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Original Article

The significance of the morphological and immunological characteristics in the diagnosis of parotid mammary analogue secretory carcinoma: five case reports and a review of the literature

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Abstract: Mammary analogue secretory carcinoma (MASC) of the salivary glands is rarely reported. In this article, the histopathological features of 5 cases of parotid MASC were retrospectively analyzed. AB/PAS and immunohistochemical staining of S-100, mammaglobin and P63 was performed, which were validated by the ETV6-fluorescent FISH detection of NTRK3 gene. The tumors were composed of two kinds of tumor cells. One kind of cells was rich in cytoplasms, transparent or vacuole-like, partial basophilic double tropism, but another type of cellular cytoplasm was acidophilic. The karyotype was consistent between two types of tumors in the vacuolar pattern. Although the tumor cells were arranged in different forms, the cystic (capsule or microcapsule) structures were constantly observed. Two kinds of tumor cells produced different secretions, which were distributed among the tumor cells. Tumor tissues were divided by hardened collagen interstitial, even in the infiltration lesion, collagen and tumor cells mass was also inseparable. The expression of mammaglobin and S-100 significantly differed between the two types of tumor cells. These characteristics contribute to the differential diagnosis of MASC. ETV6-NTRK3 gene detection can be applied in the diagnosis of atypical cases, but it should not be done routinely.

Keywords: Mammary analogue secretory carcinoma, diagnosis, morphology, immunological staining, case reports

Introduction

Mammary analogue secretory carcinoma (MASC) occurring in the salivary glands has been classified as a novel type of salivary gland tumor by WHO due to its unique morphological characteristics, genetic changes, and clinical prognosis [1, 2]. The incidence of this type of MASC is extremely low and has been rarely reported [3].

Due to an insufficient understanding and the similarities of its histological and morphological characteristics, a majority of MASC cases have been misdiagnosed as acinic cell carcinoma (AiCC) or other low-grade salivary gland tumors [4-6]. Hence, the pathological features of MASC contribute to an accurate diagnosis, epidemiological data statistics, clinical treatment, and prognosis evaluation. The pathological and morphological characteristics of MASC in this study have not been described in previous findings [4-9].

In this article, the histopathology, special staining, and immunohistochemical features of 5 cases of parotid MASC were reported, aiming to provide evidence for the accurate differential diagnosis of parotid MASC for pathologists in clinical practice.

Case report

Among the 344 cases of parotid gland tumors admitted to our hospital from August 2011 to May 2017, 63 cases were classified as malignant tumors (**Table 1**). Of these, 5 cases of parotid MASC and 5 cases of AiCC were recruited for this investigation. The histopathological features, special staining, immunohistochemis-

Table 1. Classification of malignant tumor types in 344 cases of parotid tumors

Type	Case (n)	Percentage
Parotid malignancy type	63	
Non-specific adenocarcinoma	12	19%
Malignant mixed tumor	11	17%
AiCC	8	13%
Mucous epidermoid carcinoma	7	11%
Metastatic cancer	6	10%
MASC	5	8%
Basal cell adenocarcinoma	3	5%
Myoepithelioma	3	5%
Highly-differentiated squamous cell carcinoma	3	5%
Adenoid cystic carcinoma	2	3%
Epithelial-myoepithelial carcinoma	2	3%
Adenocarcinoma of the gland	1	2%

try, and gene test results were retrospectively analyzed.

As illustrated in **Table 2**, the 5 male MASC patients were aged 34-60 years with a median age of 46 years. The MASC was located in the superficial lobe of the parotid gland in 3 cases and in an unknown location in the remaining 2 patients. The maximal diameter of the tumors was 2.0-3.5 cm. The tumors were located in the capsule of 4 cases and had invaded into the capsule in 1 patient. No lymph node metastasis was detected. The patient with the capsule-invading tumor suffered a recurrence complicated by bilateral lung metastases at 1 year post-operation. Three patients were misdiagnosed as AiCC, and 1 case was misdiagnosed as cystadenocarcinoma. All the cases were treated with adenoidectomy, and 1 case with capsular invasion underwent extended resection. Two patients received radiotherapy and chemotherapy. The follow-up time ranged from 8 to 42 months. Up until we drafted this manuscript, four cases survived well, and the remaining recurrent case received medication therapy. The histomorphological features of 5 MASC cases are illustrated in **Table 3**. Multi-nodular masses with clear boundaries were observed under a low-magnification microscope, separated into small pieces by the collagen fibers. Swelling of the surrounding tissues was noted in 4 cases, and the small lesions penetrated the capsular membrane adjacent to the rhabdomyolytic tissues in the remaining 1 patient. Interstitial collagen sclerosis was evident, and even hyalinization was evident as well Tumor tissues were divided by hardened collagen interstitial, even in the infiltration lesion, collagen and tumor cells mass was also inseparable (**Figure 1A**, **1B**). Three cases suffered from old hemorrhages.

Two types of tumor cells were observed. One type of cell was rich in cytoplasms, transparent or vacuole-like and basophilic, and the other type of tumor cell was eosinophilic. The cell nuclei were round and vacuole-like. Cell abnormalities were not obvious, and the signs of mitosis were rarely seen (**Figure 1C**, **1D**). Tumor cells were arranged in sieve-like, cystic, follicular, papillary and solid flake patterns (**Figure 1E**, **1F**). Eosinophilic or basophilic sediments were observed among the tumor cells mostly in the flow pattern, which were irregularly distributed in the tumor cells or cysts. The special staining showed that the red dye (PAS staining) was located among the eosinophilic tumor cells, and blue dye was seen among the tumor cells with vacuolar cytoplasms (AB staining), as demonstrated in **Figure 2A-C**. In 1 case, the tumor cells were arranged in a papillary pattern in 1 patient, and the tumor cells were seen as spike-like, with nuclear deviation and eosinophilic cytoplasms. A small amount of tumor cells showed light, basophilic particle deposition in 1 case.

Mammaglobin was highly expressed in the cytoplasms of the lightly-stained tumor cells, but it was lowly or even not expressed in the cytosolic eosinophilic tumor cells. S-100 was expressed in two types of tumor cells, but the expression levels and location differed (**Figure 2D**, **2E**). All the tumor cells expressed CK and vimentin, and the Ki-67 index was approximately 10%. P63 was negative in the tumor cells.

The results of immunohistochemistry and ETV6 gene test between MASC and AiCC cases are illustrated in **Table 4**. S-100 was diffusely expressed in 1 case of AiCC, but S-100, p63 and mammaglobin were not expressed in the other 4 cases (**Figure 3A-C**). Of the 5 MASC cases, ETV6 genetic rearrangement was detected in 2 MASC cases (**Figure 2D**). All 5 AiCC cases were negative for ETV6 genetic rearrangement.

Table 2. Clinical features of MASC cases

Case	1405929	1417319	1602816	1616317	1702253
Age (year)/Sex	34/Male	60/Male	46/Male	47/Male	45/Male
Size (cm)	3.0	2.0	3.5	2.1	3.0
Relationship with parotid gland membrane	Penetrate the membrane	Inside the membrane	Inside the membrane, involving the membrane without penetration	Inside the membrane	Inside the membrane, involving the membrane without penetration
Lymph node metastasis	No (0/4)	No (0/1)	No (0)	No (0/7)	No (0/3)
Recurrence and metastasis	Recurrence and metastasis with pulmonary metastasis after one year	N0	N0	N0	N0
Distant organ metastasis	Lung	NO	NO	NO	NO
Original pathological diagnosis	Cystadenocarcinoma	ACC	ACC	ACC	MASC
Treatment	Tumor and gland surgical resection + radiotherapy + chemotherapy	Tumor and gland surgical resection	Tumor and gland surgical resection	Tumor and gland surgical resection	Tumor and gland surgical resection + radiotherapy + chemotherapy
Follow-up time (months)	42 AW	36 AW	17 AW	12 AW	8 AW

AW: alive and well.

Table 3. Results of pathological morphology, immunohistochemistry and gene test

Histomorphological features	1405929	1417319	1602816	1616317	1702253
Two types of tumor cells with different cytoplasm	Yes	Yes	Yes	Yes	Yes
Two secretions by AB/PAS staining	Yes	Yes	Yes	Yes	Yes
The arrangement between secretions and tumor cells	Yes	Yes	Yes	Yes	Yes
Collagen interstitial with sclerosis and hyaline degeneration	Yes	Yes	Yes	Yes	Yes
Old hemorrhage	No	No	Yes	Yes	Yes
Immunohistochemistry					
P63	Negative	Negative	Negative	Negative	Negative
Mammaglobin					
Lighter cytoplasm, secretion of basophilic tumor cell cytoplasm	Positive	Positive	Positive	Positive	Positive
Eosinophilic tumor cell cytoplasm	Negative	Negative	Negative or weakly positive expression	Weakly positive expression	Negative
CK Diffusion	Positive	Positive	Positive	Positive	Positive
Vimentin Diffusion	Positive	Positive	Positive	Positive	Positive
Ki-67	10%	10%	10%	10%	10%
Fluorescent FISH					
ETV6 rearrangement	Positive (36%)	Positive (30%)	Positive (40%)	Positive (42%)	-

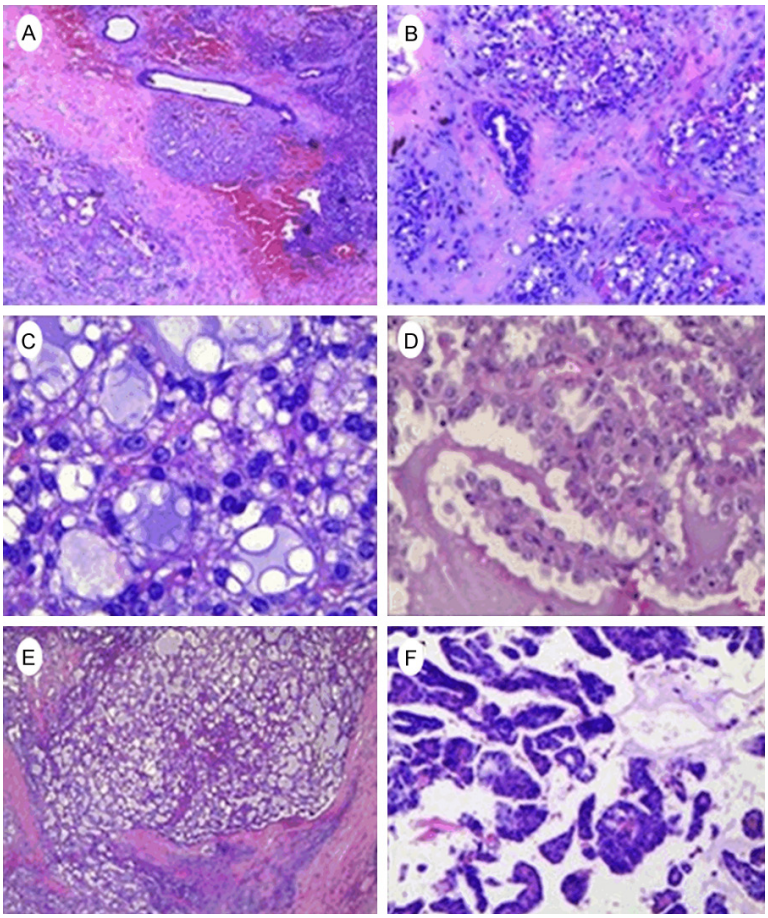

Figure 1. The tumor cells were divided and packed in collagen, even in the infiltrating tissues (A, B). Of the two types of tumor cells, one type was a cytoplasm acidophilic and the other type was a cytoplasmic empty cell mass. The nuclei were round and vacuole and the cell abnormalities were not obvious (C, D). The tumor cells were arranged in sieve-like, cystic, follicular, papillary and solid flake patterns, but microcapsular structures could be observed (E, F).

One atypical AiCC case with morphological similarities with MASC was negative for S-100, p63 and mammaglobin, but negative for the ETV6 gene (**Figure 3D**).

Discussion

It is challenging to confirm the pathological diagnosis of salivary gland tumors due to the similarity among tumor origins, the histomorphological features, the immunohistochemical expression, and even genetic changes. Therefore, it is extremely valuable for the clinicopathological diagnosis to identify the histological features and the immunohistochemical expressions of the different tumor types, In this article, the 5 cases of MASC shared similar histomorphological characteristics, which were inconsistent with previous findings [4-11]. First, the tumor tissue boundaries were relatively clear, and in the infiltration of the surrounding tissues, the glassy fibrotic tissues could be constantly observed among the tumor cells, even in the infiltration area surrounded by collagen fibers. Tumor tissues co-existed with collagen fibers. Second, the tumor tissues consisted of two kinds of tumor cells. One type of tumor cell was abundant in the cytoplasms, transparent or vacuole-like and basophilic, whereas the other type of tumor cells were eosinophilic. The proportion and arrangement of the two kinds of cells significantly differed. But the nuclei of the two types of tumor cells were seen in circular and vacuole-like patterns. The cell heteromorphism was not obvious, the mitosis was extremely rare and the expression of ki-67 was approximately 10%. The expression level and location of the mammaglobin and S-100 also significantly differed between the two types of tumor cells. A previous study [10] proposed that mammaglobin is partially expressed in MASC patients probably because the eosinophilic tumor cells dominate the tumors. However, the observation results remain to be validated. It is assumed that the origins and functions of these two cells probably differ according to the morphological features and antibody expressions. Both of the two tumor cells expressed CK and vimentin, indicating that both types of tumor cells have the characteristics of epithelia and mesenchymal differentiation. P63 was not expressed in the tumors cells, which could be utilized to differentially diagnose low-grade mucoepidermoid carcinoma [13, 14]. Third, the arrangement patterns of the tumor cells vary, and include sieve pore, cystic, follicular, papillary and solid sheet patterns, consistent with

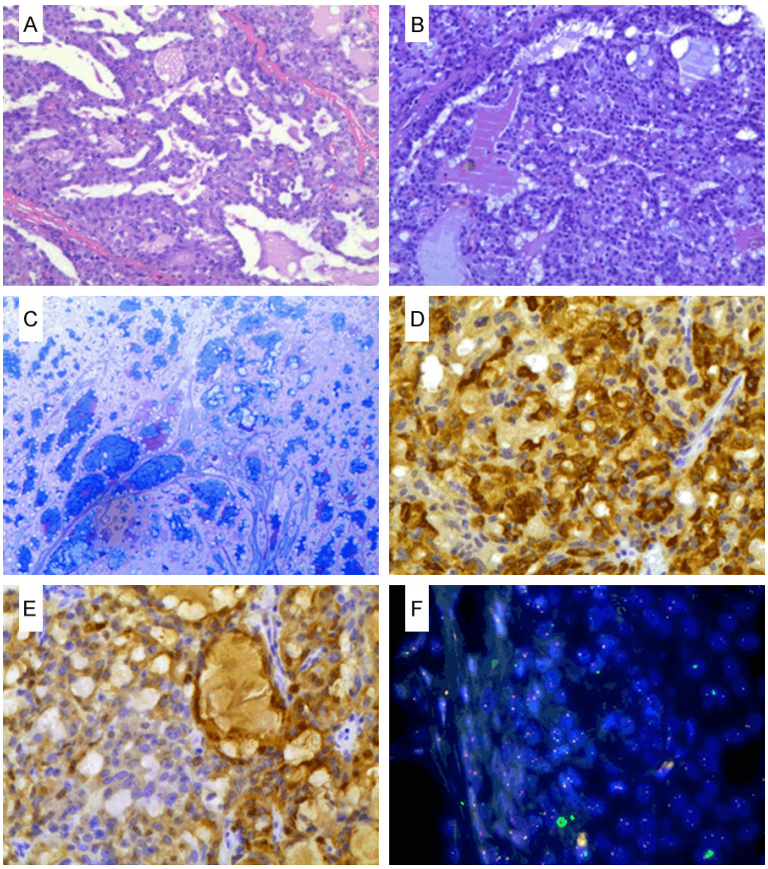

Figure 2. Eosinophilic or basophilic sediments were irregularly distributed in the tumor cells (A, B). The special staining showed that the red dye (PAS staining) was located among the eosinophilic tumor cells, and blue dye was seen among the tumor cells with vacuolar cytoplasms (AB staining) (C). The cytoplasm was lightly stained and the mammaglobin was highly expressed in the tumor cells, whereas the mammaglobin was lowly or not expressed in cytosolic eosinophilic tumor cells (D). S-100 was expressed in both types of tumor cells, but the expression level and location were inconsistent (E), ETV6 gene rearrangement was detected in MASC (F).

Table 4. Comparison of Immunohistochemistry and ETV6 gene detection between MASC and AiCC

No. of cases	MASC (n)		ACC (n)	
	5		5	
	-	+	-	+
S-100	0	5	4	1 (diffusely positive)
p63	0	5	5	0
Mammaglobin	0	5	5	0
ETV6 rearrangement	-	4	5	0

previous findings [4-8]. The cystic structures accompanied by sediments could be observed in each case. Fourth, eosinophilic or basophilic sediments were noted in each case, which were irregularly distributed among the tumors cell or within the capsule in a flow pattern. This arrangement pattern differed from the regular pattern of the basal cell tumors and the adenoid cystic carcinoma. Interestingly, a red dye substance (PAS staining) was seen among the cytoplasmic and eosinophilic tumor cells, whereas blue dye substance was noted among the partial basophilic vacuoles tumor cells (AB staining). HE staining revealed that the sediment color was similar to that of the adjacent tumor cell cytoplasms, which corresponded to the disparity of the expressions of the antibodies between the two types of tumor cells, indicating that the two kinds of tumor cells may have different biological functions. The exact reasons for this remain to be elucidated.

It is necessary to differentially diagnose MASC from low-grade cystic mucinous epidermoid carcinoma because they both equally generate alkaliphilic secretions [12]. However, the signs of typical mucoid columnar epithelial cells of cystic mucinous epidermoid carcinoma can contribute to the differential diagnosis.

Fluorescent FISH detects the characteristic ectopic fusion gene ETV6-NTRK3 in MASC patients [2, 7, 13, 15], but the gene cannot be detected in the case of MASC [1] or in secretory carcinoma of the breast [16]. Justin *et al.* [7] consider that it is not necessary to deliver genetic testing because both of them are low-grade malignancies. The misdiagnosis does not significantly affect the clinical prognosis or survival. Akeesha A. Shah et al. [11] have confirmed that molecular pathological tests are not necessary in the

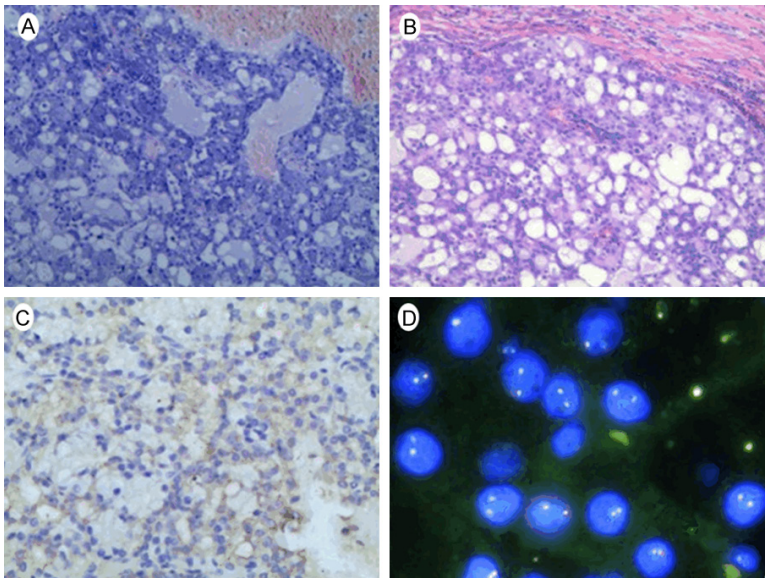

Figure 3. Microcapsule structures and purple enzyme particles full of tumor cells could be observed in the atypical AiCC (A) and MASC cases (B). Mammaglobin was negative in the MASC cases (C). No ETV6 gene rearrangement was observed in AiCC (D).

diagnosis of MASC. In this article, only two cases were confirmed using ETV6 gene rearrangement, which cannot conclusively determine the negative outcomes because it may increase the difficulty in genetic testing induced by the improper pre-treatment of specimens. Currently, FISH detection has not been conducted in many hospitals in China. Therefore, genetic testing can be applied to confirm the diagnosis rather than routine pathological examination.

Tumor cells of both MASC and ACC have identical granular cytoplasms and various structural arrangement patterns. Hence, MASC can be classified as a subtype of AiCC, but it has not been widely recognized [7]. MASCs have been misdiagnosed as AiCC in multiple retrospective studies [2, 17, 18].

In this article, 5 cases of MASC had a similar morphology. Compared with AiCC, one or two of the morphological or pathological characteristics above could also be observed in the atypical ACC. Nevertheless, no AiCC cases shared four characteristics. Besides, the disparity of immunohistochemical expression can be utilized to make a differential diagnosis [15, 19, 20]. A majority of the MASC cases have been misdiagnosed as solid-type AiCC because they lack basophilic proenzyme particles in the cytoplasm. Justin A et al. [7] have shown cystic structures that cannot be seen in the AiCC cases, which was frequently observed in the MASC cases described in this article. Additionally, S-100 and mammaglobin were not expressed in the AiCC cases. Compared with genetic testing, morphological and immunohistochemical observation play a more significant role in the differential diagnosis of MASC from AiCC, which is evidenced by the study of Akeesha [11].

The parotid gland is a frequent site of ACC because it is a pure serous gland, which is also a susceptible site for MASC, accounting for 2/3 of all MASC cases [2, 4, 14]. MASC is more likely to derive from mixed glands, such as the sublingual gland or the submandibular gland because it secretes both eosinophilic and basophilic particles. However, previous studies [2, 7] have reported that the incidence of MASC is highest in parotid glands, followed by the oral salivary glands and the submandibular glands. In this article, all the MASC cases were detected in the parotid glands, a pattern which remains to be explained by subsequent investigations.

Tumor resection and adenoidectomy were performed in this study. Four cases obtained excellent clinical prognoses. Only one patient received chemotherapy and radiotherapy after an extended resection due to the lesions invading into the capsular membrane. The patient suffered a recurrence and the cancer metastasized. These outcomes suggest that the clinical prognosis of MASC is intimately correlated with the clinical staging of the initial lesions.

Conclusion

MASC is a novel type of salivary gland tumor which resembles breast secretory carcinoma, with unique histological features and immunophenotypes. It is morphologically manifested by two different types of cytoplasms and the

same karyotype of tumor cells with cystic structures in a variety of arrangement patterns. Tumor cells amass wrapped by a collagen stroma or are seen as a cystic swelling infiltrating the surrounding tissues. The collagen and tumor cell mass co-exist. Two kinds of tumor cells produce two different categories of secretions, which are irregularly distributed among tumor cells in a flow pattern. Two types of tumor cells co-express CK and vimentin, but it is the expression of mammaglobin that significantly differs. Therefore, the histological features and immunohistochemical characteristics shared by these two diseases play a vital role in the differential diagnosis. ETV6-NTRK3 gene detection is applicable to confirm the diagnosis of complicated and atypical cases rather than a routine examination in the clinical.

Disclosure of conflict of interest

None.

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