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Steroidogenic enzymes, their related transcription factors and nuclear receptors in human sebaceous glands under normal and pathological conditions

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ABSTRACT

The sebaceous gland is a major site of steroid synthesis in human skin, but details of the status of steroidogenic enzymes and their regulation in human sebaceous glands under normal and pathological conditions have rarely been reported. Therefore, in this study, we examined the status of steroidogenic enzymes, sex steroid receptors and transcription factors in human sebaceous glands under normal and pathological conditions to explore their possible roles in in situ steroid production in human skin. Immunohistochemical analysis was performed in a total of 59 human skin specimens, including 22 normal human sebaceous glands, 12 with sebaceous nevus, 12 with sebaceous gland hyperplasia, 3 with sebaceoma and 10 with sebaceous carcinoma. Immortalised human SZ95 sebocytes were treated with forskolin or vehicle for 3 h, 6 h, 12 h or 24 h, and the mRNA levels of steroidogenic enzymes were evaluated at each time point using quantitative RT-PCR (qPCR). The results of immunohistochemistry demonstrated the immunoreactivity of 3β-HSD1, CYP11A1, StAR, 17β-HSD5, CYP17A1, 5α-red1, PRB, AR and NGFI-B in normal human sebaceous gland, with lower levels of expression in pathological sebaceous glands. The results of the *in vitro* study also indicated that the expression levels of 3β-HSD1, CYP11A1, StAR, 5α-red1 and NGFI-B were elevated by forskolin. 3β-HSD1 and other steroidogenic enzymes were expressed in sebaceous glands resulting in *in situ* androgen and progesterone synthesis and their functions.

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1. Introduction

Skin is the largest organ in the human body in terms of surface area and is an important site of synthesis and metabolism of steroid hormones [1,2]. Sebaceous glands are an important component of human skin. These glands produce and secrete sebum, which is responsible for moisturising and protecting the skin [3]. However, malfunctioning and damaged of sebaceous glands can play a central role in many dermatological conditions, including acne [3].

Abbreviations: qPCR, quantitative RT-PCR; 3β-HSD1, 3β-hydroxysteroid dehydrogenase type 1; 3β-HSD2, 3β-hydroxysteroid dehydrogenase type 2; CYP11A1, cholesterol side-chain cleavage enzymes; CYP17A1, cytochrome P45017A1; 17β-HSD5, 17β-hydroxysteroid dehydrogenase type 5; 5α-red1, 5α-reductase type 1; 5α-red2, 5α-reductase type 2; PRA, progesterone receptor A; PRB, progesterone receptor B; AR, androgen receptor; NGFI-B, nerve growth factor-induced clone B; SF-1, steroidogenic factor 1; GATA4, GATA transcription factor 4; GATA6, GATA transcription factor 6.

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Table 1
Summary of skin sample.

	Histologic type	No. cases ($n=59$)		Mean age
		Male	Female	
Normal sebaceous gland	Melanocytic nevus ($n=22$)	11	11	28.4 ± 23.71
Non-neoplasm disorders	Sebaceous nevus ($n=12$)	6	6	17.5 ± 20.56
	Sebaceous hyperplasia ($n=12$)	8	4	63.2 ± 14.51
Sebaceous neoplasm				
Benign	Sebaceoma ($n=3$)	1	2	57.7 ± 20.03
Malignant	Sebaceous carcinoma ($n=10$)	4	6	77.6 ± 10.22

Several previous clinical and experimental studies of sebaceous glands have suggested a role for alterations in steroid forming intracrine pathways in skin disorders, particularly acne [4,5]. In addition, further steroidogenic enzymes, including steroidogenic acute regulatory protein (StAR), cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) also known as CYP11A1, steroid 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), cytochrome P45017A1 (CYP17A1) and 5α-reductase (5α-red), have all reportedly been detected in human skin, including in sebaceous glands. However, their role in pathological conditions, such as hyperplasia and carcinoma, remains relatively unknown [4,6–9].

Beyond the expression of steroidogenic enzymes, the expression of the regulators of these enzymes may also be important in controlling the intracrine actions of steroids in skin biology and pathology. Transcription factors that have been reported to regulate steroidogenic enzymes in a variety of tissues, including the placenta and ovaries include nerve growth factor-induced clone B (NGFI-B), steroidogenic factor 1 (SF-1), and GATA transcription factors 4 and 6 (GATA4 and GATA6) [10,11]. Despite these known interactions, details on the status of steroidogenic enzymes and transcription factors in human sebaceous glands have only partially been evaluated in a single study, which suggested a possible role for SOX-9 in the pathogenesis of acne [12]. Therefore, in this study, we investigated the status of StAR, CYP11A1, 17β-HSD5, 5α-red1, 5α-red2, CYP17A1 and specific steroidogenic HSD3B genes as well as key transcriptional factors involved in human sebaceous glands under normal and pathological conditions to clarify the possible roles and biological significance of *in situ* steroidogenesis and its regulation in sebaceous glands.

2. Material and methods

2.1. Human skin

Fifty-nine specimens of human skin were retrieved from the surgical pathology files of Tohoku University Hospital (Sendai, Japan). The research protocol was approved by the ethics committee at Tohoku University Graduate School of Medicine (Sendai, Japan) (2013-1-65). The details of these specimens are summarised in Table 1.

2.2. Immunohistochemistry

The tissue sections were deparaffinised and rehydrated using standard protocols. The immunohistochemical analysis was performed via the streptavidin-biotin amplification method using a Histofine Kit (Nichirei, Tokyo, Japan). The antibodies and positive controls used in our present study are summarised in Table 2. The antigen–antibody complex was visualized by immersing the reacted slides in DAB solution (1 mM 3,3-diaminobenzidine), 50 mM Tris–HC1 buffer, pH 7.6 and 0.006% hydrogen peroxidase, and counterstained with hematoxylin. For negative controls, the slides were incubated with 0.01 M PBS instead of the primary

Table 2
Characteristics of the primary antibodies employed in this study.

Primary antibody	Dilution	Source	Antigen retrieval	Positive control	References
3β-HSD1	1:3000	Abnova	Microwave	Placenta	[37]
3β-HSD2	1:2000	Kyoto University	No antigen retrieval	Adrenal gland	[38]
CYP11A1	1:200	BEX	Trypsin	Adrenal gland	[39]
StAR	1:1000	Abcam	Autoclave	Adrenal gland	[40]
17β-HSD5	1:200	Sigma	Autoclave	Adrenal gland	[41]
5α-red1	1:1000	Abcam	No antigen retrieval	Liver	[41]
5α-red2	1:1000	Immunostar	No antigen retrieval	Liver	[41]
CYP17A1	1:500	BEX	Autoclave	Adrenal gland	[42]
PRA	1:200	Neomarkers (Fremont, CA)	Autoclave	Breast cancer	[43]
PRB	1:200	Neomarkers (Fremont, CA)	Autoclave	Breast cancer	[43]
AR	1:50	DAKO (Copenhagen, Denmark)	Autoclave	Prostate	[44]
NGFI-B	1:200	Geneka	Autoclave	Adrenal gland	[45]
GATA4	1:200	Santa Cruz	Autoclave	Fetal adrenal gland	[46]
GATA6	1:100	Santa Cruz	Autoclave	Adrenal gland	[47]
SF-1	1:100	Perseus	Autoclave	Adrenal gland	[48]

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