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ORIGINAL PAPER

Clinicopathological features of considerable reduction in androgen receptor expression in sebaceous gland carcinoma of the eyelid

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Abstract
Purpose This study aimed to evaluate the relationships between androgen receptor (AR) expression and clinicopathologic features of sebaceous gland carcinoma (SGC) of the eyelid.
Methods AR expression was evaluated via immunohistochemistry analysis of surgically derived samples from 11 patients with SGC of the eyelid.
Results The expression of AR was evident in 9 of 11 patients (82%). We divided patients into high AR (7 patients) and low AR (4 patients: 2 patients with low expression and 2 patients with no expression) groups. The low AR group showed significantly greater progression than the high AR group with regard to T category and exhibited a lower grade of differentiation.
Conclusion In patients with SGC of the eyelid, a marked decrease in AR expression may be associated with a poor prognosis. AR may be a prognostic factor and a potential therapeutic target in cases of SGC of the eyelid.

Keywords Androgen receptor · Differentiation · Immunohistochemistry · Pagetoid spread · Sebaceous gland carcinoma

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Introduction

Sebaceous gland carcinoma (SGC) of the eyelid is an invasive malignant tumor and often metastasizes to lymph nodes and distant organs [1]. It is relatively common in Asian countries, and it accounts for approximately 30% of malignant eyelid tumors [2]. Eyelid SGC is an aggressive tumor with vascular and perineural invasion. Metastasis occurs in 14–25% of all patients with SGC, and the most common site of metastasis is regional lymph nodes [3]. The likelihood of SGC-related mortality is reduced by early diagnosis and appropriate treatment [4], but SGC of the eyelid is frequently misdiagnosed clinically and histologically [5]. SGC can masquerade as various inflammatory lesions and other malignant tumors, such as chalazion, blepharoconjunctivitis, basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and epithelial carcinoma in situ [5, 6]; reportedly, the diagnosis of SGC may be missed in 23–77% of cases [1, 7, 8]. Recently, biomarkers such as adipophilin and androgen receptor (AR) have been evaluated via immunohistochemical staining for their potential use in the diagnosis of SGC and to distinguish it from BCC and SCC [9–13].

AR is a member of a superfamily of steroid hormone receptors and is expressed at a relatively low density in normal tissues, but can be identified in sebaceous glands, among other tissues [11, 14]. It is a sensitive marker of sebaceous differentiation in sebaceous neoplasms, including SGC [14], and sebaceous gland differentiation is controlled by AR signaling

[15]. Therefore, AR plays a diagnostic role in SGC and leads to high diagnostic accuracy. The positive rate of AR in SGC is reportedly more than 80% [12, 14, 16]. In addition, it has been reported that high AR expression is associated with a poor prognosis in periocular SGC and that AR is a promising prognostic marker [10].

We performed the current study to evaluate the relationships between AR expression and clinicopathologic features of eyelid SGC and investigated the usefulness of AR expression as a prognostic marker.

Methods

Patients and clinicopathological examination

We enrolled 11 patients with SGC of the eyelid between 2015 and 2017 at the Toyama University Hospital. All patients underwent surgical excision of the tumor. The tissues were fixed using 4% paraformaldehyde in phosphate-buffered saline. Paraffin-embedded tissues were stained with hematoxylin–eosin and examined immunohistochemically by the Pathology Institute Corporation (Toyama, Japan). Our procedures conformed to the tenets of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from the patients after provision of sufficient information about the procedures. The study was approved by the Institutional Review Board of the Toyama University Hospital.

Patient clinical data were examined, including age, sex, tumor location, tumor origin, tumor differentiation, mitosis grade, presence or absence of pagetoid spread, Ki-67 index, and T category. T category was determined using TNM stage as defined in American Joint Committee on Cancer (AJCC) 8th edition [17]. SGCs were classified into 3 groups based on differentiation: good, moderate, and poor differentiation [18]. Mitosis was classified as 0–1 per high-power microscopy field (HPF), 2–5 per HPF, or > 5 per HPF [19]. We divided the patients into 2 groups depending on the AR positivity. The high AR group included 7 patients, and the low AR group included 4 patients (2 patients with low expression and 2 patients with no expression).

Immunohistochemistry

The primary antibodies used were mouse monoclonal anti-androgen receptor (clone AR441) (M3562; 1:100 dilution, Agilent Technologies, Inc., Santa Clara, CA) and mouse monoclonal anti-Ki-67 (clone MIB-1) (1:100 dilution, Agilent Technologies, Inc., Santa Clara, CA). Immunohistochemistry was performed using Leica BOND III automation and the BOND Polymer Refine Detection System Kit (Leica Biosystems, Bannockburn, IL, USA). The protocol included in situ deparaffinization and high-pH epitope retrieval for 40 min, primary antibody incubation for 20 min, polymer for 8 min, and diaminobenzidine (DAB) as the chromogen for 10 min, followed by a 5-min hematoxylin counterstaining step. The positivity of AR and Ki-67 was measured via immunohistochemistry. Nuclear staining of tumor cells was recorded as percent positivity for AR and Ki-67 (Ki-67 index).

Statistical analysis

Differences in patient age and Ki-67 index between the groups were analyzed via Mann–Whitney U test. Comparisons of other parameters between the groups (sex, T category, differentiation, presence or absence of pagetoid spread, presence or absence of metastasis and mitosis) were analyzed via the χ^2 test or Fisher's exact test. All statistical analyses were performed with JMP 11 (SAS Institute, Cary, NC, USA).

Results

The clinicopathological characteristics of patients are presented in Table 1. Of the 11 patients, three were male and eight were female. The mean age was 74.3 ± 14.3 years (range, 36–91 years). The locations of SGCs were as follows: upper eyelid (7), lower eyelid (2), and both upper and lower eyelids (2): 4 were in the right eyelids and 7 were in the left eyelids. The mean follow-up after surgery was 13.7 ± 4.2 months (range, 7–20 months). There were 2 cases of local recurrence/metastasis in the follow-up period, and there was 1 case of metastasis detected at the first visit to the hospital. The patient distribution with respect to T category was as follows: T1 (all T1b), 5; T2, 4 (T2a: 2, T2b: 1, T2c: 1); and T3, 2 (T3a: 1, T3c: 1). Next, we examined AR positivity in the

Table 1 Clinical characteristics of patients and the positive rate of AR in SGC of eyelid

Case	Age and sex	Local recurrence	Metastasis	Follow-up after surgery (months)	T category	AR positivity rate (%)
1	91 F	–	–	13	T2b	60
2	82 F	–	–	9	T1b	50
3	68 M	–	–	7	T1b	50
4	73 F	–	–	8	T2a	3
5	90 F	–	–	15	T1b	80
6	68 M	–	–	12	T2a	80
7	81 F	–	–	16	T1b	95
8	70 F	–	+	15	T3a	0
9	80 F	+	–	19	T1b	80
10	78 F	–	–	20	T2c	0
11	36 M	–	+	17	T3c	1

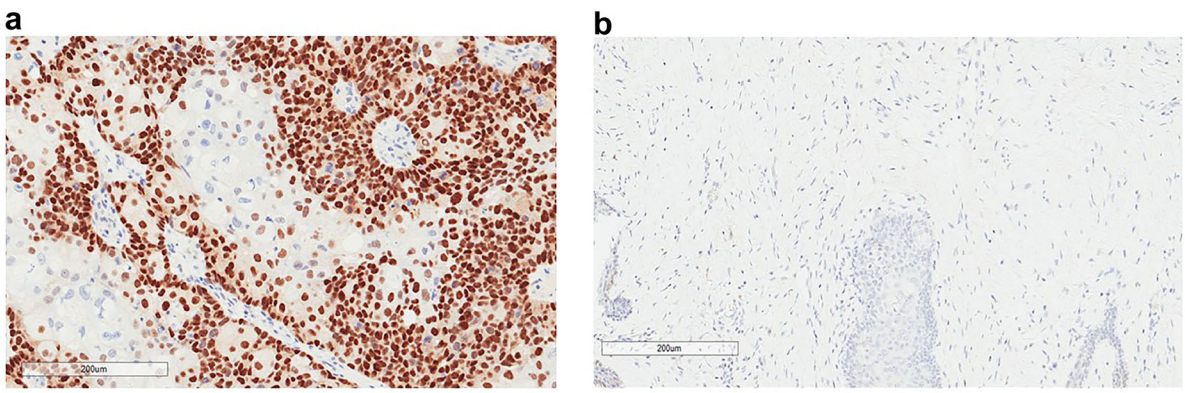

Fig. 1 Immunohistochemical analysis of androgen receptor (AR) in sebaceous gland carcinoma of the eyelid. Representative cases from the high and low AR groups are shown. AR positivity was 95% in case 7 (**a**), and it was 3% in case 4 (**b**). Scale bar = 200 μm

nucleus of SGC tumor cells using immunohistochemistry. Of the 11 patients, AR expression was detected in 9 (82%). Notably, AR positivity showed a bimodal degree of expression: either a high percentage or a low percentage. The respective positive percentages for cases 1, 2, 3, 5, 6, 7, and 9 (high AR group) were 60, 50, 50, 80, 80, 95, and 80%, and the respective positive percentages for cases 4, 8, 10, and 11 (low AR group) were 3, 0, 0, and 1%. Representative cases from the high and low AR groups are shown in Fig. 1. AR staining was confined to the nucleus and was positive in the epithelial component that differentiated into sebaceous glands (see case 7). There were also cases in which AR staining was barely positive, as in case 4.

Table 2 shows a comparison of the clinicopathological features in the high and low AR groups. There were no significant differences in age ($p = 0.144$) or sex ($p = 0.899$) between the two groups. With regard to T category, in the high AR group, there were 5 T1 cases and two T2 cases, while in the low AR group, there were two T2 cases and two T3 cases. This difference was statistically significant ($p = 0.035$). The low AR group showed significantly greater progression than the high AR group with regard to T category.

With regard to differentiation of the SGC, in the high AR group, 1 case was high grade, 3 cases were moderate grade, and 3 cases were low grade. In the low AR group, all 4 cases were low grade. There was a

Table 2 Clinicopathological features between high AR group and low AR group

Parameter	AR high (n = 7)	AR very low (n = 4)	p
Age (mean)	80.0 ± 8.6	64.3 ± 16.6	0.144
Sex			
Male	2	1	0.899
Female	5	3	
T category			
T1	5	0	0.035
T2	2	2	
T3	0	2	
Differentiation			
High	1	0	0.037
Moderate	3	0	
Low	3	4	
Pagetoid spread			
Positive	3	3	0.301
Negative	4	1	
Mitosis			
0–1/HPF	0	1	0.057
2–5/HPF	3	0	
> 5/HPF	4	3	
Metastasis/recurrence			
Positive	1	1	0.658
Negative	6	3	
Ki-67 index (mean, %)	62.7 ± 13.1	64.4 ± 14.5	0.441

significant difference between the high and low AR groups (p = 0.037). Differences in other clinicopathological features between the two groups, including the presence of pagetoid spread (p = 0.301), mitosis (p = 0.057), metastasis/local recurrence (p = 0.658), and Ki-67 index (p = 0.441), were not statistically significant, but mitosis tended to be more frequent in the low AR low group.

Discussion

AR is expressed in sebaceous glands and is a useful marker of sebaceous differentiation [14]. Therefore, immunostaining for AR has been used as a marker to distinguish SGC from SCC and BCC [9–13]. Lipid stains such as oil red O and Sudan III are useful to detect lipid accumulation, but these stains need to be performed on frozen sections from fresh tissue [19]. Therefore, immunohistochemistry is more versatile than these lipid stains. The AR positivity rate for SGC is reportedly more than 80%; however, there are cases in which AR staining is negative [16]. In the current study, because some cases were highly AR positive and some were not, we divided them into two groups and investigated each group clinically and pathologically. In the low AR group, T category progression and poor differentiation were observed. In SGC of the eyelid, tumor size [20–22] and tumor differentiation [22, 23] have been reported to be associated with prognosis, and markedly decreased AR expression may be associated with a poor prognosis.

It has previously been reported that AR positivity tends to be high in SGC of the eyelid [14, 16]. The study by Mulay et al. [10] that included 56 SGCs showed a greater recurrence, higher Ki-67 expression, and lower p53 expression in patients with a high AR expression and assessed the relationship between AR expression and tumor prognosis. On the other hand, it has been reported that AR is involved in sebaceous differentiation, and AR deficiency suggests de-differentiation and poor differentiation [15, 16, 24]. Because there are contradictory reports, the effect of AR expression on SGC of the eyelid is not yet fully understood. In the current study, AR is a useful diagnostic marker for SGC of the eyelid, whereas negative or very low levels of AR expression may be indicative of SGC with a poor prognosis. In the current study, the Ki-67 indexes were 62.7 ± 13.1 in the high AR group and 64.4 ± 14.5 in the low AR group, and the difference between the groups was not statistically significant. In a previous report, the Ki-67 index was high in AR deficient cases [15], and it has also been reported that the Ki-67 index in SGC is in the 40% range [11, 12]—but the Ki-67 index tended to be higher in the current study. Further studies may be necessary to determine the relationship between prognosis and Ki-67 index in SGC of the eyelid. In the current study, mitosis tended to be observed more frequently in the low AR group, but the difference between the high and low AR groups was not statistically significant. It has been suggested that mitosis is not a poor prognostic factor in SGC of the eyelid [10, 18], and the current study did not detect a strong association between mitosis and AR expression. It has been suggested that the presence or absence of pagetoid spread is an important prognostic

factor in SGC of the eyelid [18, 25, 26]. In the current study, there was no significant association between the proportion of pagetoid spread and AR expression. Notably, however, immunostaining of AR has been reported to be useful for the detection of pagetoid spread [11, 27], and further investigation of the relationship between pagetoid spread and AR expression is needed.

Lastly, in SGC of the eyelid, it is considered that a marked decrease in AR expression indicates a high possibility of a poor prognosis. However, the major limitation of this study is its small sample size. It is necessary to investigate this further in the future. In addition to AR being a diagnostic marker for SGC of the eyelid, the degree of its expression may be a useful prognostic factor.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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