

Steroid metabolism and profile of steroidogenic gene expression in EpiskinTM: High similarity with human epidermis

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Abstract

The skin is a well-recognized site of steroid formation and metabolism. EpiskinTM is a cultured human epidermis. In this report, we investigate whether EpiskinTM possesses a steroidogenic machinery able to metabolize adrenal steroid precursors into active steroids. EpiskinTM was incubated with [¹⁴C]-dehydroepiandrosterone (DHEA) and 4-androstenedione (4-dione) and their metabolites were analyzed by liquid chromatography/mass spectrometry (LC/MS/MS). The results show that the major product of DHEA metabolism in EpiskinTM is DHEA sulfate (DHEAS) (88% of the metabolites) while the other metabolites are 7 α -OH-DHEA (8.2%), 4-dione (1.3%), 5-androstenediol (1.3%), dihydrotestosterone (DHT) (1.4%) and androsterone (ADT) (2.3%). When 4-dione is used as substrate, much higher levels of C19-steroids are produced with ADT representing 77% of the metabolites. These data indicate that 5 α -reductase, 17 β -hydroxysteroid dehydrogenase (17 β -HSD) and 3 α -hydroxysteroid dehydrogenase (3 α -HSD) activities are present at moderate levels in EpiskinTM, while 3 β -HSD activity is low and represents a rate-limiting step in the conversion of DHEA into C19-steroids. Using realtime PCR, we have measured the level of mRNAs encoding the steroidogenic enzymes in EpiskinTM. A good agreement is found between the mRNAs expression in EpiskinTM and the metabolic profile. High expression levels of steroid sulfotransferase SULT2B1B and type 3 3 α -HSD (AKR1C2) correspond to the high levels of DHEA sulfate (DHEAS) and ADT formed from DHEA and 4-dione, respectively. 3 β -HSD is almost undetectable while the other enzymes such as type 1 5 α -reductase, types 2, 4, 5, 7, 8, and 10 17 β -HSD and 20 α -hydroxysteroid dehydrogenase (20 α -HSD) (AKR1C1) are highly expressed. Except for UGT-glucuronosyl transferase, similar mRNA expression profiles between EpiskinTM and human epidermis are observed.

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1. Introduction

Steroid metabolism plays a crucial role in skin physiology, its malfunctioning leading to diseases such as X-linked ichthyosis due to a defect of the steroid sulfatase gene [1,2] while hyperandrogenism leads to acne, hirsutism and alopecia [3–5]. Although it is well recognized that steroid metabolism is particularly active in the pilosebaceous unit [6–9], it is also known that keratinocytes metabolize steroids at a high level [10–13].

EpiskinTM is an *in vitro* epidermis model which consists of human keratinocytes deposited on a support of collagen, and let to differentiate in the air-exposed culture into a mitotic layer, a mucous Malpighian layer and a functional horny layer [14]. This model is of particular interest for studies of steroidogenic gene function in the epidermis. The activity of many steroid-metabolizing enzymes, such as 5 α -reductase, 3 α -hydroxysteroid dehydrogenase (3 α -HSD) and 17 β -HSD, has been reported in normal human keratinocytes in primary culture and in the immortalized human keratinocyte cell line HaCat [15].

EpiskinTM has been shown to be a very good replicate of the architecture of human epidermis and is being used for irritation assays of new cosmetic and chemical compounds as

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replacement of *in vivo* testing [16]. In this report, we investigate how EpiskinTM produces and metabolizes active sex steroids. We have thus incubated EpiskinTM with radiolabeled steroid precursors, namely DHEA and 4-dione and analyzed the metabolites by LC/MS/MS. In addition, we have quantified the mRNA expression levels of 47 steroid-metabolizing enzymes in Episkin using realtime PCR, and compared the steroidogenic gene expression profile with that of human epidermis.

2. Materials and methods

2.1. EpiskinTM

EpiskinTM was obtained from EPISKINTM SNC (Lyon, France) and cultured to day 16 in a 12-well plate in medium provided by the manufacturer. It consists of normal human keratinocytes that are left dividing for 3 days following deposition on a collagen support in medium containing Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham's (DHEM/HAMF12) (3:1) (HyClone, Logan, Utah), 10% fetal calf serum, 10 ng/ml EGF, 400 ng/ml hydrocortisone, 10^{-6} M isoproterenol and then differentiate in the air-exposed culture to form a well-stratified epidermis having the five typical layers, namely, the stratum basale, stratum spinosum, stratum granulosum and stratum corneum [14].

2.2. Analysis of steroid metabolism in EpiskinTM

Steroid metabolism was analysed in EpiskinTM in culture essentially as previously described for intact cells in culture [17]. [¹⁴C] DHEA and [¹⁴C]4-dione (Dupont Inc., Mississauga, Ont., Canada) were added to freshly changed culture medium to a final concentration of 0.1 μ M. After incubation for various time intervals, cell-free cultured medium (3.2 ml) was acidified with formic acid and applied to a preconditioned Strata-X, 200 mg, column (Phenomenex, Torrance, CA, USA). The column was washed with 1 ml of formic acid 0.1% and 2 ml of a solution of methanol/water (10/90) containing 0.1% formic acid, and then eluted with 7 ml of a (10/90) methanolic solution containing 0.01% ammonium hydroxide. The eluted solution was evaporated under a nitrogen stream. Then, the samples were dissolved in a 150 μ l solution of methanol/water (50/50) containing 0.3% acetic acid.

The sample prepared as described above was analyzed by high performance liquid chromatography (HPLC) using an Alliance 2695 HPLC system (Waters, MA, USA). The HPLC column was a Luna C18(2), 3 μ m, 150 mm \times 3.00 mm (Phenomenex, Torrance, CA, USA) operated at 0.3 ml/min. The following elution gradient was used: 45% A for 2 min, 45–55% A from 2 to 30 min, 55–65% A from 30 to 92 min, 65–95% A from 92 to 95 min and, finally, 95–100% A from 95 to 100 min with A, a methanol/acetic acid (99.7/0.3) solution

containing 1 mM ammonium formate and B, a water/acetic acid (99.7/0.3) solution containing 1 mM ammonium formate.

The detection and quantification of the steroid metabolites were carried out using an atmospheric pressure chemical ionization/ion trap mass spectrometer (Finnigan LCQ, ThermoQuest, SJ, USA). Both positive and negative ionization modes were used. The discharge current was set to 4 μ A. The heated capillary was maintained at 170 °C. In parallel, a flow scintillation analyzer (Radiomatic 500TR series, Packard, CT, USA) was used. A 3/1 split for mass spectrometer/flow scintillation analyzer was applied.

2.3. Preparation of human epidermis

Ten samples of human epidermis were prepared by treatment of the corresponding total breast skin sample with dispase for overnight. Briefly, skin samples of 0.5 cm \times 0.5 cm was incubated in 10 ml of Dulbecco's Modified Eagle's Medium (DMEM) medium containing 3% (v/v) gentamycin and 3.7% (v/v) antibiotic/antimycotic. Medium was changed three times at 15 min interval. After the last change, 20 ml of a medium containing 1.8 U/ml of dispase and 0.6% trypsin was added. After an overnight incubation in dispase, derm and epiderm were separated using a forceps, washed twice in PBS then incubated in RNAlater[®], a RNA stabilization solution commercialized by Ambion Inc. (Austin, TX) that help to store tissue and cells for extended periods without RNA degradation.

2.4. RNA extraction and quantification

Total RNA was extracted from EpiskinTM and epiderm using TRIzol[®] (Invitrogen, Burlington, Ont., Canada), and reverse transcribed into cDNA using Superscript II reverse transcriptase (Invitrogen). Quantification of mRNA was performed on the LightCycler realtime PCR apparatus (Hoffman-La Roche Inc., Nutley, NJ) using SYBR Green detection and the second derivative calculation method as described [18]. In brief, 30 ng of total RNA was used to perform fluorescent-based realtime PCR quantification. Reagents obtained from the same supplier were used as described by the manufacturer. The conditions for the PCR reactions were: denaturation at 94 °C for 15 s, annealing at 55 °C for 10 s and elongation at 72 °C for 35 s. The data were normalized using the mRNA expression levels of a housekeeping gene, namely ATP5o (subunit O of ATPase) as internal standard. Atp5o has been shown to have stable expression levels from embryonic life through adulthood in various tissues [19]. The mRNA expression levels are expressed as numbers of copies/ μ g total RNA using a standard curve of Cp versus logarithm of the quantity. The standard curve is established using known cDNA amounts of 0, 10^2 , 10^3 , 10^4 , 10^5 and 10^6 copies of ATP5o and a LightCy-

cler 3.5 program provided by the manufacturer (Roche Inc., Nutley, NJ).

3. Results

3.1. Analysis of DHEA metabolism

To facilitate the follow up of DHEA and 4-dione metabolism in EpiskinTM, we have indicated in Fig. 1 the steroidogenic pathways illustrating the human enzymes involved in the transformation of DHEA into active androgens and their metabolites. To determine how EpiskinTM metabolizes DHEA, the main precursor of sex steroids in peripheral tissues in the human, we have incubated EpiskinTM in culture with [¹⁴C] DHEA and identified the resulting metabolites by LC/MS/MS analysis. As illustrated in Fig. 2A and B, the metabolite produced at the highest level is DHEA sulfate (DHEA-S). At a much lower level, the presence of 4-dione, 5-androstenediol (5-diol), DHT, androsterone (ADT), 7 α -OH-DHEA, 7 β -OH-DHEA and four unknown metabolites having a retention time of 26.05, 38.90, 53.85 and 70.95

are observed. They are named according to their retention time, respectively.

3.2. Analysis of 4-androstenedione metabolism

To determine whether the low conversion of DHEA to 4-dione and their subsequent metabolites observed above is due to the low conversion of DHEA to 4-dione by 3 β -HSD, we then incubated EpiskinTM with [¹⁴C] 4-dione under the same conditions as done with [¹⁴C] DHEA. As illustrated in Fig. 3A and B, the main metabolite produced from incubation of 4-dione is ADT, thus confirming the presence of low 3 β -HSD activity and relatively high 5 α -reductase and 3 α -HSD activities that convert 4-dione to ADT. Small amounts of testosterone (testo) and androstenedione (5 α -dione) as well as many unidentified metabolites, namely 33.00, 59.95, 64.20, 65.15, 70.95, and 74.30 are observed.

To assess whether the incubation time of 48 h used in the previous experiments corresponds to saturated conditions, we then performed a time course study using incubation times of 6, 12, 24 and 48 h. As illustrated in Fig. 3C, a linear decrease of the substrate 4-dione with a corresponding increase of

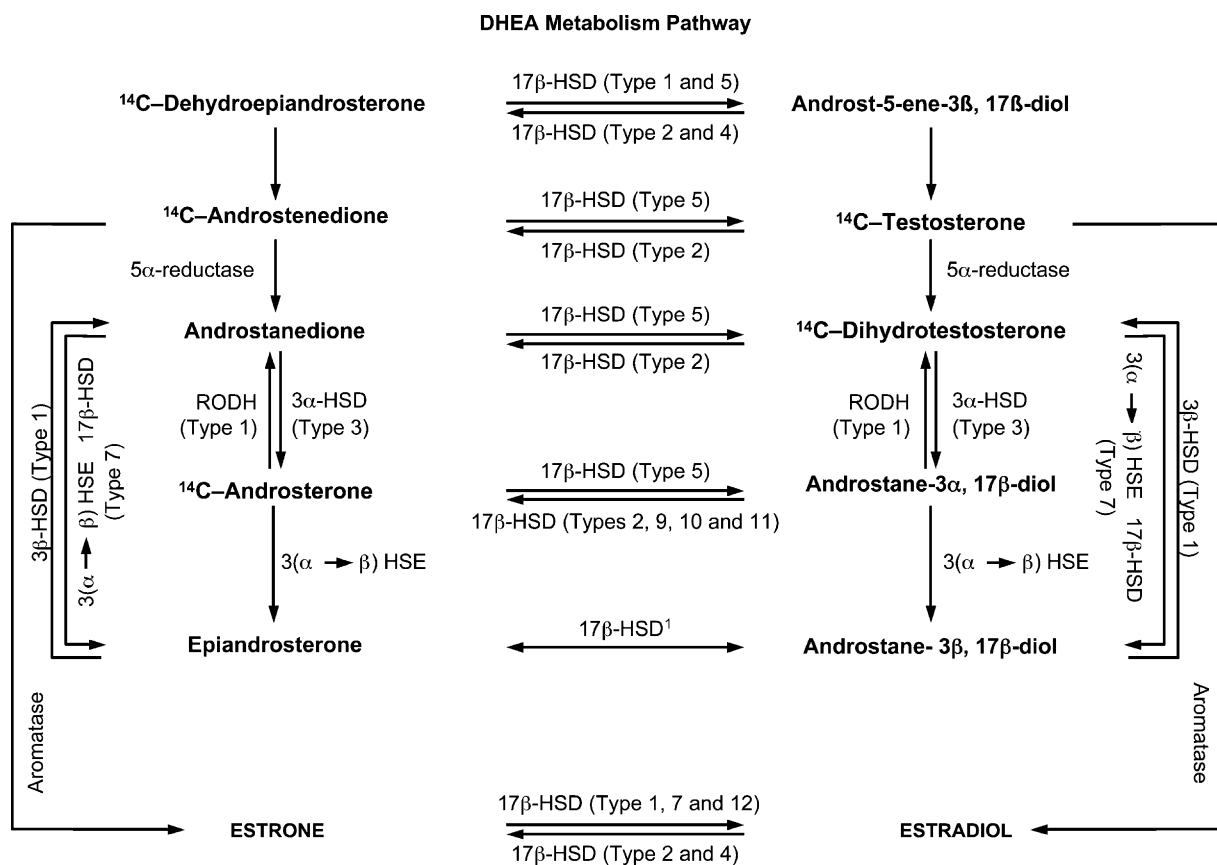


Fig. 1. Pathways of the human steroidogenic enzymes involved in DHEA metabolism: unlike conventional steroidogenic pathway which describes dehydrogenase as enzyme that possesses reversible oxido/reductase activity, in the present figure dehydrogenase is indicated as oxidase or reductase according to the activity they exert in intact transfected cells in culture [42] that is, most likely, closer to physiologic conditions. The two enzymes that possess 3-keto reductase activity able to catalyze the transformation of androstenedione and dihydrotestosterone into epiandrosterone and androstane-3 β , 17 β -diol, are 3 α (α \rightarrow β)-hydroxysteroid epimerase (3 α (α \rightarrow β)-HSE) [43] and type 7 17 β -HSD [44,45].

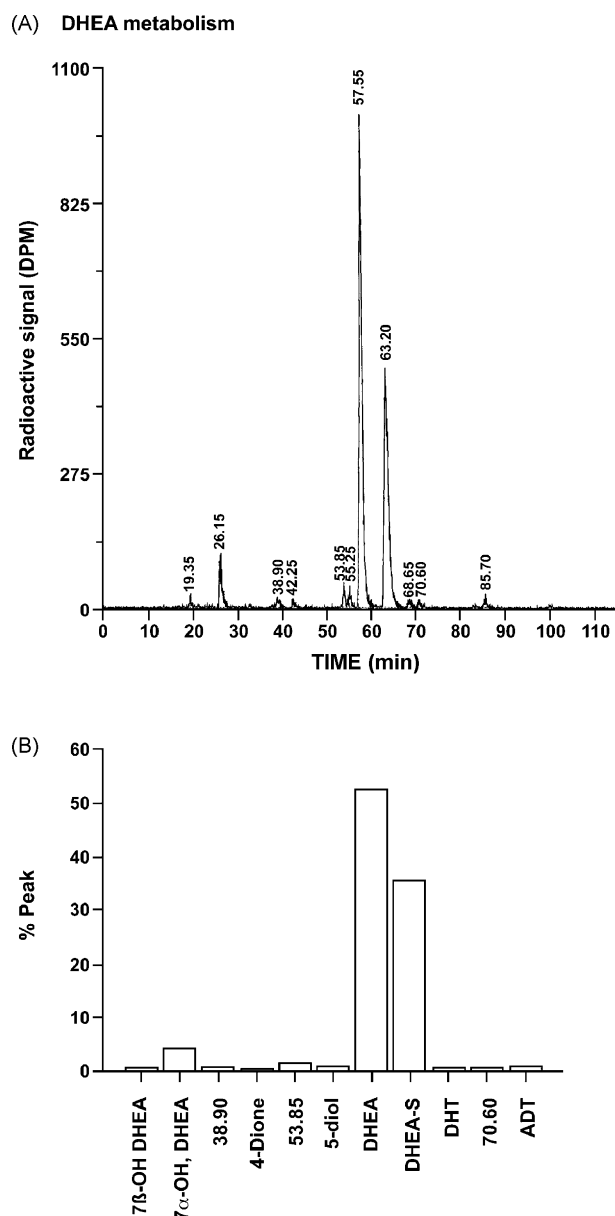


Fig. 2. Quantification of $[^{14}\text{C}]$ metabolites of $[^{14}\text{C}]$ DHEA by EpiskinTM in culture: (A) HPLC/MS metabolic profile of 0.1 μM $[^{14}\text{C}]$ DHEA incubated for 48 h with EpiskinTM cultured in 12-well plates as described under Section 2 and (B) graph showing the main metabolite obtained in (A). Unidentified metabolites are named according to their retention time while the retention time of the two most important peaks that correspond to DHEA and DHEA-S, are 57.55 and 63.20, respectively.

ADT and other minor metabolites are observed, thus indicating that the enzymatic reactions are under nonsaturated conditions.

3.3. Effect of 5 α -reductase inhibition

To determine whether the transformation of 4-dione to ADT in EpiskinTM is going through a classical 5 α -reductase step or through transformation by a yet unidentified enzymatic route, we have added to the reaction a well known

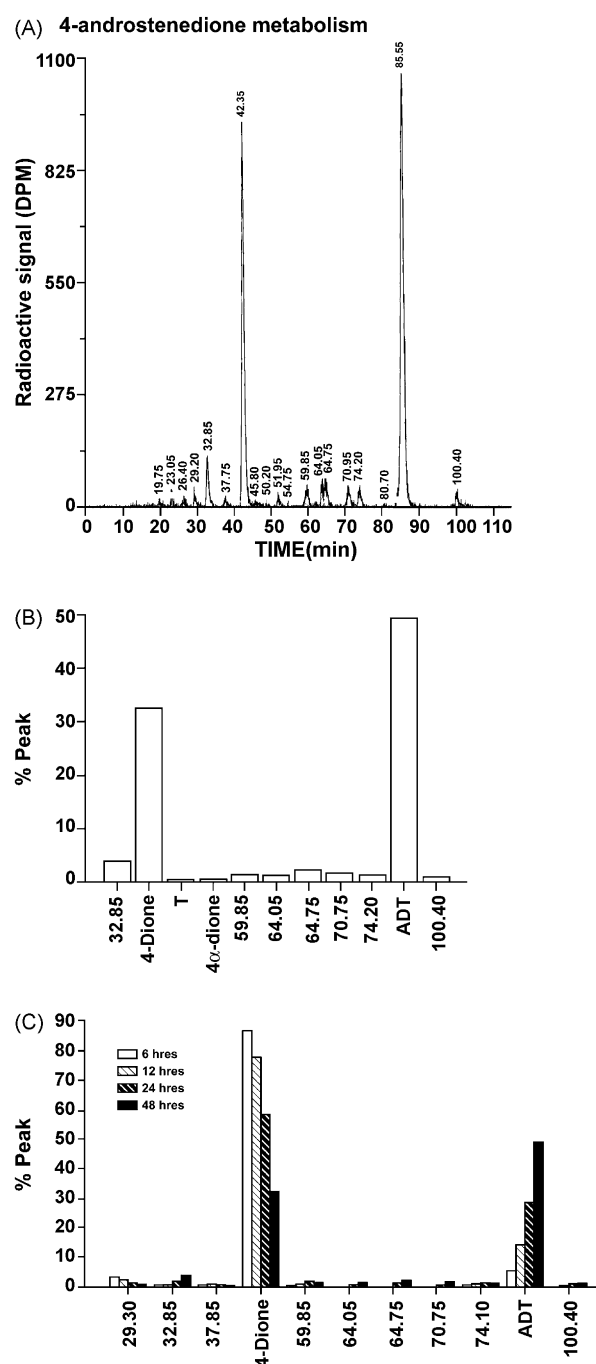


Fig. 3. Quantification of $[^{14}\text{C}]$ metabolites of $[^{14}\text{C}]$ 4-androstenedione produced by EpiskinTM in culture: (A) HPLC/MS metabolic profile of 0.1 μM $[^{14}\text{C}]$ 4-dione incubated for 48 h with EpiskinTM cultured in 12-well plates; (B) graph showing the main metabolites obtained in (A); (C) graph showing metabolic profile of 4-dione at different incubation time intervals. Experimental procedures were as described under Section 2.

inhibitor of 5 α -reductase, namely finasteride synthesized in our laboratory under the name EM-351. As expected, the level of ADT formed is markedly reduced in the presence of 10^{-6} or 10^{-7} M EM-351 (Fig. 4). This observation clearly indicates that the step of conversion of 4-dione to 5 α -dione is necessary. Since the latter compound is almost not detected

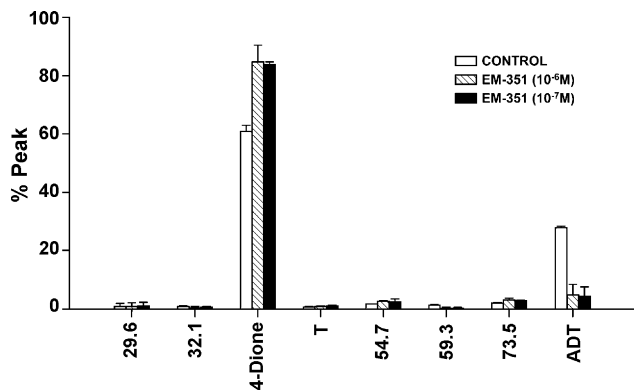


Fig. 4. Effect of 5 α -reductase inhibition on 4-androstenedione metabolism: experimental procedures were as described under Fig. 3, except that the 5 α -reductase inhibitor EM-351 was added at the indicated concentrations at the same time as the substrate. Data are expressed as means \pm S.E.M. of duplicate measurements. Black and white bars represent absence and presence of inhibitor, respectively.

under basal enzymatic conditions, it is likely that a high level of 3 α -HSD activity rapidly converts 5 α -dione into ADT.

Identification and quantification of the mRNAs encoding the steroidogenic and steroid-inactivating enzymes in the EpiskinTM and epidermis.

Since there are many isoforms for each steroidogenic enzyme, we have performed realtime PCR to identify and quantify the isoenzymes expressed in the EpiskinTM and human epidermis. As illustrated in Fig. 5, the isoforms of steroidogenic and steroid-inactivating enzymes expressed in EpiskinTM are usually expressed in the epidermis, although at different levels. Type 1 5 α -reductase and type 3 3 α -HSD are the enzymes responsible for the conversion of 4-dione into ADT, while the high expression levels of SULT2B1b corresponds to the high conversion of DHEA into DHEAS. The presence of many other steroidogenic and steroid-inactivating enzymes, such as types 1, 2, 4, 5, 7, 8 and 12 17 β -HSD as well as 20 α -HSD [20] that catalyzes the transformation of progesterone to 20 α -hydroxyprogesterone, types 1 and 2 11 β -HSD that catalyze the conversion of cortisone to cortisol and vice-versa, respectively, sulfatase [2], sulfotransferase Sult1A1 [21–23], and the enzymes of the UGT1A family [24–26] are also observed. It can be seen from the data obtained that, although the types of isoenzymes expressed in EpiskinTM and epidermis show a good correlation, the level of expression varies between the EpiskinTM and human epidermis.

4. Discussion

The metabolites obtained from DHEA and 4-dione in cultured EpiskinTM clearly indicate that EpiskinTM possesses the enzymatic machinery necessary to convert the adrenal steroid precursor DHEA into active androgens followed by their conversion into the inactive product ADT. The difference

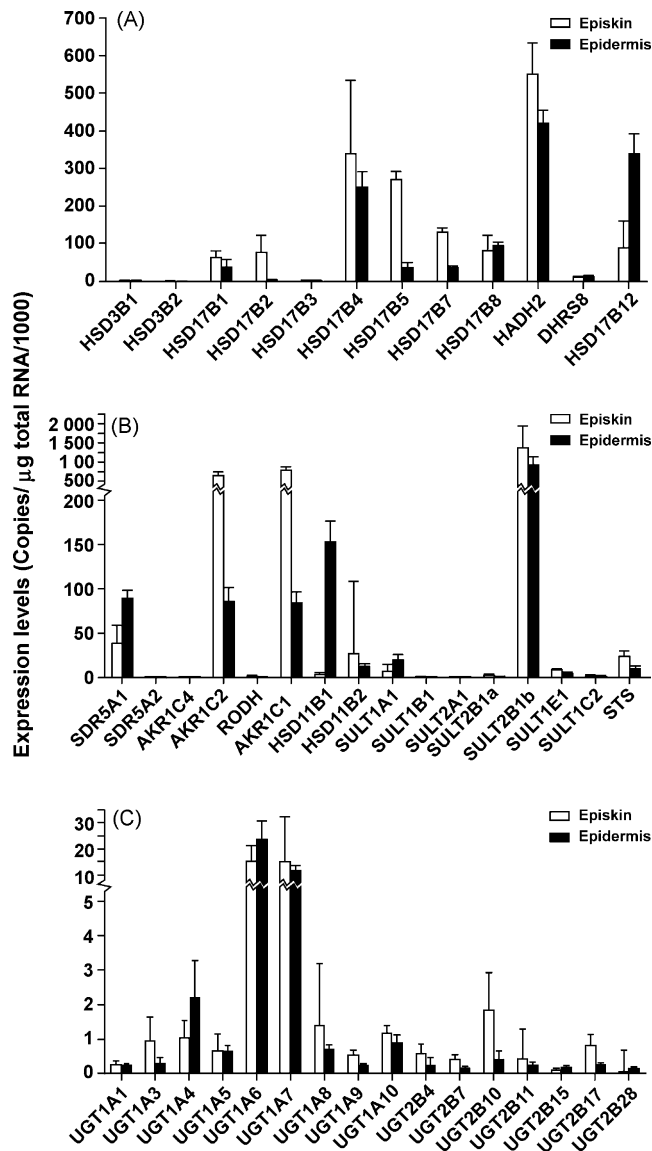


Fig. 5. Comparison of steroidogenic gene expression profiles in EpiskinTM and epidermis: 30 ng of total RNA of EpiskinTM and microdissected epidermis was used for quantification using realtime PCR. Total RNA preparation and realtime PCR quantification using second derivative calculations and the double correction method [18] with SYBR green fluorescence detection was performed as described in Section 2. RDH5, HADH2 and DHRS8 represent new names for types 9, 10 and 11 17 β -HSD, respectively. The expression level is indicated as the number of copies/ μ g of total RNA. The data are expressed as means \pm S.E.M. of duplicate measurements of three different wells in two separate preparations of Episkin (open bar), and duplicate measurements of 10 human epidermis (black bar).

observed between the profile of DHEA and 4-dione clearly suggests that the step responsible for the transformation of DHEA into 4-dione metabolism by 3 β -HSD is limiting. In fact, this is confirmed by the low mRNA expression level of type 1 3 β -HSD (Fig. 5A). However, the low conversion of DHEA into 4-dione could also be due, at least partially, to the presence of a high level of sulfotransferase SULT2B1b that competes with 3 β -HSD for the same substrate DHEA [27]. In fact, although 3 β -HSD possesses higher affinity for

DHEA than SULT2B1b (Luu-The, unpublished data [27]), the very high amount of SULT2B1b compared to 3 β -HSD (>600-fold) could dramatically reduce the amount of DHEA available for 3 β -HSD. It is noteworthy that SULT2B1b catalyzes also the transformation of pregnenolone and cholesterol into pregnenolone sulfate and cholesterol sulfate [28].

It can be seen in Fig. 5B that SULT2B1b is highly expressed in EpiskinTM and human epidermis, while SULT2B1a is expressed at a much lower level. SULT2B1a is the isoform issued from the same gene as SULT2B1b by the use of alternative promoters, thus leading to a different exon 1 [29] with an activity that prevents SULT2B1a from metabolizing cholesterol in addition to DHEA. Such data strongly suggest, however, that the target substrate for SULT2B1b in the epidermis is cholesterol and not DHEA. Type 1 3 β -HSD could thus be a key enzyme controlling the conversion of DHEA into active steroids. Since type 1 3 β -HSD is highly regulated by IL-4 and IL-13 [30,31] through Stat5 mechanisms [30–32], the cross-talk between androgens and the immune system in the epidermis could be an important issue to be considered in the future. It is noteworthy that type 5 17 β -HSD, the enzyme that converts 4-dione into DHT in peripheral tissues [18,33] is also expressed at a high level. Sulfatase, the enzyme that converts DHEA-S into DHEA has been found to be stimulated in lesioned skin [8].

It is well recognized that active steroids generally exist in a reduced form while inactive steroids are in an oxidized form, each form being finely and timely regulated by specific enzymes. This general observation agrees with the detection, in the present study, of the main metabolites present under the oxidized form. The presence of the enzymes able to reduce 17-ketosteroids into the corresponding active 17-hydroxysteroids such as types 1, 5 and 12 17 β -HSDs indicates that these enzymes produce active steroids for cell functioning at the level of the epidermis, thus making the epidermis an intracrine component of the skin [13,26,34–36]. Since types 1 and 12 are involved in the formation of estradiol [37] while type 5 17 β -HSD catalyzes the formation of testosterone [33,38], subcellular localisation of these enzymes could allow to identify cell type that is influenced by estrogen and androgen, respectively. The highly similar profiles of steroidogenic gene expression and activity between EpiskinTM and human epidermis strongly suggest that EpiskinTM could potentially be an interesting alternative model for studying steroid metabolism as well as steroid regulation and action related to keratinocyte differentiation and proliferation. Since EpiskinTM is constructed using breast skin keratinocytes, the use of this skin model should take into account heterogeneity of biological features in the skin. It is worth noting that mitotically active keratinocytes appear to be morphologically and biochemically similar irrespective of tissue origin with high expression levels of keratins K5 and K14 [39]. As keratinocytes differentiate, they down-regulate this pair of keratins and switch on expression of other pairs depending upon tissue origin [40,41].

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References

- [1] G. Lykkesfeldt, J. Muller, N.E. Skakkebaek, E. Bruun, A.E. Lykkesfeldt, Absence of testicular steroid sulphatase activity in a boy with recessive X-linked ichthyosis and testicular maldescent, *Eur. J. Pediatr.* 144 (1985) 273–274.
- [2] G. Lykkesfeldt, P. Bennett, A.E. Lykkesfeldt, S. Micic, S. Moller, B. Svenstrup, Abnormal androgen and oestrogen metabolism in men with steroid sulphatase deficiency and recessive X-linked ichthyosis, *Clin. Endocrinol. (Oxf.)* 23 (1985) 385–393.
- [3] M.E. Sawaya, A.R. Shalita, Androgen receptor polymorphisms (CAG repeat lengths) in androgenetic alopecia, hirsutism, and acne, *J. Cutan. Med. Surg.* 3 (1998) 9–15.
- [4] A.W. Lucky, Hormonal correlates of acne and hirsutism, *Am. J. Med.* 98 (1995) 89S–94S.
- [5] L. Cusan, A. Dupont, J.L. Gomez, R.R. Tremblay, F. Labrie, Comparison of flutamide and spironolactone in the treatment of hirsutism: a randomized controlled trial, *Fertil. Steril.* 61 (1994) 281–287.
- [6] R.L. Rosenfield, D. Deplewski, Role of androgens in the developmental biology of the pilosebaceous unit, *Am. J. Med.* 98 (1995) 80S–88S.
- [7] D. Deplewski, S. Liao, R.L. Rosenfield, Preputial sebocyte 5 α -reductase isoform specificity, *Endocrinology* 138 (1997) 4416–4420.
- [8] W. Chen, D. Thiboutot, C.C. Zouboulis, Cutaneous androgen metabolism: basic research and clinical perspectives, *J. Invest. Dermatol.* 119 (2002) 992–1007.
- [9] C.C. Zouboulis, K. Degitz, Androgen action on human skin—from basic research to clinical significance, *Exp. Dermatol.* 13 (Suppl. 4) (2004) 5–10.
- [10] V. Luu-The, Y. Sugimoto, L. Puy, Y. Labrie, I. Lopez Solache, M. Singh, F. Labrie, Characterization, expression, and immunohistochemical localization of 5 α -reductase in human skin, *J. Invest. Dermatol.* 102 (1994) 221–226.
- [11] M. Dumont, V. Luu-The, E. Dupont, G. Pelletier, F. Labrie, Characterization, expression, and immunohistochemical localization of 3 β -hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase in human skin, *J. Invest. Dermatol.* 99 (1992) 415–421.
- [12] W. Chen, C.C. Zouboulis, M. Fritsch, U. Blume-Peytavi, V. Kodoljia, S. Goerdts, V. Luu-The, C.E. Orfanos, Evidence of heterogeneity and quantitative differences of the type 1 5 α -reductase expression in cultured human skin cells—evidence of its presence in melanocytes, *J. Invest. Dermatol.* 110 (1998) 84–89.
- [13] F. Labrie, V. Luu-The, C. Labrie, G. Pelletier, M. El-Alfy, Intracrinology and the skin, *Horm. Res.* 54 (2000) 218–229.
- [14] E. Tinois, J. Tiollier, M. Gaucherand, H. Dumas, M. Tardy, J. Thivolet, In vitro and post-transplantation differentiation of human keratinocytes grown on the human type IV collagen film of a bilayered dermal substitute, *Exp. Cell. Res.* 193 (1991) 310–319.
- [15] S. Gingras, C. Turgeon, N. Brochu, P. Soucy, F. Labrie, J. Simard, Characterization and modulation of sex steroid metabolizing activity in normal human keratinocytes in primary culture and HaCaT cells, *J. Steroid Biochem. Mol. Biol.* 87 (2003) 167–179.
- [16] P.A. Botham, The validation of in vitro methods for skin irritation, *Toxicol. Lett.* 149 (2004) 387–390.
- [17] V. Luu-The, Y. Zhang, D. Poirier, F. Labrie, Characteristics of human types 1, 2 and 3 17 β -hydroxysteroid dehydrogenase activities: oxidation/reduction and inhibition, *J. Steroid Biochem. Mol. Biol.* 55 (1995) 581–587.

- [18] V. Luu-The, N. Paquet, E. Calvo, J. Cumps, Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction, *Biotechniques* 38 (2005) 287–293.
- [19] J.A. Warrington, A. Nair, M. Mahadevappa, M. Tsyganskaya, Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes, *Physiol. Genomics* 2 (2000) 143–147.
- [20] Y. Zhang, I. Dufort, P. Rheault, V. Luu-The, Characterization of a human 20 α -hydroxysteroid dehydrogenase, *J. Mol. Endocrinol.* 25 (2000) 221–228.
- [21] V. Luu-The, F. Bernier, I. Dufort, Steroid sulfotransferases, *J. Endocrinol.* 150 (Suppl.) (1996) S87–S97.
- [22] F. Bernier, P. Soucy, V. Luu-The, Human phenol sulfotransferase gene contains two alternative promoters: structure and expression of the gene, *DNA Cell Biol.* 15 (1996) 367–375.
- [23] N.U. Gamage, R.G. Duggleby, A.C. Barnett, M. Tresillian, C.F. Latham, N.E. Liyou, M.E. McManus, J.L. Martin, Structure of a human carcinogen-converting enzyme, *SULT1A1*. Structural and kinetic implications of substrate inhibition, *J. Biol. Chem.* 278 (2003) 7655–7662.
- [24] D.W. Hum, A. Belanger, E. Levesque, O. Barbier, M. Beaulieu, C. Albert, M. Vallee, C. Guillemette, A. Tcherno, D. Turgeon, S. Dubois, Characterization of UDP-glucuronosyltransferases active on steroid hormones, *J. Steroid Biochem. Mol. Biol.* 69 (1999) 413–423.
- [25] T. Kuuranne, M. Kurkela, M. Thevis, W. Schanzer, M. Finel, R. Kostainen, Glucuronidation of anabolic androgenic steroids by recombinant human UDP-glucuronosyltransferases, *Drug Metab. Dispos.* 31 (2003) 1117–1124.
- [26] F. Labrie, V. Luu-The, C. Labrie, A. Belanger, J. Simard, S.X. Lin, G. Pelletier, Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone, *Endocr. Rev.* 24 (2003) 152–182.
- [27] C.A. Meloche, C.N. Falany, Expression and characterization of the human 3 beta-hydroxysteroid sulfotransferases (*SULT2B1a* and *SULT2B1b*), *J. Steroid Biochem. Mol. Biol.* 77 (2001) 261–269.
- [28] H. Fuda, Y.C. Lee, C. Shimizu, N.B. Javitt, C.A. Strott, Mutational analysis of human hydroxysteroid sulfotransferase *SULT2B1* isoforms reveals that exon 1B of the *SULT2B1* gene produces cholesterol sulfotransferase, whereas exon 1A yields pregnenolone sulfotransferase, *J. Biol. Chem.* 277 (2002) 36161–36166.
- [29] C. Her, T.C. Wood, E.E. Eichler, H.W. Mohrenweiser, L.S. Ramagli, M.J. Siciliano, R.M. Weinshilboum, Human hydroxysteroid sulfotransferase *SULT2B1*: two enzymes encoded by a single chromosome 19 gene, *Genomics* 53 (1998) 284–295.
- [30] S. Gingras, R. Moriggl, B. Groner, J. Simard, Induction of 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase type 1 gene transcription in human breast cancer cell lines and in normal mammary epithelial cells by interleukin-4 and interleukin-13, *Mol. Endocrinol.* 13 (1999) 66–81.
- [31] S. Gingras, J. Simard, Induction of 3beta-hydroxysteroid dehydrogenase/isomerase type 1 expression by interleukin-4 in human normal prostate epithelial cells, immortalized keratinocytes, colon, and cervix cancer cell lines, *Endocrinology* 140 (1999) 4573–4584.
- [32] S. Gingras, S. Cote, J. Simard, Multiple signaling pathways mediate interleukin-4-induced 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase type 1 gene expression in human breast cancer cells, *Mol. Endocrinol.* 14 (2000) 229–240.
- [33] I. Dufort, P. Rheault, X.F. Huang, P. Soucy, V. Luu-The, Characteristics of a highly labile human type 5 17beta-hydroxysteroid dehydrogenase, *Endocrinology* 140 (1999) 568–574.
- [34] C. Labrie, A. Belanger, F. Labrie, Androgenic activity of dehydroepiandrosterone and androstenedione in the rat ventral prostate, *Endocrinology* 123 (1988) 1412–1417.
- [35] C. Labrie, J. Simard, H.F. Zhao, A. Belanger, G. Pelletier, F. Labrie, Stimulation of androgen-dependent gene expression by the adrenal precursors dehydroepiandrosterone and androstenedione in the rat ventral prostate, *Endocrinology* 124 (1989) 2745–2754.
- [36] F. Labrie, *Intracrinology*, *Mol. Cell Endocrinol.* 78 (1991) C113–C118.
- [37] V. Luu-The, P. Tremblay, F. Labrie, Characterization of type 12 17beta-hydroxysteroid dehydrogenase, an isoform of type 3 17beta-hydroxysteroid dehydrogenase responsible for estradiol formation in women, *Mol. Endocrinol.* 20 (2006) 437–443.
- [38] V. Luu-The, I. Dufort, G. Pelletier, F. Labrie, Type 5 17beta-hydroxysteroid dehydrogenase: its role in the formation of androgens in women, *Mol. Cell Endocrinol.* 171 (2001) 77–82.
- [39] W.G. Nelson, T.-T. Sun, The 50- and 58-kdalton keratin classes as molecular markers for stratified squamous epithelia: cell culture studies, *J. Cell Biol.* 97 (1983) 244–251.
- [40] R. Moll, W.W. Franke, B. Volc-Platzer, R. Krepler, Different keratin polypeptides in epidermis and other epithelia of human skin: a specific cytokeratin of molecular weight 46,000 in epithelia of the pilosebaceous tract and basal cell epitheliomas, *J. Cell Biol.* 95 (1982) 285–295.
- [41] E. Fuchs, H. Green, Changes in keratin gene expression during terminal differentiation of the keratinocyte, *Cell* 19 (1980) 1033–1042.
- [42] V. Luu-The, Analysis and characteristics of multiple types of human 17beta-hydroxysteroid dehydrogenase, *J. Steroid Biochem. Mol. Biol.* 76 (2001) 143–151.
- [43] X.F. Huang, V. Luu-The, Molecular characterization of a first human 3(alpha-beta)-hydroxysteroid epimerase, *J. Biol. Chem.* 275 (2000) 29452–29457.
- [44] S. Torn, P. Nokelainen, R. Kurkela, A. Pulkka, M. Menjivar, S. Ghosh, M. Coca-Prados, H. Peltoketo, V. Isomaa, P. Vihko, Production, purification, and functional analysis of recombinant human and mouse 17beta-hydroxysteroid dehydrogenase type 7, *Biochem. Biophys. Res. Commun.* 305 (2003) 37–45.
- [45] H. Liu, A. Robert, V. Luu-The, Cloning and characterization of human form 2 type 7 17beta-hydroxysteroid dehydrogenase, a primarily 3beta-keto reductase and estrogen activating and androgen inactivating enzyme, *J. Steroid Biochem. Mol. Biol.* 94 (2005) 173–179.