

CASE REPORT

Stroma-poor Warthin's tumor with significant oncocytic hyperplasia: case presentation and considerations regarding its histogenesis

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Abstract

Although Warthin's tumor is one of the common tumors of the salivary glands, Warthin's tumors with a prominent component of nodular oncocytic hyperplasia reminiscent of oncocytoma are rare. Here we report such a tumor, measuring 3 cm in diameter, found in the parotid gland of an 81-year-old man. Histologically, approximately 70% of the mass was a component of nodular oncocytic proliferation, and the remaining portion was a component of conventional Warthin's tumor. We performed immunohistochemical analysis to explore what factors determined the morphogenesis of the two components in the single mass. Cytokeratin (CK) 5/6-positive tumor cells, which represent basal cells, were aligned in a layer in the conventional Warthin's tumor component, whereas they were localized around blood vessels in the nodular oncocytic hyperplasia component. Immunostaining for CD34 showed that capillaries were sparsely present beneath the bilayered epithelia in the former component, while blood vessels resembling sinusoids separated the trabeculae of the tumor cells in the latter component. Ki-67 labeling index was slightly higher in the latter component. Double immunostaining for CK5/6 and Ki-67 revealed that most of Ki-67-positive proliferating tumor cells were CK5/6-positive, suggesting that CK5/6-positive population contained proliferative progenitor cells of the tumor. These findings imply that the regional difference in the distribution pattern and proliferative activity of CK5/6-positive putative progenitor cells along with the difference in the pattern of vascular network occurred during the tumorigenic process of the tumor and determined one region to become conventional Warthin's tumor morphology and the other to become nodular oncocytic hyperplasia.

Keywords: Warthin's tumor, oncocytoma, oncocytic hyperplasia, salivary gland, histogenesis, basal cell.

Introduction

Oncocytes are large epithelial cells with granular eosinophilic cytoplasm, which contains abundant mitochondria. In salivary gland tumors, Warthin's tumor and oncocytoma are two major tumors composed of a proliferation of oncocytes. Warthin's tumor is characterized by a papillary and cystic proliferation of bilayered epithelia that consist of luminal oncocytic cells and small basal cells [1, 2]. The stroma contains a variable amount of lymphoid tissue, and Seifert *et al.* subclassified Warthin's tumors into typical, stroma-poor, stroma-rich, and metaplastic, depending mainly on the ratio of epithelial tumor component to lymphoid stroma [3]. On the other hand, oncocytoma is a well-demarcated tumor exclusively composed of oncocytes. It shows a solid, trabecular or tubular pattern without lymphoid stroma, and basal cells are not readily identifiable by routine Hematoxylin–Eosin-stained sections [1, 2]. Although the pathogenesis of these oncocytic lesions remains unclear, a possible link between these two tumors has been implied because, in rare cases, Warthin's tumors may contain a component of nodular proliferation of oncocytes, which is so prominent that the distinction from oncocytoma might be problematic [2]. Analysis of such cases is important since it

may bring us an insight into what factors determine the morphogenesis of the two components, namely the component of conventional Warthin's tumor and that of nodular oncocytic hyperplasia, in a single lesion. However, to the best of our knowledge, detailed immunohistochemical studies of such cases toward elucidation of their histogenesis have not been reported previously.

Here we report a case of stroma-poor Warthin's tumor of the parotid gland with a prominent component of nodular oncocytic hyperplasia, and our immunohistochemical analysis has revealed possible histogenetic factors underlying the morphogenesis of the lesion.

Materials and Methods

Sections of the formalin-fixed paraffin-embedded tissue were cut, and stained with Hematoxylin–Eosin. Single-color immunostaining was performed by standard immunoperoxidase method using an automated immunostaining system BenchMark XT (Ventana Medical Systems, Tucson, AZ, USA, Product code N750-BMKXT-FS). Primary antibodies and antigen retrieval methods used in this study are listed in Table 1.

3,3'-Diaminobenzidine (DAB) was used as a chromogen, and the sections were counterstained with Hematoxylin.

Ki-67 (MIB-1) labeling index was defined as the percentage of immunoreactive tumor cells divided by the total number

of tumor cells analyzed. The index was analyzed in a total of 1000–1500 tumor cells from representative three areas.

Table 1 – Antibodies used for immunohistochemistry

Antibody to	Clone	Dilution	Antigen retrieval*	Source**	Product code
Cytokeratin (CK) 7	OV-TL 12/30	1:100	ProK	Biocare Medical	CM 061
Cytokeratin (CK) 5/6	D5/16 B4	1:100	Heat	Dako	M7237
Cytokeratin (CK) 14	LL002	1:50	Heat	Biocare Medical	CM 185
Epithelial membrane antigen (EMA)	E29	1:100	ProK	Dako	M0613
Carcinoembryonic antigen (CEA)	II-7	1:50	Heat	Dako	M7072
p63	7JUL	1:25	Heat	Novocastra	NCL-p63
S100 protein	Polyclonal	1:600	Heat	Dako	Z0311
Smooth muscle actin (SMA)	1A4	1:50	–	Dako	M0851
CD34	QBEnd/10	Prediluted	Heat	Biocare Medical	ACR 084
Ki-67 (mouse monoclonal)	MIB-1	1:50	Heat	Dako	M7240
Ki-67 (rabbit monoclonal)	EPR3610	1:2000	Heat	Epitomics	2746-1

*ProK, digestion with proteinase K; Heat, boiling in antigen retrieval buffer (pH 8). **Biocare Medical, Concord, CA, USA; Dako, Glostrup, Denmark; Novocastra, Newcastle Upon Tyne, UK; Epitomics, Burlingame, CA, USA.

Double immunostaining for cytokeratin (CK) 5/6 and Ki-67 was performed using MACH 2 Double Stain 1 (a secondary antibody cocktail containing alkaline phosphatase-conjugated anti-mouse IgG and peroxidase-conjugated anti-rabbit IgG antibodies; Biocare Medical, Concord, CA, USA, Product code BRR523) according to the manufacturer's instructions. Briefly, deparaffinized sections were boiled in 10 mM Tris-HCl buffer, pH 8, treated with 0.3% H₂O₂ solution, and incubated with a primary antibody cocktail (mouse anti-CK5/6 antibody diluted at 1:100 and rabbit anti-Ki-67 antibody diluted at 1:2000). Then, the sections were reacted with MACH 2 Double Stain 1, and color was developed with Vulcan Fast Red (Biocare Medical, Product code BRR805) for CK5/6 and DAB (Dako, Glostrup, Denmark, Product code K3468) for Ki-67. The sections were counterstained with Hematoxylin.

To confirm the specificity of the immunostaining, negative controls were performed by omitting the primary antibodies.

Case presentation

An 81-year-old man came to the hospital due to a painless mass in his right parotid gland. He had smoked one pack per day of cigarettes until 61 years old. He had no previous history of radiation exposure.

Magnetic resonance (MR) imaging revealed a well-circumscribed mass in the superficial lobe of the right parotid gland. The mass was slightly hyperintense to muscle on T1-weighted MR images, and slightly hyperintense to muscle and partially cystic on T2-weighted images. The mass showed early enhancement with washout on postcontrast T1-weighted images. The fine-needle aspiration (FNA) cytology of the mass showed small lymphocytes and clusters of oncocyte-like epithelial cells, suggesting Warthin's tumor. Resection of the mass was

performed. The patient is well eight months after the resection without recurrence.

Grossly, a well-circumscribed, encapsulated, tan-brown mass measuring 3 cm in diameter was found in the parotid gland (Figure 1a). Approximately 70% of the mass was solid, and the remaining 30% of the mass was cystic.

Histologically, in the cystic component of the mass, a papillary and cystic proliferation of bilayered epithelia composed of luminal oncocytic cells and small basal cells was found (Figure 1b).

The stroma contained lymphoid tissue. This component was thought to be a conventional Warthin's tumor component. In the solid component of the mass, oncocytic cells with round vesicular nuclei proliferated in a trabecular or organoid growth pattern (Figure 1c).

The trabeculae were separated by thin stromal tissue mainly composed of small blood vessels. Lymphoid tissue was absent in this component. Anaplastic cells or mitotic figures were not observed. This component was considered to be nodular oncocytic hyperplasia. The boundary between these two components showed abrupt transition from bilayered glands with lymphoid stroma to trabecular structure without lymphoid tissue (Figure 1d).

In the parotid gland tissue outside this tumor, neither oncocytic tumor nor oncocytosis was found.

Immunohistochemically, the luminal and basal layers of the bilayered epithelia in the conventional Warthin's tumor component and the tumor cells in the nodular oncocytic hyperplasia were positive for CK7, and negative for S100 protein and smooth muscle actin (SMA). Epithelial membrane antigen (EMA) was faintly positive in the apical portions of the luminal cells in the conventional Warthin's tumor component (Figure 2a).

On the other hand, EMA was intensely positive in the dot-like or small tubular pattern in the component of nodular oncocytic hyperplasia, suggesting inconspicuous glandular lumina (Figure 2b).

Additionally, carcinoembryonic antigen (CEA) was focally positive in these inconspicuous glandular lumina. Immunostaining for CD34 revealed that capillaries were sparsely present beneath the bilayered epithelia in the conventional Warthin's tumor component (Figure 2c).

In the nodular oncocytic hyperplasia component, CD34-positive blood vessels resembling sinusoids separated the trabeculae of the tumor cells (Figure 2d).

Ki-67 (MIB-1) labeling index was 1.7% and 3.1% in the conventional Warthin's tumor and nodular oncocytic hyperplasia components, respectively. It has been known that p63-, CK5/6- or CK14-positive cells represent basal cells in normal and neoplastic salivary gland tissues [4–6].

While the basal layer of the bilayered epithelia in the conventional Warthin's tumor component was uniformly positive for p63 (Figure 2e), CK5/6 and CK14 (Figure 2g), tumor cells in perivascular locations were positive for p63 (Figure 2f), CK5/6 and CK14 (Figure 2h) in the nodular oncocytic hyperplasia component.

These immunohistochemical findings suggested that the distribution patterns of the basal cells as well as the morphology of vascular network were closely related to the structural difference of the two components.

Since CK5-positive basal cells were reported to be an epithelial progenitor population in normal development of murine salivary glands [7], it is possible that CK5/6-positive basal cells in the present tumor might play a progenitor-like role in the tumor growth. To test this possibility, we performed double immunostaining for CK5/6 and Ki-67.

As shown in Figure 3, most of Ki-67-positive cells were found in CK5/6-positive population of the tumor cells. When we counted 164 Ki-67-positive cells in the tumor cells, 151 (92.1%) cells were CK5/6-positive. These data implied that, in the present tumor, the tumor cells with CK5/6-positive phenotype contained a proliferative population while most of the oncocytic (non-basal) tumor cells constituted a terminally differentiated, non-proliferative population.

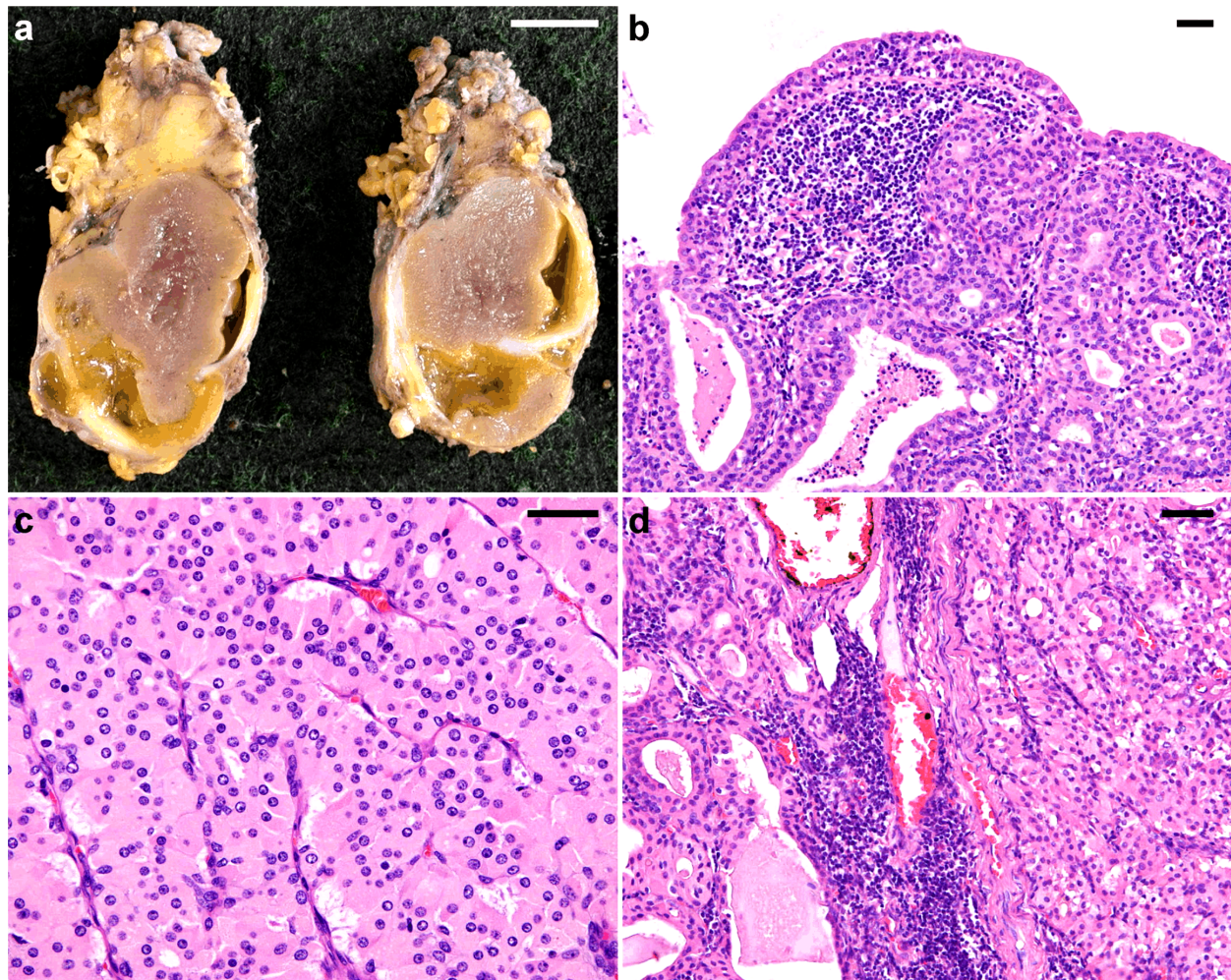


Figure 1 – Gross and histological findings of the parotid tumor. (a) Grossly, the tumor was well circumscribed and encapsulated. Approximately 70% of the tumor was solid and 30% was cystic. **(b)** Histological findings of the cystic component. This component was thought to be a conventional Warthin's tumor component. **(c)** Histological findings of the solid component. This component was considered to be nodular oncocytic hyperplasia. **(d)** The boundary between the two components showed abrupt transition from bilayered glands with lymphoid stroma (left) to trabecular structure without lymphoid tissue (right). **(b–d)** Hematoxylin–Eosin staining. Scale bars: (a) 1 cm; (b–d) 50 μ m.

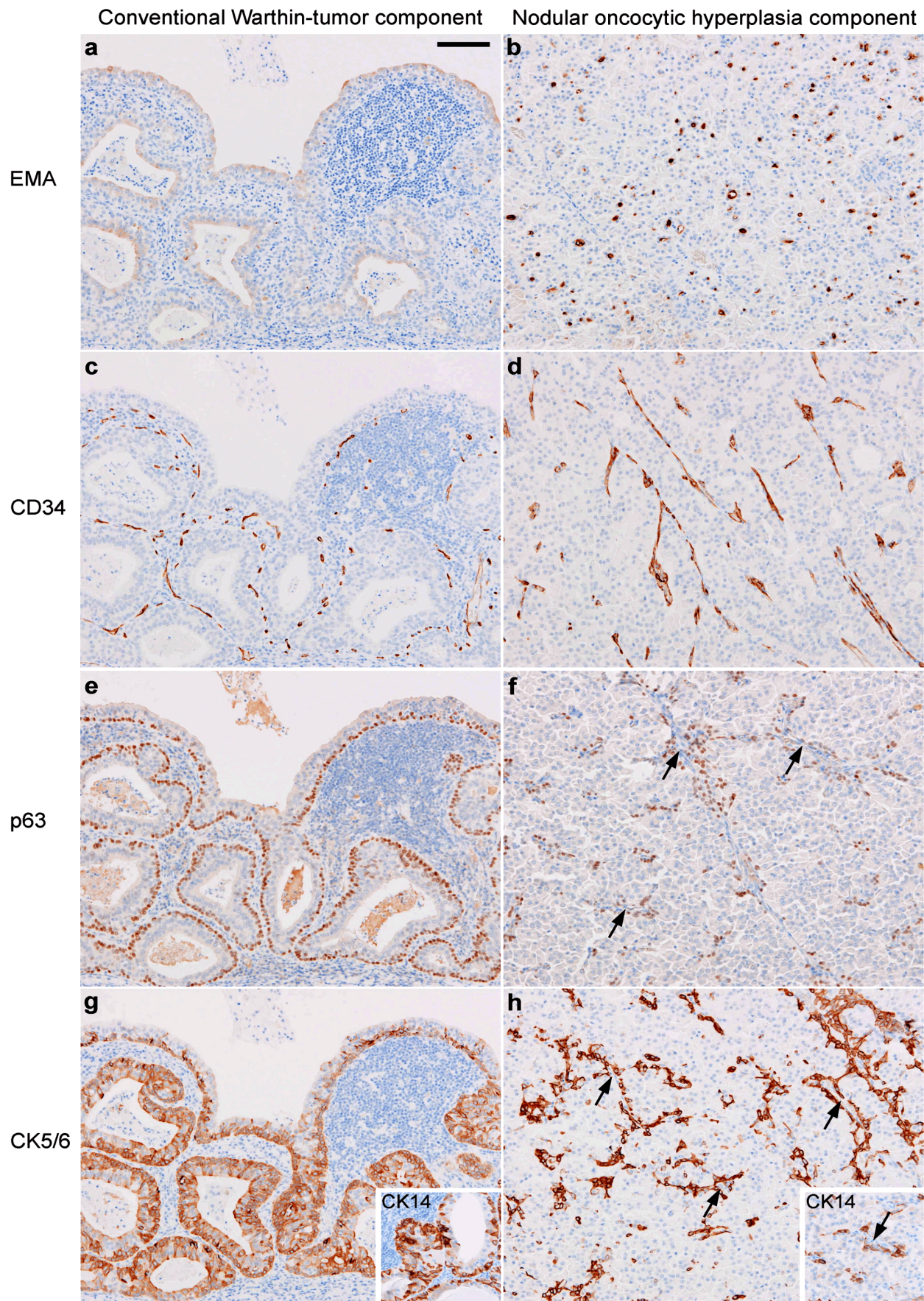


Figure 2 – Comparison of the immunohistochemical findings of the conventional Warthin's tumor and nodular oncocytic hyperplasia components of the tumor. Serial sections of the former component (a, c, e and g) and the latter component (b, d, f and h) immunostained for epithelial membrane antigen (EMA) (a and b), CD34 (c and d), p63 (e and f), cytokeratin (CK) 5/6 (g and h) and CK14 (insets in g and h) are shown. The magnifications of all the photographs are the same. Scale bar: 100 μ m. Arrows in (f) and (h) indicate blood vessels.

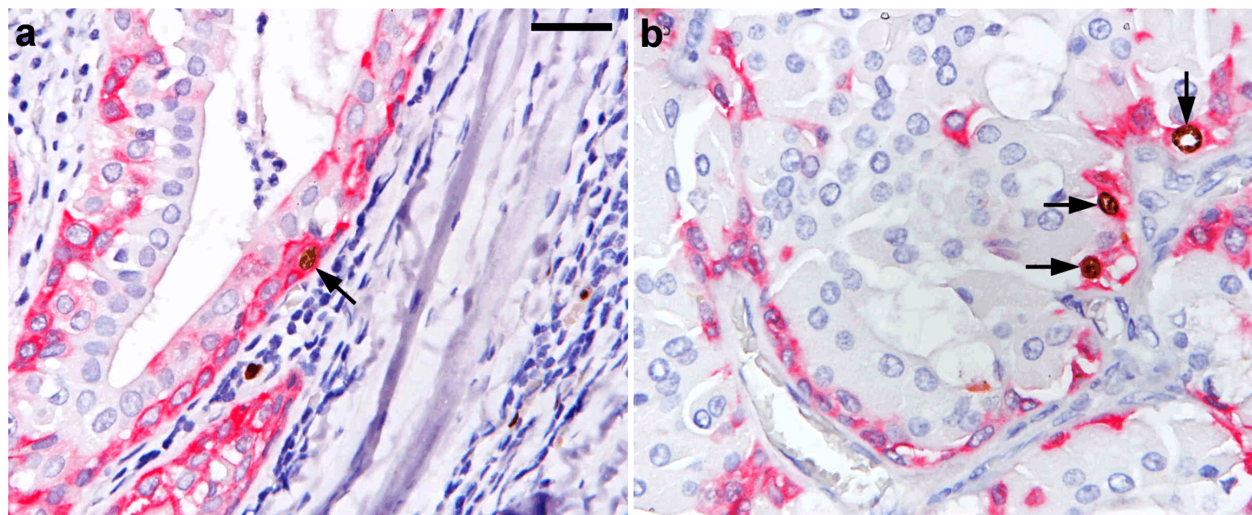


Figure 3 – Double immunostaining for CK5/6 (red) and Ki-67 (brown) of the tumor tissue. The conventional Warthin's tumor component (a) and the nodular oncocyctic hyperplasia component (b) are shown. Most of the Ki-67-positive cells (arrows) were CK5/6-positive basal cells. The magnifications of the photographs are the same. Scale bar: 25 µm.

Discussion

The present case is a rare and interesting one because the conventional Warthin's tumor component and a prominent component of nodular oncocyctic hyperplasia comprised the single tumor. It is conceivable that the two components shared a common pathway of early tumorigenesis and then differentiated into the two morphologically distinct components by the intratumoral difference in certain factors. Thus, the analysis of this case provides us with a unique opportunity to explore the key histogenetic factors of the conventional Warthin's tumor and nodular oncocyctic hyperplasia.

In the conventional Warthin's tumor component of the present tumor, both of the luminal and basal layers of the epithelia were positive for CK7, and only the basal layer was p63-, CK5/6- and CK14-positive. The tumor cells were negative for S100 protein and SMA. These immunohistochemical features were compatible with the previous reports about conventional Warthin's tumors [4, 5]. The component of nodular oncocyctic hyperplasia in the present tumor also showed positivity for CK7, and negativity for S100 protein and SMA. The dot-like or small tubular staining patterns for EMA suggested inconspicuous glandular lumina. A small fraction of the tumor cells in perivascular locations were positive for p63, CK5/6 or CK14, which were regarded as basal cells. These findings indicated that this solid component might be formed by highly compact and back-to-back proliferation of oncocyctic epithelia.

Stem/progenitor cells play important roles in normal organogenesis and maintenance of normal tissue structures. Appropriate distribution pattern and activity of stem/progenitor cells are considered to be essential to the morphogenesis of various tissues. In neoplastic lesions, it is assumed that neoplastic counterparts of stem/progenitor cells exist and may function as a determinant of the architecture or growth pattern of the tumor tissues. Although little is known about stem/progenitor cells in salivary gland tumors, our results of the double immunostaining of the present tumor suggested that CK5/6-positive basal cells in the tumor tissue contained a population of

proliferating progenitors. Thus, the distribution pattern and activity of the CK5/6-positive cells might directly influence the tissue architecture. We found that CK5/6-positive cells were aligned in a layer in the conventional Warthin's tumor component, while they were located in perivascular locations in the component of nodular oncocyctic hyperplasia. Ki-67 labeling index, which reflects proliferative activity, was slightly higher in the latter component than in the former component. We speculate that the regional difference in the distribution pattern and proliferative activity of CK5/6-positive progenitor cells along with the difference in the pattern of vascular network occurred during the tumorigenic process of the present tumor and determined one region to become conventional Warthin's tumor morphology and the other to become morphology of nodular oncocyctic hyperplasia.

There have been two major theories about the histogenesis of Warthin's tumor [1]. In the first theory, the tumor originates in heterotopic salivary duct tissue within intra- or peri-parotid lymph nodes. The lymphoid stroma of the tumor is derived from residual lymph-node tissue in this theory. The second theory regards Warthin's tumor as an epithelial proliferation, which induces lymphoid reaction. It has been described that aberrant expression of major histocompatibility complex (MHC) class II antigens, cytokines, or viral antigens by epithelial cells of Warthin's tumor might activate lymphocytes and induce the formation of lymphoid stroma [8]. Some researchers suggested that Warthin's tumor initially develops as an epithelial proliferation followed by lymphocytic infiltration [9]. It has also been reported that the specialized blood vessels in the stroma of Warthin's tumor might play a role in lymphocyte recruitment (homing) to the lymphoid stroma [10]. At present, it remains unclear whether CK5/6-positive cells directly regulate the induction of lymphoid stroma or the patterning of blood vessels. Further studies are needed to fully explain the histogenetic mechanism of these oncocyctic lesions.

Conclusions

We reported a case of stroma-poor Warthin's tumor

of the parotid gland with a prominent component of nodular oncocytic hyperplasia. This case highlighted the important role of the CK5/6-positive putative progenitor cells and the blood-vessel pattern in the histogenesis of the lesion.

Conflict of interests

The authors declare that they have no conflict of interests.

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