Evidence of Heterogeneity and Quantitative Differences of the Type 1 5α-Reductase Expression in Cultured Human Skin Cells – Evidence of its Presence in Melanocytes

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Steroid 5α-reductase is of crucial importance in androgen physiology because it catalyzes the conversion of testosterone into the more potent 5α-dihydrotestosterone in androgen-regulated target tissues. The enzyme occurs in two isoforms, whereby type 1 isozyme exists mainly in the skin and type 2 in the prostate. By using human cell cultures, we examined cutaneous expression and subcellular localization of type 1 5α-reductase *in vitro*. In immunocytochemistry, type 1 5α-reductase was detected in the cytoplasm of cultured human sebocytes, keratinocytes, fibroblasts, dermal microvascular endothelial cells, hair dermal papilla cells, and melanocytes. In western blot studies, two closely lying bands of 21–27 kDa were detected, possibly indicating heterogeneity of the type 1 5α-reductase in all the cell types tested, with the exception of beard dermal papilla cells. Northern blot studies revealed most abundant type 1 mRNA in neonatal foreskin keratinocytes, followed by adult facial sebocytes. Occipital hair dermal papilla cells presented higher levels of type 1 5α-reductase mRNA than those of beard. These findings were confirmed by semiquantitative reverse transcriptase polymerase chain reaction coupled with high performance liquid chromatography analysis. Taken together, it seems likely that in cultured human skin cells there exist (i) heterogeneity of type 1 5α-reductase protein and (ii) quantitative differences in its transcriptional and translational expression levels. *Key word: skin cell cultures.*

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Steroid 5α-reductase is the enzyme that catalyzes the conversion of testosterone (T) to 5α-dihydrotestosterone (DHT). The conversion of T to DHT amplifies the androgenic signal through two mechanisms: (i) DHT, unlike T, cannot be aromatized to estrogen, and thus its effect remains purely androgenic; and (ii) *in vitro* DHT binds to the human androgen receptor with greater affinity than does T, and the DHT-receptor complex appears to be more stable (Anderson and Liao, 1968; Ito and Horton, 1971; Kovacs *et al*, 1984).

Studies in the 1970s and 1980s have revealed that the skin, in addition to the prostate, is rich in 5α-reductase (Hay and Hodgins, 1973; Sharp *et al*, 1976; Takayasu *et al*, 1980). In this respect, it appears very likely that 5α-reductase functions as an autocrine mediator in skin growth and differentiation. Comparison of the expression and distribution of 5α-reductase in normal *versus* diseased skin could also help to further elucidate the etiopathogenesis of androgen-dependent skin disorders, such as acne vulgaris, hirsutism, androgenetic alopecia, and seborrhea.

In the last decade, molecular cloning studies have characterized two genes that encode two isozymes of 5α-reductase, namely, type 1 and type 2 (Andersson and Russell, 1990; Andersson *et al*, 1991). The former exists predominantly in the skin, whereas the latter exists in the prostate (Jenkins *et al*, 1992). The cutaneous distribution of type 1 isozyme *in vivo* was identified immunohistochemically to be in the sebaceous glands, epidermis, eccrine sweat glands, apocrine sweat glands, and hair follicles (outer root sheath cells, dermal papilla cells, matrix cells) as well as in the endothelial cells of small vessels and the Schwann cells of cutaneous myelinated nerves (Luu-The *et al*, 1994; Eicheler *et al*, 1995). The subcellular localization, however, is still controversially described. The type 1 isozyme was found by some authors to be localized in the nuclei of skin cells (Itami *et al*, 1990; Eicheler *et al*, 1995) and by others to be localized in the cytoplasm (Luu-The *et al*, 1994; Patel *et al*, 1996). Moreover, the relative amounts of the enzyme in different cells or in the same cell type from different skin areas remain to be determined.

In the present study, by using human cell cultures we have demonstrated immunocytochemically the presence of type 1 5α-reductase in the cytoplasm of all the skin cell types tested, including melanocytes. Evidence of heterogeneity of the enzyme protein was observed in western blotting study. Quantitative differences in the isozyme expression among the cells were shown at the transcriptional as well as at the translational levels.

MATERIALS AND METHODS

Cell cultures Unless otherwise stated, all the cells were cultuted in 100 mm Petri dishes (Falcon 3003 Becton Dickinson, Plymouth, U.K.) at 37°C in a humidified atmosphere containing 5% CO_2. The medium was replaced every 3 d.

Sebocyte culture Primary sebocyte cultures were obtained as previously described (Xia *et al*, 1989). Skin specimens from the facial area of middle-to

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Abbreviations: cDNA, complementary DNA; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; T, testosterone.

old-aged patients (50–70 y old) undergoing surgical excision were cut in small fragments and incubated for 20 h in 3 U dispase (Boehringer, Mannheim, Germany) per ml at 4°C to separate epidermis from dermis. Epidermis was then maintained for 15 min in 0.02% desoxyribonuclease (Sigma, Deisenhofen, Germany) at 37°C. Intact sebaceous glands were microdissected and the isolated lobules were cultivated on mitomycin C (Sigma)-inactivated 3T3 cells in sebocyte medium consisted of Dulbecco's modified Eagle's medium/Ham's F 12 medium (3:1, Biochrom, Berlin, Germany), 10% fetal calf serum (Seromed, Berlin, Germany), 10 ng epidermal growth factor (Sigma) per ml, 10 ng keratinocyte growth factor (Boehringer) per ml, 10^{-9} M cholera toxin (Sigma), 3.4 mM L-glutamine (Seromed), 100 IU penicillin (Seromed) per ml, and 100 μg streptomycin (Seromed) per ml. Outgrowths from the periphery of the gland lobules were usually first seen in 5–7 d. The cells of the first two passages were used in this study.

Culture of human dermal papilla cells Individual hair follicles were isolated by microdissection from skin specimens of human occipital scalp and beard area. This was achieved by using a scalpel blade to cut through the skin at the dermo-subcutaneous fat interface. The intact hair follicles were then removed from the subcutaneous fat by gently gripping the outer root sheath of the follicle with microforceps and pulling the hair follicle from the subcutaneous fat. The proximal one-third of hair follicles containing the hair bulbs were dissected in Dulbecco's modified Eagle's medium (Biochrom) supplemented with 10% fetal calf serum, 100 IU penicillin per ml, 100 μg streptomycin per ml, 2 mM L-glutamine, and 50 ng amphotericin B (Sigma) per ml. The follicular fragments were then treated with 30 U dispase solution per ml for 15 min at 37°C (Chiu *et al*, 1993) and transferred to a Petri dish containing the same medium with 20% fetal calf serum. Each follicle was carefully microdissected, to separate out the dermal papilla, and three to five isolated dermal papillae were individually transferred to one 35-mm Petri dish (Messenger, 1984). Primary cultures were incubated undisturbed for 5–7 d, then the medium was changed every 3 d. Subcultures were performed after 4–5 wk, when sufficient cells were present. Cells of the third passage were used for the study.

Fibroblast culture Fibroblasts were cultured by explant outgrowth from small pieces of dermal tissue taken from skin sites in occipital scalp, chin, and shoulders, in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 IU penicillin per ml, 100 μg streptomycin per ml, and 2 mM L-glutamine.

Keratinocyte culture Keratinocytes from neonatal foreskin and adult non-genital skin regions like flank or arm were cultured in serum-free keratinocyte medium (Gibco BRL, Berlin, Germany) supplemented with 5 ng epidermal growth factor per ml and 50 μg bovine pituitary extract (Clonetics, San Diego, CA) per ml. Cells of the second passage were used for these experiments.

Melanocyte culture Normal human melanocytes were obtained and prepared as described before (Krasagakis *et al*, 1991). Primary cultures were maintained in modified MCDB 153 medium (Seromed) with amino acids and the following supplements: 2 mM Ca^{2+}, 10 μg human transferrin per ml, 5 μg insulin (Sigma) per ml, 0.4% vol/vol whole bovine pituitary extract, 2 ng bovine basic fibroblast growth factor (Boehringer) per ml, 10^{-9} M cholera toxin, 50 μM hydrocortisone (Sigma), 100 IU penicillin per ml, and 100 μg streptomycin per ml. Ten per cent fetal calf serum was added within the first 2 d of the primary culture and the first 24 h of each subculture. After ≈3 wk in culture any keratinocytes had degenerated and detached leaving an essentially pure growth of melanocytes with no detectable contamination by other cell types.

Culture of dermal microvascular endothelial cells A cell line of dermal microvascular endothelial cells was used (generous offer by Dr. E.W. Ades, Atlanta, Emory University, GA; Ades *et al*, 1992). The cells were cultured in endothelial basal medium supplemented with 2% fetal calf serum, 10 ng epidermal growth factor per ml, 1 μg hydrocortisone per ml, 50 μg gentamicin per ml, 50 ng amphotericin B per ml, and 3.0 mg bovine brain extract per ml (all Clonetics).

Immunocytochemistry The cultured cells were cytocentrifuged (Cytospin 2, Shandon, Frankfurt, Germany) on microscopic slides at a density of 25,000 cells per 200 μl and fixed with cold acetone for 15 min. The primary and secondary antibodies used were rabbit serum anti-human type 1 5α-reductase antibody (Luu-The *et al*, 1994) and alkaline phosphatase anti-alkaline phosphatase complex-conjugated goat-anti-rabbit IgG (H + L) monoclonal antibody (Dianova, Hamburg, Germany), respectively, both at dilutions of 1:100 in solutions containing 10% RPMI 1640 (Biochrom) and 10% fetal calf serum, pH 7.4.

RNA and protein isolation Total RNA and proteins were simultaneously isolated in a single-step reaction using a mono-phase solution of phenol, guanidine thiocyanate, buffer, and solubilizing agents (TRIzol, Gibco BRL/Life Technologies, Gaithersburg, MD) (Chomczynski, 1993). Cells in monolayer culture were first washed with phosphate-buffered saline twice and directly lysed in culture dishes by the addition of the reagent. In order to rule out the contamination of genomic DNA, the RNA yield was treated with RNAse-free DNAse (Boehringer Ingelheim, Heidelberg, Germany) for 10 min at 37°C. The amount of RNA was spectrophotometrically determined with OD_{260}/OD_{280} ratio above 1.8. By use of bicinchoninic acid (Pierce BCA protein assay reagent, Rockford, IL), the protein concentration was quantitated by measuring the absorbance at 550 nm with an enzyme-linked immunosorbent assay reader (Dynatech MR5000, Denkendorf, Germany).

Western blot analysis Aliquots (5–10 μg) of total protein isolated from the cultured cells were heated for 15 min at 95°C to denaturize proteases (Johnstone and Thorpe, 1987). Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of each sample was performed on a 12.5% gel. Proteins were transferred to a immobilon-P PVDF transfer membrane (Millipore, Eschborn, Germany), using a semi-dry blotting system (Bio-Rad, Munich, Germany). The blots were probed with a 1:1000 dilution of rabbit anti-serum raised against human type 1 5α-reductase and then with alkaline phosphatase anti-alkaline phosphatase complex-conjugated goat anti-rabbit antibody in a final concentration of 0.5 μg per ml (Oncogene Science, Cambridge, MA). After thorough washing, the signal was visualized using a combination of nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates for the phosphatase reaction (Zymed, San Francisco, CA).

Northern blot analysis Ten μg total RNA isolated from the cultured cells was denaturized and size-fractionated by electrophoresis over 1.2% agarose gel containing 3-(N-morpholino)propanesulfonic acid/formaldehyde and then transferred to a supported, positively charged nylon membrane (GeneScreen Plus, Dupont, Boston, MA). After baking for 2 h at 80°C, the blots were sequentially hybridized against ^{32}P-labeled probes for human type 1 5α-reductase, β-actin, and glyceraldehyde 3-phosphate dehydrogenase (G3PDH), respectively, which were prepared by recovery of polymerase chain reaction (PCR) products from amplification of corresponding primers. Exposure to autoradiography film (Kodak X-OMAT, Sigma, Germany) was done at –70°C (Sambrook *et al*, 1989). The results were analyzed by densitometry (Bio Profil, Vilber Lourmat, Marne La Vallèe, France).

Semiquantitative reverse transcriptase PCR coupled with high performance liquid chromatography (HPLC) analysis The kinetic method was utilized in this experiment. In order to avoid tube-to-tube variations, the primer pair for a 368-bp type 1 5α-reductase fragment (forward 5′-TGG CGC TTC TCT ATG GAC TT-3′, reverse 5′-GGA AGC AAC ACT GCA GTT GA-3′) (sequences kindly provided by Drs. Eicheler and Hoffmann, Marburg, Germany) and a second primer pair of a 227-bp housekeeping gene β-actin fragment (forward 5′-GGG AAA TCG TGC GTG ACA TT-3′, reverse 5′-GGA GTT GAA GGT AGT TTC GTG-3′), as endogenous internal standard, were mixed together from the beginning in the same tube during PCR amplification (Noonan *et al*, 1990; Van Hille *et al*, 1995). In the preceding step, we decided the relative concentrations of each primer pair and the appropriate range of PCR cycles. The aim was to establish a linear logarithmic relationship between the PCR amplicons and the cycle numbers. PCR amplification of the complementary DNA (cDNA) was carried out using the "hot start" technique for 14–28 consecutive cycles in 50 μl of amplification buffer (Perkin-Elmer, Weiterstadt, Germany) containing 1.25 U per reaction Tag DNA polymerase (Perkin-Elmer), and 5′ and 3′ primers in concentration of 0.5 μM and 0.125 μM for type 1 5α-reductase and β-actin, respectively. The thermal profile was initially 94°C for 5 min, followed by the indicated number of cycles at 94°C for 45 s, at 60°C for 45 s, at 72°C for 90 s, and finally at 72°C for 10 min in a programmable thermal controller (PTC-100, MJ Research, Watertown, MA). The total amount of end products in 50 μl were directly loaded on the HPLC system (LKB Bromma, Pharmacia Biotech, Freiburg, Germany) set up with a diethylaminoethyl anion guard column (5.0 mm long × 4.6 mm internal diameter; Perkin-Elmer) and diethylaminoethyl anion exchange column (3.5 cm long × 4.6 mm internal diameter, Perkin-Elmer). The mobile phase consisted of various volume proportions of buffer A (25 mM/Tris-HCl, pH 9.0, plus 1 M NaCl) and buffer B (25 mM/Tris-HCl, pH 9.0) in the following gradient program (A/B): 0 min, 30/70; 0.5 min, 50/50; 4.0 min, 56/44; 14.0 min, 61/39; 14.5 min, 100/0; 19.5 min, 100/0; 20.0 min, 30/70; 25.0 min, 30/70. The total flow rate was 1 ml per min. ϕ X174 RF DNA/Hae III fragments (Gibco BRL) were used as external DNA standards. The signal was detected with 260 nm ultraviolet light. Utilizing a computer program, we assessed the original amount of the mRNA of interest and that of standard β-actin. The ratio in between was used to assess the quantitative differences between different cell types under examination.

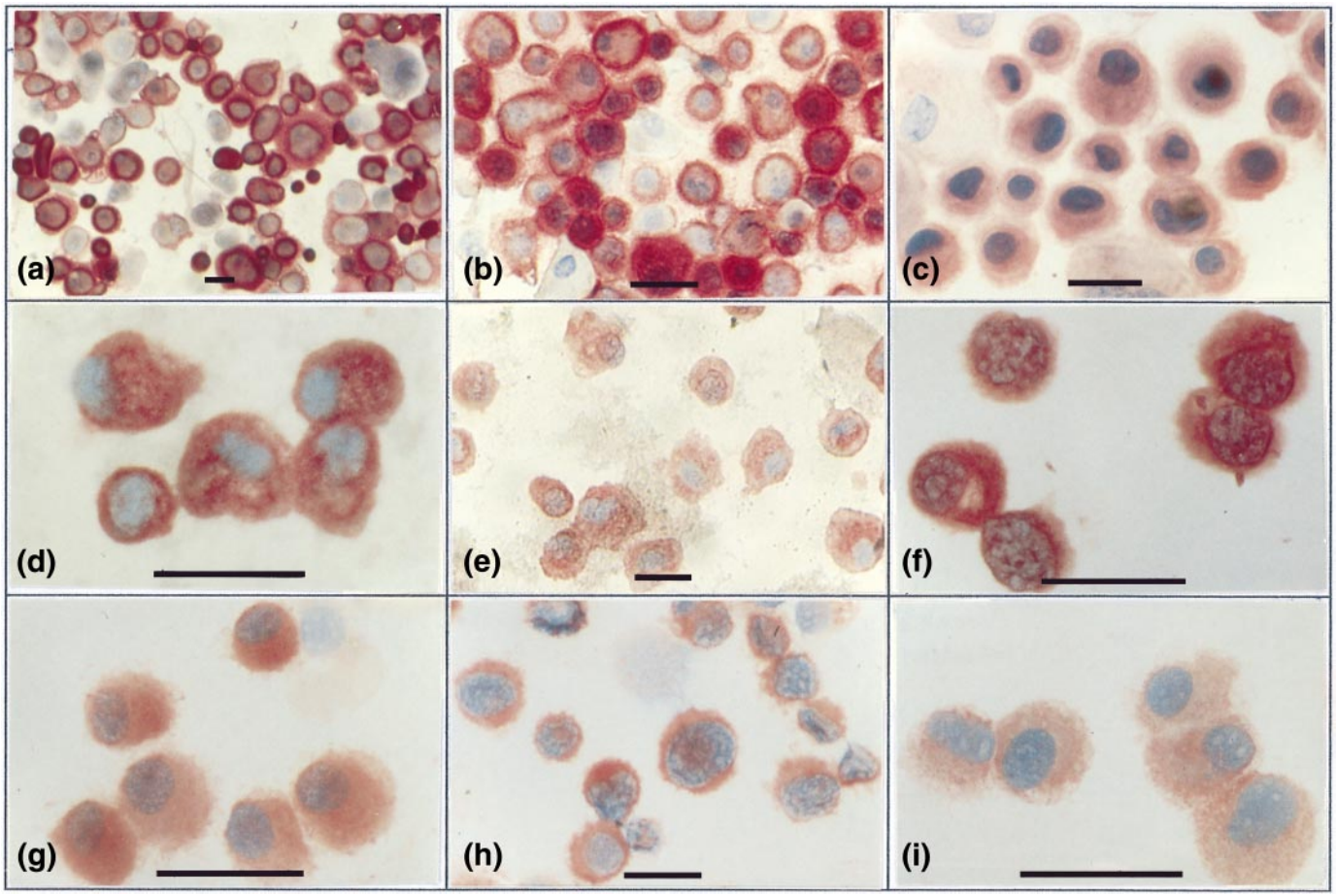

Figure 1. Results of immunocytochemistry using rabbit anti-serum raised against human type 1 5α-reductase. (*a*) Adult facial sebocytes; (*b*) neonatal foreskin keratinocytes; (*c*) adult nongenital keratinocytes (flank); (*d*) occipital dermal papilla cells; (*e*) beard dermal papilla cells; (*f*) occipital fibroblasts; (*g*) melanocytes; (*h*) dermal microvascular endothelial cells; (*i*) chin fibroblasts. *Scale bars*, 50 μm.

RESULTS

Immunocytochemistry The staining procedure was first established on skin tissue with sebaceous glands, which are known to express type 1 5α-reductase. The enzyme protein was located in the cytoplasm, not in the nucleus, of all the cell types tested (**Fig 1**). The intensity of the expression could be broadly divided into three groups, in order of decreasing intensity: (i) adult facial sebocytes and neonatal foreskin keratinocytes, (ii) occipital dermal papilla cells, occipital fibroblasts, shoulder fibroblasts, keratinocytes from nongenital area (flank), and (iii) beard dermal papilla cells, chin fibroblasts, melanocytes, dermal microvascular endothelial cells. Moreover, among sebocytes, the smaller, undifferentiated sebocytes were more strongly stained than the larger, differentiated ones (**Fig 2**).

Western blotting The molecular weight of type 1 5α-reductase protein is between 28 and 29 kDa (Andersson and Russell, 1990); however, in sodium dodecyl sulfate/polyacrylamide gel electrophoresis, the isozymes show aberrant electrophoretic mobility because of high hydrophobic amino acid contents, and migrate with molecular weights of 21–27 kDa (Russell and Wilson, 1994). When 5 μg per protein sample was loaded, only sebocytes and neonatal foreskin keratinocytes showed positive results. When 10 μg per protein samples were examined and concentration of the sodium dodecyl sulfate gel was elevated above 10%, two corresponding bands lying closely together were demonstrated in all the cultured cells under examination, with the exception of beard dermal papilla cells (**Fig 3**). The expressional intensity of type 1 5α-reductase was stronger in neonatal foreskin keratinocytes than in keratinocytes from adult nongenital areas, and it was also stronger in occipital dermal papilla cells than in beard dermal papilla cells.

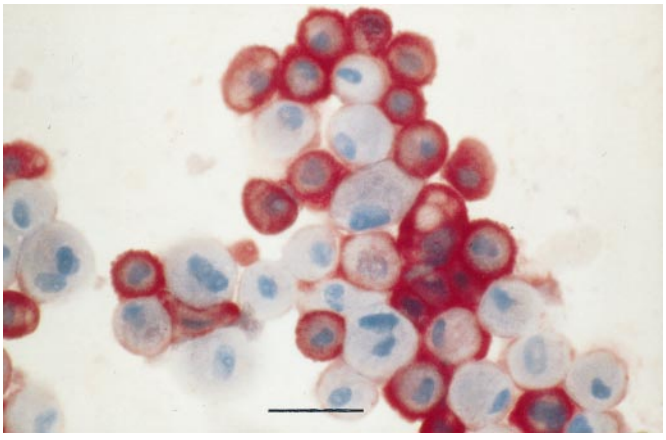

Figure 2. Immunocytochemistry of adult facial sebocytes from the fourth passage in culture. The smaller, less differentiated sebocytes are more strongly stained with anti-type 1 5α-reductase antibody as compared with larger, well-differentiated ones. *Scale bar*, 50 μm.

Northern blotting The sizes of the type 1 5α-reductase, β-actin, and G3PDH mRNA were estimated to be 2.4 kb, 1.7 kb, and 1.3 kb, respectively, which are compatible with those described in the literature (Nakajima-Iijima *et al*, 1985; Allen *et al*, 1987; Andersson and Russell, 1990). Densitometrical analysis revealed that the expression of type 1 5α-reductase mRNA, corrected by the amount of G3PDH, was (i) most abundant in neonatal foreskin keratinocytes, followed by adult

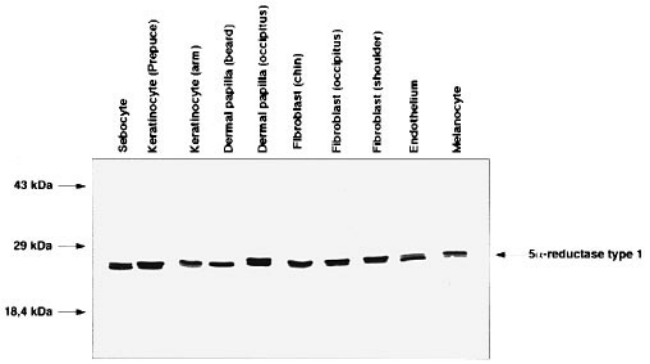

Figure 3. Results of western blot study with 12.5% sodium dodecyl sulfate/polyacrylamide gel electrophoresis. With the exception of beard dermal papilla cells, all the cells under examination show two closely lying bands in the range of molecular weight 21–27 kDa.

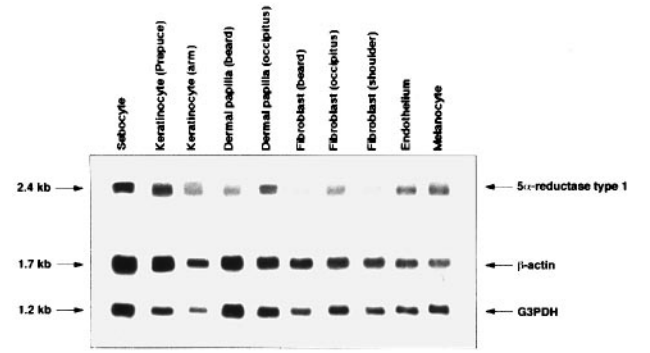

Figure 4. Results of northern blot study with 1.2% MOPS/fomaldehyde agarose gel. Quantitative differences are observed among different cell types, after correction by β-actin and G3PDH, respectively, which show a parallel relationship.

Table I. Densitometrical data of northern blot analysis (the 5α-reductase/G3PDH ratio in sebocytes was assessed = 1)

	5α-reductase/G3PDH ratio
Sebocyte	1
Keratinocyte (foreskin)	3.83
Keratinocyte (flank)	0.66
Dermal papilla (occipitus)	0.41
Dermal papilla (beard)	0.08
Fibroblast (occipitus)	0.70
Fibroblast (chin)	0.19
Fibroblast (shoulder)	0.44
Melanocyte	0.31
Endothelium	0.24

facial sebocytes, (ii) stronger in occipital dermal papilla cells as compared with that in beard, and (iii) different in fibroblasts from different skin areas (**Fig 4** and **Table I**).

Semiquantitative reverse transcriptase PCR The initial amounts of target mRNA (type 1 5α-reductase and β-actin) were obtained by using linear regression of the logarithmic area under concentration values of the serially individual HPLC peak (**Fig 5**). The ratios inbetween, i.e., type 1 5α-reductase/β-actin, from three experiments ranged from 1.5- to 10-fold higher in neonatal foreskin keratinocytes as compared with that in adult facial sebocytes (**Fig 6**), which verified the results of our northern blot study.

DISCUSSION

The expression and distribution of 5α-reductase isozymes in human skin have been investigated mainly *in vivo* by means of (i) performing enzyme assay in isolated tissues or cultured cells (Voigt *et al*, 1970;

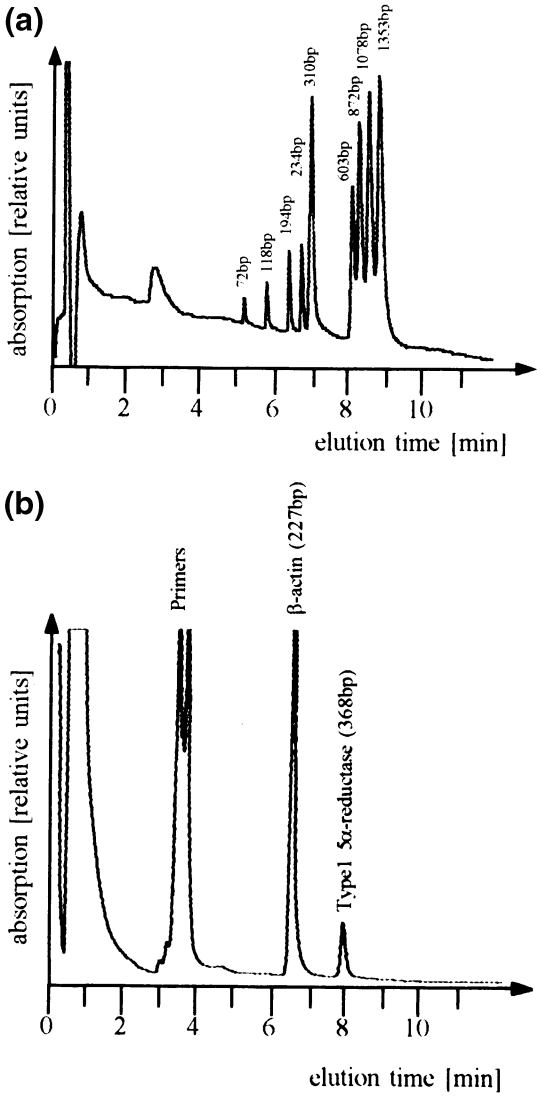

Figure 5. (*a*) DNA standards; (*b*) β-actin and type 1 5α-reluctase PCR products. (*a*) HPLC analysis of PCR products from 22 cycles amplification. cDNA of β-actin and type 1 5α-reductase were amplified in the same tube, whereby the concentration of β-actin primers was one-fourth of that of type 1 5α-reductase primers (0.125 μM *vs* 0.5 μM). (*b*) Elution of PCR products was correlated to size in accordance with HPLC analysis of DNA standards.

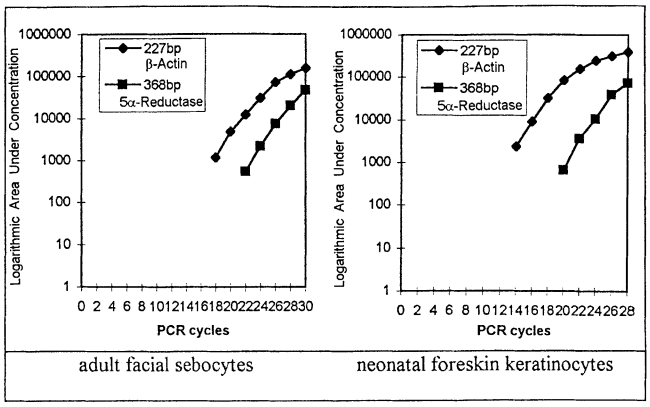

Figure 6. Linear correlations between PCR cycles and area under concentration of HPLC analysis. The appropriate cycles for type 1 5α-reductase and β-actin were shown to be 22–28 and 14–20, respectively. The value of type 1 5α-reductase in neonatal foreskin keratinocytes, when corrected with β-actin, is about 1.5–10 higher that of adult facial sebocytes.

Takayasu and Adachi, 1972; Hay and Hodgins, 1973; Sharp *et al*, 1976; Takayasu *et al*, 1980) or (ii) detecting the enzyme localization on skin specimens by specific antibodies (Luu-The *et al*, 1994; Eicheler *et al*, 1995). To verify and compare the *in vivo* results, we have used skin cell cultures *in vitro* for cytochemical and molecular biologic studies.

Because the antibodies described in the literature were raised against different peptide sequences in different animals, the results regarding the cutaneous distribution of the 5α-reductase isozymes vary among different research groups (Thigpen *et al*, 1993; Luu-The *et al*, 1994; Eicheler *et al*, 1995; Patel *et al*, 1996). There is agreement on the expression of type 1 isozyme in sebaceous glands, keratinocytes, and eccrine sweat glands. In contrast, the isozyme expression in different parts of hair follicles remains controversial and no data are available about its expression in melanocytes and Langerhans cells. Additional questions include the subcellular localization of the enzyme and whether there exist quantitative differences in the same cell type from different skin regions as well as differences between healthy persons and diseased subjects.

Our findings clarify the following issues: (i) the presence of type 1 5α-reductase in the cytoplasm of all cultured skin cell types under examination; (ii) the possibility of the existence of heterogeneous human type 1 5α-reductase proteins; (iii) the strongest expression of type 1 5α-reductase in facial sebocytes among skin cells from adult subjects; (iv) the stronger expression of type 1 5α-reductase in neonatal foreskin keratinocytes than in adult facial sebocytes; (v) the regional variance of type 1 5α-reductase expression in fibroblasts and keratinocytes; (vi) the presence of type 1 5α-reductase in dermal papilla cells of both beard and occipital scalp, and the stronger expression in the latter; and (vii) the expression of type 1 5α-reductase in cultured melanocytes.[1]

To explain the two closely lying bands on western blotting, the following possibilities should be considered: (i) the presence of heterogeneity of human type 1 isozyme protein; (ii) the enzyme existence in two states, activated or inactivated, through phosphorylation or dephosphorylation; and (iii) a partial protein denaturation during the process of extraction/isolation. The first observation is supported by a recent study that revealed the presence of two forms of mRNA species for rat type 1 5α-reductase (Lopez-Solache *et al*, 1996). The newly isolated cDNA clones encoded a longer protein with four additional amino acids in the NH_2-terminal region, and the protein had a higher substrate affinity than the previously reported one. The presence of heterogeneous type 1 protein isozymes in all the cells tested except beard dermal papilla cells, could be of the following significance: (i) it may help to explain the discrepancy of the available findings with regard to enzyme distribution and subcellular localization; (ii) it may elucidate the different responses of hair follicles from different skin areas (beard, occipital, and frontal scalp) to androgens; and (iii) it should be taken into account in perspective of treatment of androgenetic alopecia. It is unlikely that the type 1 5α-reductase anti-serum used cross-reacted with type 2 5α-reductase, because sebocytes that expressed two bands in western blotting were found not to express type 2 5α-reductase at the mRNA level (data not shown).

The strongest expression of type 1 5α-reductase in sebocytes among the adult skin cells examined confirms our previous hypothesis that the sebaceous gland would be the main source of high concentration of tissue-active androgens in the pilosebaceous unit (Zouboulis *et al*, 1994; Zouboulis *et al*, in press). When we consider that the sebocytes examined in this study originated from older subjects, who have been shown to exhibit a lower sebocyte activity as compared with younger individuals (Pochi and Strauss, 1974), we may expect even higher 5α-reductase expression in sebocytes deriving from young subjects.

The abundance of type 1 5α-reductase in neonatal foreskin keratinocytes highlights the importance of DHT in the growth and differentiation of epidermis. In this respect, the DHT secreted by keratinocytes may be of autocrine as well as paracrine function for the development of skin and skin appendages in the period of time from shortly after birth until puberty, whereas the sebaceous glands cease to work. It is worth examining further (i) whether the expression of type 1 5α-reductase in keratinocytes decreases with aging, and (ii) the role of androgens in the physiologic function of epidermis, such as maintenance of the integrity of skin barrier.

By studying patients with familial incomplete male pseudohermaphroditism type 2 (now termed type 2 5α-reductase deficiency syndrome), it was demonstrated that fibroblasts from nongenital areas possess type 1 isozyme, whereas those from the genital area display type 2 isozyme (Moore *et al*, 1975; Moore and Wilson, 1976). Our results further point out the existence of quantitative differences in the expression of type 1 5α-reductase in fibroblasts among nongenital skin areas (chin, scalp, shoulder).

The expression pattern of the type 1 *versus* type 2 isozymes in human hair dermal papilla cells has been the subject of vigorous discussion. The results of early studies in the 1970s, which were obtained by incubating the plucked human hair follicles with radiolabeled T and other androgen precursors, do not provide valuable information, because the plucked human hairs usually leave the dermal papilla in the skin (Fazekas and Lanthier, 1971; Takayasu and Adachi, 1972; Schweikert and Wilson, 1974). Itami *et al* (1990) showed that the 5α-reductase of beard dermal papilla cells exhibited an pH optimum at 5.5, which corresponds to type 2 isozyme, whereas the enzyme of occipital dermal papilla cells showed a broad and low plateau between pH 6.0 and 9.0, which is optimal for type 1 isozyme. Thornton *et al* (1993) showed that significant amounts of DHT were recovered inside beard dermal papilla cells, but not in occipital dermal papilla cells. There were no significant differences in the substrate specificity between these two types of cells. Harris *et al* (1992) showed that the 5α-reductase in human scalp obtained during hair transplantation (site not clearly defined, possibly fronto-parietal area) has a broad pH optimum centered at pH 7.0 (corresponding to type 1 isozyme). Two 4-azasteroids and a 3-carboxyandrostadione were shown to be potent inhibitors of the prostate reductase but weak on the scalp reductase. Immunohistochemical studies, however, showed controversial results; the type 1 isozyme was demonstrated by Eicheler *et al* (1995) in the dermal papilla cells from the scalp (skin region unknown), whereas it was detected by Patel *et al* (1996) only in the sebaceous glands in balding scalp. In the present study, we have demonstrated the presence of type 1 5α-reductase in both cultured occipital and beard dermal papilla cells, whereby a stronger expression was observed in the former. There was no heterogeneity of type 1 5α-reductase in beard dermal papilla cells under examination.

The expression of 5α-reductase in melanocytes is an additional interesting finding, the significance of which is unknown. Because the maturation or transformation of vellous hair to terminal hair involves the melanin production by melanocytes situated in the hair dermal papilla (Randall, 1994) and the outgrowth of pigmented terminal hairs in hirsutism, it could be supposed that the presence of 5α-reductase in melanocytes and the local production of DHT may play a role in the melanin production. The effect of DHT on the tyrosinase activity remains to be determined. On the other hand, it is well documented that women with melanoma present a better prognosis compared with male subjects (Shaw *et al*, 1980; Karjalainen and Hakulinen, 1988). An *in vitro* study recently showed that addition of flutamide, an antiandrogen agent, to cultured IIB-MEL-J melanoma cells in the presence of serum significantly inhibited cell proliferation in a dose-dependent manner (Morvillo *et al*, 1995). When cells were incubated with 10 nM DHT and 1% charcoal-adsorbed serum, a significant stimulation of growth was observed and this was inhibited by 4 μM OH-flutamide. This observation, together with our finding, raises the question of the roles of androgens and 5α-reductase in the biology of melanoma cells.

In summary, by using skin cell cultures *in vitro*, we have demonstrated the cytoplasmic localization of type 1 5α-reductase. We have also detected possible heterogeneity and quantitative differences of type 1 5α-reductase in skin cells, including melanocytes. This finding may help to explain some of the discrepancies of previous results. Further examination and comparison of the type as well as the amount of 5α-reductase isozymes between normal and diseased subjects in various

[1]Since this paper was written another work (Tadokoro *et al*, 1997) has been published confirming 5α-reductase type 1 expression in human melanocytes from genital skin.

stadium (acne vulgaris, androgenetic alopecia, hirsutism) could help us better understand the etiopathogenesis of these androgen-dependent skin disorders.

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