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CASE REPORT

**Naohiko Kamio · Youichi Tanaka · Makio Mukai
Eiji Ikeda · Shigeru Kuramochi · Masato Fujii
Yasuhiro Hosoda**

A hybrid carcinoma: adenoid cystic carcinoma and salivary duct carcinoma of the salivary gland

An immunohistochemical study

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Abstract Hybrid tumours of the salivary glands are very rare entities composed of two different tumours, each of which conforms with an exactly defined category. We describe an unusual hybrid carcinoma of the palate; it was comprised of an adenoid cystic carcinoma and a salivary duct carcinoma with a transitional region. These two different compartments showed different characteristics as regards cellular differentiation, proliferative activity, and expression of oncogene and tumour suppressor oncogene proteins, as revealed by using markers for muscle actin, keratin, vimentin, S-100 protein, GFAP, Ki-67, p53, and c-erbB-2 proteins. This case is the first reported with overexpression of p53 and c-erbB-2 proteins in the tumour entities. Salivary gland tumours consist of heterogeneous histological groups, and each has morphological diversity. This case indicates that some of the oncogene and tumour suppressor oncogene proteins may help to produce the histological heterogeneity of the salivary gland tumour.

Key words Salivary gland · Hybrid tumour · Adenoid cystic carcinoma · Salivary duct carcinoma · Tumour markers

Introduction

Hybrid salivary tumours are very rare entities and are composed of two different tumour entities, each of which conforms with an exactly defined category. The entities are not separate, but have an identical origin in the same topographical area [9]. We describe a case of a hybrid carcinoma of the palate comprised of a tubular or cribriform type of adenoid cystic carcinoma and a salivary duct carcinoma. We demonstrated a transitional zone between the two compartments, making it highly probable that they have an identical origin.

The development and progression of various malignant human neoplasms are regulated by the expression of various oncogenes and tumour suppressor genes. Salivary gland carcinoma is a relatively rare neoplasm, and the molecular genetic alterations that result in malignant transformation or progression are not well understood. In a previous report, we analysed 63 malignant salivary gland tumours, including the case in this report, with focus on the expression of p53, c-erbB-2 proteins, and Ki-67. Overexpression of the p53 protein correlated closely with the overexpression of the c-erbB-2 protein, and co-expression of both proteins appeared to play an important part in tumour progression [5]. In the present study, we have analysed an interesting case, which as already reported in brief [5], has a transitional region suggesting an identical origin of the two compartments. To clarify the degree of cellular differentiation of each tumours, we investigated the expression of various proteins, including muscle actin, keratin, vimentin, S-100 protein, and glial fibrillary acidic protein (GFAP) by immunohistochemistry.

N. Kamio (✉) · M. Mukai · E. Ikeda · S. Kuramochi · Y. Hosoda
Department of Pathology, Keio University School of Medicine,
35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan
Fax.: (81) 3-3353-3290, Tel. (81) 3-3353-1211 (ext. 2677)

M. Fujii
Department of Otolaryngology,
Keio University School of Medicine, 35 Shinanomachi,
Shinjuku-ku, Tokyo, Japan

Y. Tanaka
Tokyo Dental College, Ichikawa General Hospital,
Clinical Laboratory, Chiba, Japan

Case report

A 51-year old man had a painless mass on the right side of the palate, which was first noticed in August 1988. He complained of swelling in the right side of the neck; his initial symptoms progressed, and he was admitted to the Department of Otolaryngology, Keio University Hospital in March 1989. CT and MRI revealed a soft tissue mass in the right side of the palate (Fig. 1) and right cervical lymphadenopathy. A general examination did not show any evidence of metastatic disease. On hospital day 7, partial resection of the palate, radical neck dissection, and reconstructive surgery were performed. Intraoperative evaluation revealed a 30×45 mm mass involving the pterygopalatine fossa and the infe-

Table 1 Primary antibodies and staining methods used in this study

Antibody	Clone	Clonality	Source[a]	Method[b]	Dilution
Muscle actin	HHF-35	Mono.	Dako	Indirect	×100
Keratin	AE1/AE3	Mono.	Boehringer	Indirect	×100
Vimentin	V9	Mono.	Dako	Indirect	×100
S-100 protein		Poly.	Dako	Indirect	×100
GFAP	6F2	Mono.	Dako	Indirect	×100
Ki-67	MIB-1	Mono.	Immunotech	Antigen retrieval and indirect	×100
p53	DO-1	Mono.	O-S	Antigen retrieval and indirect	×100
	PAb1801	Mono.	O-S	Antigen retrieval and indirect	×20
c-erbB-2		Poly.	Dako	Antigen retrieval and indirect	×100
	CB11	Mono.	Novocastra	ABC	×40

[a] O-S, Oncogene Science, Cambridge, USA; Dako, Dako A/S, Glostrup, Denmark; Novocastra, Novocastra Laboratories, Newcastle, UK; Immunotech, Immunotech S.A. France; Boehringer, Boehringer Mannheim, Indianapolis, Ind., USA

[b] Indirect, indirect method; ABC, avidin–biotin complex–peroxidase method; antigen retrieval, antigen retrieval by microwave heating

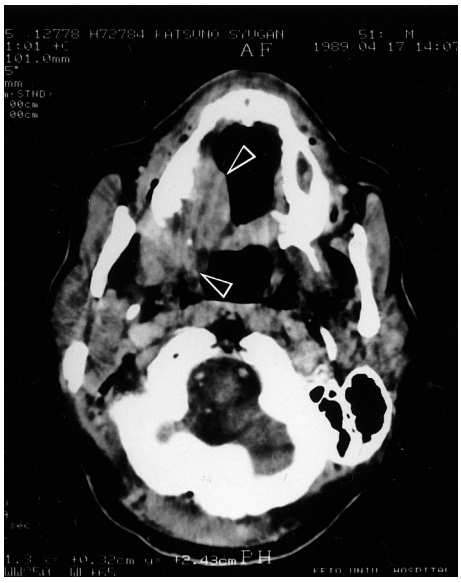

Fig. 1 Computerized tomography showed a soft density mass of the right soft palate invading the pterygoid muscle

rior wall of the maxillary sinus. Right cervical lymph node metastases were found on pathology. Although recurrence of the neck swelling was recognized in August 1989, postoperative management was not performed, at the patient's wish. He was readmitted to the Shizuoka Red Cross Hospital near the place where he was born. His general condition deteriorated and he died in September 1990, 19 months after diagnosis. An autopsy was not performed.

Materials and methods

For light microscopic evaluation, resected surgical specimens were fixed in 10% buffered formalin, and 5-mm-thick tissue sections were cut and paraffin-embedded. All sections were stained with haematoxylin–eosin, and selected sections were stained with alcian blue, pH 2.5, and periodic acid–Schiff (PAS) stain with and without diastase digestion. Selected sections were immunostained by the avidin–biotin–peroxidase complex (ABC) technique or indirect method, using the primary antibodies listed in Table 1.

In the examination of p53 expression, only tumour cells with distinct nuclear immunostaining for p53 were regarded as positive. In evaluation of the expression of c-erbB-2 protein, intense staining on cell surface membranes was interpreated as positive. For evaluation of Ki-67, nuclei showing an intense homogeneous brown colou or granular staining were recognized as positive.

Results

An oval elastic and hard tumour measuring 30×45×30 mm was present under the palatal mucosa. On section, the tumour showed a poorly demarcated margin with a yellow–white cut surface and scattered dark red necrotic areas.

Microscopically, the tumour was surrounded by the salivary gland tissue, and it was found to consist of distinct two histological types. One was consistent with a tubular or cribriform type of adenoid cystic carcinoma and the other with a salivary duct carcinoma (Fig. 2a). A transitional region was recognized between the two portions of the tumour (Fig. 2b).

The adenoid cystic carcinoma region consisted of epithelial cell nests permeated by numerous cylindrical spaces. Most of these were pseudocysts, which showed positivity for alcian blue stain. A small number of true cysts, which showed positivity for PAS stain before and after diastase digestion, and for mucicarmine were present. In the salivary duct carcinoma there was a solid growth pattern showing central necrosis resembling comedocarcinoma of the breast and a thin trabecular or small nest pattern (Fig. 2c). Tumor cells of the salivary duct carcinoma had only a few cells that were positive for alcian blue, PAS, and mucicarmine stains. Severe nuclear pleomorphism and frequent mitoses were present in the region of the salivary duct carcinoma. Perineural and vascular invasion was evident. In the metastatic lesion of the cervical lymph node only salivary duct carcinoma was found.

Fig. 2 **a** Two distinct histological types, an adenoid cystic carcinoma and a salivary duct carcinoma, were observed. ×105. **b** Transitional region between the two portions of the tumour. ×200. **c** Salivary duct carcinoma was comprised in parts of thin trabecular and small nests patterns. ×200

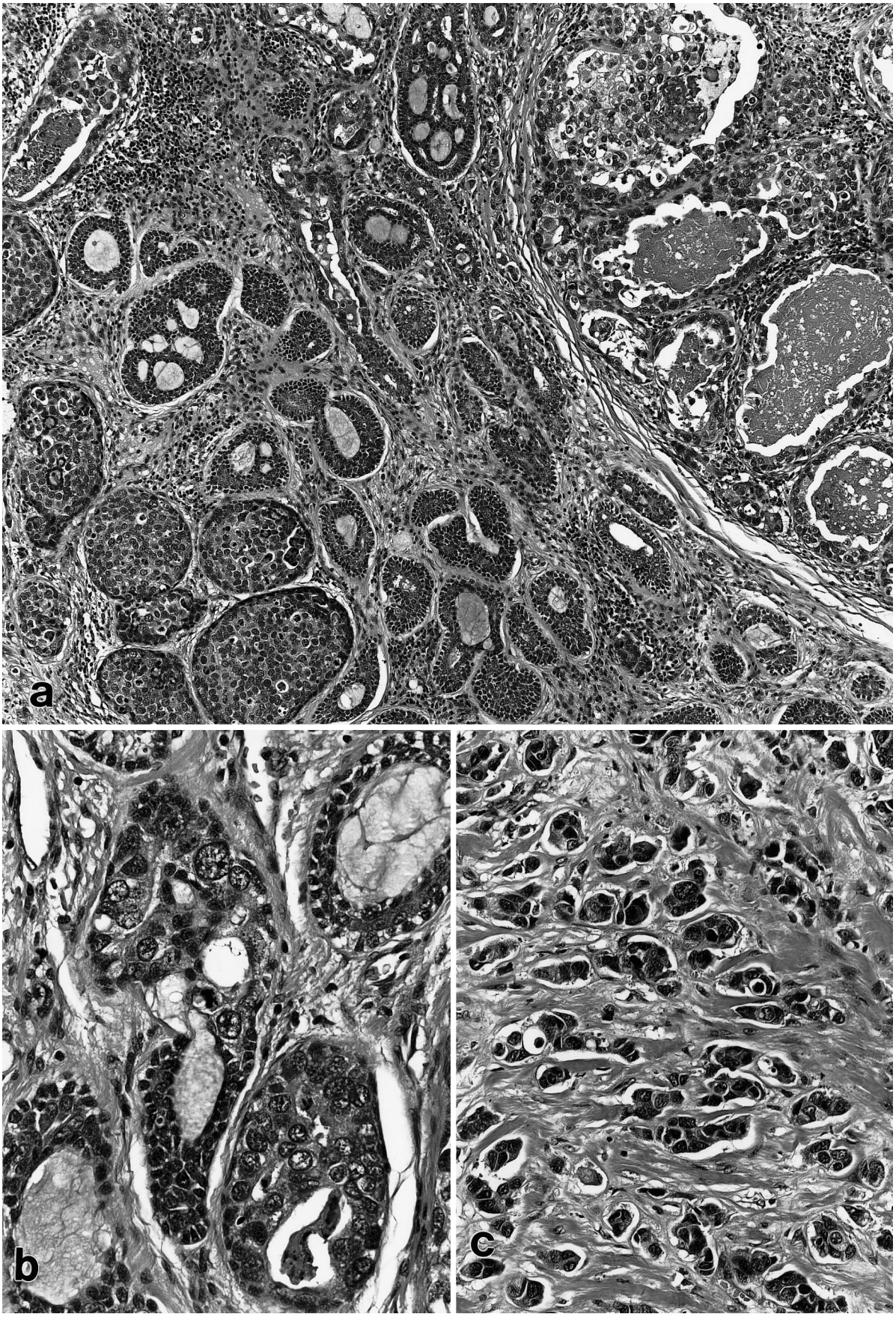
a
b
c

Table 2 Immunohistochemical characteristics of the tumour [(++) intensely reactive, (+) moderately reactive, (+–) faintly reactive, (–) nonreactive, *GFAP* glial fibrillary acidic protein]

Antibodies	Muscle actin	Keratin	Vimentin	S-100 protein	GFAP	Ki-67	p53	c-erbB-2
Salivary duct carcinoma	(–)	(++)	(–)	(++)	(–)	(++)	(+)	(+)
Adenoid cystic carcinoma								
Inner layer	(–)	(+)	(–)	(+–)	(–)	(+)	(–)	(–)
Outer layer	(+)	(–)	(+–)	(+–)	(–)	(+)	(–)	(–)

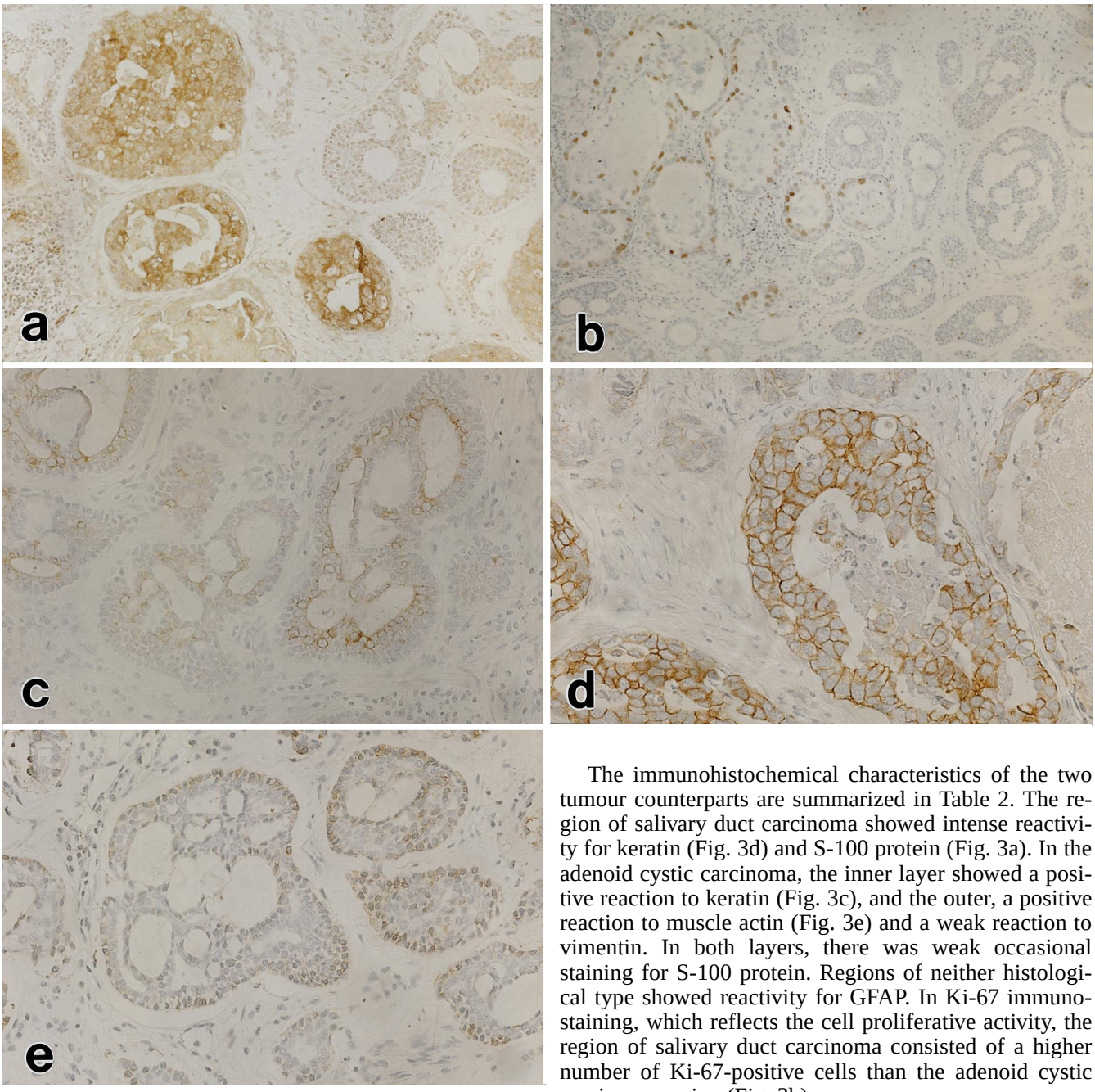

Fig. 3 **a** A salivary duct carcinoma showed intense reactivity for S-100 protein. ×40. **b** A salivary duct carcinoma contained a larger number of Ki-67-positive cells than an adenoid cystic carcinoma. ×25. **c** Inner layer cells of adenoid cystic carcinoma were positive for keratin. ×66. **d** A salivary duct carcinoma was positive for keratin. ×66. **e** Outer layer cells of adenoid cystic carcionoma were positive for muscle actin. ×66

The immunohistochemical characteristics of the two tumour counterparts are summarized in Table 2. The region of salivary duct carcinoma showed intense reactivity for keratin (Fig. 3d) and S-100 protein (Fig. 3a). In the adenoid cystic carcinoma, the inner layer showed a positive reaction to keratin (Fig. 3c), and the outer, a positive reaction to muscle actin (Fig. 3e) and a weak reaction to vimentin. In both layers, there was weak occasional staining for S-100 protein. Regions of neither histological type showed reactivity for GFAP. In Ki-67 immunostaining, which reflects the cell proliferative activity, the region of salivary duct carcinoma consisted of a higher number of Ki-67-positive cells than the adenoid cystic carcinoma region (Fig. 3b).

The two monoclonal antibodies used to evaluate overexpression of p53 protein showed similar levels of reactivity. The majority of tumour cells in the salivary duct carcinoma regions showed overexpression of p53 (Fig. 4a). No immunostaining was recognized in the adenoid cystic carcinoma region or the unaffected salivary

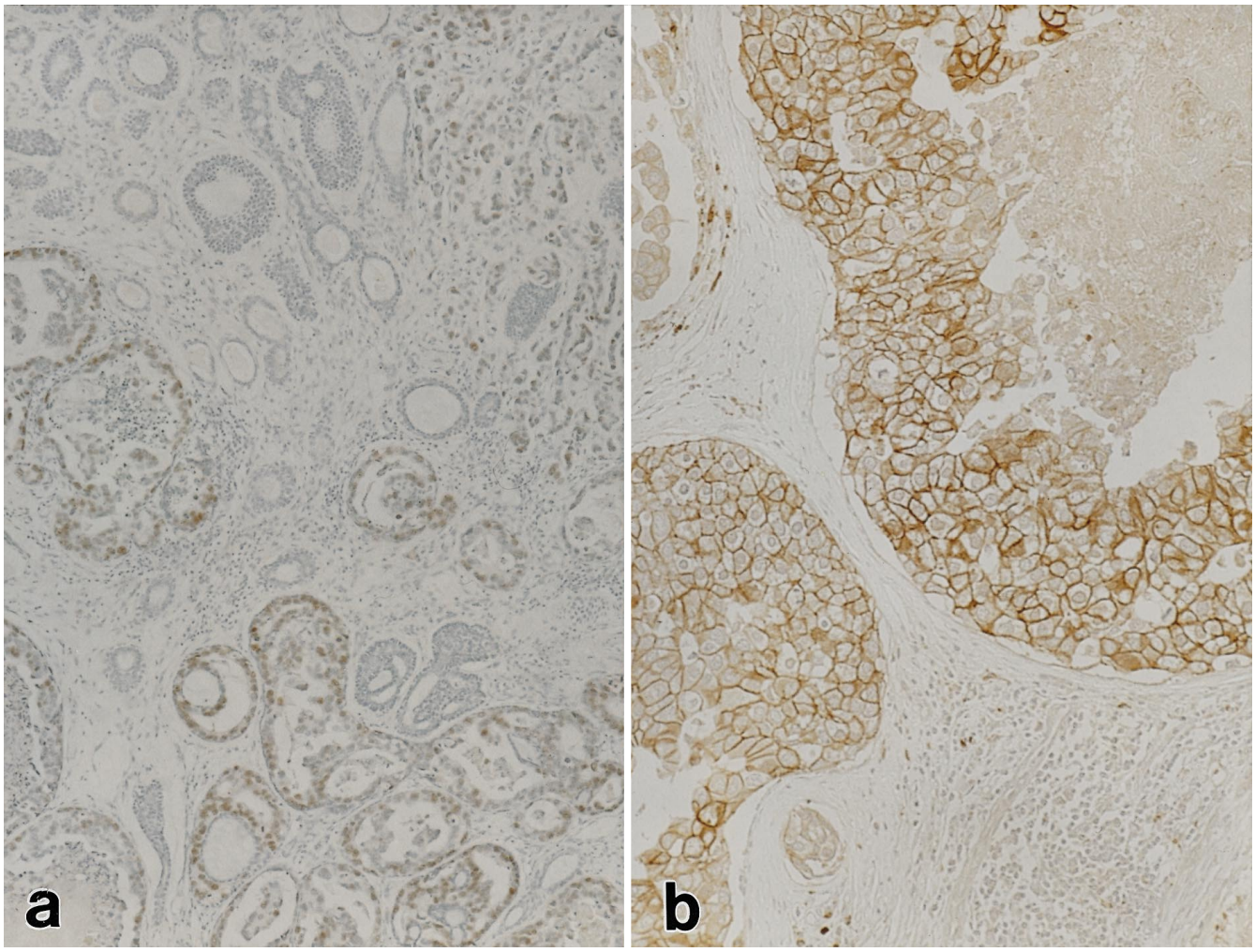

Fig. 4 **a** A salivary duct carcinoma was positive for p53, while an adenoid cystic carcinoma was negative. ×20. **b** A salivary duct carcinoma was positive for c-erbB-2 protein. ×40

gland tissue surrounding the tumour. The staining patterns with monoclonal and polyclonal antibodies against c-erbB-2 were similar. In areas of advanced disease, and particularly at sites of lymphatic invasion of salivary duct carcinoma, strong immunostaining against c-erbB-2 protein was found (Fig. 4b), while no cell membrane immunoreactivity was observed in the adenoid cystic carcinoma region or unaffected salivary gland tissues.

Discussion

We diagnosed the tumour under scrutiny as hybrid carcinoma of the salivary gland. The tumour was composed of two different entities, which were indistinguishable from the tubular or cribriform type of adenoid cystic carcinoma and salivary duct carcinoma on light microscopic observation [2, 8]. Furthermore, the presence of the transitional zone of the two counterparts suggested that the two portions have an identical origin and this case was not a collision tumour. Immunohistochemical analysis using markers for muscle actin, keratin, vimentin, S-100 protein, and GFAP indicated that the two tumours had different differentiation characteristics. Immunohistochemical staining of adenoid cystic carcinoma reveals the presence of two cell populations; ductal cells and myoepithelial cells. Chen et al. have shown that the ductal cells of adenoid cystic carcinoma express keratin and S-100 protein; the myoepithelial cells express muscle-specific actin, and occasionally keratin and S-100 protein [1]. In the present case, immunohistochemical staining in the adenoid cystic carcinoma was consistent with their results. However, the salivary duct carcinoma showed intense expression of keratin and S-100 protein, but no expression of muscle actin. This indicates that the cells in the salivary duct carcinoma have mainly a ductal-type differentiation, rather than the myoepithelial type.

The development and progression of cancer are thought to be regulated by the expression of various oncogenes and tumour suppressor genes. In the salivary gland carcinomas, p53 oncoprotein expression may be an independent indicator of clinical aggressiveness in carcinoma of the parotid gland [4]. Press et al. [7] and Sugano et al. [10] demonstrated that overexpression of c-erbB-2

protein was associated with a poor prognosis. In the present study, the region of salivary duct carcinoma showed overexpression of both p53 and c-erbB-2 proteins, in contrast to the negative reactivity for both proteins in the region of adenoid cystic carcinoma. Ki-67 immunostaining indicated that in the region of salivary duct carcinoma cell proliferative activity was higher with high histological grades including severe nuclear atypia, the presence of necrosis, and more frequent mitoses than in the adenoid cystic region. Our marker studies of cellular differentiation, proliferation, and expression of oncogene and tumour suppressor oncogene proteins demonstrate distinct characteristics in the two components, suggesting progression from the adenoid cystic carcinoma to the salivary duct carcinoma. In colon [3] or liver [6], a genetic model of multi-stage carcinogenesis has been proposed and specific genetic alterations involved in different stage of tumour progression have been defined. Although additional molecular studies are needed, investigation of the overexpression of gene products, which are thought to reflect gene alterations in hybrid tumours, may clarify the molecular basis of carcinogenesis or progression in salivary gland tumours. Salivary gland tumours consist of heterogeneous histological groups, and each tumour has real morphological diversity. The present case also indicates that some genetic changes may be involved in the histological heterogeneity of salivary gland tumours.

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