Role of retinol dehydrogenase 12 in the biosynthesis of 5α-androstane-3β,17β-diol in the skin

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Abstract

Using RealTime PCR, we found that RDH12 is highly expressed in the skin of *Macaca fascicularis* (mf). It has been known that RDH12 is a retinol dehydrogenase that catalyzes the reduction of retinal into retinol and play an important role in the visual cycle. Indeed, its deficiency is the cause of Leber's congenital amaurosis 3, a genetic disorder characterized by retinal dystrophy affecting both rods and cones. Previously, we have shown that many of the members of the retinol dehydrogenase family, such as RDH1, 5, 11 and 16 could also metabolize 5α-reduced steroids, including DHT, the most potent natural.

In order to determine the possible role of RDH12 in a non-visual cycle and especially in the intracrine metabolisation of DHT in the skin, we perform Real-Time PCR to quantify its expression levels and *in situ* hybridization to localise its expression in the skin. To determine its activity, we construct expression vectors that express the coding region of human, mf and mouse RDH12 under the control of CMV promoter, and stably transfect the resulting vectors into HEK-293 cells. Using cells stably expressed human, mouse and mf RDH12 in culture without addition of co-factor, we show that the enzyme catalyzes effectively the transformation of DHT into 3β-diol and 5α-androstane-3,17-dione (5α-dione) into 5α-androstane-3β-ol-17-one (epi-ADT). *In situ* hybridization shows that RDH12 is highly expressed in mf sebocytes. These data strongly suggest that RDH12 could play an important role in the skin, especially in sebocytes by controlling the intracrine concentration of DHT and retinoic acid levels. RDH12 could thus play an important in acne seborrhea, a disease due to altered sebaceous glands secretions and is influenced by DHT and retinoic acid. In addition, 3β-diol could also have a yet determined effect in the skin and sebocytes due to its ability to modulate ERβ.

Results

Figure 1

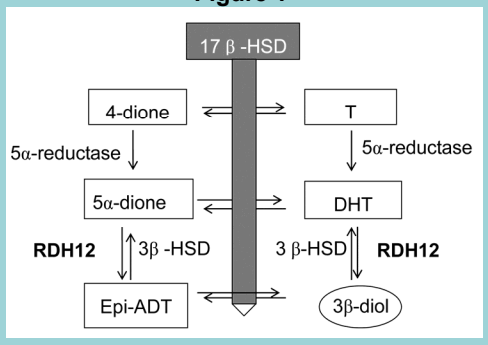


Figure 1. Pathway of 3β-diol biosynthesis

Figure 2

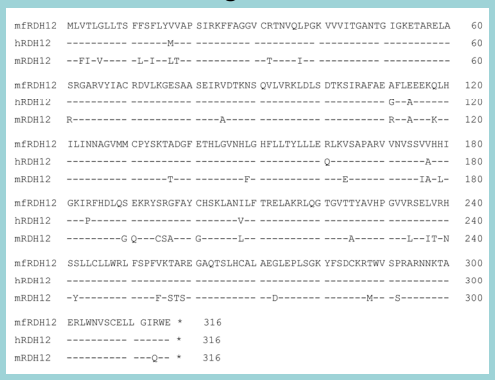
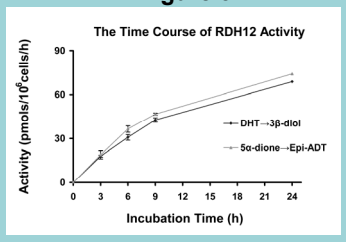


Figure 2. Alignment of amino acid sequences of mf, human and mouse RDH12
Amino acids are indicated by conventional letters. Dash (-) and point (.) represent identical and missing amino acid, respectively.

Figure 5. Localization of RDH12 mRNA in the mf skin by *in situ* hybridization.
(A-B) are consecutive sections through a female breast skin. (A), negative control section hybridized with sense RDH12 riboprobe. (B), adjacent section hybridized with the antisense RDH12 riboprobe. SG: sebaceous gland. S: stroma. E: epithelium of hair follicular. × 600.

Figure 3



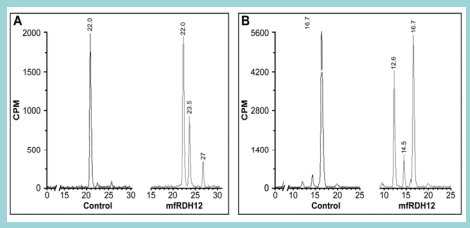


Figure 3. Quantification by phospho Imager (above) and Identification by HPLC (below) of metabolites obtained using RDH12 expressed in HEK-293 cells. Metabolites obtained after incubation with [^{14}C]5α-dione (A) and [^{14}C]DHT (B) in non transfected (Control) and transfected (mfRDH12) cells.

Figure 4

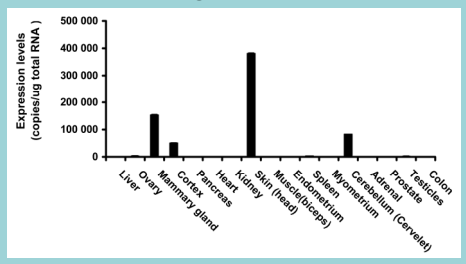
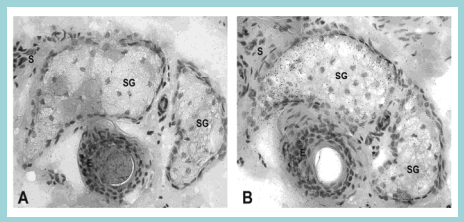


Figure 4. mRNA expression levels of RDH12 in various human tissues



Materials and Methods

Animal tissues. Tissues of adult Cynomolgus monkeys (*Macaca fascicularis*, mf) weighing 5-7 kg in good health were collected for total RNA extraction and mRNA localization by hybridization *in situ* . Animals were maintained and handled in accordance with the Canadian Council on Animal Care and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Protocol was approved by the Ethical Committee for Animal Protection of the CHU de Québec.

Construction of vector for mf RDH12
The coding region of mf RDH12 was amplified by PCR using total RNA obtained from mf kidney. The resulting amplified cDNA fragments were then subcloned into a pCMVneo vector and the resulting plasmid transfected into HEK-293 cells using Jet Prime transfection reagent (VWR International, Montreal, QC, Canada). HEK-293 cells stably expressing the enzymes were selected among positive clones that are resistant to G418.

RealTime PCR quantification
Total RNA from various mf tissues were extracted using RNeasy kit (Qiagen, Missisauga, Canada). 20 ng of total RNA was used to perform RealTime PCR quantification with the LightCycler 480 (Roche Diagnostics, NJ), SYBR Green detection and our improved quantification method based on second derivative detection and double correction using the mRNA expression levels of a housekeeping gene, ATP5o as internal and external standard.

Enzymatic assays
Cells were incubated with ^{14}C-labeled steroids and freshly changed cultured medium for 24h. After incubation, steroids were extracted using solid phase column Strata-X, 200 mg, column (Phenomenex, Torrance, CA, USA). Steroid metabolites were separated and quantified using phospho Imager. Substrates and metabolites were identified by comparison with reference steroids.

Conclusion

- We show for the first time that RDH12 catalyzes efficiently the transformation of DHT into 3β-diol and 5α-dione into epi-ADT in cultured transfected cells.
- Since 3β-diol has been shown to have the ability to modulate ERβ, RDH12 that is expressed in the skin, mammary glands, cerebellum and cortex, could thus play an important role in these tissues.
- The localisation of RDH12 in the sebaceous gland suggests that 3β-diol could have a yet determined role in acne seborrhea, a disease due to altered sebaceous glands secretions and is influenced by DHT and retinoic acid.