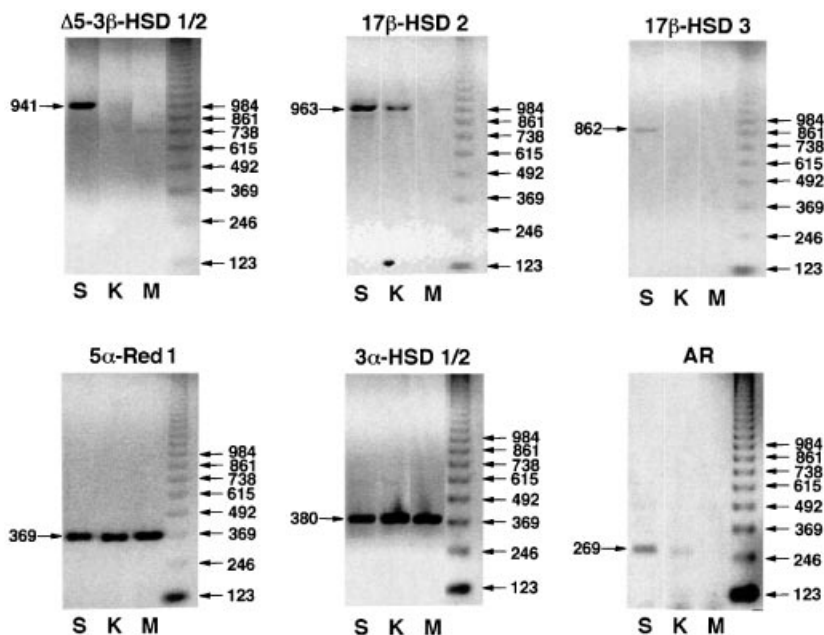


Figure 1. RT-PCR of androgen metabolizing enzymes and the androgen receptor (AR). mRNA was isolated from half-confluent sebocytes (SZ95, S), keratinocytes (HaCaT, K), and melanoma cells (MeWo, M) and RT-PCR was performed as described in *Materials and Methods*. Sizes of the expected PCR products and of the standard DNA ladder are given in base pairs and indicated on the left and right margins of the figures, respectively. The TIFF pictures of the agarose gels were inverted for better visibility (e.g., dark bands on white background instead of white bands on dark background). 5 α -Red, 5 α -reductase.

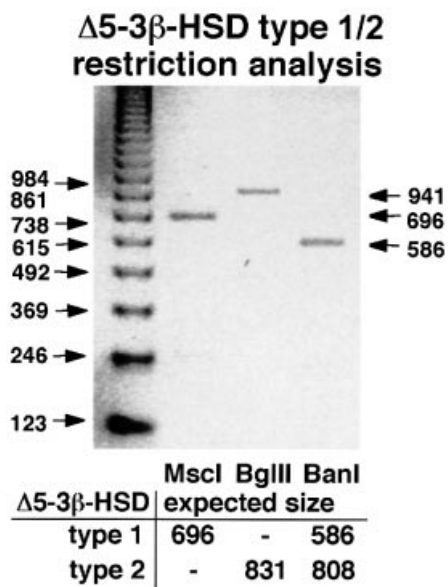


Figure 2. Restriction analysis of the Δ^5 -3 β -HSD RT-PCR product. The restriction enzymes MscII (lane 2), BglIII (lane 3), and BamI (lane 4) have been used. The expected size of the largest restriction fragment is indicated. The appropriate products could only be seen in digests with type 1 specific restriction enzymes. The TIFF picture of the agarose gel was inverted for better visibility (e.g., dark bands on white background instead of white bands on dark background).

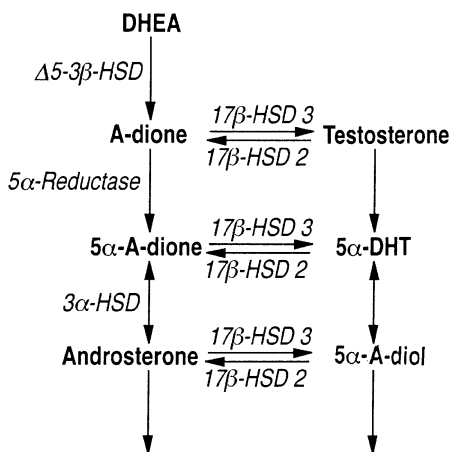


Figure 3. Peripheral androgen metabolism and the involved enzymes. The schematic metabolism of androgens in peripheral tissue is depicted. The reactions catalyzed by Δ^5 -3 β -HSD and 5 α -reductase are irreversible. Under physiologic conditions, 17 β -HSD 2 oxidizes and 17 β -HSD 3 reduces 17-hydroxy to 17-keto compounds, respectively, e.g., testosterone to A-dione, 5 α -DHT to 5 α -A-dione, 5 α -A-diol to androsterone and vice versa.

and Methods, we determined sites sensitive for specific endonucleases in the mRNA/cDNA sequence. The theoretically expected single cut fragments and the results of this experiment for Δ^5 -3 β -HSD are shown in Fig 2. We could show Δ^5 -3 β -HSD mRNA to be exclusively of type 1 in SZ95 sebocytes. Analysis for 3 α -HSD isotypes did not lead to a definitive result (data not shown).

3 H-DHEA metabolism in SZ95 sebocytes and HaCaT keratinocytes To complete our investigations with studies at

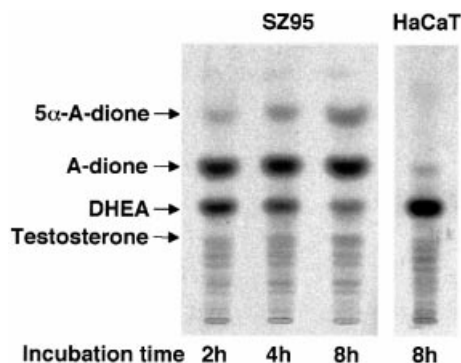


Figure 4. Metabolism of 3 H-DHEA by Δ^5 -3 β -HSD in SZ95 sebocytes. SZ95 sebocytes, but not HaCaT keratinocytes, metabolized DHEA to A-dione. The picture is produced from a TIFF file received by the bio-imaging analyzer.

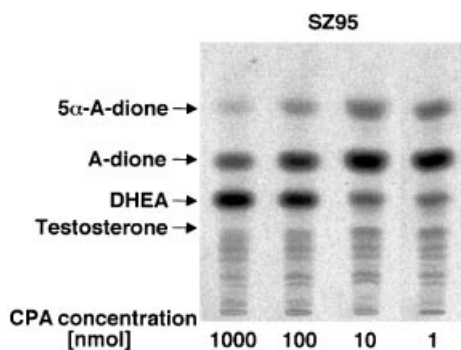


Figure 5. Inhibition of Δ^5 -3 β -HSD activity in SZ95 sebocytes by CPA. Significant dose-dependent inhibition of Δ^5 -3 β -HSD activity by CPA could be observed. The picture is produced from a TIFF file received by the bio-imaging analyzer.

the protein level, expression and activity of the enzymes participating in the peripheral androgen metabolism were determined by evaluating 3 H-DHEA metabolism in SZ95 sebocytes and HaCaT keratinocytes. The pathways of peripheral androgen metabolism are depicted in Fig 3. The results of 3 H-DHEA metabolism are shown in Fig 4. In SZ95 sebocytes, DHEA was rapidly converted to A-dione via Δ^5 -3 β -HSD and further to 5 α -A-dione and testosterone via 5 α -reductase and 17 β -HSD, respectively. In contrast, almost no turnover of DHEA could be detected in HaCaT keratinocytes. Quantitative evaluation of the data for SZ95 sebocytes revealed $78.6 \pm 2.3\%$ 3 H-DHEA metabolism to A-dione, $4.3\% \pm 0.4\%$ to testosterone, and $8.1 \pm 0.2\%$, $p < 0.001$; after 8 h ($n = 3$ experiments). These data fit well to the high mRNA expression of Δ^5 -3 β -HSD found in SZ95 sebocytes.

Inhibition of Δ^5 -3 β -HSD activity in SZ95 sebocytes Δ^5 -3 β -HSD activity in SZ95 sebocytes was significantly inhibited by CPA (Fig 5), confirming previous data (Dumont *et al*, 1992; Tóth *et al*, 1997). At 1000 nM CPA, $15.5 \pm 1.5\%$ 3 H-DHEA was converted to A-dione compared to $78.6 \pm 2.3\%$ of the untreated control, $p < 0.001$; $3.1 \pm 1.5\%$ to testosterone compared to $4.3\% \pm 0.4\%$, ns; and $2.3 \pm 0.5\%$ to 5 α -A-dione compared to $8.1 \pm 0.2\%$, $p < 0.001$; after 8 h ($n = 3$ experiments). No complete inhibition of Δ^5 -3 β -HSD activity could be achieved, however, indicating that CPA is a partial inhibitor of this enzyme. A 50% inhibitory concentration (IC_{50}) of 100 nM CPA was assessed.

Figure 6. Time-dependent metabolism of ^3H -testosterone in SZ95 sebocytes. *Left panel:* Cells were incubated with ^3H -testosterone for the times indicated. The metabolites were indicated from the medium and separated on HPTLC plates. The picture is a TIFF file from a scanned autoradiograph. *Right panel:* The counts from the HPTLC plate were quantified and are shown as a percent of the total recovered radioactivity. The standard deviation from triple experiments was lower than 5% and is not indicated. \blacktriangle , A-dione; \bullet , 5 α -A-dione; \blacksquare , androsterone; \circ , 5 α -DHT; \square , 5 α -A-diol.

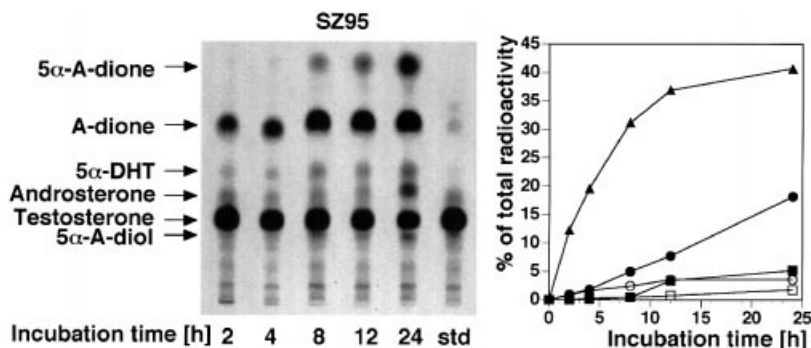


Figure 7. Time-dependent metabolism of ^3H -testosterone in primary sebocyte cultures. *Left panel, lanes 1-4:* Cells were incubated with ^3H -testosterone for the times indicated. The metabolites were isolated from the medium and separated on HPTLC plates. *Lanes 5, 6:* Cells were incubated for 24 h with 5 α -reductase type 2 (dihydrofinasteride, HF) and type 1 (MK386, MK) selective inhibitors at a concentration of 10^{-9} M, respectively. Metabolites were isolated and separated as described. The picture is a TIFF file from a scanned autoradiograph. *Right panel:* The counts from the HPTLC plate were quantified and are shown as a percentage of total recovered radioactivity. The standard deviation from triple experiments was lower than 5% and is not indicated. \blacktriangle , A-dione; \bullet , 5 α -A-dione; \blacksquare , androsterone; \circ , 5 α -DHT; \square , 5 α -A-diol.

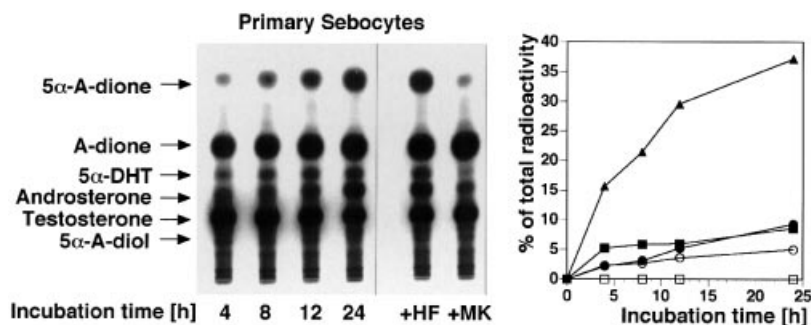
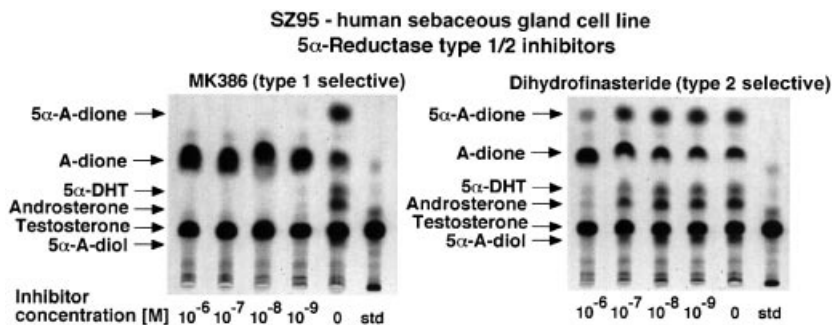


Figure 8. Inhibition of 5 α -reductase activity in SZ95 sebocytes by type 1 and 2 selective inhibitors. Cells were incubated for 24 h with ^3H -testosterone and the inhibitors at the concentrations indicated. The picture is a TIFF file from a scanned autoradiograph: 0, control culture without inhibitor; std, ^3H -testosterone without cells for 24 h.



^3H -testosterone metabolism in SZ95 sebocytes and in primary sebocyte cultures Resolution of DHEA, 5 α -DHT, and androsterone on HPTLC plates in ^3H -DHEA experiments was not sufficient because of their neighboring distance of migration values. To evaluate the metabolic activity of 17 β -HSD, 5 α -reductase, and 3 α -HSD, the irreversibility of the reaction catalyzed by Δ^5 -3 β -HSD was exploited. Therefore, SZ95 sebocytes and primary sebocyte cultures were incubated with ^3H -testosterone. The time-dependent results with SZ95 sebocytes over a 24 h period are seen in **Fig 6**. The main metabolite obtained through the activity of 17 β -HSD type 2 enzyme was A-dione, which seemed to reach a plateau level after 24 h of incubation with testosterone. A-dione was further converted to 5 α -A-dione by 5 α -reductase. In contrast to A-dione products, the 5 α -reductase product of testosterone, 5 α -DHT, could be detected at much lower levels. In addition, we could show 3 α -HSD enzyme activity through the detection of 5 α -A-diol and androsterone. In primary sebocyte cultures, ^3H -testosterone metabolism was essentially identical to that detected in SZ95 sebocytes, e.g., A-dione and 5 α -A-dione were the main products identified (**Fig 7**). As in SZ95

sebocytes, 5 α -DHT and androsterone could be found as minor products.

The total amount of 5 α -reductase metabolites (e.g., 5 α -DHT, 5 α -A-dione, androsterone, and 5 α -A-diol) was approximately 29% of total substrate in SZ95 sebocytes and 23% in primary sebocyte cultures after 24 h of treatment. Of the total amount of substrate available for 3 α -HSD (e.g., the sum of all 5 α -reductase products) in SZ95 sebocytes and primary sebocyte cultures, 24% and 37% were converted to the 3 α -HSD products androsterone and 5 α -A-diol, respectively.

Inhibition of 5 α -reductase activity in SZ95 sebocytes and in primary sebocyte cultures To evaluate testosterone metabolism under 5 α -reductase inhibition, SZ95 sebocytes and primary sebocyte cultures were incubated with ^3H -testosterone and increasing concentrations of the selective 5 α -reductase type 1 and 2

²Seiffert K, Fritsch M, Seltmann H, Orfanos CE, Zouboulis ChC: Inhibition of 5 α -reductase activity in immortalized human sebocytes and keratinocytes *in vitro*. *J Invest Dermatol* 114:811, 2000 (abstr.)

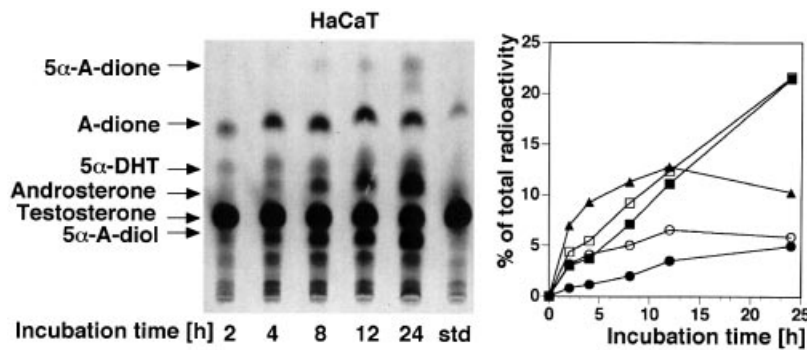


Figure 9. Time-dependent metabolism of ^3H -testosterone in HaCaT keratinocytes. *Left panel:* Cells were incubated with ^3H -testosterone for the times indicated. The metabolites were isolated from the medium and separated on HPTLC plates. The picture is a TIFF file from a scanned autoradiograph. *Right panel:* The counts from the HPTLC plate were quantified and are shown as a percentage of total recovered radioactivity. The standard deviation from triple experiments was lower than 5% and is not indicated. \blacktriangle , A-dione; \bullet , 5 α -A-dione; \blacksquare , androsterone; \circ , 5 α -DHT; \square , 5 α -A-diol.

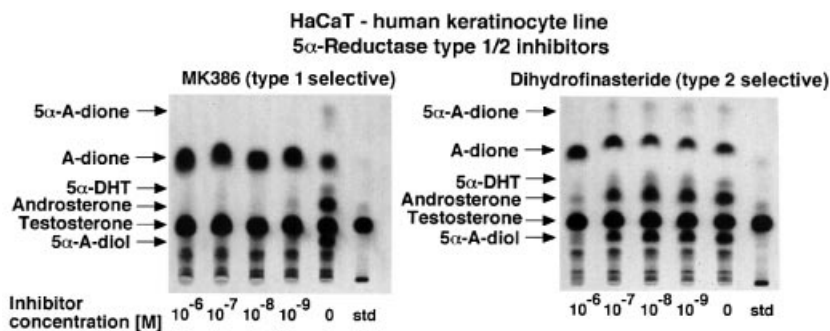


Figure 10. Inhibition of 5 α -reductase activity in HaCaT keratinocytes by type 1 and 2 selective inhibitors. Cells were incubated for 24 h with ^3H -testosterone and the inhibitors at the concentrations indicated. The picture is a TIFF file from a scanned autoradiograph: 0, control culture without inhibitor; std, ^3H -testosterone without cells for 24 h.

isozyme inhibitors, MK386 and dihydrofinasteride, respectively (Figs 7, 8). 5 α -Reductase activity was inhibited by low concentrations of the type 1 selective inhibitor MK386 in both SZ95 sebocytes ($\text{IC}_{50} < 10^{-9}$ M) and primary sebocyte cultures. In contrast, dihydrofinasteride showed a 5 α -reductase inhibition in SZ95 sebocytes at high concentrations only (IC_{50} approx. 4×10^{-7} M), e.g., at a 400-fold higher concentration than that of MK386. Interestingly, inhibition of 5 α -reductase activity by both agents was associated with an increased A-dione/testosterone ratio.²

^3H -testosterone metabolism in HaCaT keratinocytes
Experiments with HaCaT keratinocytes were performed as described for sebocytes. As in SZ95 sebocytes, all enzymes detected in HaCaT keratinocytes by the RT-PCR studies were metabolically active, e.g., their products could be detected. In comparison to SZ95 sebocytes, HaCaT keratinocytes secreted much lower amounts of A-dione and 5 α -A-dione, but higher levels of the 3 α -HSD products 5 α -A-diol and androsterone (Fig 9).

5 α -Reductase products added up to 54% of total substrate in our experiments with HaCaT keratinocytes after 24 h of treatment. Of the total amount of substrate available for 3 α -HSD (e.g., the sum of all 5 α -reductase products) in HaCaT keratinocytes 80% was converted to the 3 α -HSD products androsterone and 5 α -A-diol.

Inhibition of 5 α -reductase activity in HaCaT keratinocytes

The experiments performed were equivalent to those described using sebocytes. As in SZ95 sebocytes, the 5 α -reductase type 1 inhibitor MK386 almost completely blocked the enzyme activity in HaCaT keratinocytes at 10^{-9} M ($\text{IC}_{50} < 10^{-9}$ M), whereas the type 2 inhibitor dihydrofinasteride only exhibited its effect at much higher concentrations (IC_{50} approx. 7×10^{-6} M) (Fig 10). The inhibition of 5 α -reductase activity in HaCaT keratinocytes by both agents was also associated with an increased A-dione/testosterone ratio.

DISCUSSION

In this study, we investigated the mRNA expression of enzymes that are responsible for intracellular activation and inactivation of androgens in different human skin cell types. Furthermore, the expression patterns obtained were correlated with the enzyme

activities with and without use of selective enzyme inhibitors. Whereas human skin seen in total possesses all the enzymes necessary for androgen synthesis and catabolism indicating that it is an independent peripheral endocrine organ, there is an allocation of duties to different skin cells concerning the presence and the activity of androgen-metabolizing enzymes.

Using RT-PCR, we detected Δ^5 -3 β -HSD mRNA exclusively in SZ95 sebocytes compared with HaCaT keratinocytes and MeWo melanoma cells. Metabolism of DHEA to A-dione in SZ95 sebocytes and not in HaCaT keratinocytes confirmed Δ^5 -3 β -HSD mRNA translation to an active enzyme in human sebocytes only. Δ^5 -3 β -HSD in the SZ95 sebocytes was shown to be of the type 1 isoform by means of restriction analysis of the RT-PCR products. Our *in vitro* results in SZ95 sebocytes are in agreement with earlier *in vivo* studies that detected the occurrence of Δ^5 -3 β -HSD type 1 in human skin and localized Δ^5 -3 β -HSD exclusively in the sebaceous glands (Milne, 1969; Dumont *et al*, 1992; Courchay *et al*, 1996). These findings indicate that human sebocytes are exclusively responsible for intracellular activation of adrenal androgens in the skin. Therefore, it is likely that the sebaceous gland is the key regulator of androgen-dependent skin changes in peripheral hyperandrogenism due to adrenal hyperplasia (Orfanos, 1982; Kuttann *et al*, 1985).

In inhibition experiments, we could show Δ^5 -3 β -HSD activity in SZ95 sebocytes to be repressed by CPA with an IC_{50} of approximately 100 nM. Earlier reports in whole skin homogenates also showed CPA to inhibit Δ^5 -3 β -HSD activity with an IC_{50} of 150 nM for Δ^5 -3 β -HSD activity (Dumont *et al*, 1992; Tóth *et al*, 1997). Therefore, antagonism of adrenal androgen activation is another antiandrogenic mechanism of CPA (Mowszowicz *et al*, 1984).

Messenger RNA of 17 β -HSD type 2, an isozyme that catalyzes oxidation of testosterone to A-dione, was found highly expressed in SZ95 sebocytes, whereas the signal was weaker in HaCaT keratinocytes and undetectable in MeWo melanoma cells. Messenger RNA of 17 β -HSD type 3, an isozyme that activates A-dione metabolism to testosterone, could be detected in SZ95 sebocytes only. The strong 17 β -HSD type 2 isozyme expression in SZ95 sebocytes and HaCaT keratinocytes correlates with our metabolic data showing strong oxidation of testosterone to A-dione

in both cell lines and in primary sebocyte cultures. These data are in agreement with results in sebaceous glands *ex vivo* and human keratinocytes *in vitro* showing 17 β -HSD type 2 isozyme to be predominantly expressed and active (Sonoda *et al*, 1993; Thiboutot *et al*, 1998). In contrast, in whole skin homogenates, reduction of A-dione to testosterone is dominant over 17 β -HSD type 2 oxidation of testosterone to A-dione (Martel *et al*, 1992). Our results and the current report by Ando *et al* (1999) who detected 17 β -HSD type 3 mRNA expression in beard and axillary dermal papilla cells but 17 β -HSD type 2 mRNA expression in dermal papilla cells of occipital scalp make it likely that the direction of the reversible testosterone to A-dione metabolic reaction differs among the distinct skin cell populations and/or is dependent on the skin area of their origin.

5 α -Reductase type 1 mRNA was expressed in SZ95 sebocytes, HaCaT keratinocytes, and MeWo melanoma cells, whereas mRNA of the type 2 isozyme could not be detected. The 5 α -reductase inhibition experiments confirmed the RT-PCR-derived data, whereas the total amount of 5 α -reductase metabolites was similar in SZ95 sebocytes and primary sebocyte cultures after 24 h of treatment, being lower than in HaCaT keratinocytes. Predominant expression of 5 α -reductase type 1 isozyme has previously been reported in several skin cell populations *in vitro*, such as primary human sebocytes, keratinocytes, axillary dermal papilla cells, fibroblasts, and melanocytes (Luu-The *et al*, 1994; Chen *et al*, 1998; Ando *et al*, 1999). In contrast, dermal papilla cells from beard and occipital skin express both 5 α -reductase isozymes (Ando *et al*, 1999). Also the activity of the enzyme in sebaceous glands *ex vivo* and in whole skin specimens is almost exclusively of type 1 isoform (Thiboutot *et al*, 1995). In human scalp skin, predominant expression of the 5 α -reductase type 1 isozyme has been located immunohistochemically in sebaceous glands and of the type 2 within the infundibulum and root sheath of hair follicles (Bayne *et al*, 1999). Therefore, 5 α -reductase type 1 isozyme can be considered as the predominant "skin type" 5 α -reductase (Chen *et al*, 1996; 1998).

3 α -HSD type mRNA expression was found in all three cell populations, but it was stronger in MeWo melanoma cells and HaCaT keratinocytes than in SZ95 sebocytes. The isozyme type could not be identified. Furthermore, markedly higher rates of 3 α -HSD metabolites were detected in HaCaT keratinocytes than in SZ95 sebocytes and primary sebocyte cultures. A high 3 α -HSD activity has also been reported in primary keratinocytes *in vitro* (Sonoda *et al*, 1993). These findings indicate that sebocytes may be less responsible for androgen degradation in human skin, a duty that is fulfilled by other skin cell populations, such as the keratinocytes.

A requirement for the *in vivo* effects of androgen signal transduction is the presence of androgen receptors in the target cells (Liao *et al*, 1989). In our RT-PCR experiments, we could detect expression of androgen receptor mRNA in SZ95 sebocytes and HaCaT keratinocytes but not in MeWo melanoma cells. The apparent contradiction of the latter finding to the detection of androgen receptors in genital melanocytes (Tadokoro *et al*, 1997) may be due to the different location of cell origin or the loss of androgen receptor expression during the procedure of melanocyte transformation.

The early steps of testosterone metabolism in human sebocytes and keratinocytes mainly occur through synthesis of A-dione, the 17 β -HSD type 2 oxidation product, and not through synthesis of 5 α -DHT, the 5 α -reductase product (Flamigni *et al*, 1971). The opposite may have been expected from the strong 5 α -reductase and weaker 17 β -HSD type 2 mRNA expression pattern in SZ95 sebocytes and, especially, in HaCaT keratinocytes. The results are compatible with the different enzyme kinetic values [K_m (μ M)/ V_{max} (nmol per min per mg protein)] of the enzymes involved. The measured K_m/V_{max} values of 5 α -reductase type 1 for A-dione (0.3/5.7) and of 17 β -HSD type 2 for testosterone (0.4/45) are both in favor of the reaction of testosterone via A-dione to 5 α -A-dione when compared with the value of 5 α -reductase type 1 for testosterone (1.7/2.8) (Thigpen *et al*, 1993; Wu *et al*, 1993).

Therefore, at the physiologic testosterone concentration used in our metabolic experiments (20 nM), we have confirmed that testosterone is preferentially metabolized to A-dione and 5 α -A-dione in human sebocytes and keratinocytes. A single report with controversial data showing 5 α -DHT – and not A-dione – as the main testosterone metabolite in keratinocytes (Milewich *et al*, 1986) employed nonphysiologic high testosterone concentrations (20 μ M), which may stimulate testosterone metabolism by 5 α -reductase. Moreover, these findings support the reported unidirectional reactions in intact cells (Andersson and Moghrabi, 1997), whereas in homogenates of human sebaceous glands the specific activity of 5 α -reductase was shown to be greater than that of 17 β -HSD (Thiboutot *et al*, 1999). In gland homogenates, enzymes can work in both directions when cofactors are supplied.

The high metabolic rate of 3 α -HSD in HaCaT keratinocytes in contrast to primary sebocyte cultures and SZ95 sebocytes and the unique expression of Δ^5 -3 β -HSD in the latter are hints for differentially programmed duties of these two cell populations in androgen metabolism. Catabolism of tissue-active androgens seems to be more efficiently performed in keratinocytes. In contrast, sebocytes are capable of synthesizing androgen receptor binding and tissue-active compounds from the adrenal androgen DHEA that circulates in high concentrations in human blood. These androgens not only are important for the intracrine regulation of sebocytes (Labrie, 1991) but also may influence the function of other skin cells via a paracrine pathway (Horton, 1992). On the other hand, the high 17 β -HSD oxidation activity in sebocytes could be associated with the expected maintenance of androgen homeostasis in these cells (Hay and Hodgins, 1978), which are the major target of tissue-active androgens. They probably utilize 17 β -HSD type 2 isozyme to inactivate testosterone and avoid its metabolism to excessive intracellular 5 α -DHT levels.

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REFERENCES

- Andersson S, Moghrabi N: Physiology and molecular genetics of 17 beta-hydroxysteroid dehydrogenases. *Steroids* 62:143–147, 1997
- Ando Y, Yamaguchi Y, Hamada K, Yoshikawa K, Itami S: Expression of mRNA for androgen receptor, 5 α -reductase and 17 β -hydroxysteroid dehydrogenase in human dermal papilla cells. *Br J Dermatol* 141:840–845, 1999
- Azzolina B, Ellsworth K, Andersson S, Geissler W, Bull HG, Harris GS: Inhibition of rat α -reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition. *J Steroid Biochem Molec Biol* 61:55–64, 1997
- Baillie AH, Thomson J, Milne JA: The distribution of hydroxysteroid dehydrogenase in human sebaceous glands. *Br J Dermatol* 78:451–457, 1966
- Bayne EK, Flanagan J, Einstein M, *et al*: Immunohistochemical localization of types 1 and 2 5 α -reductase in human scalp. *Br J Dermatol* 141:481–491, 1999
- Berkovitz GD, Brown TR, Fujimoto M: Aromatase activity in human skin fibroblasts grown in cell culture. *Steroids* 50:281–295, 1987
- Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE: Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol* 106:761–771, 1988
- Chen W-C, Zouboulis ChC, Orfanos CE: The 5 α -reductase system and its inhibitors; recent development and its perspective in treating androgen-dependent skin disorders. *Dermatology* 193:177–184, 1996
- Chen W-C, Zouboulis ChC, Fritsch M, Blume-Peytavi U, Kordelja V, Goerdts S, Orfanos CE: Evidence of heterogeneity and quantitative differences of the type 1 5 α -reductase expression in cultured human skin cells – first evidence of its presence in melanocytes. *J Invest Dermatol* 110:84–89, 1998
- Courchay G, Boyera N, Bernard BA, Mahe Y: Messenger RNA expression of steroidogenesis enzyme subtypes in the human pilosebaceous unit. *Skin Pharmacol* 9:169–176, 1996
- Diamond P, Cusan L, Gomez JL, Bélanger A, Labrie F: Metabolic effects of 12-

- month percutaneous dehydroepiandrosterone replacement therapy in postmenopausal women. *J Endocrinol* 150:S43-S50, 1996
- Dufort I, Soucy P, Labrie F, Luu-The V: Molecular cloning of human type 3 3α -hydroxysteroid dehydrogenase that differs from 20 α -hydroxysteroid dehydrogenase by seven amino acids. *Biochim Biophys Res Commun* 228:474-479, 1996
- Dumont M, Luu-The V, Dupont E, Pelletier G, Labrie F: Characterization expression, and immunohistochemical localisation of 3β -hydroxysteroid dehydrogenase/ $\Delta 5$ - $\Delta 4$ isomerase in human skin. *J Invest Dermatol* 99:415-421, 1992
- Eicheler W, Dreher M, Hoffmann R, Happle R, Aumüller G: Immunohistochemical evidence for differential distribution of 5α -reductase isoenzymes in human skin. *Br J Dermatol* 133:371-376, 1995
- Ellsworth K, Azzolina B, Baginsky W, et al: MK386: a potent, selective inhibitor of the human type 1 5α -reductase. *J Steroid Biochem Molec Biol* 58:377-384, 1996
- Flamigni C, Collins WP, Koullapis EN, Craft I, Dewhurst CJ, Sommerville IF: Androgen metabolism in human skin. *J Clin Endocrinol* 32:737-743, 1971
- Geissler WM, Davis DL, Wu L, et al: Male pseudohermaphroditism caused by mutations of testicular 17β -hydroxysteroid dehydrogenase 3. *Nature Genet* 7:34-39, 1994
- Grino PB, Griffin JE, Wilson JD: Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone. *Endocrinology* 126:1165-1172, 1990
- Harris G, Azzolina B, Baginsky W, et al: Identification and selective inhibition of an isozyme of steroid 5α -reductase in human scalp. *Proc Natl Acad Sci USA* 89:10787-10791, 1992
- Hay JB, Hodgins MB: Distribution of androgen metabolizing enzymes in isolated tissues of human forehead and axillary skin. *J Endocrinol* 79:29-39, 1978
- Horton R: Dihydrotestosterone is a peripheral paracrine hormone. *J Androl* 13:23-27, 1992
- Hughes SV, Robinson E, Bland R, Lewis HM, Stewart PM, Hewison M: 1,25-dihydroxyvitamin D3 regulates estrogen metabolism in cultured keratinocytes. *Endocrinology* 138:3711-3718, 1997
- Imperato-McGinley J, Gautier T, Cai L-Q, Yee B, Epstein J, Pochi P: The androgen control of sebum production. Studies of subjects with dihydrotestosterone deficiency and complete androgen insensitivity. *J Clin Endocrinol Metab* 76:524-528, 1993
- Khanna M, Qin K-N, Wang R-W, Cheng K-C: Substrate specificity, gene structure, and tissue-specific distribution of multiple human 3α -hydroxysteroid dehydrogenases. *J Biol Chem* 270:20162-20168, 1995
- Kuttenn F, Couillin P, Girard F, et al: Late-onset adrenal hyperplasia in hirsutism. *N Engl J Med* 313:224-231, 1985
- Labrie F: Intra-crinology. *Mol Cell Endocrinol* 78:C113-C118, 1991
- Labrie F, Simard J, Luu-The V, Bélanger A, Pelletier G: Structure function and tissue-specific gene expression of 3β -hydroxysteroid dehydrogenase/5-ene-4-ene isomerase enzymes in classical and peripheral intracrine steroidogenic tissues. *J Steroid Biochem Molec Biol* 43:805-826, 1992
- Labrie F, Luu-The V, Lin S-X, Labrie C, Simard J, Breton R, Bélanger A: The key role of 17β -hydroxysteroid dehydrogenases in sex steroid biology. *Steroids* 62:148-158, 1997
- Liao S, Kokontis J, Sai T, Hiipakka RA: Androgen receptors: structures, mutations, antibodies and cellular dynamics. *J Steroid Biochem* 34:41-51, 1989
- Lookingbill DP, Horton R, Demers LM, Egan N, Marks JG Jr, Santen RJ: Tissue production of androgens in women with acne. *J Am Acad Dermatol* 12:481-487, 1985
- Luu-The V, Sugimoto Y, Puy L, Labrie Y, Lopez Solache I, Singh M, Labrie F: Characterization expression, and immunohistochemical localization of 5α -reductase in human skin. *J Invest Dermatol* 102:221-226, 1994
- Martel C, Rhéaume E, Takahashi M, Trudel J, Luu-The V, Simard J, Labrie F: Distribution of 17β -hydroxysteroid dehydrogenase gene expression and activity in rat and human tissues. *J Steroid Biochem Molec Biol* 41:597-603, 1992
- Messenger AG: The control of hair growth: an overview. *J Invest Dermatol* 101:4S-9S, 1993
- Milewich L, Kaimal V, Shaw CB, Sontheimer RD: Epidermal keratinocytes: a source of 5α -dihydrotestosterone production in human skin. *J Clin Endocrinol Metab* 62:739-746, 1986
- Milne JA: The metabolism of androgens by sebaceous glands. *Br J Dermatol* 81 (Suppl. 2):23-28, 1969
- Mowszowicz I, Wright F, Vincens M, et al: Androgen metabolism in hirsute patients treated with cyproterone acetate. *J Steroid Biochem* 20:757-761, 1984
- Orfanos CE: Antiandrogenos en dermatologia. *Arch Argent Dermat* 32:51-55, 1982
- Patel S, Einstein M, Geissler W, Wu L, Andersson S: Immunohistochemical analysis of steroid 5α -reductase type 1 in human scalp and prostate. *Ann N Y Acad Sci* 784:27-39, 1996
- Peltoketo H, Nokelainen P, Piao Y-S, Vihko R, Vihko P: Two 17β -hydroxysteroid dehydrogenases (17HSDs) of estradiol biosynthesis: 17HSD type 1 and type 7. *J Steroid Biochem Molec Biol* 69:431-439, 1999
- Pochi PE, Strauss JS: Sebaceous gland response in man to the administration of testosterone, Δ^4 -androstenedione, and dehydroisoandrosterone. *J Invest Dermatol* 52:32-36, 1969
- Puerto AM, Mallol J: Regional differences of the androgenic metabolic pattern in subjects affected by male pattern baldness. *Rev Esp Fisiol* 46:289-296, 1990
- Russell DW, Wilson JD: Steroid 5α -reductases: two genes/two enzymes. *Annu Rev Biochem* 63:25-61, 1994
- Sansone G, Reisner RM: Differential rates of conversion of testosterone to dihydrotestosterone in acne and in normal human skin - a possible pathogenic factor in acne. *J Invest Dermatol* 56:366-372, 1971
- Sawaya ME, Penneys NS: Immunohistochemical distribution of aromatase and 3β -hydroxysteroid dehydrogenase in human hair follicle and sebaceous gland. *J Cutan Pathol* 19:309-314, 1992
- Schürer N, Kohne A, Schliep V, Barlag K, Goerz G: Lipid composition and synthesis of HaCaT cells, an immortalized human keratinocyte line, in comparison with normal human adult keratinocytes. *Exp Dermatol* 2:179-185, 1993
- Sonoda T, Itami S, Kurata S, Takayasu S: Testosterone metabolism by cultured human beard outer root sheath cells in comparison with epidermal keratinocytes. *J Derm Sci* 6:214-218, 1993
- Sperling LC, Heimer WL: Androgen biology as a basis for the diagnosis and treatment of androgenic disorders in women. *J Am Acad Dermatol* 28:669-683, 1993
- Tadokoro T, Itami S, Hosokawa K, Terashi H, Takayasu S: Human genital melanocytes as androgen target cells. *J Invest Dermatol* 109:513-517, 1997
- Thiboutot D, Harris G, Iles V, Cimis G, Gilliland K, Hagari S: Activity of the type 1 5α -reductase exhibits regional differences in isolated sebaceous glands and whole skin. *J Invest Dermatol* 105:209-214, 1995
- Thiboutot DM, Knaggs H, Gilliland K, Hagari S: Activity of type 1 5α -reductase is greater in the follicular infundibulum compared with the epidermis. *Br J Dermatol* 136:166-171, 1997
- Thiboutot D, Martin P, Volikas L, Gilliland K: Oxidative activity of the type 2 isozyme of 17β -hydroxysteroid dehydrogenase predominates in intact and homogenized human sebaceous glands. *J Invest Dermatol* 111:390-395, 1998
- Thiboutot D, Gilliland K, Light J, Lookingbill D: Androgen metabolism in sebaceous glands from subjects with and without acne. *Arch Dermatol* 135:1041-1045, 1999
- Thigpen AE, Cala KM, Russell DW: Characterization of chinese hamster ovary cell lines expressing human steroid 5α -reductase isozymes. *J Biol Chem* 268:17404-17412, 1993
- Tóth I, Szécsi M, Julesz J, Faredin I: Activity and inhibition of 3- β -hydroxysteroid dehydrogenase/ $\Delta 5$ - $\Delta 4$ -isomerase in human skin. *Skin Pharmacol* 10:160-168, 1997
- Wright CJ, Liepe IJ, White PJ, Hibbs AR, Werther GA: Intracellular localization of insulin-like growth factor binding protein-3 (IGFBP-3) during cell division in human keratinocytes. *J Invest Dermatol* 111:239-242, 1998
- Wu L, Einstein M, Geissler WM, Chan HK, Elliston KO, Andersson S: Expression cloning and characterization of human 17β -hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20 α -hydroxysteroid dehydrogenase activity. *J Biol Chem* 268:12964-12969, 1993
- Xia L, Zouboulis Ch, Detmar M, Mayer-da-Silva A, Stadler R, Orfanos CE: Isolation of human sebaceous glands and cultivation of sebaceous gland-derived cells as an *in-vitro* model. *J Invest Dermatol* 93:315-321, 1989
- Zhang Y, Word RA, Fesmire S, Carr BR, Rainey WE: Human ovarian expression of 17β -hydroxysteroid dehydrogenase types 1, 2, and 3. *Endocrinology* 81:3594-3598, 1996
- Zouboulis ChC, Xia L, Akamatsu H, et al: The human sebocyte culture model provides new insights into development and management of seborrhoea and acne. *Dermatology* 196:21-31, 1998
- Zouboulis ChC, Seltmann H, Neitzel H, Orfanos C: Establishment and characterization of an immortalized human sebaceous gland cell line (SZ95). *J Invest Dermatol* 113:1011-1020, 1999