Histol Histopathol (2023) 38: 155-163

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE **Open Access**

A diagnostic pitfall; small cell carcinoma-like features in basaloid squamous cell carcinoma of the esophagus

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Summary. Esophageal basaloid squamous cell carcinoma may resemble small cell carcinoma biopsy specimens and cause difficulties in pathology diagnosis. We aimed to clarify the clinicopathological significance of small cell carcinoma-like morphologies in basaloid squamous cell carcinoma. Thirty biopsy specimens of esophageal basaloid squamous cell carcinoma were reviewed and compared with 13 matched surgical specimens. Small cell carcinoma-like features, such as diffuse growth, nuclear molding, or nuclear crush artifact, were identified in 80% (24/30) of the biopsies and in 77% (10/13) of the surgery specimens, but in a proportionally much smaller area in the surgical specimens than in the biopsy samples. The presence of a small cell carcinoma-like feature had no impact on patients´ outcome. Immunohistochemically, synaptophysin and chromogranin A were consistently negative, while CD56 was expressed in 42% (10/24) of basaloid squamous cell carcinomas with small cell carcinoma-like features. p16, a highly sensitive marker for small cell carcinoma, was also expressed in 8% (2/24). p40 was expressed in all cases of basaloid squamous cell carcinoma. In conclusion, small cell carcinoma-like features are frequent and conspicuous in biopsies, which are probably caused by exogenous factors such as friction and external pressure that occur in biopsy procedure and in the tumor environment. Small cell carcinoma-like features may lead to a misinterpretation of a true small cell carcinoma, if CD56 is the only neuroendocrine marker expressed. p16 expression may also be detected in basaloid squamous cell carcinoma.

Key words: Esophagus, Biopsy, Basaloid squamous cell carcinoma, Small cell carcinoma, Diagnostic pitfall

Introduction

Basaloid squamous cell carcinoma is a histological variant of squamous cell carcinoma, first reported in 1986 by Wain et al. in tongue, hypopharynx, and larynx (Wain et al., 1986). Reported prevalence of basaloid squamous cell carcinoma of the esophagus is 0.07–11.8% of all esophageal malignancies (Bellizzi et al., 2009; Chen et al., 2012; Wang et al., 2013; Watanabe et al., 2021). Patients' clinical outcome of esophageal basaloid squamous cell carcinoma is poorer than that of conventional esophageal squamous cell carcinoma (Chen et al., 2012).

Basaloid squamous cell carcinoma shows distinctive histological features from conventional squamous cell carcinoma. Besides the conventional features of squamous cell carcinoma, basaloid squamous carcinoma consists of large solid nests of the carcinoma with a palisading arrangement, comedo-like necrosis with hyalinized and/or mucin-like material without prominent keratinization. The tumor cells have scant and basophilic

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DOI: 10.14670/HH-18-497

Abbreviations. BSCC, basaloid squamous cell carcinoma; C, cribriform arrangement; DG, diffuse growth; DSS, disease specific survival; H, hyalinized stroma; NC, nuclear crush artifact; NM, nuclear molding; P, peripheral palisading of nuclei; SmCC, small cell carcinoma; SqCC, squamous cell carcinoma.

cytoplasm with round-to-oval-shaped and dense hyperchromatic nuclei. These morphological features share the spectrum of the "small blue round cell"-like appearance and may mimic small cell carcinomas.

Esophageal small cell carcinoma accounts for 0.05-3.1% of all esophageal malignancies (Feng et al., 2013; Watanabe et al., 2021) and shows a poor clinical course characterized by early dissemination and a high metastatic potential (Pantvaidya et al., 2002; Zhu et al., 2014; Ishida et al., 2017). Small cell carcinomas are composed of highly cellular and diffusely proliferating tumor cells with scant cytoplasm, hyperchromatic and coarsely granular nuclei with inconspicuous nucleoli (Brown et al., 2019). In addition, small cell carcinomas often show characteristic morphological features, such as nuclear molding or nuclear crush artifact. Nuclear molding is an appearance that neoplastic cells directly attach to and push adjacent nuclei due to scant and almost unrecognizable cytoplasm. Nuclear crush artifact is an appearance that chromatin-like basophilic materials are observed in a streaming manner.

The treatment strategies of patients with small cell carcinomas are different from those of basaloid squamous cell carcinomas. Neoadjuvant chemotherapy with cisplatin plus 5-fluorouracil and subsequent radical surgery are performed for resectable basaloid squamous cell carcinoma patients, while cisplatin-based chemotherapy is commonly used for patients with advanced esophageal small cell carcinoma (Yau et al., 2007; Sawabata et al., 2011; Nakajima et al., 2012; Nakamura et al., 2013; Ishida et al., 2019). Therefore, it is essential to accurately distinguish the two malignancies, despite the morphological similarities that are usually seen in the biopsy specimens (Crapanzano et al., 2011; Brown et al., 2019).

In this study, we aimed to explore the pathological features of basaloid squamous cell carcinomas in biopsy specimens that mimic small cell carcinoma and to clarify their diagnostic and prognostic features. In addition, we discuss the possible pathogenesis of this unique appearance by comparing with the surgical specimens obtained from the corresponding patients. This study will contribute to draw attention to an important pitfall in pathology diagnosis in endoscopic biopsy specimens of the esophagus.

Materials and methods

Acquisition of tumor specimens and clinical data

Endoscopic biopsy specimens and the clinicopathological data were retrieved from 30 patients with basaloid squamous cell carcinoma of the esophagus diagnosed at Tohoku University Hospital between 2002 and 2020. Thirteen (38%) of the patients underwent surgery and their surgical specimens were also studied. TNM staging was determined according to the eighth edition of the American Joint Committee on Cancer / Union for International Cancer Control TNM staging system for esophageal carcinoma (Brierley et al., 2017). Disease specific survival was defined as the time from the date of initial pathological diagnosis to the date of disease-related death or last censor. Patients with the neoplasm located at the esophagogastric junction or concomitant Barrett's esophagus were excluded from the study. The study protocol was approved by the ethics committee of Tohoku University and informed consents were obtained from all patients examined.

Pathology diagnosis

All the biopsy specimens and the surgical specimens of the patients were reviewed by three pathologists (AK, FF, and HS) in an independent fashion. Diagnosis of basaloid squamous cell carcinoma was made according to the presence of basaloid features, i.e., round-to-oval-shaped nuclei, hyalinized material and scant basophilic cytoplasm (Brown et al., 2019) and positive staining for p40 by immunohistochemistry as previously described (Ishida et al., 2017).

Determination of small cell carcinoma-like features

To identify basaloid squamous cell carcinoma mimicking small cell carcinoma, the following morphological features were documented; presence or absence of 1) diffuse growth pattern, 2) nuclear molding, 3) nuclear crush artifact, 4) cribriform arrangement, 5) peripheral palisading of nuclei, 6) hyalinized stroma, 7) solid cell nests, and 8) necrosis in biopsies as well as in the surgical specimens (Brown et al., 2019). If the above-mentioned findings were identified, the percentage of the area with the findings to the total tumor area was recorded. A small cell carcinoma-like feature was defined, when at least one of the three following findings was observed: 1) diffuse growth pattern, 2) nuclear molding and 3) nuclear crush artifact (Brown et al., 2019; Brambilla et al., 2021). Findings including cribriform arrangement, peripheral palisading of nuclei, or hyalinized stroma were regarded as the key histological features for basaloid squamous cell carcinoma. Clinicopathological and immunohistochemical features were compared between basaloid squamous cell carcinoma with and without small cell carcinoma-like features.

Immunohistochemical analysis

Specimen preparation and immunohistochemistry of synaptophysin, chromogranin A, CD56, Ki-67, p40, and p16 were performed in all cases as previously described (Kasajima et al., 2011, 2021; Ishida et al., 2017). Labelling index of Ki-67, p40, and p16 was quantified in a 0.04 mm^2 area each using the Histoquest semi-automated image analysis software (TissueGnostics, Tarzana, Los Angeles, CA, USA), and the percentage of immunopositive nuclei in total tumor cells was subsequently obtained (Ishida et al., 2017, 2021).

Cytoplasmic staining was considered as a specific expression for synaptophysin, chromogranin A, membranous expression for CD56 and a nuclear expression for p40 and Ki-67. A strong and diffuse cytoplasmic and nuclear expression was considered as positive for p16. We confirmed the accuracy of immunohistochemical staining using external or internal positive controls (non-neoplastic pancreatic islet cells for synaptophysin, chromogranin A and CD56, reactive stromal cells, lymphocytes and/or non-neoplastic epithelial cells for p40/p16 and tonsils for Ki-67) (Ishida et al., 2021).

Statistical analysis

JMP Pro version 16.0.0 software (SAS Institute, Inc., Cary, NC, USA) was used for all statistical analyses. Continuous data were analyzed using the Mann–Whitney U-test. Differences between clinicopathological factors and immunoreactivity were evaluated using Pearson's χ^2-test, Fisher's exact test, or the Mann–Whitney U-test. Disease specific survival was calculated using the log-rank test. A *P*-value of <0.05 was considered statistically significant.

Results

Small cell carcinoma-like features in biopsy specimens

Overall, 24 of 30 (80%) basaloid squamous cell carcinomas had small cell carcinoma-like features including diffuse growth pattern in 14 (47%), nuclear molding in 12 (40%), and crush artifact in 19 (63%) cases (Table 1, Figure 1a-c). Among the 24 cases with small cell carcinoma-like features, all the three findings of small cell carcinoma-like features were observed in 38% (9/24). In these biopsies, basaloid characteristic morphologies, i.e., cribriform arrangement, peripheral palisading of nuclei or hyalinized stroma, were also observed in 62% (15/24), while in 38% (9/24) basaloid characteristic morphologies were not detected. The six cases without small cell carcinoma-like features showed at least one of the basaloid morphologies. Cribriform arrangement, peripheral palisading of nuclei and hyalinized stroma were detected in 37% (11/30), 50% (15/30) and 33% (10/30), respectively (Table 1, Figure

Table 1. Morphological features in biopsy specimens obtained from 30 patients with basaloid squamous cell carcinoma of the esophagus and their clinical characteristics and outcome.

Case	Basaloid SqCC small cell carcinoma-like or conventional	Age	Sex	Small cell carcinoma-like features	Other findings	Location	TNM Stage
1	small cell carcinoma-like	76	M	DG, NM, NC		Lower	I
2	small cell carcinoma-like	57	M	DG, NM, NC		Middle	IVA
3	small cell carcinoma-like	66	M	DG, NM, NC		Upper	IVB
4	small cell carcinoma-like	78	M	DG, NC		Lower	III
5	small cell carcinoma-like	65	M	DG, NC		Middle	II
6	small cell carcinoma-like	67	M	NM		Middle	I
7	small cell carcinoma-like	8	M	NM		Middle	II
8	small cell carcinoma-like	62	M	NC		Upper	I
9	small cell carcinoma-like	70	M	NC		Lower	II
10	small cell carcinoma-like	61	M	DG, NM, NC	H	Middle	III
11	small cell carcinoma-like	69	F	DG, NM, NC	C, P	Middle	II
12	small cell carcinoma-like	60	M	DG, NM, NC	C, P	Middle	I
13	small cell carcinoma-like	86	M	DG, NM, NC	P, H	Middle	IVB
14	small cell carcinoma-like	68	M	DG, NM, NC	C, P, H	Middle	I
15	small cell carcinoma-like	65	M	DG, NM, NC	C, P, H	Lower	I
16	small cell carcinoma-like	59	F	NM, NC	P, H	Middle	I
17	small cell carcinoma-like	79	M	DG, NC	C, P, H	Middle	I
18	small cell carcinoma-like	63	M	DG	P	Upper	I
19	small cell carcinoma-like	83	M	NC	C	Middle	II
20	small cell carcinoma-like	69	M	NC	P	Middle	I
21	small cell carcinoma-like	54	M	NC	H	Middle	I
22	small cell carcinoma-like	74	M	DG	P, H	Middle	III
23	small cell carcinoma-like	74	M	DG	C, P, H	Middle	II
24	small cell carcinoma-like	57	F	NC	C, P, H	Middle	I
25	conventional	65	M		C	Upper	III
26	conventional	79	M		C	Lower	I
27	conventional	75	M		P	Middle	I
28	conventional	80	M		P	Middle	I
29	conventional	82	M		C, P	Upper	II
30	conventional	61	M		*	Upper	II

SqCC, squamous cell carcinoma; M, male; F, female; DG, diffuse growth; NM, nuclear molding; NC, nuclear crush artifact; C, cribriform arrangement; P, peripheral palisading of nuclei; H, hyalinized stroma. *, Diagnosis of basaloid squamous cell carcinoma was made according to the presence of comedo-necrosis and solid nest.

1d-f).

Comparison of small cell carcinoma-like features in biopsy and surgical specimens

Small cell carcinoma-like features were detected in 10 of 13 surgically resected specimens of esophageal basaloid squamous cell carcinoma (Table 2). Relevant findings were detected in the biopsy specimens of the corresponding patients, but the percentage of the findings in a total tumor area in the surgical specimens was lower than in that in biopsy specimens as follows. A diffuse growth pattern was observed in 57% of the total tumor area in biopsy specimens (Figure 2a), while in surgical specimens solid-nested pattern was well-recognized, and diffuse growth pattern was observed only in 29% of the tumor areas (Figure 2c). Nuclear molding and nuclear crush artifact were observed in 1% and 2% of the total area in surgical specimens, respectively, while 4% and 17% in biopsy specimens, respectively. In biopsy specimens, these findings were often seen on the outer edge of the tissue and in the periphery of the tumor nests (Figure 2b) but in surgical tissues, they were confined to the mucosal surface of the tumor and the tumor cell shape was well-preserved in invading tumor cell nests (Fig. 2d). The basaloid characteristic morphology was also detected in smaller area in biopsy specimens than in surgery specimens of the corresponding patient (cribriform 14% versus 20%, peripheral palisading 25% versus 53%, hyalinized stroma 16% versus 22%) (Table 2, Fig. 2).

Immunohistochemical features in biopsy specimens

Neuroendocrine markers, synaptophysin and chromogranin A, were not expressed in any of the biopsy specimens (Fig. 3a). CD56, in contrast, was expressed in 42% of the cases (10/42) and the expression was detected in 5-50% of the total tumor area (Fig. 3b). p40 was expressed, by definition, in all cases and the expression was diffuse and strong in all specimens (Fig. 3c). p16, which is a highly sensitive marker for small cell carcinomas (Svajdler et al., 2018), was observed in two cases of basaloid squamous cell carcinomas with small cell carcinoma-like features, as strong cytoplasmic and nuclear expression (Fig. 3d). No other difference was detected in the immunohistochemical features between the basaloid squamous cell carcinomas with and

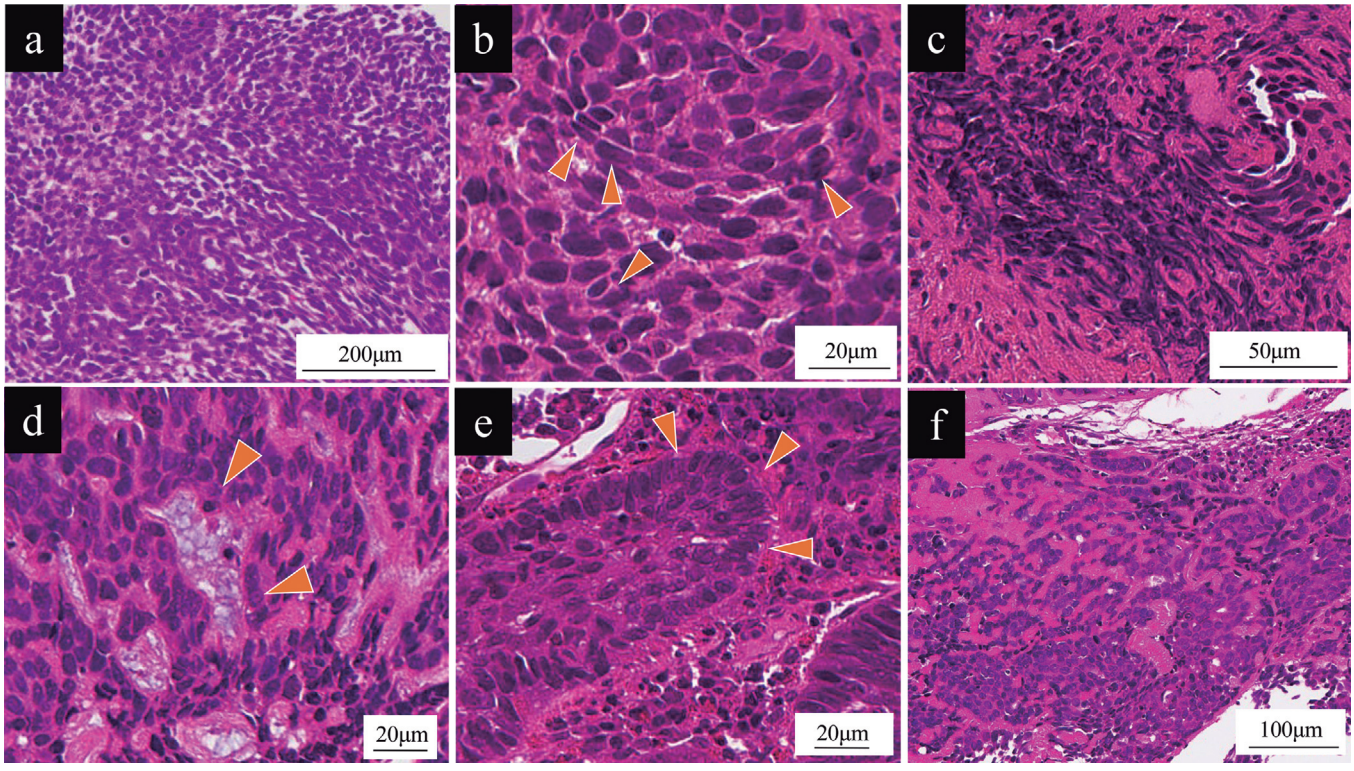

Fig. 1. Microscopic images of biopsy specimens of esophageal basaloid squamous cell carcinoma that mimic small cell carcinoma **(a, b, c)** and conventional basaloid morphology **(d, e, f)**. **a.** Dense sheets of small round-to-oval-shaped cells were detected. No specific architectural pattern was recognized and it was regarded as a diffuse growth pattern. **b.** Carcinoma cells with hyperchromatic nucleus and inconspicuous nucleolus. Arrows point to a "nuclear molding" phenomenon that appears to attach to and overlap with the neighboring nuclei due to scant cytoplasm of the cells. **c.** Due to crush artifact showing chromatin-like basophilic materials in a streaming manner, morphologies of carcinoma cells are hardly assessable. **d.** Carcinoma cell nests with a cribriform arrangement with mucinous material (arrows). **e.** Peripheral cells with nuclear palisading arrangement on the edge of the solid tumor nests (arrows). **f.** Hyalinized stroma with eosinophilic amorphous area.

without small cell carcinoma-like features. Mean Ki-67 index of the two groups were 51% and 47%, respectively, and no statistical difference was detected.

Clinical characteristics

No significant differences were detected in age and gender of the patients as well as location and TNM stage of patients with basaloid squamous cell carcinomas with and without small cell carcinoma-like features (Table 3). No difference was observed in the 2- and 5-year disease specific survival of the basaloid squamous cell carcinoma patients with and without small cell carcinoma-like features.

Discussion

Clinical significance of small cell carcinoma-like features

In this study, we identified that 80% of endoscopic biopsy specimens of basaloid squamous cell carcinoma morphologically resembled small cell carcinoma due to the presence of diffuse growth pattern, nuclear molding, and/or crush artifact. After precise re-evaluation of morphological, clinical and immunohistochemical features of the basaloid squamous cell carcinomas with and without small cell carcinoma-like features, we saw no significant differences in clinical outcome or aggressiveness between the two groups. Thus, small cell carcinoma-like features do not represent the aggressive nature of the carcinoma.

Possible development mechanisms of small cell carcinoma-like features

Basaloid squamous cell carcinoma mostly had middle to large sized tumor nests and a "true" diffuse growth pattern is only focally observed. In biopsy specimens, however, the large solid nests are not always appreciated. The large nests are probably compressed or fragmented and consequently the cell nests appear as diffuse cell proliferation in small specimens. Indeed, diffuse growth pattern was observed in smaller areas in the surgical specimens than in biopsy specimens (mean 29% versus 57% of the total tumor areas). However, a small number of the cases showed predominantly diffuse growth pattern in the surgical specimens (100% in case 4, 70% in case 18, see Table 2), suggesting that the artifacts through the specimen processing are not the only mechanism, but also reflect the individual tumor morphology. Nuclear crush artifact may be also caused by compression through sampling by endoscopic forceps

Table 2. Comparison of histological patterns detected in biopsy and surgical specimens from corresponding patients.

Case	Specimens	Small cell carcinoma-like feature (%)			Conventional BSCC feature (%)				
		Diffuse growth	Nuclear molding	Nuclear crush artifact	Cribridform arrangment	Peripheral palisading	Hyalinized palisading	Solid cell nest	Necrosis
Pt. 4	Biopsy	100	0	30	0	0	0	0	0
Pt. 4	Resected	100	0	5	5	10	5	10	15
Pt. 5	Biopsy	60	0	30	0	0	0	40	0
Pt. 5	Resected	50	0	0	10	60	10	50	5
Pt. 11	Biopsy	100	5	20	20	50	0	0	0
Pt. 11	Resected	0	0	0	30	70	20	100	30
Pt. 12	Biopsy	30	5	15	10	20	0	20	0
Pt. 12	Resected	20	0	0	15	50	20	80	0
Pt. 14	Biopsy	60	5	15	20	20	15	0	0
Pt. 14	Resected	20	0	0	15	50	50	80	15
Pt. 15	Biopsy	30	30	25	40	20	30	70	0
Pt. 15	Resected	0	5	5	60	60	30	100	0
Pt. 16	Biopsy	80	0	20	0	10	20	20	0
Pt. 16	Resected	10	0	5	0	90	20	80	15
Pt. 17	Biopsy	30	0	10	20	20	30	30	0
Pt. 17	Resected	10	0	3	20	80	60	30	20
Pt. 18	Biopsy	100	0	0	0	30	0	0	0
Pt. 18	Resected	70	0	0	50	50	20	15	5
Pt. 20	Biopsy	0	0	40	0	90	0	80	0
Pt. 20	Resected	40	5	5	10	80	10	60	0
Pt. 22	Biopsy	90	0	0	0	40	10	10	0
Pt. 22	Resected	0	0	0	40	30	20	100	0
Pt. 23	Biopsy	60	0	0	40	10	30	40	0
Pt. 23	Resected	50	0	0	0	40	10	50	10
Pt. 24	Biopsy	0	0	10	30	20	70	100	0
Pt. 24	Resected	0	0	0	10	20	10	20	3
Mean (%)	Biopsy	57	4	17	14	25	16	32	0
Mean (%)	Resected	29	1	2	20	53	22	60	9

BSCC, basaloid squamous cell caricinoma; Pt., Patient.

and proceeding tissue preparation, mostly detected in small biopsy or cytology specimens by fine-needle aspiration specimens (De Las Casas et al., 2004). However, nuclear crush artifact was not necessarily a specific finding in biopsy samples, observed in 5 out of 13 surgical specimens. An interesting observation was that in the biopsy specimens, the nuclear crush artifact was, in addition to being on the outer edge of the tissue (where is it affected by friction and external pressure), at the periphery of the tumor cell nests, while in the center of the cell nests tumor cells were well-preserved. In the surgical specimens, in contrast, the nuclear crush artifact was limited only to the surface of the tumor and the tumor cell morphology was well-maintained in the deeper part, therefore the findings did not cause diagnostic difficulty. It is notable that a crush artifact was observed in the surgical specimens, only if it was detected in the corresponding biopsy specimens, suggesting that exogenous factors such as friction and external pressure may occur both in physiological circumstances and by biopsy procedure (Lee et al., 1992). Nuclear molding is considered as a highly characteristic finding in small cell carcinoma (Sturgis et al., 2000; Cakir et al., 2010; Crapanzano et al., 2011; Maleki, 2011) but is also seen in basaloid squamous cell carcinomas with a reported detection rate reaching up to 100% (Crapanzano et al., 2011; Marks et al., 2013). In our study, nuclear molding was observed often together with nuclear crush artifact, where elongated tumor cells with narrow cytoplasm lie densely together.

Pitfalls in the interpretation of immunohistochemistry

As a definition by the WHO classification of gastrointestinal tract tumor (Brown et al., 2019), at least one of the neuroendocrine markers, synaptophysin and chromogranin A, is positive in small cell carcinoma. In contrast, CD56, a representative neuroendocrine marker, is often expressed in non-neuroendocrine neoplasms. Indeed, CD56 was detected in 37% of biopsy specimens of basaloid squamous cell carcinoma in our present study and in 42% of surgically resected specimens in our database (data not shown). Similarly, CD56 immunoreactivity in basaloid squamous cell carcinoma of anus was reported (6.3%), but no CD56 expression in that of head and neck (Lewis et al., 2018; Madahian et al., 2021). Therefore, we might often encounter basaloid squamous cell carcinoma of the esophagus with CD56 immunoreactivity compared to that of the other organs, and its expression without accompanying expression of synaptophysin or chromogranin A must be carefully interpreted (Brown et al., 2019; Kasajima and Klöppel, 2020). p40 is a squamous differentiation marker, constantly expressed in basaloid squamous cell

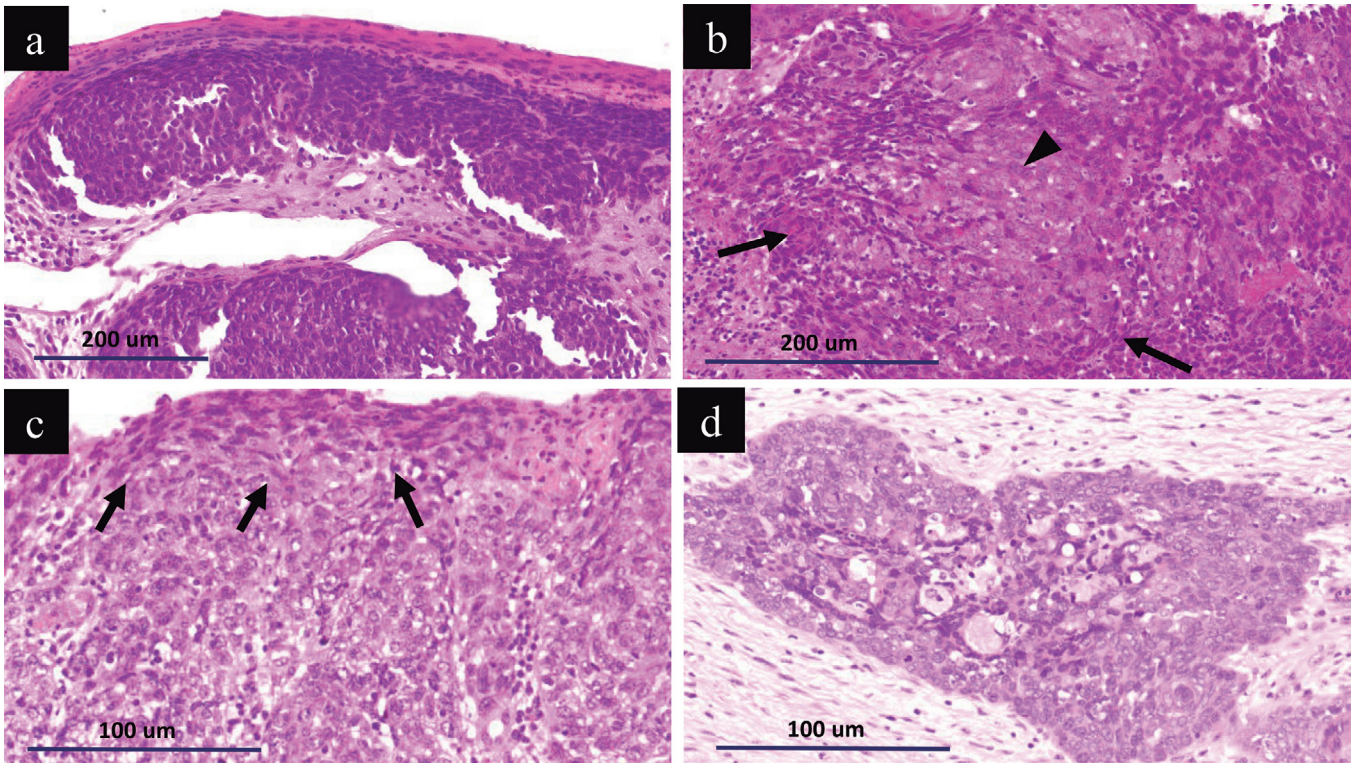

Fig. 2. Microscopic images of esophageal basaloid squamous cell carcinoma in biopsy specimens **(a, b)** and in surgery specimens **(c, d)**. **a.** Carcinoma cells do not form distinct cell nests and are recognized as diffuse growth pattern. **b.** Carcinoma cells in periphery of the cell nests show nuclear crushed artifact (arrow), while cell morphology is maintained in the center of the cell nest (arrowhead). **c.** Nuclear crush artifact in surgical specimens is confined to the tumor surface (arrow). **d.** Cell morphology is well-maintained in nested cell nests in surgical specimens.

carcinoma. p16 is overexpressed in small cell carcinoma by deregulation of tumor suppressor gene *RB1* as positive feedback (Alos et al., 2016; Ishida et al., 2021), which also plays a pivotal role in neuroendocrine differentiation (Dosaka-Akita et al., 2000; Beasley et al., 2003; Sutherland et al., 2011, Park et al., 2011; Ishida et al., 2017; 2021; Svajdler et al., 2018). p16 expression in small cell carcinoma is not associated with an HPV-infection and is a strong diagnostic tool for small cell carcinoma (Ishida et al., 2017, 2021; Svajdler et al., 2018). Unfortunately, we were not able to clarify the HPV-infection status in the two p16 expressing basaloid squamous cell carcinomas due to a limited tumor sample, because none of the patients were surgically treated. HPV-related esophageal squamous cell carcinomas have been reported in 0-63% of the cases in Japan (Rajendra et al., 2020). An interesting and well-known association of HPV-associated squamous cell carcinoma with a basaloid morphology in head and neck squamous cell carcinomas has so far not been recognized in the esophageal squamous cell carcinoma.

Conclusion

In summary, the presence of the small cell carcinoma-like features is a frequent finding in basaloid squamous cell carcinoma of the esophagus and is an important pitfall in the pathological diagnosis of the carcinoma. The small cell carcinoma-like features do not represent aggressive nature of the neoplasm and are likely caused by exogenous factors both in vivo and during the biopsy procedure. Patients with small cell

Table 3. Comparison of clinical features of basaloid squamous cell carcinomas with small cell carcinoma-like features and conventional basaloid squamous cell carcinomas in biopsy specimens.

	Basaloid squamous cell carcinoma		
	small cell carcinoma-like	conventional	*P* value
Total	24 (80)	6 (20)	
Age (Mean±SD)	69±9	74±9	0.22
Sex			0.36
Male	21	6	
Female	3	0	
Location			0.11
upper	3	3	
middle	17	2	
lower	4	1	
Stage			0.67
I/II	18	5	
III/IV	6	1	

SD, standard deviation.

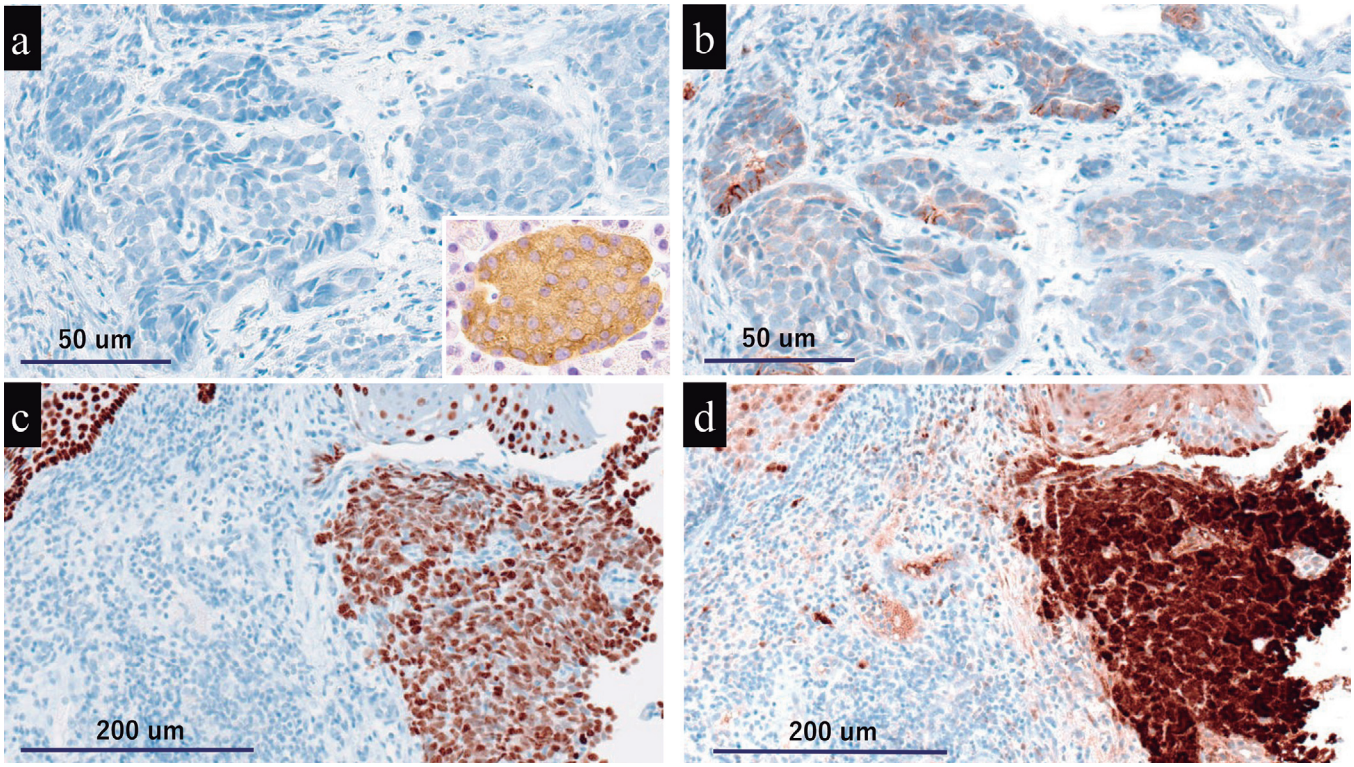

Fig. 3. Immunohistochemical images of basaloid squamous cell carcinoma. **a.** A negative synaptophysin immunoreactivity (positive control in left bottom). **b.** Focal membranous expression of CD56. **c.** Strong and diffuse nuclear expression of p40. **d.** Strong cytoplasmic and nuclear p16 expression.

carcinoma demonstrate a different clinical outcome and are treated differently from basaloid squamous cell carcinomas. Thus, basaloid squamous cell carcinoma must be accurately differentiated from small cell carcinomas by a strong p40 expression and lack of synaptophysin and chromogranin A expression. CD56 expression alone among neuroendocrine markers is also observed in basaloid squamous cell carcinoma and should not be used as the basis for the diagnosis of small cell carcinoma. p16, which is usually expressed in small cell carcinomas, may also be expressed in basaloid squamous cell carcinomas and are a pitfall in pathology diagnosis.

Acknowledgements. The authors would like to acknowledge Ms. Kazue Ise, Ms. Erina Iwabuchi, Mr. Katsuhiko Ono, and Ms. Yasuko Furukawa (Tohoku University, Miyagi, Japan) for their excellent technical support.

Conflict of interest statement. The authors have no conflicts of interest to disclose.

Funding. This work was supported in part by the Manfred Stolte-Stiftung (to A.K).

Ethics approval. The study protocol was approved by the ethics committee of our institution (Accession number of Tohoku University Hospital 2018–1–151).

Consent to participate/for publication. Informed consents were obtained from all patients for participating and publication.

Authors' contributions. The conception of the study was designed by Hirotaka Ishida and Atsuko Kasajima. Material preparation was performed by Hirotaka Ishida, Takuro Yamauchi, Ryujiro Akaishi, Shunsuke Ueki and Fumiyoshi Fujishima. Immunohistochemical staining was performed by Hirotaka Ishida, Atsuko Kasajima, Takuro Yamauchi, Ryujiro Akaishi and Shunsuke Ueki. Hirotaka Ishida, Atsuko Kasajima, Fumiyoshi Fujishima and Hironobu Sasano evaluated morphological features of the neoplasms examined in this study and the results of immunohistochemistry. Data collection was performed by Hirotaka Ishida, Takuro Yamauchi, Ryujiro Akaishi, Shunsuke Ueki, Yusuke Taniyama, Tomoyuki Koike and Takashi Kamei. The data was analyzed by Hirotaka Ishida and Atsuko Kasajima. Hironobu Sasano, Takashi Kamei and Alfred King-Yin Lam supervised the project. The first draft of the manuscript was written by Hirotaka Ishida and Atsuko Kasajima, and all authors commented on the manuscript. Manuscript editing was performed by all authors. All authors approved the final version of the manuscript.

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Accepted July 21, 2022