Expression of Peroxisome Proliferator-Activated Receptor and CCAAT/Enhancer Binding Protein Transcription Factors in Cultured Human Sebocytes

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Lipid synthesis and accumulation represent a major step in sebocyte differentiation and it may be of importance for sebocytes to express two families of transcription factors, CCAAT/enhancer binding proteins (c/EBPs) and peroxisome proliferator-activated receptors (PPARs), which were found to play a crucial role in the differentiation of adipocytes. Using the immortalized human sebaceous gland cell line SZ95 we examined the expression of the molecules before and after treatment with testosterone, 5α-dihydrotestosterone, dexamethasone, 17β-estradiol and genistein, at 6, 12, 24, and 48 h, respectively. Reverse transcription-PCR analysis showed expression of peroxisome proliferator-activated receptors -α, −δ, −γ1, -γ2 and CCAAT/enhancer binding proteins-α, −β, −γ −δ in native SZ95 sebocytes. In western blot studies, high levels of CCAAT/enhancer binding proteins-α and -β, and peroxisome proliferator-activated receptors-γ were expressed at 6, 24, and 12 h, respectively. Immunostaining of the cultured sebocytes showed the CCAAT/enhancer binding proteins-α and -β mainly localized within nuclei, whereas peroxisome proliferator-activated receptors-γ in the cytoplasm. Strong staining of sebocytes was immunohistochemically revealed in the basal layer of sebaceous glands in human scalp and sebaceous nevus. Genistein downregulated the expression of CCAAT/enhancer binding proteins-α and -β, and peroxisome proliferator-activated receptors-γ on the protein level. Treatment with linoleic acid for 48 h induced further differentiation of sebocytes leading to abundant lipid synthesis. ***Key words: androgens/genistein/sebaceous glands/steroids/transcription factors. J Invest Dermatol 121:441–447, 2003***

Sebaceous lipogenesis, leading to accumulation of lipid droplets and finally to sebum excretion, represents a major step of the differentiation process in sebaceous gland cells (Downie and Kealey, 1998). Excessive sebum production is supposed to be of crucial importance in the pathogenesis of acne (Kligman, 1974). Over the past decade, considerable progress has been made in our understanding of the molecular events regulating adipocyte differentiation. Several transcription factors have been identified, which act cooperatively and sequentially to trigger the terminal differentiation program in cultured pre-adipocyte cell lines, such as the NIH 3T3 fibroblast cell line (MacDougald and Lane, 1995; Mandrup and Lane, 1997). Among them, CCAAT/enhancer binding proteins (c/EBP) and peroxisome proliferator-activated receptors (PPAR) are thought to play crucial parts. Whereas high levels of c/EBP-β and c/EBP-δ are expressed in the early induction phase, c/EBP-α maintains terminal adipocyte differentiation (Mandrup and Lane, 1997). Expression of c/EBP-γ is ubiquitous and constitutive and the murine c/EBP-γ was shown to act as a transdominant negative regulator on the transcriptional ability of c/EBP-β and c/EBP-α (Cooper *et al*, 1995).

Subtypes of human PPAR, including PPAR-α, PPAR-δ, and PPAR-γ, share a high degree of amino acid sequence as well as functional similarities and can bind to retinoid X receptor α to form heterodimers (MacDougald and Lane, 1995). Among PPAR, PPAR-γ was found to be the most adipogenic, followed by PPAR-α, whereas PPAR-δ was rather inactive (Brun *et al*, 1996). Alternative splicing of one gene product yields two transcripts of PPAR-γ; whereas PPAR-γ1 was found to predominate in adipose tissue and large intestine, PPAR-γ2 seemed to play a minor part even in adipose tissue (Auboeuf *et al*, 1997). The ratio of PPAR-γ2/-γ1 in adipose tissue, however, was found to be correlating with the extent of human obesity (Vidal-Puig *et al*, 1997). PPAR-δ expression was found to be activated early in the differentiation and its levels remained relatively constant during the differentiation process (Mandrup and Lane, 1997).

c/EBP and PPAR interact closely to conduct terminal differentiation of adipocytes. c/EBP-δ may, by synergizing with c/EBP-β, enhance the level of PPAR-γ expression (Wu *et al*, 1996). c/EBP-β is believed to be a transactivator of the c/EBP-α gene and equally activates the PPAR-γ expression. c/EBP-α, whose expression coincides with the later stages of differentiation, cooperates with PPAR-γ in inducing additional target genes and sustains a high level of PPAR-γ in the mature adipocyte as part of a feed-forward loop (Fajas *et al*, 1998).

Specific activators of PPAR have been identified and tested for their effect on the adipocyte differentiation (Hihi *et al*, 2002).

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Abbreviations: c/EBP, CCAAT/enhancer binding proteins; DHT, 5α-dihydrotestosterone; E2, 17β-estradiol; LIN, linoleic acid; PPAR, peroxisome proliferator-activated receptors.

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Table I. Primers used for reverse transcription–PCR analysis of transcription factors PPAR and c/EBP in cultured transformed human sebocytes

Gene	Primer	Sequence (5′–3′)	Annealing temperature (°C)	Product size (bp)
β-actin	Sense	AGC GGG AAA TCG TGC GTG	58	309
	Anti-sense	CAG GGT ACA TGG TGG TGG TGC C		
PPAR-γ1	Sense	TCT CTC CGT AAT GGA AGA CC	55	474
	Anti-sense	GCA TTA TGA GAC ATC CCC AC		
PPAR-γ2	Sense	GCG ATT CCT TCA CTG ATA C	58	580
	Anti-sense	GCA TTA TGA GAC ATC CCC AC		
PPAR-α	Sense	TCA TCA AGA AGA CGG AGT CG	55	211
	Anti-sense	CGG TTA CCT ACA GCT CAG AC		
PPAR-δ	Sense	TCC CTC TTT CTC AGT TCC TC	58	287
	Anti-sense	CAG GAG ACA GAA GTG AGG AC		
c/EBP-α	Sense	GAA CAC GAA GCA CGA TCA G	55	268
	Anti-sense	CCA AAA CCA AAA GGA AAG GGA G		
c/EBP-β	Sense	TGA TAA ACT CTC TGC TCT CCC	55	191
	Anti-sense	AAA CAT CAA CAG CAA CAA GCC		
c/EBP-γ	Sense	ACC CTG CTC TCA TTT CTA CC	55	103
	Anti-sense	ACA CTA ATT CCG TTC ACC CC		
c/EBP-δ	Sense	AAC GAC CCA TAC CTC AGA C	55	240
	Anti-sense	ACA AAT GTA CCT TAG CTG CAT C		

Strong PPAR-α ligands include the physiologically existent leukotriene B4 and synthetic hypolipemic compounds of fibrates (e.g., WY-14643, clofibrate, fenofibrate). Linoleic acid (LIN) is a natural PPAR-δ/β ligand. 15-deoxy-δ(12,14)-prostaglandin J_2 is a natural PPAR-γ ligand, as are also the synthetic insulin sensitizing compounds thiazolidinediones (ciglitazone, pioglitazone, rosiglitazone).

Phytestrogens, such as the soya isoflavones genistein and daidzein, are associated with a reduced risk of many hormone-dependent tumors, such as prostate and breast cancer (Barnes *et al*, 2000). The effect of isoflavones on androgen-dependent dermatoses, however, remains to be determined.

In this study we examined and compared the expression of these adipogenesis-related genes in human sebocytes, in order to understand better the molecular control of human sebocyte differentiation and sebum production. The experiments were aimed to study: (1) the sequential expression of c/EBP (c/EBP-α, -β, -δ, -γ) and PPAR (PPAR-γ1, -γ2, -α, -δ); (2) the regulatory effects of testosterone, 5α-dihydrotestosterone (DHT), dexamethasone, 17β-estradiol (E2), and genistein; and (3) the effect of different PPAR agonists on lipogenesis in sebocytes.

MATERIALS AND METHODS

Cell culture and treatment The immortalized sebaceous gland cell (sebocyte) line SZ95 was used (Zouboulis *et al*, 1999). The cells were initially grown in Sebomed basal medium (Biochrom, Berlin, Germany) containing 10% heat inactivated fetal bovine serum, and 5 ng human recombinant epidermal growth factor per mL. Before treatment, the culture medium was switched to serum-free, phenol red-free Sebomed ready-to-use medium (Biochrom) containing 50 μg bovine pituitary extract per mL, 5 ng human recombinant epidermal growth factor per mL, 1 mg fatty acid-free bovine serum albumin per mL, and 0.042 μg LIN per mL (about 1.5×10^{-7} M).[1] The cells were treated with testosterone, DHT, dexamethasone, E2, and genistein at 10^{-6} M for 0, 6, 12, 24, and 48 h, respectively; 10^{-6} M WY-14643 and ciglitazone (both from AG Scientific, San Diego, California) and 10^{-4} M LIN (Sigma-Aldrich, Deisenhofen, Germany), specific ligands for PPAR-α, -γ, and -δ/β , respectively, were also administered to the cells in serum-free medium for 48 h to evaluate their effects on lipid synthesis of cultured sebocytes (Rosenfield *et al*, 1998).

Reverse transcription–polymerase chain reaction (reverse transcription–PCR) studies Total RNA was extracted using a commercial kit (StrataPrep, Stratagene, La Jolla, California), including a specific DNA removal step with DNAse. Two micrograms of total RNA was reverse transcribed using oligo(dT) primers and AMV reverse transcriptase (Promega, Madison, Wisconsin). The oligonucleotide primers used for reverse transcription–PCR, synthesized by Gibco-BRL (Tokyo, Japan) are listed in **Table I**. PCR amplification of the complementary DNA was carried out for 35 consecutive cycles in 50 μL of amplification buffer (Promega) containing 2.5 U per reaction Tag polymerase (Promega), and 5′ and 3′ primers each in concentration of 0.5 μM. The thermal profile began with one cycle 94°C for 5 min, and 35 consecutive cycles at 94°C for 30 s, at indicated hybridization temperature (see **Table I**) for 45 s, at 72°C for 45 s and finally at 72°C for 10 min in a programmable thermal controller (GeneAmp, PCR system 2400, Applied Biosystems, Foster City, California).

Western blot analysis Protein extraction was performed using M-PER mammalian protein extraction reagent (Pierce, Rockford, Illinois). Aliquots (10 μg) of total protein isolated from the cultured cells were heated for 15 min at 95°C in a buffer to denaturize proteases. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of each sample was performed on 12.5% gels. Proteins were transferred on to a PVDF transfer membrane (PolyScreen, NEN, Boston, Massachusetts), using a semi-dry blotting system (Bio-Rad, Hercules, California). The blots were primarily probed with goat polyclonal IgG (anti-human PPAR-γ) and rabbit polyclonal IgG (anti-human c/EBP-α and -β) (all from Santa Cruz, Santa Cruz, California) at a concentration of 1:500, respectively, and visualized using a chromophore-conjugated anti-IgG antibody (1:5000) and enhanced chemiluminescence reagents (ECL Western blotting luminol reagents, Santa Cruz) followed by exposure to BioMax light film (Kodak, Rochester, New York). The results were analyzed by densitometry (Bio-1D Version 99, Vilber Lourmat, France).

***In vivo* and *in vitro* immunochemical studies** Frozen sections (6 μm thick) of skin specimens taken from occipital scalp of a 34 y old male and from sebaceous nevus of a 10 y old boy during surgical excision were fixed for 15 min in cold acetone, followed by treatment in 0.3% (vol/vol) H_2O_2 in methanol for 15 min to block endogenous peroxidase activity, and then incubation with 0.1% bovine serum albumin for 30 min to block nonspecific staining. The SZ95 sebocytes were cultured in eight-well tissue culture chambers (Nunc, Naperville, Illinois) until 50% confluence, washed with PBS twice, and then fixed and treated in the same way. Incubations with the aforementioned primary antibodies diluted to 1:250 and 1:1000 in antibody diluent (DAKO, Glostrup, Denmark) for cultured

[1]Seltmann H, Hornemann S, Orfanos CE, Zouboulis ChC: Linoleic acid induces accumulation of neutral lipids in undifferentiated human sebocytes and reduces spontaneous IL-8 secretion. *Arch Dermatol Res* 291:181, 1999 (Abstr.)

cells and for tissues, respectively, were carried out at room temperature for 1 h, followed by sequential 10 min incubations with the respective biotinylated link antibodies and peroxidase-labeled streptavidin (DAKO). Staining was completed after incubation with diaminobenzidine/chromogen solution (DAKO) and mounted in aqueous mounting medium.

Cell proliferation The cultured SZ95 sebocytes were seeded in 96-well culture plates (Nunc, Naperville, Illinois) at cell densities of 10,000 cells per well and the sebocyte proliferation before and after treatment was measured by a colorimetric assay (tetrazolium salt WST-1 based) (Roche, Mannheim, Germany) and the fluorescence was read on an Emas precision microplate reader (Molecular Devices, Sunnyvale, California) with absorbance at 590 nm.

Nile red staining and flow cytometry SZ95 sebocytes maintained in Sebomed basal medium supplemented with 10% heat inactivated fetal bovine serum, 5 ng human recombinant epidermal growth factor per mL, 1 mM $CaCl_2$, and 50 μg gentamycin per mL were grown over six passages with and without testosterone (10^{-11} M, 10^{-9} M, 10^{-7} M, 10^{-5} M). At subconfluence, the sebocytes were subcultured and the medium was changed every 2 d with fresh supplemented testosterone concentrations. Adherent SZ95 sebocytes of subconfluent cultures were detached from flasks with Accutase (PAA, Cölbe, Germany), resuspended in complete medium and centrifuged at $250 \times g$ for 10 min. Viable cells were then resuspended in PBS and stained with Nile red, a fluorescent lipophilic dye (Sigma). The Nile red stock solution (1 mg per mL in acetone) was diluted in PBS, then added immediately to the cell suspension to achieve a final concentration of 1 μg Nile red per ml. To assess the lipid content by flow fluorometry, 10,000 cells per sample were analyzed using a FACScalibur (Becton Dickinson, Plymouth, U.K.) equipped with a 15 mW argon-ion laser device operated at 488 nm. A short-pass beam splitter transmitted emission less than 560 nm. Nile red fluorescence was collected through a 530 ± 30 nm and 585 ± 21 nm band-pass filter, respectively.

Statistical analysis Growth studies were assessed in sixplicate wells of 96 well plates. All other experiments were performed in triplicate dishes. Statistical significance of the data was evaluated by the two-sided Student's t test. Mean differences were considered to be significant when $p < 0.05$.

RESULTS

mRNA and protein expression of PPAR-α, -δ, -γ1, and -γ2 and c/EBP-α, -β, -γ, and -δ in SZ95 sebocytes Reverse transcription–PCR analysis showed presence of PPAR-α, -δ, -γ1, and -γ2, and c/EBP-α, -β, -γ, and -δ in SZ95 sebocytes (**Fig 1**). No dynamic alteration could be observed in the native cells during the time course of culture from 6 to 48 h. Treatment with various steroid hormones (testosterone, DHT, E2, dexamethasone) and genistein for up to 48 h seemed to exert little regulatory effect on the mRNA expression of the molecules under examination. In western blot studies, high levels of c/EBP-α, PPAR-γ, and c/EBP-β were observed at 6, 12, and 24 h, respectively (**Fig 2**). The expression of PPAR-γ, reaching its peak at 12 h, was generally weaker than that of c/EBP-α and -β. Immunocytochemistry detected the presence of c/EBP-α and c/EBP-β within nuclei of cultured sebocytes, whereas PPAR-γ was seen mainly in the cytoplasm (**Fig 2**). Immunohistochemistry showed strong *in vivo* expression of c/EBP-α and c/EBP-β in the sebocytes, especially those in the basal layer of sebaceous glands (**Fig 3**). Immunostaining appeared to be stronger in sebocytes from sebaceous nevus than those from occipital scalp. Low levels of PPAR-γ were detected. Among the hormones tested, genistein downregulated the expression of the three molecules under examination at different time points, with maximal rates of –33% for c/EBP-α at 48 h, –64% for c/EBP-β at 24 h, and merely –18% for PPAR-γ at 24 h (**Fig 4**). **Table II** summarized the densitometrical data of western blot analysis.

Effects of hormones and PPAR agonists on SZ95 sebocytes There was no regulatory effect of testosterone, DHT, dexamethasone, E2, and genistein at a concentration of 10^{-6} M on SZ95 sebocyte proliferation and no long-term effect of testosterone on lipid synthesis of SZ95 sebocytes (**Fig 5*A***). Sebocytes cultured in serum-free medium seemed to produce more lipids than cultured in serum-containing medium (**Fig 5*B***). Whereas both WY-14643 and ciglitazone at 10^{-6} M showed no regulatory effect on cultured sebocytes, treatment with 10^{-4} M LIN for 48 h significantly induced lipid accumulation in SZ95 sebocytes (**Fig 5*C***) and enlarged lipid droplets (**Fig 5*D***), which was also morphologically reflected in **Fig 6**, where increased cell size was also observed after LIN treatment (**Fig 6*B***).

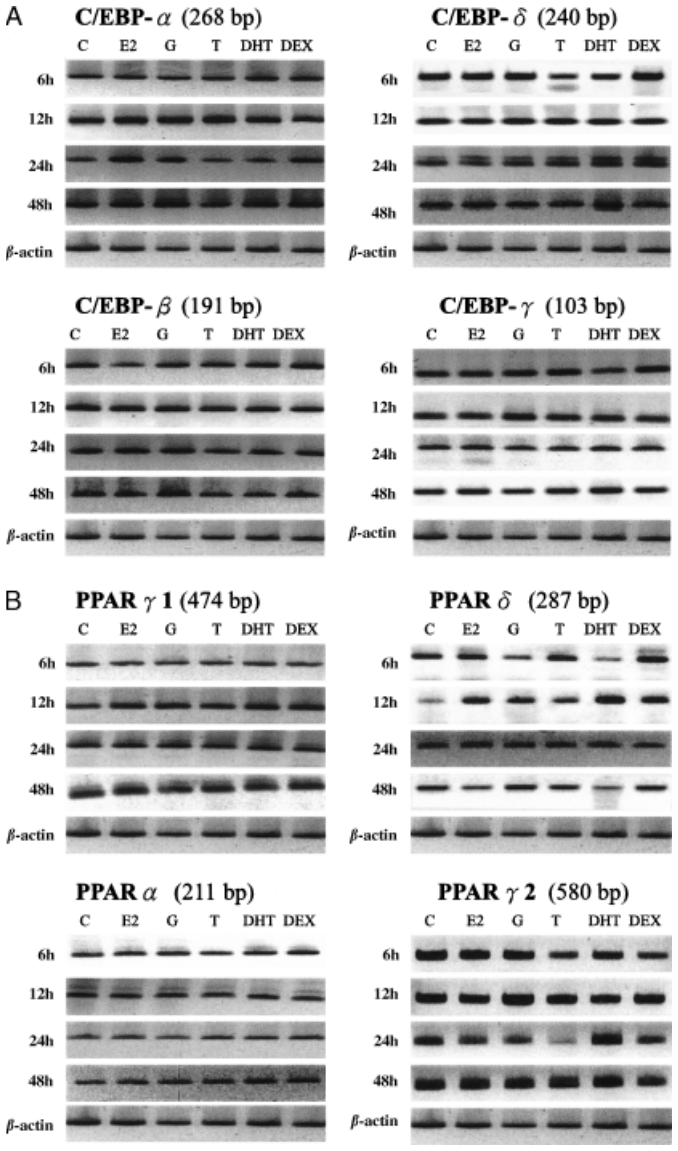

Figure 1. Reverse transcription–PCR study of the mRNA expression of c/EBP-α, -β, -γ, and -δ (*A*), and PPAR-α, -δ, -γ1, and -γ2 (*B*) in cultured human SZ95 sebocytes. Expression of all molecules investigated was detected, and no marked regulatory effect of testosterone (T), 5α-dihydrotestosterone (DHT), dexamethasone (DEX), 17β-estradiol (E2), and genistein (G) was found at 10^{-6} M for up to 48 h, as compared with untreated cells group (C).

DISCUSSION

Cutaneous expressions of c/EBP-α, c/EBP-β, and c/EBP-δ have been demonstrated in normal mouse epidermis (Oh and Smart,

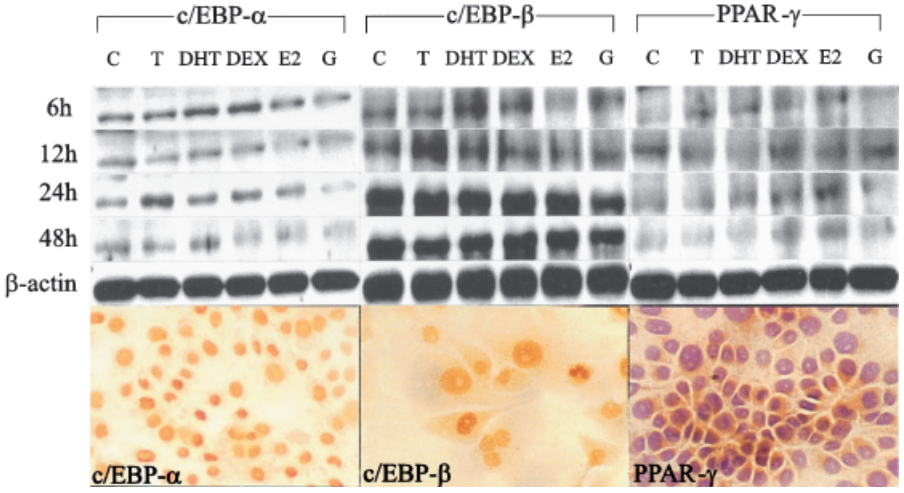

Figure 2. Western blot and immunocytochemical studies of protein expression. The smaller, less differentiated sebocytes were more strongly stained for c/EBP-α than the larger well differentiated cells (*left below*), whereas c/EBP-α was localized in cell nucleus. High levels of c/EBP-α were already detected in the early stage of cell growth at 6 h, then decreased and persisted from 12 h to 48 h at lower levels (*left above*). c/EBP-β was also localized intranuclearly (*middle below*), beginning to be expressed at 6 h and presenting a crescendo regulation to peak at 48 h (*middle above*). PPAR-γ was seen mainly in the cytoplasm (*right below*) and its expression was generally weaker than that of c/EBP-α and c/EBP-β, reaching its highest levels at 12 h (*right above*).

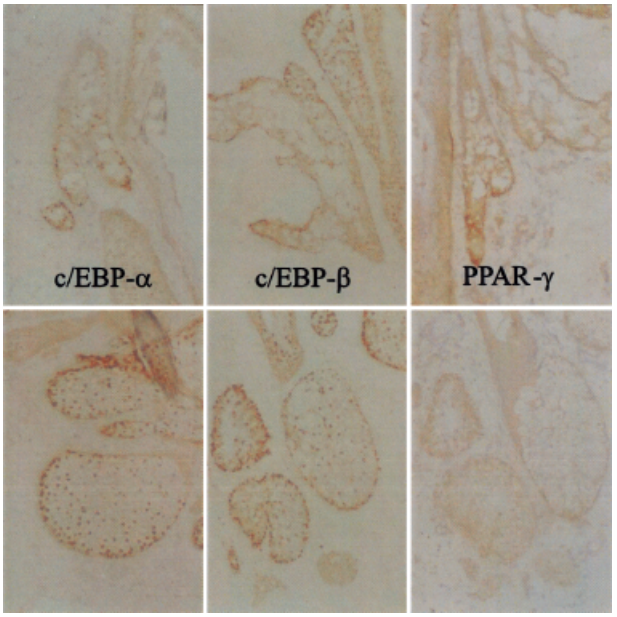

Figure 3. Immunohistochemical staining for c/EBP-α, c/EBP-β, and PPAR-γ in adult scalp (*upper panel*) and sebaceous nevus (*lower panel*). Strong expression was mainly seen in the basal layer of sebaceous glands. Nevus cells were more intensely stained than normal sebocytes. c/EBP-α and c/EBP-β had stronger *in vivo* expression than PPAR-γ at the same antibody dilution (1:1000).

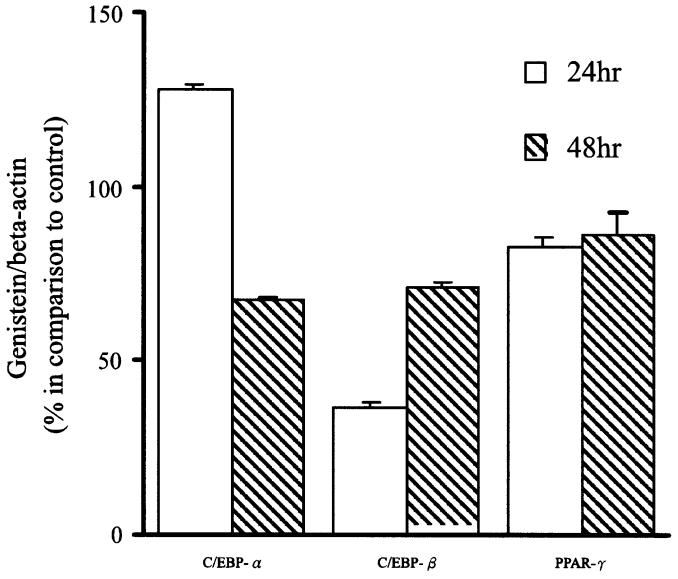

Figure 4. Regulation of c/EBP-β expression by genistein. Genistein downregulated the expression of c/EBP-β at 24 h and c/EBP-β at 48 h. The results are presented as percentages of genistein expression standardized to β-actin in comparison with control.

1999). Whereas PPAR-δ mRNA was shown to be expressed in both rat preputial gland cells and epidermal cells, PPAR-γ1 mRNA was demonstrated only in rat preputial glands (Rosenfield *et al*, 1999). Recent studies using PPAR-α, -β, and -γ mutant mice species demonstrated that PPAR-α and -β are important for the rapid epithelialization of wounded skin; whereas PPAR-α is mainly involved in the early inflammatory phase of healing, PPAR-β is implicated in the control of keratinocyte proliferation (Michalik *et al*, 2001). The PPAR-β mutant primary keratinocytes showed impaired adhesion and migration properties (Michalik *et al*, 2001). Activated PPAR-β/-δ regulates the expression of genes associated with apoptosis resulting in an increased resistance of cultured keratinocytes to cell death (Tan *et al*, 2001).

As for human skin, expression of c/EBP-α, -β, and -δ, especially high levels of c/EBP-β, have been observed in hair follicles and sebaceous glands (Maytin and Habener, 1998; Bull *et al*, 2002). c/EBP-α was concentrated in the upper epidermis in a predominantly cytoplasmic location within cells, whereas the highest level of c/EBP-β was seen in the mid-epidermis, mainly within nuclei (Maytin and Habener, 1998). PPAR-α, -δ, and -γ were also detected immunohistochemically in both dermal and epithelial cells of the human hair follicle (Billoni *et al*, 2000). In cultured human keratinocytes and in reconstructed epidermis alike, the gene expression of PPAR-α and -γ has been shown to increase during keratinocyte differentiation, whereas that of PPAR-δ was not modified (Rivier *et al*, 1998). Our *in vitro* as well as *in vivo* studies showed the immunostaining of c/EBP-α and -β mainly in the nuclei, whereas PPAR-γ was expressed in the cytoplasm of sebocytes (**Figs 2 and 3**). The much higher levels of c/EBP-α and -β in the basal layer of sebaceous glands, especially in the nevus cells (sebaceous nevus) as compared with the sebocytes from normal adult sebaceous glands imply their importance in the early differentiation of sebocytes (**Fig 3**). Our reverse transcription–PCR studies showed consistent mRNA expression of PPAR-α, -δ, -γ1, -γ2, and c/EBP-α, -β, -γ, and -δ in cultured sebocytes. The demonstration of PPAR-γ2, in addition to PPAR-γ1, in human

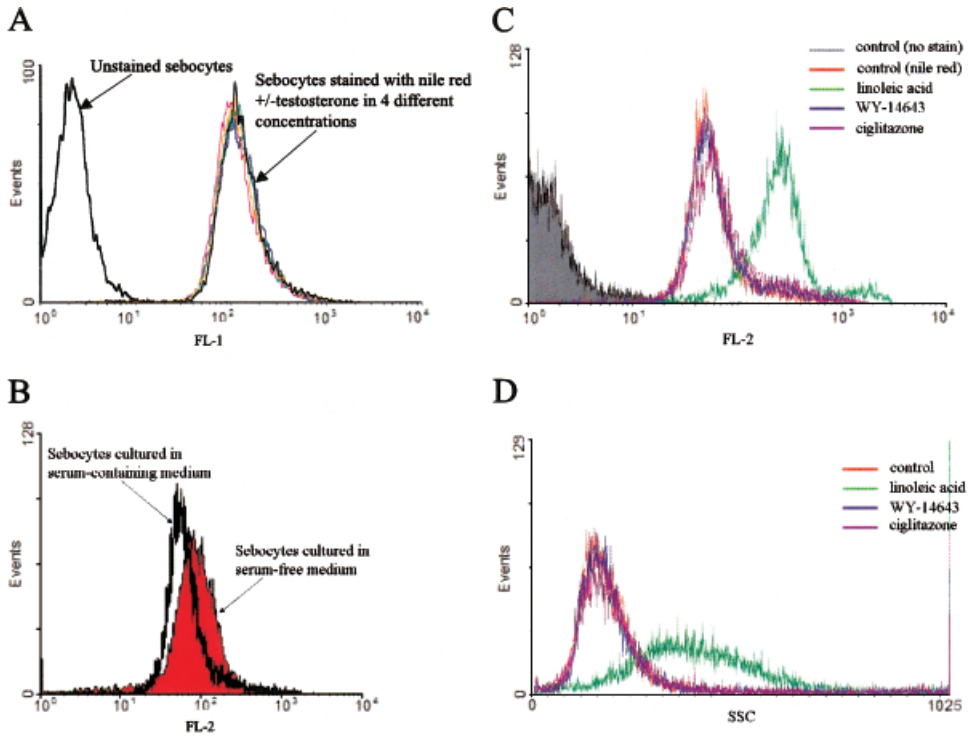

Figure 5. Flow cytometry of SZ95 sebocytes labeled with Nile red. The entire group of cultured SZ95 sebocytes was identified to contain lipid droplets. (*A*) Testosterone did not modify lipid content of viable SZ95 sebocytes in a long-term experiment (six passages) at any concentration tested (right curves: control, testosterone 10^{-11} M, 10^{-9} M, 10^{-7} M, and 10^{-5} M). (*B*) Sebocytes cultured in serum-containing medium produced slightly less lipids than those in serum-free medium. (*C*) Treatment with 10^{-4} M linoleic acid induces differentiation of cultured sebocytes, leading to higher rates of lipid production and (*D*) larger lipid droplets. FL-1 (500–560 nm) measures mainly the neutral lipids, whereas FL-2 (564–627 nm) measures additionally also the phospholipids. SSC is related to the internal granularity or complexity of a particle, which represents the lipid droplets in the present experiment.

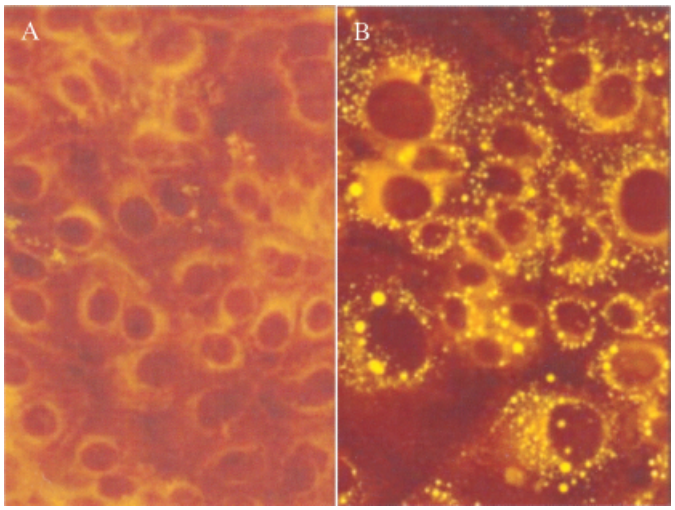

Figure 6. Nile red staining performed on the SZ95 sebocytes cultured in chamber slides, before (*A*) and after treatment with 10^{-4} M linoleic acid for 48 h (*B*). Note the increased cell size and the accumulating bigger lipid droplets in the cytoplasma (*B*).

sebocytes implicates its significance in sebocyte differentiation (Rosenfield *et al*, 1999). The biologic role of c/EBP-γ expression in human sebocytes remains unknown. In western blot analysis, high levels of c/EBP-α and PPAR-γ appeared in the early stage of cell growth, whereas c/EBP-β expression turned out to be stronger in a later stage. This finding seems to imply, at least partially, a different differentiation program in sebocytes as compared with adipocytes, where high levels of c/EBP-β are expressed in the early phase and c/EBP-α maintains the terminally differentiated state (Mandrup and Lane, 1997). A possible explanation for this discrepancy may be related to the different final destination of sebocyte *versus* adipocyte lipids, whereas in the former lipids are excreted from the cells and in the latter fat will be stored intracellularly. Better quantitative methods, e.g., real-time reverse transcription–PCR technique, are needed to corroborate our observation and to find if there also exists a dynamic expression of the examined molecules at the transcriptional level.

Growth and maturation of sebocytes are known to be profoundly influenced by various steroids, especially testosterone and its metabolite 5-DHT. Our experiments could identify low levels of regulatory effects of sex steroid hormones on the proliferation and lipid synthesis of cultured SZ95 sebocytes. As shown in previous work, androgens are only able to stimulate proliferation of normal and SZ95 sebocytes when the cells are grown in nutritionally deficient culture conditions that partially or totally inhibit steady-state sebocyte proliferation (Akamatsu *et al*, 1992; Zouboulis *et al*, 1999). Interestingly, androgens, applied as single agents *in vitro*, seem to influence mainly proliferation but not lipid synthesis in SZ95 sebocytes, in short- and long-term experiments, and that of rat preputial gland cells (Rosenfield *et al*, 1998; and this study). This is also evidenced by the observation that sebocytes cultured in serum-free medium (Sebomed ready-to-use medium) produce slightly more lipids than those cultured in 10% fetal bovine serum-containing medium (**Fig 5*B***). The low level of LIN (10^{-7} M) in the former medium may also induce lipid production. These data indicate that the pronounced regulation of androgens on sebaceous gland physiology *in vivo* may need cofactor(s) to become apparent.

In accordance with previous studies on rat preputial gland cells as well as on human sebocytes[1] (Rosenfield *et al*, 1998, 1999; Seltmann *et al*, 1999), we showed the strong stimulatory effect of LIN on lipid production (**Fig 5*C*,*D***), indicating the role of PPAR-δ in

Table II. Densitometrical data of western blot analysis (the target molecules/β-actin in untreated sebocytes was assessed as 1)

		Control	Testosterone	DHT	Dexamethasone	E2	Genistein
c/EBP-α	6 h	1	0.89	0.86	0.90	0.84	0.87
	12 h	1	0.99	1.24	1.12	1.14	1.88
	24 h	1	1.35	1.06	1.12	1.10	1.28
	48 h	1	0.98	0.88	1.06	1.30	0.67
c/EBP-β	6 h	1	1.08	1.36	1.07	0.93	1.08
	12 h	1	1.31	0.98	1.07	0.91	0.91
	24 h	1	1.11	0.85	1.10	0.75	0.36
	48 h	1	0.77	1.01	1.09	0.85	0.71
PPAR-γ	6 h	1	1.06	1.01	1.02	1.15	0.96
	12 h	1	0.94	0.73	0.90	0.72	1.03
	24 h	1	0.91	1.01	0.92	0.96	0.82
	48 h	1	0.94	0.93	1.12	1.07	0.86

the terminal differentiation of sebocytes. Moreover, the treated cell population seemed to increase in their cell size (**Fig 6**). On the other hand, the lipid-inducing effect of specific ligands for PPAR-α and -γ in cultured rat preputial gland cells, respectively, was not observed in human sebocytes (**Fig 5*C*,*D***). In addition to a species-specific difference, it is possible that the regulatory effect of different ligands (BRL-49653 used in rat preputial sebocytes *versus* ciglitazone used in human sebocytes) also varies for certain PPAR receptors (e.g., PPAR-γ).

Although clinicians are familiar with the occurrence of steroid acne as a long-term steroid effect but also with the beneficial short-term effect of corticosteroids in severe inflammatory acne, the exact effect of glucocorticoids on growth and differentiation of sebaceous glands has not been clearly defined. In this study, treatment of SZ95 sebocytes with 10^{-6} M dexamethasone for up to 48 h did not influence cell growth, whereas in previous work hydrocortisone induced a concentration-dependent increase of sebocyte proliferation cultured in a serum-free medium for 7 d (Zouboulis *et al*, 1998). In another, yet unpublished study, potent glucocorticoids seem to decrease significantly the formation of lipids within SZ95 sebocytes in a 7 d culture system.

Genistein has been shown to exert anti-lipogenic action and to augment basal lipolysis in isolated rat adipocytes (Szkudelska *et al*, 2000). Besides that, genistein inhibited proliferation of preconfluent pre-adipocytes in a time- and concentration-dependent manner (Harmon and Harp, 2001). When added to 2 d postconfluent pre-adipocytes during the induction of differentiation, genistein inhibited mitotic clonal expansion, triglyceride accumulation, and PPAR-γ expression. In this study, genistein at 10^{-6} M exerted a downregulatory effect on the expression of c/EBP-α, c/EBP-β, and PPAR-γ at the protein level, whereas it did not affect sebocyte proliferation. Further studies are needed to evaluate the detected regulatory effect on sebocyte differentiation under long-term culture condition. Should genistein be further shown to be able to antagonize the stimulatory effect of LIN on lipid production in cultured sebocytes, this may indicate that the decreased intake of many genistein-rich phytestrogen-containing nutrition products (e.g., soybeans) might be related to the increased prevalence and severity of acne over the past decades in Taiwan (personal observation by W. Chen, Taiwan).

In conclusion, the major transcriptional factors c/EBP and PPAR were demonstrated in cultured human sebocytes. The dynamics of their expression seemed to vary from that observed in adipocytes. In a short-term culture system for up to 48 h, genistein inhibited mainly c/EBP-β and c/EBP-α expression. In contrast to adipocytes, PPAR-δ seems to play an important part in the terminal differentiation of sebocytes. Experiments on long-term culture systems of human sebocytes may add valuable information. To observe better the treatment response of the cultured cells, the use of charcoal-stripped medium may be an alternative way to eliminate the confounding effect caused by the serum-containing steroid hormones. Better understanding of the molecular control of sebocyte differentiation and sebum production may lead to advanced pharmacologic development of more potent agents for effective treatment of acne.

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