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Expression of Anti-apoptotic Protein BAG3 in Human Sebaceous Gland Carcinoma of the Eyelid

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Abstract. *Background: Bcl-2-associated athanogene 3 (BAG3), a co-chaperone of heat shock protein 70 (HSP70), has been shown to play a role in anti-apoptosis of various malignant tumors. In this study, the expression of BAG3 was examined in human sebaceous gland carcinoma of the eyelid. Materials and Methods: The expression of BAG3 was evaluated by immunohistochemistry of surgical samples from 5 patients with sebaceous gland carcinoma in the eyelid. Results: BAG3 was positive diffusely in the cytoplasm in all patients. The average positive rate of BAG3 was 73.0±26.0% in tumor cells of all patients. Conclusion: BAG3 was highly expressed in sebaceous gland carcinoma of the eyelid. BAG3 may play an important role in the pathogenesis and progression of sebaceous gland carcinoma of the eyelid.*

Sebaceous gland carcinoma of the eyelid is a highly aggressive malignant tumor that is relatively common in Asian countries, and it comprises about 30% of malignant eyelid tumors (1). The fundamental treatment for sebaceous gland carcinoma is surgical excision of the tumor, but it can exhibit aggressive local behavior and can metastasize to regional lymph nodes and distant organs (2). Furthermore, sebaceous gland carcinoma of the eyelid is often misdiagnosed as other benign and malignant lesions, thereby making early detection and treatment difficult (3). Successful management of eyelid sebaceous gland carcinoma is dependent on several factors, including tumor size (4, 5) and pagetoid spread (diffuse epithelial involvement) (6, 7). However, despite many efforts, the outcomes of patients are mostly unpredictable, and there are still few useful markers for predicting prognosis. Accordingly, research focused on predicting the risk of recurrence and the effectiveness of adjuvant therapy is valuable for the patients with sebaceous gland carcinoma of the eyelid.

Heat shock proteins (HSPs) function as molecular chaperones and exert cytoprotective effects. Among the HSPs, proteins from the HSP70 family play central roles as molecular chaperones. Bcl-2-associated athanogene 3 (BAG3) belongs to a family of co-chaperones that interacts with the ATPase domain of HSP70 (8). Although BAG3 is down-regulated in normal cells, it is upregulated in various malignant tumors (9-13). It has been reported that BAG3 plays a role in anti-apoptotic properties through pro-survival response to various stresses in cancer cells (14-16).

The relationships between human sebaceous gland carcinoma and BAG3 are poorly understood. In this study, we examined these relationships immunohistochemically in human surgically removed sebaceous gland carcinoma of the eyelid.

Materials and Methods

Patients and clinical materials. We enrolled 5 patients with sebaceous gland carcinoma of the eyelid between 2014 and 2016 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). All patient details are summarized in Table I. 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.

Immunohistochemistry. The primary rabbit monoclonal BAG3 antibody (ab92309; Abcam, Cambridge, MA, USA) was used at a dilution of 1:400. Immunohistochemistry was performed using Leica BOND III automation and BOND Polymer Refine Detection System Kit (Leica Biosystems, Bannockburn, IL, USA). The protocol included *in situ* deparaffinization and high-pH epitope

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retrieval for 40 min, primary antibody incubation for 20 min, polymer for 8 min, and DAB as the chromogen for 10 min, followed by a 5-min hematoxylin counterstaining.

BAG3 scoring. Positivity was graded by the membranous and cytoplasmic staining. Staining intensity was applied for the immunohistochemistry results: 3+, strongly positive, 2+, moderately positive, 1+, weakly positive, and negative. The total amount of positive rate (1+~3+) was set as the tumor positive rate of the case.

Results

We examined the expression of BAG3 in surgically removed eyelid tissues from 5 patients with sebaceous gland carcinoma of the eyelid by immunohistochemistry. The clinical characteristics of patients are presented in Table I. Altogether, 5 patients were enrolled (1 male, 4 female; mean age=76.8±8.0 years, range 67-89=years; 4 with upper eyelid affected, one with lower eyelid affected). All patients had nodule-type tumors. The tumor category, as defined by the American Joint Committee on Cancer (AJCC) was T2a in 4 and T2b in 1. The mean follow-up period after surgery was 20.4±4.1 months (range=16-28 months). In the follow-up period, there was 1 case of local recurrence and 1 case of metastasis to lymph nodes.

BAG3 was positive diffusely in the cytoplasm of all patients (Figure 1). However, the rate of BAG3 positivity differed by case. The positive rate for Case 1 and Case 2 was 100%, and the positive rate of Cases 3-5 was 80%, 45%, 40%, respectively. On average, the BAG3 positivity rate was 73.0%±26.0% in all patients. Furthermore, the rate of strongly-positive was 10.0%±13.0% (range=0-35), moderately-positive was 26.0%±29.6% (range 0-80), and weakly-positive was 37.0%±23.1% (range=0-60) (Table II).

Table I. *Patient characteristics*.

	Case 1	Case 2	Case 3	Case 4	Case 5
Age	89	67	80	69	79
Gender	F	M	F	F	F
Location	L upper	L lower	L upper	R upper	R upper
T category	T2a	T2a	T2a	T2a	T2b
Tumor pattern	nodule	nodule	nodule	nodule	nodule
Follow-up after surgery (months)	20	28	20	16	18
Local recurrence	–	–	–	+	–
Lymph node metastasis	–	–	–	–	+

F: Female, M: male, L: left, R: right.

Discussion

Sebaceous gland carcinoma of the eyelid is a high-grade malignancy that metastasizes to regional lymph nodes and distant organs (2). Chemotherapy has been used for metastatic sebaceous gland carcinoma, but it is not effective (17, 18). Accordingly, the study of sebaceous gland carcinoma-specific biomarkers is important for improving prognosis and treatment outcomes. In this study, we confirmed that anti-apoptotic protein BAG3 was highly expressed in sebaceous gland carcinoma of the eyelid histopathologically. To the best of our knowledge, this is the first report of the overexpression of BAG3 in the sebaceous gland carcinoma of the eyelid.

Previous reports have shown that overexpression of BAG3 was observed in human cancer cells of various origins (9-13), and it has been demonstrated that this overexpression is related to the development of cancer, invasiveness, metastasis, angiogenesis, tumor adhesion, migration, and resistance to chemotherapy (19-23). BAG3 shows anti-apoptotic activity by interacting with different molecular partners, such as Bcl-2 family member BAX, Mcl-1, IKKγ, and BRAF (14-16, 24, 25). At present, the detailed molecular mechanisms underlying the relationship between BAG3 and sebaceous gland carcinoma of the eyelid are unknown. Up-regulated BAG3 expression may contribute to apoptosis-resistant properties through several mechanisms, and may become to a useful therapeutic strategy for anti-cancer therapy for patients with sebaceous gland carcinoma of the eyelid. We should validate molecular targets of BAG3 as a novel strategy though inhibition of BAG3. Further investigations will be needed to clarify this issue.

In this study, the rate of BAG3 positive rate varied by case. Case 1 and Case 2, in which the positive rate of BAG3 was 100%, did not show local recurrence or lymph node metastasis. Several authors have reported that significant correlations between BAG3 protein levels and prognosis were observed (26, 27). Therefore, BAG3 overexpression may correlate with poor prognosis of patients with sebaceous gland carcinoma of the eyelid. However, the relationship between recurrence or metastasis and BAG3 positivity is not clear at present. Accordingly, we must examine more cases over a longer follow-up period.

In conclusion, BAG3 is overexpressed in human sebaceous gland carcinoma of the eyelid. Our findings suggest that BAG3 may offer a therapeutic target for patients with sebaceous gland carcinoma, and plays a role in the pathogenesis and progression of sebaceous gland carcinoma.

Conflicts of Interest

There is no conflict of interest.

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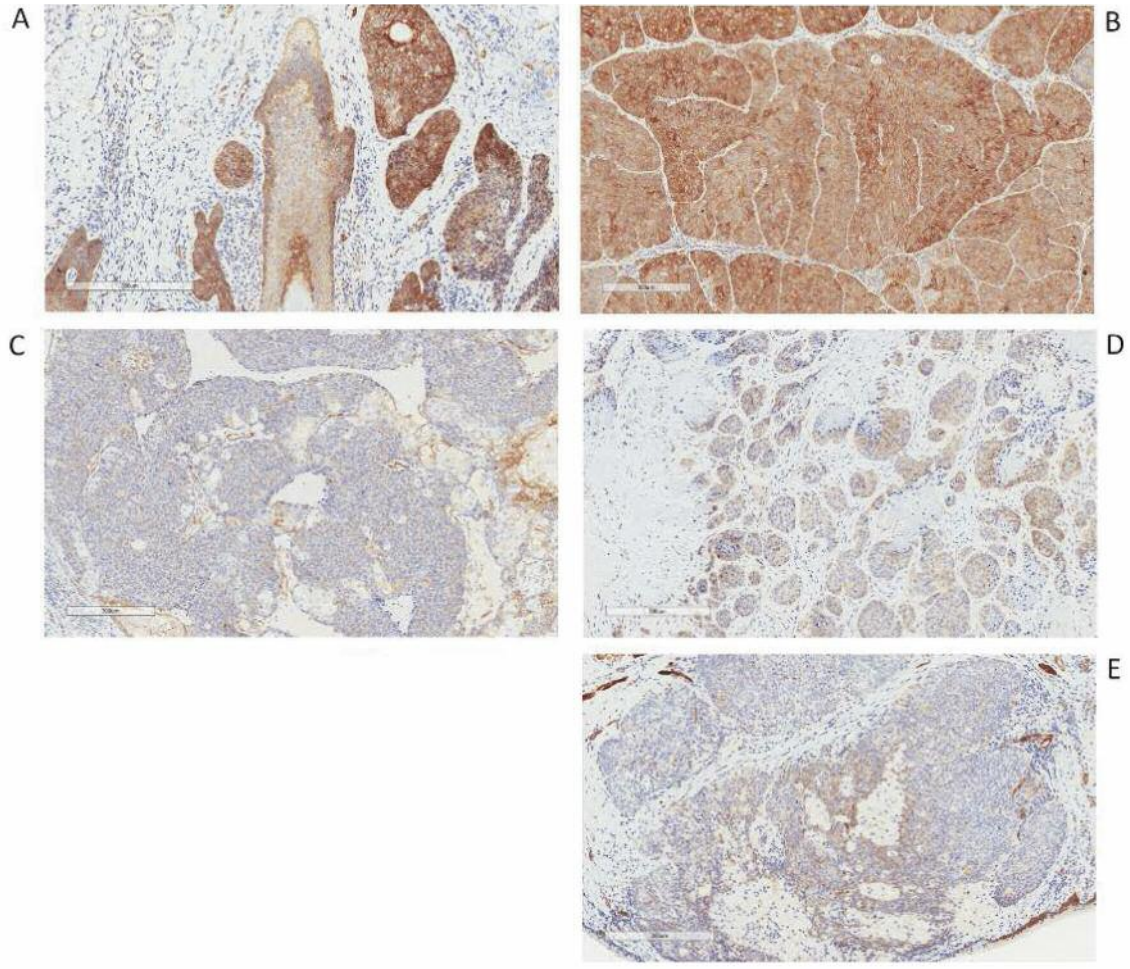

Figure 1. *Immunohistochemical analysis of BAG3 expression in sebaceous gland carcinoma of the eyelid. Paraffin sections from human surgically removed sebaceous gland carcinoma of the eyelid were analyzed by immunohistochemistry using anti-BAG3 antibody. A: Case 1 (original magnification ×40). B: Case 2 (original magnification ×40). C: Case 3 (original magnification ×40). D: Case 4 (original magnification ×40). E: Case 5 (original magnification ×40).*

Table II. *BAG3 expression in sebaceous gland carcinoma*

Case	BAG3 strongly positive 3+ (%)	BAG3 moderately positive 2+ (%)	BAG3 weakly positive 1+ (%)	BAG3 negative rate (%)	BAG3 positive rate A total of 1+~3+ (%)
1	35	35	30	0	100
2	10	80	10	0	100
3	0	0	80	20	80
4	5	10	30	55	45
5	0	5	35	60	40

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