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Original Article

# Clinicopathological analysis of 18 cases of secretory carcinoma of the salivary glands

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Carbonic anhydrase VI

**Abstract** *Background/purpose:* Secretory carcinoma (SC) is a rare salivary gland tumor that featured by *ETV6::NTRK3* gene fusion, and was included in the WHO Classification of Head and Neck Tumors since 2017. Nevertheless, the description of SCs by WHO is still vague. This study examined 18 SC cases by using both histomorphology and molecular pathology for diagnostic determination, especially immunohistochemical features of SCs.
*Materials and methods:* Based on WHO characteristics, 18 patients with SC admitted between 2001 and 2022 were included in this study. Main histomorphological patterns, FISH analyses of the *ETV6::NTRK3* gene fusion, and immunohistochemical analyses of S100, mammaglobin, DOG1, ADFP, CA6 and Ki-67 were performed.
*Results:* Among the 18 SC patients, the median age of onset was 39.22 years. Grossly, the average tumor size in 2.96 cm with various texture from soft to tough. The majority patients were positive for S100, mammaglobin, and negative for DOG1, except for one patient negative for S100 (*Case 18*). All patients were positive for ADFP, and the majority patients were negative for CA6, except for *Case 9*. Two cases were found recurrence, and the tumor were found both in parotid gland with local invasion.
*Conclusion:* Combined with the results of previous studies, we proposed that the combination of all five markers, S100, mammaglobin, DOG1, ADFP and CA6, could contribute more to differential diagnosis of SCs with other salivary carcinomas, especially with AciCC. The prognosis of SCs is optimistic in most cases, but larger patient cohort and long-term follow-up are still needed.

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## Introduction

The secretory carcinoma (SC) of the salivary glands was first described by Skalova et al., in 2010, and has been included in the World health organization (WHO) Classification of Head and Neck Tumours since 2017.[1] The development of SCs is slightly higher in males than in females (58.7% vs. 41.3%), and the average age onset of SCs is 45 years (range from 5 to 87 years).[2] It occurs most frequently in the parotid gland, followed by the submandibular gland, and usually presents as a painless, slow-growing mass. The microscopic structures of SCs are diverse, including solid-type, papillary cystic, glandular tubular, and striated structures, infiltration of surrounding tissue and ulceration of the skin or mucosal surface could also be observed.[2–4]

WHO noted that the immunohistochemical profile of SCs is characterized by the co-expression of S100 and mammaglobin, and negative for DOG1. *ETV6::NTRK3* gene fusion,[1] which is mainly caused by chromosome t (12:15) (p13:q25) translocations,[5] has also been shown with high sensitivity and specificity for the diagnosis of SCs. Recent studies have further identified the fusion of *ETV6* with *RET*,[6] *MET*,[7] and *MAMML3* of SCs.[8]

Nevertheless, the differential diagnosis of SCs includes, but is not limited to acinic cell carcinoma (AciCC),[2] polymorphous adenocarcinoma (PAC), mucoepidermoid carcinoma (MEC), and intraductal carcinoma (IDC),[2] noting that the diagnosis feature of SCs is remained to be enriched. The mere diagnosis features provided by WHO might not adequately assist differential diagnosis of SCs from these carcinomas. For clinical practice, co-expression of S100 and mammaglobin had a sensitivity of up to 95% for the diagnosis of SCs.[2,9] Nevertheless, Guilmette et al. reported a case of SC with positive ETV6 gene isolation, but the immunohistochemical results were focally positive for mammaglobin in one part, and only single-positive for S100 in the other part, indicating that the possibility of a diagnosis of SC could not be excluded when both S100 and mammaglobin were not co-positively expressed.[8]

Adipophilin, also known as ADFP, is normally expressed in sebaceous gland cells, and is closely related to adipocyte differentiation.[10] Previous studies proposed that 90% of the positive expression of ADFP could be observed in SCs, indicating ADFP might represent as a specific marker for SCs.[11] Carbonic anhydrase VI (CA6), which is involved in the regulation of pH homeostasis and taste, is present in the cytoplasm in association with zymogen granules, and is a specific marker of glandular vesicle cells, as it binds only to plasmocytic differentiated glandular vesicle cells.[12,13] Its negative expression contributed to the definitive diagnosis of SCs in a manner similar to that of DOG1. Hence, the combination of these two markers with the previously recognized three markers (S100, mammaglobin and DOG1) might shed light on accurate and easier diagnose of SCs.

Hence, we performed a study regarding the clinicopathologic and molecular characterization of 18 SC cases identified via screening. According to WHO, FISH analysis of *ETV6::NTRK3* gene fusion, immunohistochemical staining analyses for S100, mammaglobin and discovered on gastrointestinal stromal tumor 1 (DOG1) were performed. In addition, immunohistochemical staining analyses for ADFP, carbonic anhydrase VI (CA6), Ki-67 were also performed to assist future diagnose of SC.

## Materials and methods

### Case selection

In accordance with the characteristic features described by the WHO for the classification of head and neck tumors and the recent studies on SCs, 18 SC patients who were admitted between 2001 and 2022 were screened at the Department of Pathology, West China Hospital of Stomatology, at Sichuan University. Frozen section was performed on these cases. This study was approved by the Ethics Committee of West China Hospital of Stomatology, Sichuan University (project approval number: WCHSIRB-2022-3910).

### Immunohistochemical analysis

Tumor tissues were fixed in 10% formalin, embedded in paraffin, and sliced into sections with a thickness of 3 μm. Immunohistochemical analysis was performed using the following antibodies: S100 (1:400 diluted; Beyotime Biotechnology, Shanghai, China), mammaglobin (1:100 diluted; San Ying Biotechnology, Wuhan, China), DOG1 (1:200 diluted; San Ying Biotechnology), ADFP (1:400 diluted; San Ying Biotechnology), CA6 (1:2000 diluted; Abcam, Shanghai, China), and ki-67 (1:800; Proteintech, Wuhan, China). Immunoreactions were performed using labeled streptavidin and biotin, and by incubating the reaction mixture overnight. 3,3′-diaminobenzidine was used as the chromogen. The results of the semi-quantitative immunoreactivity analysis were evaluated by two experienced investigators. Cut-offs of 0%, 10%, 50%, and 80% were used to record the positive cell ratio with a score of 0–4. The staining intensity was recorded with a score of 0 (unstained), 1 (mildly stained), 2 (moderately stained), and 3 (strongly stained). A final immunostaining score was calculated by multiplying the positive cell ratio and staining intensity (0, unstained; 1–4, mildly stained; 5–8, moderately stained; 9–12, strongly stained).

### Fluorescence in situ hybridization testing

We extracted 3 mm-thick tumor tissue sections that were fixed with formalin from paraffin-embedded tissue blocks and placed them on slides. The sections were deparaffinized in xylene 3 times for 5 min each at 68 °C and washed in 100% ethanol for 5 min at room temperature (RT). Then, tissues were heated at 90 °C in Triton X-100 (Wuhan HealthCare Biotechnology Co, Wuhan, China) for 20 min and subsequently cooled in deionized water at 37 °C for 3 min. Ready-made 1 × pepsin (Wuhan HealthCare Biotechnology Co.) was used for digestion for 10 min at RT. The slides were then immersed into deionized water twice for 5 min each and dehydrated via gradient immersion in 70%, 85%, and 100% ethanol for 2 min at RT. Next, all specimens were air-dried.

An *ETV6* gene break-apart probe (Wuhan HealthCare Biotechnology Co.) was applied on each slide. For detection of the *ETV6::NTRK3* gene fusion, the following primers were used: ETV6 forward primer P385: 5′-ACCACATCATGGTCTCTGTCTCCC-3′ and NTRK3 reverse primer P386: 5′-CAGTTCTCGCTTCAGCAC GATG-3'. All the slides were covered with a glass coverslip and sealed using rubber cement. Samples were incubated with co-denaturation parameters at 85 °C for 5 min and hybridization parameters at 42 °C for 16 h. Under dark conditions, the slides were air-dried after washing them in 2 × sodium saline citrates (SSC) for 1 min at RT and in 0.4 × SSC for 2 min at 68 °C. Subsequently, the slides were cooled using deionized water at 37 °C for 1 min and air-dried with lightproof treatment. Eventually, the slides were counterstained with 4,6-diamino-2-phenylindole, covered with a cover slip, and evaluated immediately.

Processed samples were first observed using a 10× lens to confirm the location of tumor cells and then observed with a 40× and 100× lens. ETV6 was imaged as two complete signals (i.e., the yellow signal formed by the superposition of orange and green signals), while positive ETV6 gene segregation was performed by a nucleus with one yellow fusion signal and one orange and green signal that were separated by at least 2 signal diameters. A positive result was reported when at least 15% of tumor nuclei appeared to fit the split-signal pattern.

### Collection of clinical features of 18 cases

Clinical data were collected for further analysis, including age, gender, tumor size, site of occurrence, tumor texture, TNM stage, tumor margins, symptoms, treatment administered, recurrence. For patients with recurrence, apart from the above information, tumor histomorphological type, tumor invasion, exist of dedifferentiation and necrotic foci were also collected.

### Statistical analysis

SPSS statistical software (version 25, IBM, Armonk, NY, America) was used to summarize the original data and carry out correlation statistical analysis. Descriptive analyses of the results and frequencies were conducted.

## Results

### Clinical features of 18 cases of secretory carcinoma

Basic clinical feature of all cases was shown in Table 1. The age of onset ranged from 7 to 68 years (median 39.22 years). 12 (66.67%) of the patients were male and 6 (33.33%) were female. 16 (88.89%) SCs occurred in the parotid gland, followed by 2 (11.11%) SCs in the palate gland.

Grossly, the average tumor size in 2.96 cm with various texture from soft to tough. SCs were mostly tough and nodular, with a gray or gray-brown surface. Most of the masses had a capsule, but 5 (20.8%) patients exhibited SCs with unclear borders.

5 patients (27.78%) had T1 tumors, 11 (61.11%) had T2 tumors, and 2 (11.11%) had T3 tumors. Only one patient presented with regional lymph node metastasis; none of the patients had distant metastasis at diagnosis. 12 patients reported painless and 3 patients experienced various types of painful sensations, including itchy-like and pinprick-like pain, together with 1 patient experienced pain under slight pressure.

Case 8 and 13 were reported recurrence 5 month and 6 years after surgery, and patients of Case 8 was treated by surgery, patient of Case 13 was treated by both surgery and radiotherapy. Details were shown in Table 2. Both patients were male, and the occurred in the parotid gland. Local invasion, exist of dedifferentiation, and non-existence of necrotic foci of the tumor were the common features.

**Table 1** Clinical features of 18 cases of secretory carcinoma.

| Variables | Results |
|---|---|
| Median age, in years [range] | 39.22 [7–68] |
| Gender, n (%) | |
| Male | 12 (66.67%) |
| Female | 6 (33.33%) |
| Mean tumor size, in cm [range] | 2.96 [0.40–11.00] |
| Site of occurrence | |
| Parotid gland | 16 (88.89%) |
| Palate gland | 2 (11.11%) |
| Texture of SC tumor, n (%) | |
| Soft | 1 (5.56%) |
| Medium | 2 (11.10%) |
| Tough | 10 (55.56%) |
| Unknown | 5 (27.78%) |
| TNM stage, n | |
| T1/T2/T3/T4 | 5/11/2/0 |
| N0/N1/N2/N3 | 17/0/1/0 |
| M0/M1 | 18/0 |
| Pain feature of SC tumor, n (%) | |
| Painless | 12 (66.67%) |
| Sensitive to touch | 1 (5.56%) |
| Itchy-like pain | 1 (5.56%) |
| Pinprick-like pain | 1 (5.56%) |
| Unknown | 3 (16.65%) |
| Recurrence of SC tumor, n (%) | 2 (11.11%) |

SC: secretory carcinoma.

**Table 2** Details of two recurrence of secretory carcinoma.

| Features | Case 8 | Case 13 |
|---|---|---|
| Age | 53 | 29 |
| Gender | Male | Male |
| Tumor size (cm) | 11.0 | 4.0 |
| Site of occurrence | Parotid gland | Parotid gland |
| Texture of SC tumor | — | hard |
| TNM stage | T3N0M0 | T2N0M0 |
| Margins of SC tumor | — | unclear |
| Pain feature of SC tumor | — | painless |
| Histological type | Solid-type | Solid-type |
| Tumor invasion | Local invasion | Local invasion |
| Exist of dedifferentiation | Exist | Exist |
| Exist of necrotic foci | Non-existence | Non-existence |
| Treatment procedure | Surgery | Surgery + Radiotherapy |

SC: secretory carcinoma.

### Histomorphological features of 18 cases of secretory carcinoma

The histomorphological and cell types of all SC samples were examined and exhibited solid-type (8 patients, 44.44%), papillary cystic type (7 patients, 38.89%), and mixed-type (3 patients, 16.67%) (Fig. 1). Hemorrhage, necrosis, and cholesterol crystal clefts were seen in some cases. Most of the SC tissues included in this study exhibited low heterogeneity and rare nuclear pleomorphism. Only 5 patients (27.78%) displayed dedifferentiation with obvious nuclear pleomorphism, significant nucleoli, and pathological nuclear pleomorphism, while 3 patients (27.78%) displayed the existence of necrotic foci (Fig. 1).

### Immunohistochemical findings and fluorescence in situ hybridization analysis

Immunohistochemical results have been shown in Figs. 2 and 3. All samples showed positive reactivity for mammaglobin, ADFP and negative for DOG1 (Fig. 2A—D). 17 out of 18 SC tissues (94.44%) showed immunoreactivity towards S100 (Fig. 2A), where 15 (88.24%) showed strong (+++) immunoreactivity, and 2 (11.76%) showed moderate (++) immunoreactivity. For mammaglobin, 4 (22.22%) showed moderate (++) immunoreactivity (Fig. 2B). For ADFP, 2 (11.11%) exhibited weak (+) immunoreactivity, and 1 (5.56%) exhibited moderate (++) immunoreactivity, while the others showed strong (+++) immunoreactivity (Fig. 2C). The vast majority of tissues were negative for CA6, where only patient in case 9 showed susceptibility (±). All tissues had a Ki-67 reactivity below 20%, which manifested specifically in 13 out of the 24 patients within a range of 1—3%, while that for the others ranged between 5% and 10% (Fig. 2E).

Fig. 3 displayed a combination of immunoreactivity of 5 markers. Compared with the combination of previous recognized markers, the combination of 5 markers assists more accurate diagnosis of SCs, especially for *Case 18*.

A total of 18 SC patient samples were analyzed for *ETV6* gene breaks via FISH, and all of them were positive for a rearrangement of *ETV6* with *NTRK3* (Fig. 4).

## Discussion

SCs are generally low-grade salivary gland carcinoma, which is featured by morphological resemblance to mammary secretory carcinoma and *ETV6::NTRK3* gene fusion.[3,14] SCs are generally developed in adults with a slightly higher ratio in male, but could also occur in children and adolescents.[2,15] In the present study, the age of onset of patients with SCs ranged from 7 to 68 years (median: 39.22 years). Higher ratio of onset in male were also found (male: female = 2:1). The parotid gland is the most common site of SCs, which was also found in the present study. To note, 2 cases of SCs of palate gland-origin were observed. In the previous study, the small salivary gland in the buccal and upper lip were also reported as the site of SCs.

SCs usually represent as a slow-growing, painless tumor with a size of less then 1 cm in diameter.[2,15,16] Consistent with the present study, 12 (66.67%) patients presented with a painless mass, only a few presented as sensitive to touch, itchy-like pain or pinprick-like pain. Only 2 patients presented with T3 tumors, while the others presented with T1/T2 tumors. No T4 tumor was reported. The majority patients presented with N0 tumors, and only 1 patient presented with N2 tumors, which highlighted SCs are low-grade tumor in consistent with the previous conclusions. Moreover, the texture of SCs tumors varied from soft to tough.

Upon reviewing published data, the microscopic structures of SCs were found diverse, including solid types, papillary cystic, glandular tubular, and striated structures.[2,17,18] Tumor cells were eosinophilic and round or ovoid with abundant cytoplasm, and were usually multivesicular and lipid-rich.[9] Malignant SCs usually structurally presented as solid-type or trabecular-type with larger tumor cells, together with more visible necrosis, indicating its heterogeneity.[19,20] In the present study, most patients presented as SCs with solid-type or papillary cystic type, while 3 patients presented with mixed-type. Hemorrhage, necrosis, and cholesterol crystal clefts were also observed.

SCs are frequently classified as AciCC owing to their nearly identical histomorphological growth patterns, and were also mimics other salivary carcinomas, such as IDC, PAC and MEC.[6,8,21] Hence, histomorphological features were needed.

As described by WHO, SCs are featured by co-expression of S100 and mammaglobin, and negative expression of DOG1.[1] S100 is a soluble acidic protein that is mainly located in the cytoplasm and nucleus.[2,22] Mammaglobin involved in cellular secretory, and has been identified as a marker of breast cancer.[23,24] DOG1 is a protein of calcium-activated chloride channel, and was considered as a marker for gastrointestinal mesenchymal tumors.[13] The co-expression of S100 and mammaglobin had a sensitivity of up to 95% for the diagnosis of SCs.[1] Guilmette et al. also reported a case featured with *ETV6::NTRK3* gene fusion,

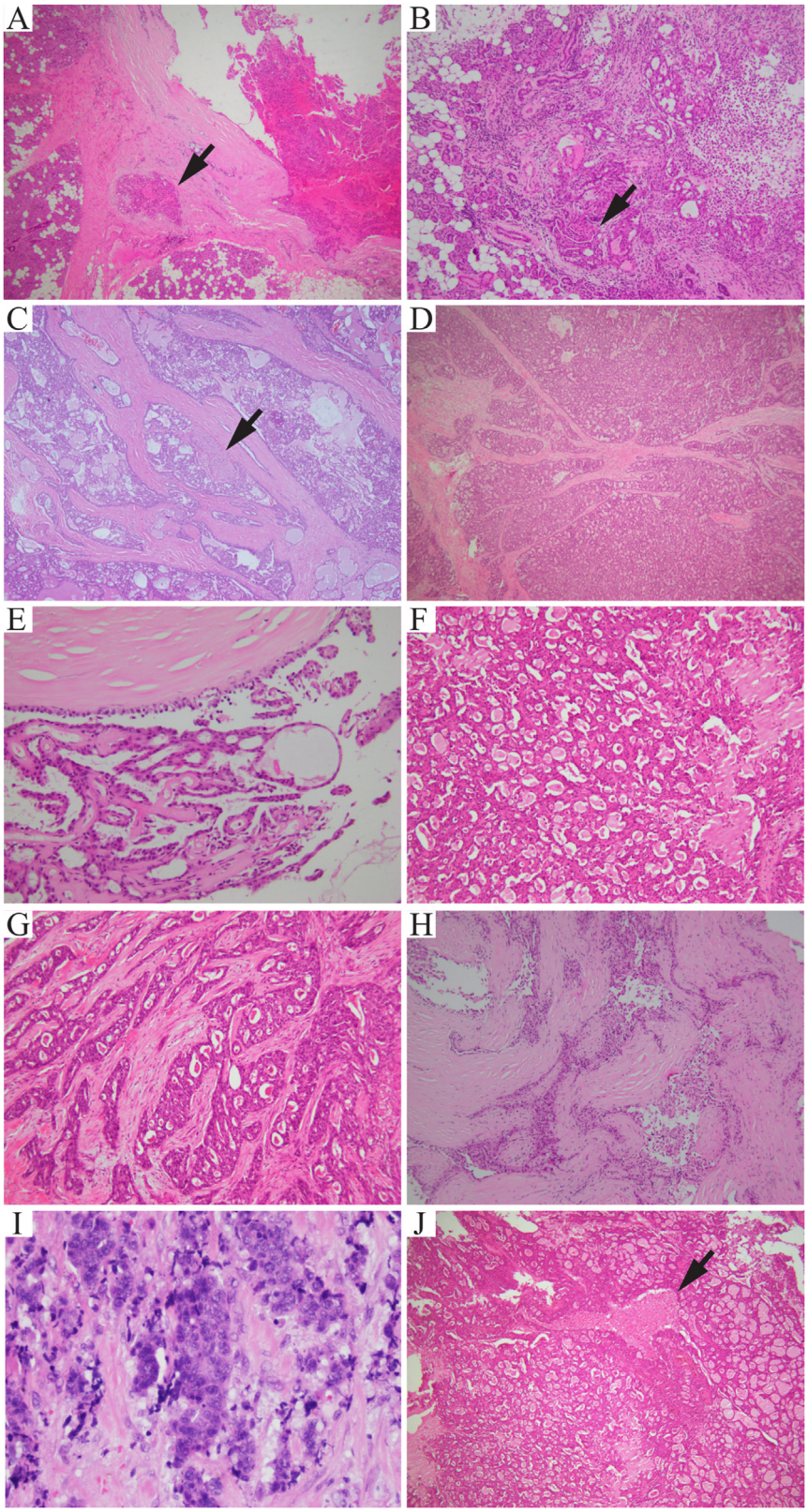


**Figure 1** Morphological features of SC. (A) Invasion of the capsule (black arrow, H&E, original magnification ×40). (B) Local invasion of glands (black arrow, H&E, original magnification ×100). (C) Invasion of the nerve (black arrow, H&E, original magnification ×40). (D) Structure of solid type (H&E, original magnification ×40). (E) Structure of papillary capsule type (H&E, original magnification ×200). (F) Structure of microcapsule type (H&E, original magnification ×100). (G) Structure of glandular duct-like type (H&E, original magnification ×100). (H) Structure of striatal type (H&E, original magnification ×40). (I) Exist of dedifferentiation (H&E, original magnification ×400). (J) Exist of necrotic foci (black arrow, H&E, original magnification ×100).

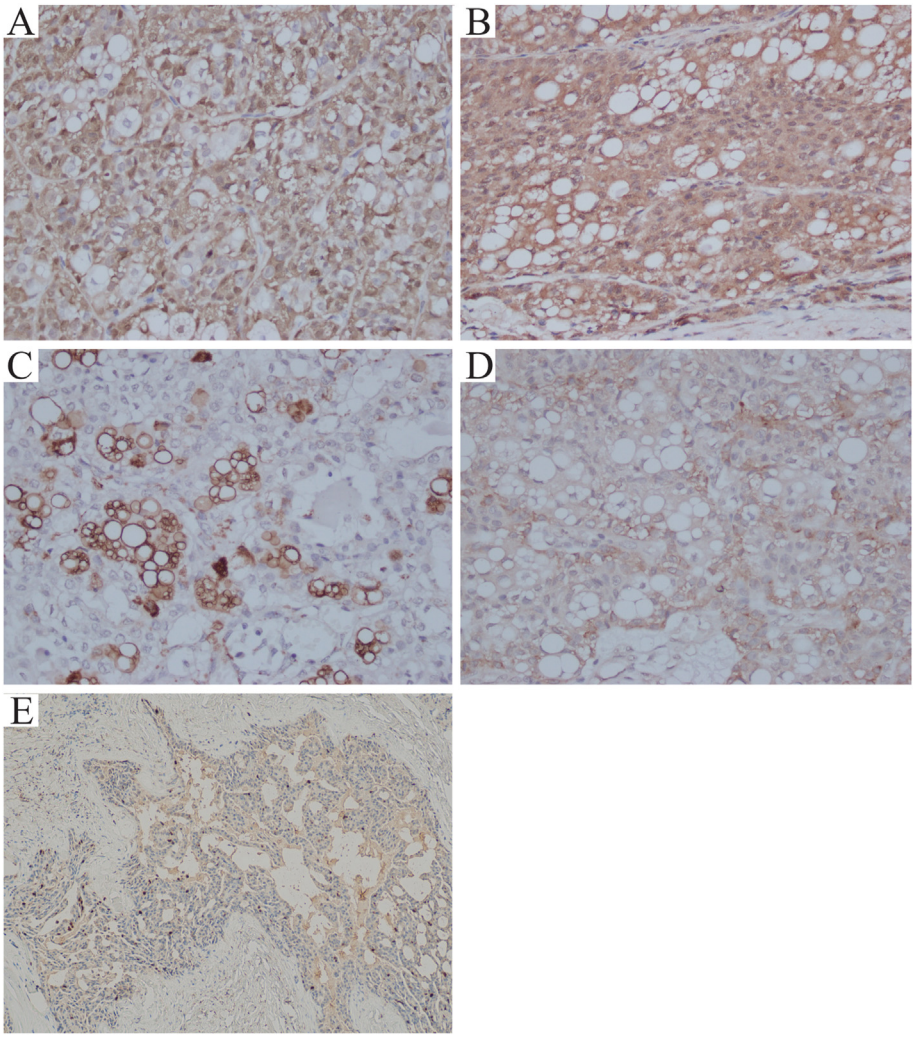


**Figure 2** Immunohistochemical results of SC regarding S100, mammaglobin, ADFP, DOG1, CA6 and ki-67. (A—C) Tumor showed diffuse S100, mammaglobin and ADFP reactivity (original magnification ×400). (D) Tumor was negative for DOG1 (original magnification ×400). (E) Nonuniformity of cells positive for ki-67 could be seen (original magnification ×200).

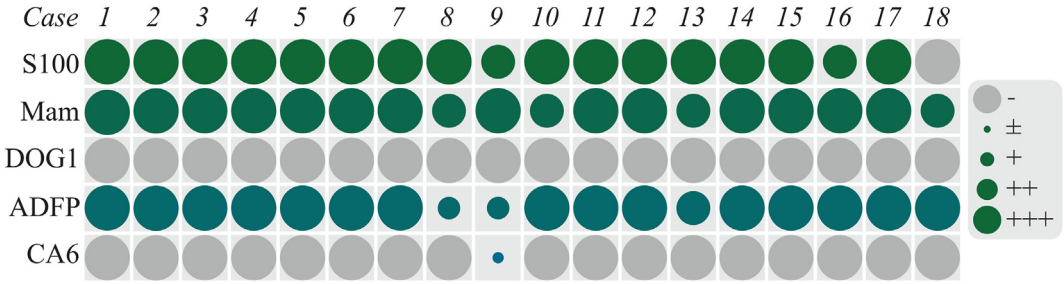


**Figure 3** General findings of SC regarding S100, mammaglobin, ADFP, DOG1, CA6 and *ETV6::NTRK3* gene fusion. All cases were found positive for *ETV6::NTRK3* gene fusion. All cases were found positive for mammaglobin and ADFP in varying degrees. The majority was tested positive for S100, negative for DOG1 and CA6.

but focally single-positive for S100 and mammaglobin were showed,[8] in the present study, 94.44% cases showed co-expression of S100 and mammaglobin, indicating the diagnosis of SCs could not be excluded when co-expression of S100 and mammaglobin was not observed.

Upon reviewing the previous study, ADFP and CA6 has also present as markers of SCs. ADFP is a surface component of intracellular fat glomeruli that are normally expressed in sebaceous gland cells, adipocytes, adrenal cortical cells, mammary lactating epithelia, and the basal part of some

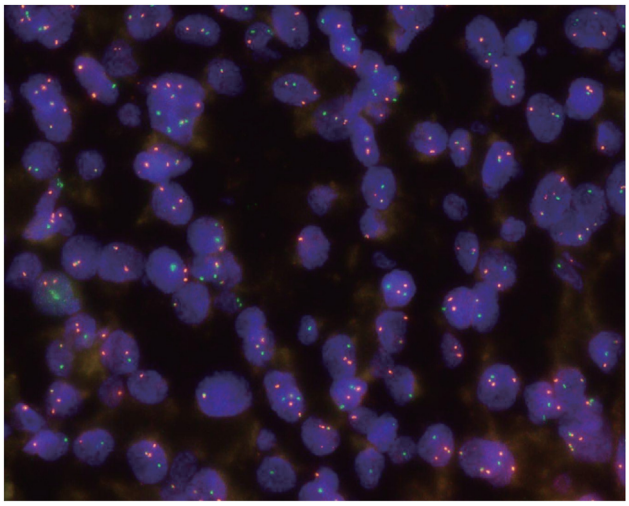

**Figure 4** FISH analysis of *ETV6::NTRK3* gene fusion. FISH test of ETV6 showing fused (yellow) and split (red and green) signal indicative of ETV6 translocation (original magnification ×100).

histiocytes and small intestinal absorptive epithelial cells, and is closely related to adipocyte differentiation.[10] Its expression is not found in normal salivary parenchyma, except for occasional duct cells with sebaceous differentiation.[10] CA6, which is involved in the regulation of pH homeostasis and taste, is present in the cytoplasm in association with zymogen granules, and is a specific marker of glandular vesicle cells, as it binds only to plasmocytic differentiated glandular vesicle cells.[13] CA6 present in secretory granules of serous cells in mammalian and human parotid glands.[12]

The combination of five markers, S100, mammaglobin, DOG1, ADFP and CA6, contributed to differential diagnosis of SCs with other salivary carcinomas, especially with AciCC. As defined, AciCC is negative for both S100 mammaglobin, and positive for both DOG1 and CA6. In a previous study, 1 out of 14 cases of SC, and 2 out of 28 cases of AciCC were triple-positive for S100, mammaglobin and DOG1, only CA6 marker showed all-positive in AciCC cases and all-negative in SC cases.[13] Another study showed that only in SCs and sebaceous carcinoma did ADFP surround large droplets in neoplastic cells, and all cases of SCs showed diffuse and strong staining for ADFP, while in other salivary carcinomas these ADFP droplets were minute.[10]

Combined with the present study, we proposed that the combination of five Immunohischemical markers, S100, mammaglobin, DOG1, ADFP and CA6 could facilitate diagnosis of SCs, and separate it with other salivary carcinomas, especially AciCC.

Approximately 90% of SCs exhibited characteristic *ETV6::NTRK3* gene fusion. In the present study, all cases showed *ETV6::NTRK3* gene fusion.[8] In addition to *NTRK3*, fusion genes of *ETV6* in SCs included *RET*, *MET*, *MAMML*.[6–8] Guilmette et al. identified two structural sites for two types of gene fusions in a patient with SC; one had adenoid and solid-type structures, which were diffusely positive for S100 and focally positive for mammaglobin. The other site showed a sieve-like, papillary cystic structure, and pseudostratified cuboidal columnar cells with hyaline cytoplasm and occasional nuclear division. This area was negative for both S100 and mammaglobin. Follow-up tests showed the coexistence of two fusion genes *ETV6::NTRK3* and *ETV6-MAMML3*, suggesting that the fusion genes could influence the specific expression of immunohistochemistry and microscopic structures.[8] Black et al. reported a case of a sieve and papillary cystic SCs with two gene fusions, *ETV6-RET* and *ETV6-SEPT14*.[16] Novel gene fusions such as *CTNNA1-ALK* and *VIM-RET* had also been found in SCs.[6,25] All of these conclusions suggested that complex conditions that result in genetic alterations could exist in SCs, and the clinical detection and application were yet to be explored.

SCs are mostly treated surgically. Selective or radical cervical dissection was considered when lymph node metastases presented, while adjuvant treatment such as chemotherapy and radiotherapy were considered if tumor invaded surrounding tissues, blood vessels and nerves.[2] Complete surgical resection with cervical lymphatic dissection should be performed for high-grade transformed SCs, and should supplemented with postoperative radiotherapy. For patient with recurrent metastatic or in operable SCs, targeted therapy should be considered.[2] The prognosis of SCs is optimistic in most previous reports, with few reported cases of recurrence, metastasis and death.[2,15,26] Similarly, in this study, distant metastasis had not occurred and recurrence occurred only in two patients receiving primary surgery treatment (*Case 8* after 5 month and *Case 13* after 6 years). As concluded in Table 2, both cases were founded as male, parotid gland-origin, solid-type, local invasion-existing and dedifferentiation-existing. However, due to the small sample size, such commonalities did not have statistically significance. Follow-up studies could further conduct statistical analysis of SCs recurrence cases in the published studies.

This study is not without limitations. Although we have included newly diagnosed and re-diagnosed SC cases in the past two decades, the number of cases shown is still relatively small, more attention needs to be paid to the characteristics and differential diagnosis of SC cases. Secondly, although we present a new combination of immunohistochemical diagnosis, there is still limited improvement in diagnosis, and there is an urgent need for new cases to be incorporated for further validation.

In conclusion, SC is a rare type of low-grade malignant salivary gland tumor that commonly occurs in the parotid gland with good prognosis. Histomorphological and immunohistochemical features are essential for separating SCs with other salivary carcinomas. In the 18 cases involved in the present study, 94.44% patients showed co-expression of S100 and mammaglobin, and negative for CA6, all patients were positive for ADFP and negative for DOG1. Combined with the results of previous studies, we proposed that the combination of all five markers could contribute more to differential diagnosis of SCs with other salivary carcinomas, especially with AciCC. For SCs with classic morphology, FISH analysis is not always necessary. The prognosis of SCs is optimistic in most cases, but its relevant features are remained to be explored, as cases of recurrence, metastasis and death were reported. The followed are the prospective for further studies regarding SCs:

1. As a rare type of low-grade tumor, more cases of SCs should be reported to summarize its epidemiological features. Before SC is clearly defined, attention should be paid to cases that were misdiagnosed as AciCC.
2. Exploration of more markers of SCs, especially immunohistochemical markers, should be encouraged. These markers could not only facilitate diagnosis of SCs by separating it with other salivary carcinomas, but also assist treatment. With the emergence of bioinformatic studies, RNA-seq and single-cell analysis could be performed to explore differential expressing gene (DEG) and other features at gene and molecular levels.
3. Larger patient cohort and long-term follow-up should also be performed to ascertain the optimal treatment and outcomes.

## Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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