Sex Steroid Hormone Receptors in Human Skin Appendage and its Neoplasms

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Abstract. Sex steroids have been postulated to influence pathophysiology of human skin through various skin appendages. The presence of sex steroid receptors has been also reported in adnexal tumors but its details still remained unknown. Therefore, in this study, we immunolocalized sex steroid receptor protein (estrogen receptor (ER) α , ER β , progesterone receptor (PR)A, PRB and androgen receptor (AR)) in 23 cases of non-pathological skin (male: 10, female: 13) and in 50 cases of skin adnexal tumors (male 24, female 26; 38 benign and 12 malignant). ER α immunoreactivity was detected exclusively in basal cells of sebaceous glands of non-pathological skin. AR and PRB immunoreactivity was detected in both differentiated and basal cells of sebaceous gland. AR and ER β immunoreactivity was also detected in sebaceous and eccrine sweat glands but not in outer root sheath of hair follicles. In sebaceous gland neoplasms, the number of ER α positive cases was significantly lower in skin appendage neoplasms than non-pathological skin. ER β immunoreactivity was not detected in any of sebaceous gland neoplasms examined. There were no significant differences in PRA, PRB and AR immunoreactivity between non-pathological sebaceous gland and its neoplasm. In sweat gland neoplasms, the number of AR positive cases was significantly lower in benign neoplasms than their non-pathological counterpart. Therefore sex steroids are considered to play important roles in regulation of non-pathological skin appendage function and pathogenesis and/or development of its neoplasm. In addition, the status of the great majority of sex steroid hormone receptors was maintained throughout the process of neoplastic transformation of skin appendages, except for AR and ER α in sweat and sebaceous gland neoplasms.

Key words: Hormone receptor, Sex steroid hormone, Skin appendage, Neoplasm, Immunohistochemistry

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IT is well-known that sex steroids play important roles in development and/or physiology of human mammary glands [1–3]. In addition, abnormalities of sex steroid metabolism are also considered to be involved in development and/or biological behavior of mammary gland neoplasms. Human sweat gland is analogous to the mammary gland in its development and/or physiology [4]. Therefore, non-pathological human sweat glands and their neoplasms may share similar characteristics with mammary glands and its tumors in terms

of influence by sex steroids. Sex steroids have been implicated in the development of acne in puberty [5–8], moisturizing of skin or epidermis in women [9–12] and others. In addition, previous studies demonstrated that some skin appendage tumors were positive for sex steroid hormone receptors [13–22]. The studies of Thornton *et al.* and Shin *et al.* both demonstrated that immunoreactivity of some sex steroid hormone receptors in human scalp skin [23, 24]. However, the precise distribution of steroid hormone receptors in non-pathological human skin and/or skin appendage neoplasms has remained virtually unknown. Therefore, in this study, we immunolocalized ER (ER α and ER β), PR (PRA and PRB), and androgen receptor (AR) in normal human skin appendages from both male and female subjects obtained from surgery as well as vari-

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ous skin appendage tumors in order to examine the possible involvement of sex steroids in physiology and/or pathology of human skin appendage and its neoplasms.

Material and Methods

Fifty cases of skin appendage neoplasms (38 benign and 12 malignant) and 23 cases of non-pathological skin tissue were retrieved from surgical pathology files at Tohoku University Hospital, Sendai, Japan between 1990 and 2001. Cases of skin appendage tumors examined were 24 males and 26 females, with a mean age of 59.0 years (range: 0 to 86, median: 66). Non-pathological skin tissue specimens were obtained from various parts of the body and from 10 male and 13 female subjects with a mean age of 44.8 years (range: 1 to 80, median: 49). The histological subtypes of skin appendage neoplasms examined in this study are summarized in Table 1. Non-pathological skin tissue specimens were obtained from patients undergoing surgical excision of skin lesion with informed consent of the patients. This study protocol was approved by the Ethics Committee of Tohoku University School of Medicine, Sendai, Japan (protocol #2001–240).

All specimens had been fixed in 10% formalin-fixed for 24 to 36 hours at room temperature, embedded in paraffin, sliced at 3.0 μ m thickness, and mounted on MAS-coated glass slides (Matsunami Co. Ltd., Tokyo,

Japan). The antibodies used in this study are summarized in Table 2. Anti-ER α (6F11, mouse monoclonal antibody) was purchased from Novocastra (Newcastle upon Tyne, UK). Anti-ER β (14C8), mouse monoclonal antibody, was purchased from GeneTex (San Antonio, TX). Antibodies against the two isoforms of PR, PRA (hPRa7) and PRB (hPRa2), both mouse monoclonal antibodies, were purchased from NeoMarkers Co. Ltd. (Fremont, CA). Anti-AR (AR411), mouse monoclonal antibody, was purchased from DAKO (Glostrup, Denmark). The slides were then immersed in methanol containing 0.3% hydrogen peroxide for 30 min at room temperature in order to block endogenous peroxidase activity. Autoclave treatment for 5 min at 120°C in 0.01 mol/L citrate buffer, pH 6.0, was employed after deparaffinization for antigen retrieval [25]. Normal rabbit or goat serum was incubated for 30 min at room temperature in order to suppress nonspecific staining. Tissue sections were then incubated with the primary antibodies for 18 hr at 4°C. After washing, tissue slides were incubated with biotinylated anti-mouse immunoglobulin for 30 min at room temperature, and subsequently incubated with peroxidase-conjugated streptavidin for 30 min at room temperature, using Histofine Kit (Nichirei, Tokyo, Japan). The antigen-antibody complexes were visualized by immersion in 3,3'-diaminobenzidine (DAB) solution (0.001 M DAB, 0.05 M Tris-HCl buffer, pH 7.6 0.01 M sodium azide and 0.006% hydrogen peroxidase). The tissue sections were subsequently counterstained with hematoxylin.

For negative controls of immunostain, the sections were incubated with 0.01 mol/L phosphate-buffered saline or normal mouse IgG instead of the primary antibodies. No specific immunoreactivity was detected in these tissue sections (data not shown).

Table 1. Summary of skin appendage neoplasms, examined

	Histologic type	Number
Sweat gland tumor (n = 42)		
Benign (n = 30)	Eccrine hidrocystoma Mixed tumor Eccrine poroma Syringoma Eccrine spiradenoma Clear cell hidradenoma Syringocystadenoma papilliferum Apocrine adenoma	6 6 6 3 3 3 2 1
Malignant (n = 12)	Extramammary Paget's disease Sweat gland carcinoma Microcystic adnexal carcinoma	7 4 1
Sebaceous gland tumor (n = 8)		
Benign (n = 8)	Sebaceous adenoma Sebaceoma Sebaceous hyperplasia	3 3 2

Table 2. Summary of primary antibodies employed in this study

Antigens	Antibody clone	Source	Dilution	Antigen retrieval
ER α	6F11	Novocastra (Newcastle, UK)	1:40	Autoclave
ER β	14C8	GeneTex (San Antonio, TX)	1:4000	Autoclave
PRA	hPRa7	NeoMarkers (Union City, CA)	1:200	Autoclave
PRB	hPRa2	NeoMarkers (Union City, CA)	1:200	Autoclave
AR	AR411	DAKO (Glostrup, Denmark)	1:100	Autoclave

Nuclear, not cytoplasm, staining on particular skin appendages and skin appendage neoplasm were considered positive in this study [26]. For statistical analysis, we used the Mann-Whitney's U test and Kruskal-Wallis test to compare the ratio of the positive cases between the groups. $P < 0.05$ was defined as statistically significant difference.

Results

Non-pathological skin appendages and epidermis

The ratio of sex steroid receptor positive cases is summarized in Table 3 and 4. We could examine only eccrine glands, since normal apocrine glands were not available for examination in this study. The status of sex steroid receptor immunoreactivity in non-pathological skin appendages varied markedly among the cases examined. Both PRB and AR were present in

sebaceous glands, and outer root sheath of hair follicles were completely devoid of ER β and AR (Fig. 1). The basal cells of sebaceous glands were positive for ER α in 19 (82.6%) of 23 cases (Fig. 2). ER α immunoreactivity in basal cells was significantly more frequent than sebaceous lobules (differentiated cells of sebocytes) and other skin appendages. ER β immunoreactivity varied in sebaceous glands (both differentiated and basal cells) and secretory cells of eccrine sweat glands, and not detected in outer root sheath cells of hair follicles. PRA immunoreactivity was frequently positive in secretory cells of eccrine sweat glands than any other structures of skin appendages examined, but the differences did not reach statistical significance. PRB immunoreactivity was the most frequently detected sex steroid receptor in all non-pathological human skin appendages and among sex steroid receptors examined in this study. AR immunoreactivity was also frequently detected in both sebaceous glands and secretory cells of eccrine glands, but outer root sheath cells

Table 3. The proportion of hormone receptor positive cases in normal skin appendages

	Differentiated cells of sebaceous glands No. (%)	Basal cells of sebaceous glands No. (%)	Eccrine sweat glands No. (%)	Outer root sheath of hair follicles No. (%)	Epidermis No. (%)
ER α	2/21 (9.5) ^a	19/23 (82.6) ^{a,b,c}	6/21 (28.6) ^b	9/21 (42.9) ^c	11/23 (47.8)
ER β	8/18 (50.0) ^d	4/20 (20.0) ^e	9/23 (39.1) ^f	0/22 (0) ^{d,e,f}	4/23 (17.4)
PRA	6/19 (31.6)	3/21 (14.3)	13/22 (59.1)	5/19 (26.3)	5/23 (21.7)
PRB	20/20 (100)	22/22 (100)	21/22 (95.5)	16/17 (94.1)	22/23 (95.7)
AR	20/20 (100) ^g	22/22 (100) ^h	21/23 (91.3) ⁱ	0/21 (0) ^{g,h,i}	7/23 (30.4)

a, b, c: $P < 0.01$ d, e, f: $P < 0.05$ g, h, i : $P < 0.01$

Table 4. The proportion of hormone receptor positive cases in normal skin appendages of male and female

	Differentiated cells of sebaceous glands No. (%)	Basal cells of sebaceous glands No. (%)	Eccrine sweat glands No. (%)	Outer root sheath of hair follicles No. (%)	Epidermis No. (%)
ER α	1/9 (11.1)	9/10 (90.0)	3/8 (37.5)	4/8 (50.0)	4/10 (40.0)
	1/12 (8.3)	10/13 (76.9)	3/13 (23.1)	5/13 (38.4)	7/13 (53.8)
ER β	4/8 (50.0)	3/9 (33.3)	4/10 (40.0)	0/10 (0)	2/10 (20.0)
	4/10 (40.0)	1/11 (9.1)	5/13 (38.5)	0/12 (0)	2/13 (15.4)
PRA	2/8 (25.0)	1/9 (11.1)	8/10 (80.0)	3/9 (33.3)	3/10 (30.0)
	4/11 (36.3)	2/12 (16.7)	5/12 (41.6)	2/10 (20.0)	2/13 (15.4)
PRB	9/9 (100)	10/10 (100)	10/10 (100)	7/8 (87.5)	10/10 (100)
	11/11 (100)	12/12 (100)	11/12 (91.7)	9/9 (100)	12/13 (92.3)
AR	9/9 (100)	10/10 (100)	10/10 (100)	0/9 (0)	4/10 (40.0)
	11/11 (100)	12/12 (100)	11/13 (84.6)	0/12 (0)	3/13 (23.1)

upper low: male; under low: female

Fig. 1. Immunostaining of normal skin appendages with PRB. Nuclear staining of secretory cells of hair follicles and sebaceous glands. Original magnification ($\times 200$).

Fig. 3. Immunostaining of normal skin appendages with AR. Nuclear staining of sebaceous glands and secretory cells sweat glands, but negative staining in outer root sheath cells of hair follicle. Original magnification ($\times 200$).

Fig. 2. Immunostaining of normal sebaceous glands with ER α . ER α was positive in nuclei of basal cells but completely negative in nuclei of sebaceous lobule. Original magnification ($\times 400$).

of hair follicles were negative in all the cases examined (Fig. 3, 4a). There were no significant differences between male and female subjects (Table 4).

In epidermis, immunoreactivity of all steroid hormone receptors was detected, with PRB immunoreactivity being the most frequently detected.

Sweat gland neoplasms

Results are summarized in Table 5. All of the sweat glands neoplasms, except for one case of extramammary Paget's disease, were positive for PRB, as in non-pathological sweat glands. PRA was positive in 86.7% (26/30) of the benign neoplasms and 50% (6/12) of malignant tumors arising from sweat glands. In skin

appendage neoplasms, both ER α and ER β immunoreactivity tended to be relatively low. The ratio of AR positive cases in normal sweat glands (91.3%) was significantly higher than in benign sweat gland neoplasms (66.7%) ($P < 0.05$) (Fig. 4b). AR-positive cases in malignant sweat gland neoplasms were not different from benign tumors (66.7%) and normal sweat glands in this study (Fig. 4c). The proportion of the sex steroid hormone receptor positive cases in sweat gland neoplasms was summarized in Table 6.

Sebaceous gland neoplasms

Results were summarized in Table 7. ER α immunoreactivity was significantly lower in benign tumors (25.0%) than basal cells of normal sebaceous glands (82.6%) ($P < 0.01$) (Fig. 5). ER β immunoreactivity was negative in any of sebaceous glands neoplasms examined in this study. But there were no significant differences between non-pathological sweat glands and neoplasms. The number of PRA positive cases was relatively low (25.0%). The great majority (87.5%) of benign sebaceous neoplasms was positive for AR and PRB, but there were no significant differences between benign tumors and non-pathological sebaceous glands.

Discussion

In this study, we examined immunoreactivity of various steroid hormone receptors in non-pathological

Fig. 4. Immunostaining of normal sweat glands and sweat gland neoplasms with AR. Variable staining of normal sweat glands (a), but negative staining in benign (syringoma) (b) and malignant (extramammary Paget's disease) (c) sweat gland neoplasms. Original magnification ($\times 400$).

skin appendages from both sexes and skin appendage neoplasms. Expression of sex steroid hormone receptors examined in this study has been reported in various normal human organs [27–32]. In the skin, Thornton

Table 5. The proportion of the sex steroid hormone receptor positive cases in normal sweat glands and sweat gland neoplasms

	Normal sweat glands No. (%)	Benign sweat gland neoplasms No. (%)	Malignant sweat gland neoplasms No. (%)
ER α	6/21 (28.6)	6/30 (20.0)	4/12 (33.3)
ER β	9/23 (39.1)	8/30 (26.7)	3/12 (25.0)
PRA	13/22 (59.1)	26/30 (86.7)	6/12 (50.0)
PRB	21/22 (95.5)	30/30 (100)	11/12 (91.7)
AR	21/23 (91.3)*	20/30 (66.7)*	8/12 (66.7)

*: $P < 0.05$

Fig. 5. Immunostaining of sebaceous gland neoplasms with ER α . Negative staining in benign (sebaceoma) (a), sebaceous adenoma (b) sebaceous gland neoplasms. But variable staining of basal cells of normal sebaceous glands (Fig. 2). Original magnification ($\times 200$).

et al. demonstrated the presence of these receptors in scalp skin [23]. But to the best of our knowledge the systemic evaluation of these steroid receptors in human skin and skin appendage neoplasms have yet to be

Table 6. The proportion of the sex steroid hormone receptor positive cases in each sweat gland neoplasms

	ER α	ER β	PRA	PRB	AR
<i>Eccrine/benign</i>					
Eccrine poroma	0/6 (0)	0/6 (0)	5/6 (83.3)	6/6 (100)	3/6 (50.0)
Eccrine hidrocystoma	1/6 (16.7)	3/6 (50.0)	5/6 (83.3)	6/6 (100)	4/6 (66.7)
Eccrine spiradenoma	0/3 (0)	2/3 (66.7)	3/3 (100)	3/3 (100)	1/3 (33.3)
Clear cell hidradenoma	1/3 (33.3)	0/3 (0)	1/3 (33.3)	3/3 (100)	2/3 (66.7)
Syringoma	1/3 (33.3)	1/3 (33.3)	3/3 (100)	3/3 (100)	1/3 (33.3)
<i>Apocrine/benign</i>					
Mixed tumor	3/6 (50.0)	2/6 (33.3)	6/6 (100)	6/6 (100)	6/6 (100)
Syringocystadenoma papilliferum	0/2 (0)	0/2 (0)	2/2 (100)	2/2 (100)	2/2 (100)
Apocrine adenoma	0/1 (0)	0/1 (0)	1/1 (100)	1/1 (100)	1/1 (100)
<i>Eccrine/malignant</i>					
Microcystic adnexal carcinoma	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
<i>Apocrine/malignant</i>					
Extramammary Paget's disease	2/7 (28.6)	1/7 (14.3)	4/7 (57.1)	6/7 (85.7)	5/7 (71.4)
Sweat gland carcinoma	1/4 (25.0)	1/4 (25.0)	1/4 (25.0)	4/4 (100)	2/4 (50.0)

(%)

Table 7. The proportion of sex steroid hormone receptor positive cases in normal sebaceous gland and sebaceous gland neoplasms

	Differentiated cells of sebaceous glands No. (%)	Basal cells of sebaceous lobules No. (%)	Benign sebaceous gland neoplasms No. (%)
ER α	6/21 (28.6)	6/30 (20.0)	4/12 (33.3)
ER β	9/23 (39.1)	8/30 (26.7)	3/12 (25.0)
PRA	13/22 (59.1)	26/30 (86.7)	6/12 (50.0)
PRB	21/22 (95.5)	30/30 (100)	11/12 (91.7)
AR	21/23 (91.3)*	20/30 (66.7)*	8/12 (66.7)

*: $P < 0.01$

reported.

Human sweat gland is analogous to the mammary gland in its development [4]. Therefore, sweat gland neoplasms of the skin, especially malignant neoplasms, sometimes stimulate histological features of ductal carcinoma of the breast. Differential diagnosis between primary sweat gland carcinoma and metastatic breast carcinoma to the skin is sometimes difficult in histopathological examination. The differentiation between these lesions, which is crucial for clinical staging, management, and prognosis of the patient, also can be occasionally difficult [34–36]. Several investigators proposed the use of sex steroid receptors to differentiate primary sweat gland neoplasms from metastatic breast cancer to the skin [13, 35, 36]. However, it is

also very important to recognize that primary neoplasms of skin appendage also expressed sex steroid receptors.

In this study, apocrine glands were not available for examination in any of the tissue sections of normal skin examined. Choudhry *et al.* reported AR-positive cases were high in apocrine sweat gland, as well as eccrine sweat gland [26]. This finding also suggests that functions of both apocrine and eccrine sweat glands may be modulated by sex steroids. AR was detected in the great majority of normal eccrine sweat glands (91.3%) examined in our present study. PRB immunoreactivity was also detected in non-pathological eccrine sweat glands in 95.5% of the cases, but further investigations are required to clarify the possible roles of progesterone in regulation of human skin appendage functions.

Both ER and PR have been reported to be detected in some of the sweat gland neoplasms [13–19], but their isoforms have not been examined. ER β , which is widely distributed throughout the body [28], was detected in some sweat gland neoplasms. On the other hand, PRB was almost always expressed in sweat gland neoplasms in our series, and the ratio was relatively higher than that of PRA. However, the number of PRA and PRB positive cases was not significantly different between non-pathological sweat glands and malignant sweat gland neoplasms. This finding also suggests that progesterone does not necessarily influence growth

of malignant sweat gland neoplasms. PRA immunoreactivity was frequently more positive in benign sweat gland tumors than in non-pathological sweat glands. Wallace *et al.* [33] demonstrated that PR was detected in 89% of syringoma, and that progesterone may influence growth of syringoma cells. Therefore these findings in our present study also suggest that progesterone may influence growth of these benign sweat gland neoplasms through PRA but it awaits further investigations for clarification.

AR immunoreactivity was detected in 71% of the cases of extramammary Paget's disease. Previous studies also demonstrated that AR was detected in 54% [14] and 80% [15] of extramammary Paget's disease. ER α was detected in 29% of extramammary Paget's disease in our series. However, the positivity of AR in sweat gland neoplasms both benign and malignant including extramammary Paget's disease was not necessarily high. Immunoreactivity tended to be lower than that in non-pathological sweat glands. These findings indicated that AR has an important role in maintenance of normal sweat gland, and the decrease of AR immunoreactivity may result in development of neoplasm.

Sebaceous glands have been considered one of the target organs of androgen and estrogen [5]. In addition, progesterone may modulate the function of normal sebaceous structures based on the results of our present study. In our present study, AR immunoreactivity was detected in all basal and differentiated cells. This finding is consistent with the results of other studies which identified AR in sebaceous glands of normal skin [26, 36, 37]. These results also indicated that androgen is considered the dominant hormone influencing the development and/or physiology of sebaceous glands. PRB immunoreactivity was consistently detected in sebaceous glands, both in differentiated glandular cells and basal cells. ER α immunoreactivity was frequently detected only in basal cells of normal sebaceous glands in our study. Therefore, not only androgen but also progesterone and/or estrogen, may regulate hormone-related physiology and/or development of sebaceous glands, especially through PRB and ER α .

AR was also shown to be expressed in sebaceous gland neoplasms, including adenomas, carcinomas, and sebaceous nevi [18, 38]. Hamilton *et al.* [38]

reported that congenital hamartoma with sebaceous glands consistently expressed AR during puberty. Our results also demonstrated that both AR and PRB were well preserved even in benign neoplasms, but the frequency of ER α was decreased. Androgen is normally considered the major regulator of human hair growth with paradoxically different effects on hair follicles depending on their body site. For instance, androgens incrementally stimulate the size of hair follicles on the face and trunk but not in other portions of the body [7, 39]. In addition, several studies demonstrated the correlation between androgen and hair growth, especially alopecia [39, 40]. Hence, neither the previous report [26] nor our present study demonstrated the AR expression in outer root sheath of normal hair follicles. On the other hand, ER α (42.9%), PRA (26.3%) and PRB (94.1%) were detected in structures of outer root sheath of hair follicles. These discrepancies of androgenic action may be explained as follows; 1) The expression level of AR was low in our study, and examination in the disease status, such as alopecia, may clarify possibility of correlation between androgen and hair growth; 2) AR expression may be influenced by the body sites examined. AR was reported as positive on non-genital skin [26]; 3) androgenic actions may be indirectly regulated by other hormones, including estrogen and progesterone, because ER α , PRA and PRB are all expressed in these cells.

Sex steroids are considered to play important roles in regulation of normal skin appendage function and pathogenesis and/or development of its neoplasm and the status of the great majority of sex steroid hormone receptors was maintained throughout the process of neoplastic transformation of skin appendages, except for AR and ER α in sweat and sebaceous gland neoplasms.

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