Case report

Giant cribriform adenocarcinoma of the tongue showing PRKD3 rearrangement

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Cribriform adenocarcinoma of the tongue and minor salivary glands (CAMSG) was first described 16 years ago. It typically presents as a mass at the base of the tongue with early spread to lymph nodes, but without potential for distant metastases. In the 2005 World Health Organization Classification of Tumors the entity was classified as a possible variant of polymorphous low-grade adenocarcinoma (PLGA). Since then, more than 40 cases have been described in the English literature. Recently, *PRKD1-3* translocation was found in more than 80% of CAMSGs. In some of those cases *ARID1A* or *DDX3X* was the translocation partner.
We reviewed 183 primary carcinomas of major and minor salivary glands, resected at the Medical University of Gdańsk, Poland, in the period 1992-2012, and identified only one case of CAMSG.
A giant tumor developed at the base of the tongue in a 76-year-old man. The primary tumor was resected with multiple bilateral cervical lymph node metastases. The patient received radiotherapy but died 10 months after the surgery due to causes not related to the primary cancer. The tumor presented *PRKD3* rearrangement as confirmed by FISH.
As the tumor is extremely rare (it represented only 0.5% of salivary gland tumors in our series), the controversy on its nosological status is still unresolved. This is the first report in the world literature of a patient who died in the course of CAMSG.

Key words: CAMSG, CAT, cribriform adenocarcinoma, salivary gland, tongue.

Introduction

Cribriform adenocarcinoma of the tongue (CAT) was first described by Michal *et al*. in 1999 as a morphologically distinctive adenocarcinoma with early spread to lymph nodes but without potential for distant metastases [1]. In the 2005 World Health Organization Classification of Tumors the entity was classified as a possible variant of polymorphous low-grade adenocarcinoma (PLGA) [2]. Since then, more than 40 cases have been described in the English literature [1, 3, 4, 5, 6, 7, 8]. Although originally designated as CAT and considered to be restricted to the tongue, it was later shown to arise from the minor salivary glands of the soft palate, buccal mucosa, area of the palatine tonsils, upper lip and even epiglottis [4]. Therefore, the tumor was renamed as "cribriform adenocarcinoma of the tongue and minor salivary glands" (CAMSG) [4, 9, 10].

In 2014 Weinreb *et al*. by means of next generation RNA sequencing, Fusion Seq data analysis, and *in situ* hybridization (FISH) showed that 80% of CAMSGs harbor recurrent translocations in the *PRKD1-3* gene family [11]. Nevertheless, the study left some doubt as to whether PRKD1-3 translocation is specific for CMASGs as nearly half of tumors showing morphological overlap between CAT and PLGA exhibited these translocations. In contrast, only one of 18 typical PLGAs, with unusual localization in the parotid gland, revealed a translocation in *PRKD2*.

Material and methods

We reviewed all the primary carcinomas of major and minor salivary glands resected at the Medical University of Gdańsk (Departments of Otolaryngology and Maxillofacial Surgery) between 1992 and 2012, as described previously [12]. The total historical cohort of 183 salivary gland carcinomas was reviewed and reclassified according to the criteria published by WHO 2005 (HM and AS) [2]. Based on histomorphology, one case of CAMSG was identified. The neoplasm was screened by FISH for *ARID1*, *DDX3X* and *PRKD1-3* gene rearrangements as described elsewhere [11].

Fluorescent *in situ* hybridization (FISH)

Molecular genetic analysis was performed at the Memorial Sloan Kettering Cancer Center in New York, USA [11]. For the preparation of *ARID1*, *DDX3X* and *PRKD1*, *PRKD2* and *PRKD3* probes the custom bacterial artificial chromosome (BAC) probes were used as previously described [11]. The BAC clones were obtained from the BACPAC resources of the Children's Hospital of Oakland Research Institute. DNA from individual BACs were isolated according to the manufacturer's instructions and labeled with different fluorochromes in a nick translation reaction [13]. FISH was performed on 4 μm thick sections of formalin-fixed paraffin-embedded tissue. Two hundred randomly selected non-overlapping tumor cell nuclei were examined for the presence of yellow (normal) or green and red (chromosomal breakpoint) fluorescent signals. The sample was considered positive if more than 20% of nuclei showed split signals (e.g. red and green signals were separated by two signal diameters) or single red signals (3') were observed.

Case description

A 76-year-old man was admitted to the Department of Otolaryngology Medical University of Gdansk in September 2007, due to severe dysphagia caused by a large pharyngeal tumor. The patient had been complaining of increasing difficulties in swallowing for the last 2 years. He had undergone an incomplete resection of the tumor at the base of the tongue 6 years earlier in a small district hospital, with the tumor diagnosed at that time as a pleomorphic adenoma. In addition, the patient suffered from benign prostatic hyperplasia and chronic coronary disease. He had been smoking 10 cigarettes a day for 20 years. He denied any allergies. On examination a large firm tumor was identified at the base of the tongue filling the left epiglottic vallecula and obstructing the throat. It was confirmed by magnetic resonance imaging (Fig. 1). In addition, bilateral cervical lymphadenopathy was also found. Chest X-ray and ultrasound of the abdomen did not show distant metastases.

Lateral pharyngotomy with excision of the polypoid bluish tumor and bilateral cervical lymph node dissection was performed. The tumor had a rough surface and was attached to the base of the tongue on a wide peduncle. The resected tumor measured 6 × 6 × 4 cm and contained beige-colored, solid infiltrate that grossly was estimated as removed incompletely. Neither hemorrhages nor areas of necrosis were identified on the cut surface. Architecturally, the tumor showed a cribriform, solid, papillary and microcystic pattern of growth, arranged in lobules divided by thin fibrous or myxoid-mucinous septa (Fig. 2 A, B). Dilated cystic spaces contained cribriform and papillary structures and were filled with delicate pale pink proteinaceous material (Fig. 2B). The tubular or microcystic growth patterns were less obvious (Fig. 3A). In scattered solid nodules of tumor small necrotic foci were identified (Fig. 3B). The tumor infiltrated widely the surrounding muscle, adipose and fibrous tissues. Surgical margins of resection were infiltrated by carcinoma. The overlying squamous epithelium was intact (Fig. 4). The neoplasm undermining the superficial epithelium neither infiltrated nor destroyed it. Multiple tumor emboli were visible in the lymphatic vessels beneath the epithelium (Fig. 5A, B). Cytologically, the tumor cells were monomorphous; they contained overlapping, pale, vesicular nuclei with ground glass appearance. Nuclear grooves were present in some of them (Fig. 6). Cellular atypia was mild and there were single mitotic figures. Tumor cells showed expression of keratin (CK) AE1/AE3, CK7 and S100; GFAP and smooth muscle actin (SMA) were focally positive. Thyroglobulin and TTF1 were absent. Metastases were identified in all lymph nodes. Extranodal extension was absent. A final diagnosis of cribriform adenocarcinoma of the tongue with local lymph node involvement was established.

The patient underwent radiotherapy of the tumor bed and cervical lymph node basin. The postoperative period was complicated by local pain, difficulties in swallowing and dyspnea. Difficulty in breathing, due to radiotherapy-related laryngeal edema, was

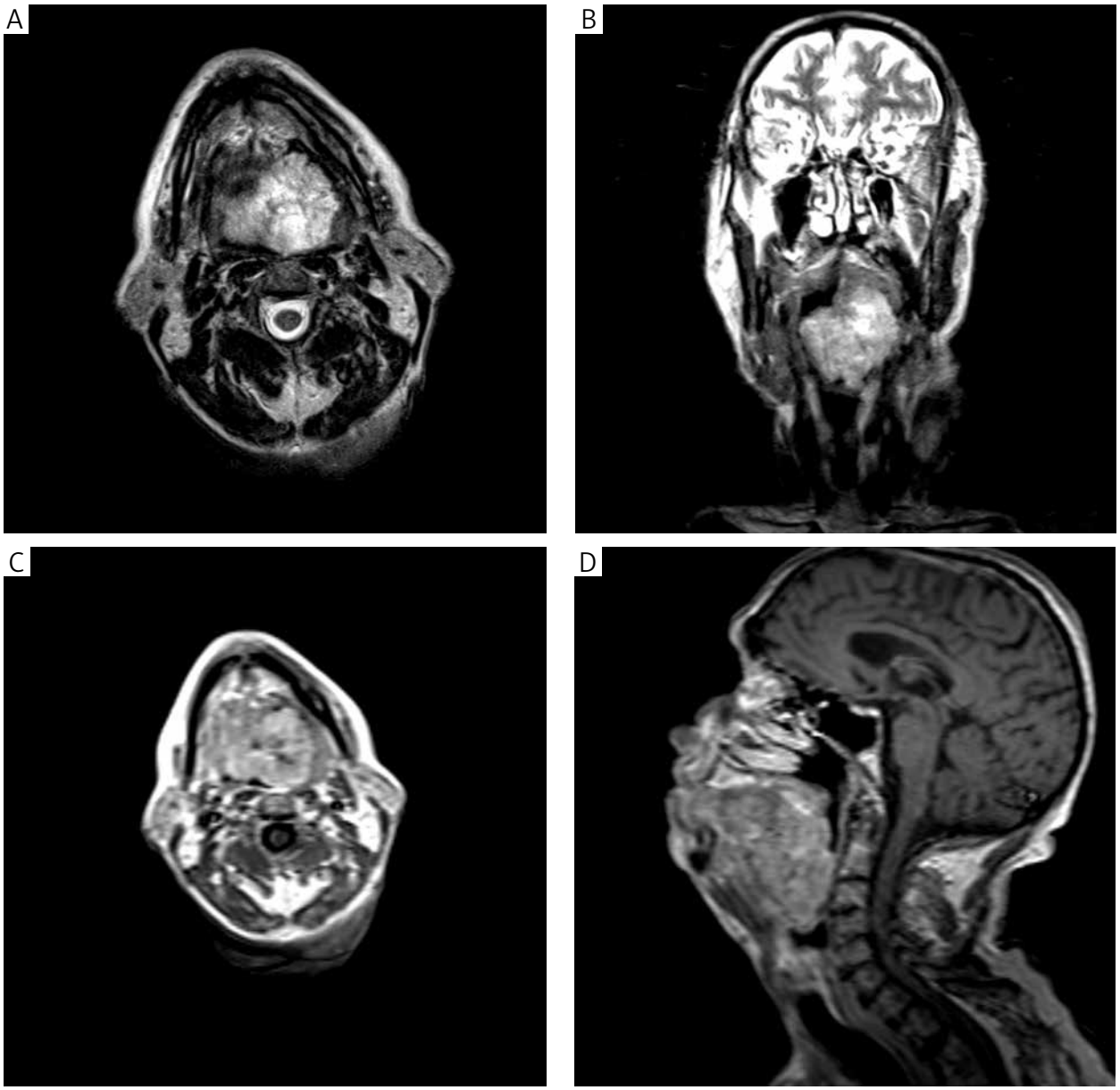

Fig. 1. Axial (A) and coronal (B) T2-weighted MR images revealed a hyperintense mass at the base of the tongue extending to the oropharynx. Axial (C) and sagittal (D) post-gadolinium T1-weighted images show inhomogeneously enhancing tumor causing displacement of the epiglottis

a recurrent problem. Finally, tracheostomy was performed and feeding by a gastric tube was initiated. Acute bronchogenic pneumonia developed in the patient 10 months after the operation, and he died 2 months later without signs of recurrent cancer.

In the multicentre retrospective study, the *PRKD3* rearrangement was found to be positive in the tumor (case no. 8, Table I) (Fig. 7) [11].

Discussion

Cribriform adenocarcinoma of the tongue was originally described by Michal *et al*. in 1999 [1]. They reported eight cases, all of which were located at the base of the tongue. Therefore, thyroglossal duct anlage was considered as a potential source of origin of this malignancy [1]. Since then, more that 40 well-documented cases have been described in the English literature [3, 4, 5, 6, 7, 8, 14, 15, 16]. In Poland the first case of this tumor was described by Borowski-Borowy *et al*. in 2011 [5]. Most cases were located at the base of the tongue, but some developed at other sites populated by minor salivary glands. It is currently believed that they are the site of origin of this neoplasm. This hypothesis is supported by the fact that, until now, no CAT has developed in a large salivary gland. Therefore, a new term widely used in the literature is cribriform adenocarcinoma of a minor salivary gland (CAMSG) [4, 10].

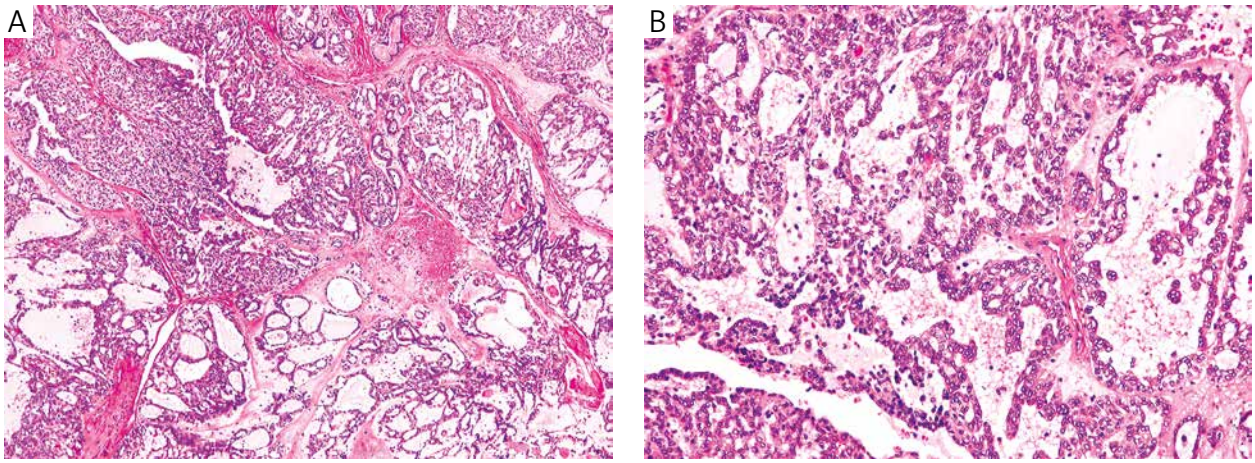

Fig. 2. The tumor is composed of lobules predominantly of cribriform architectural pattern divided by thin fibrous septa (HE, magnification 20×) (A). Cystically dilated spaces contain papillary structures and are filled with delicate pale pink proteinaceous material (HE, magnification 100×) (B)

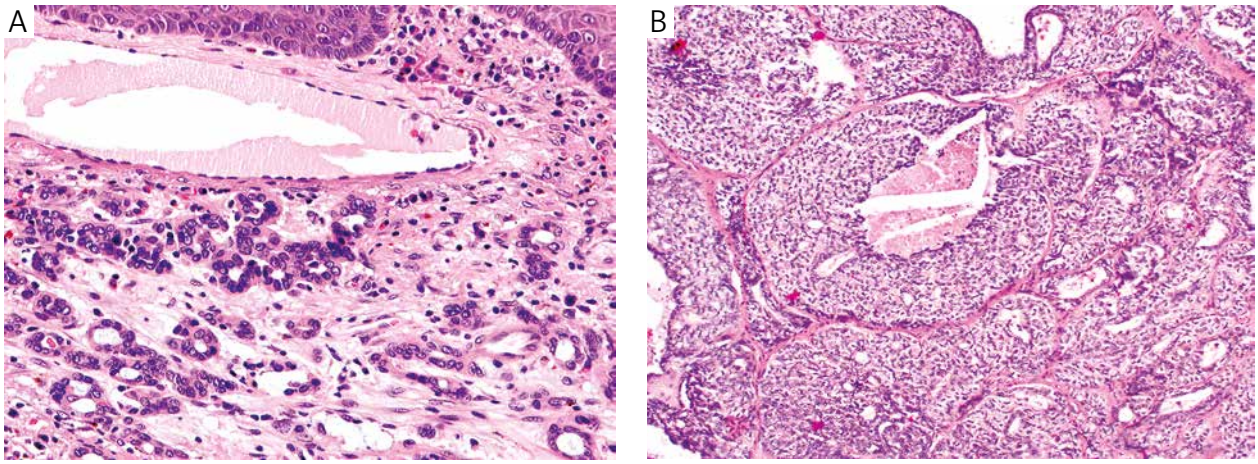

Fig. 3. Tubular architectural pattern (HE, magnification 200×) (A) and focal necrosis within the solid nests (HE, magnification 100×) (B)

CAMSG is a malignant but not a highly aggressive neoplasm. Clinically, it is an infiltrative, poorly circumscribed lesion, presenting as a submucosal tumor. All but one CAMSG developed in adults, with a wide age range (from 21 to 85 years of age) [1, 5, 6, 7, 15, 16]. Recently, a CAT in a 13-year-old patient was reported [15]. CAMSG does not show any gender predilection. More than half of patients had cervical lymph node metastases at the time of diagnosis and one had a recurrent disease. Including our case, only four patients developed bilateral metastases. Generally, early clinical metastasis to cervical lymph nodes is a consistent clinical finding. Clinical follow-up ranged from 2 months to 13 years. None of the patients developed distant metastases. In the case of positive surgical margins, patients had a higher risk of developing recurrences (2 patients, including our case).

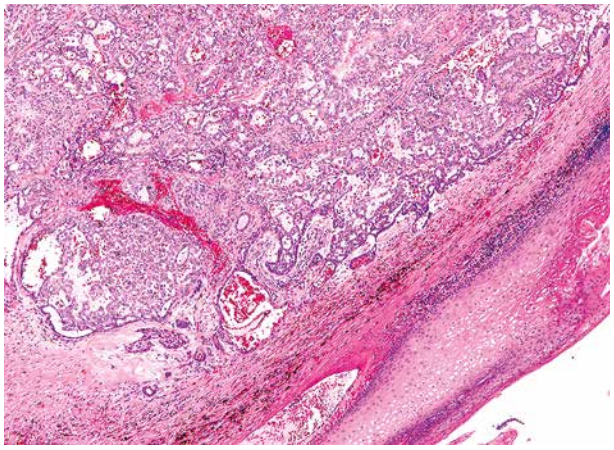

Fig. 4. The lesion is covered by non-ulcerated intact squamous epithelium (HE, magnification 20×)

The overall survival of our patient after the primary surgery was approximately seven years, and it is consistent with the literature data. Moreover, he developed bilateral metastases, probably due to the large size of the tumor, widely crossing the midline.

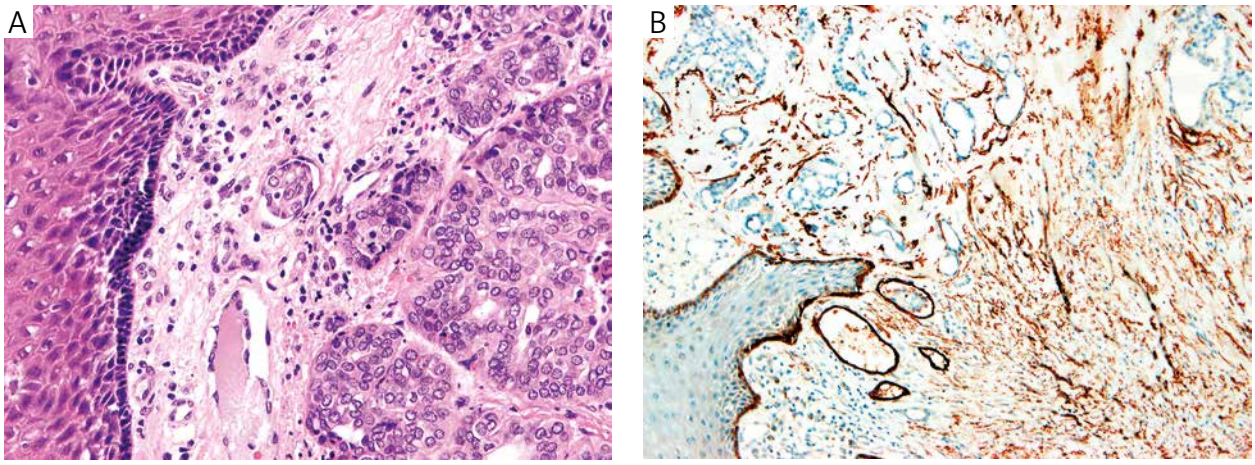

Fig. 5. Neoplastic emboli are found in the lumen of lymphatic vessels beneath the epithelium (HE, magnification 200×) (A); immunohistochemical staining with podoplanin (D 2-40) magnification 100×) (B)

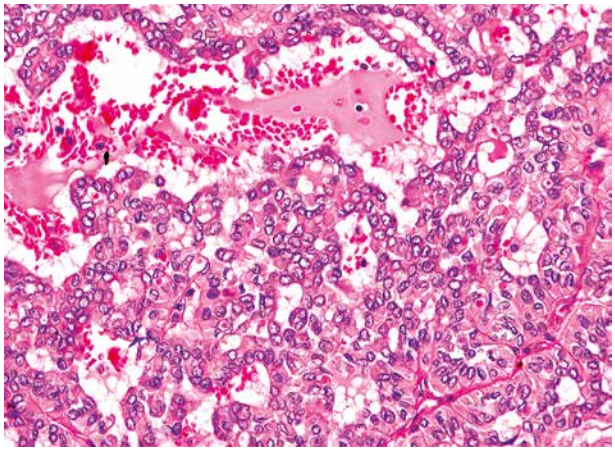

Fig. 6. The characteristic appearance of "papillary thyroid carcinoma-like" ground glass and overlapping nuclei (HE, magnification 400×)

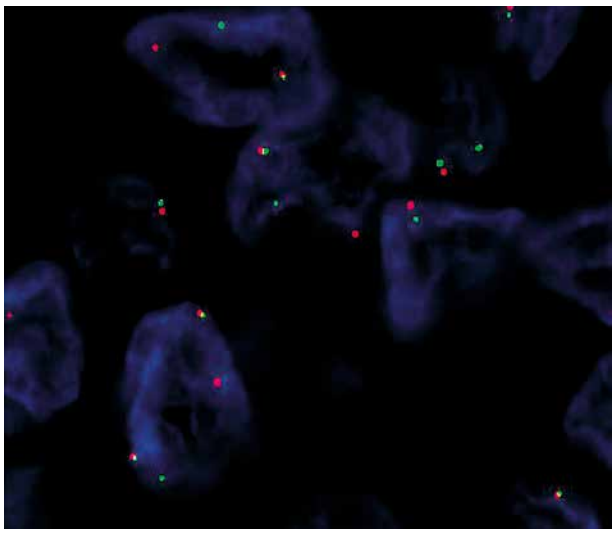

Fig. 7. Fluorescent *in situ* hybridization with PRKD3 break apart probe. Nuclei with split red and green signals indicate PRKD3 break. Chromosomes with normal PRKD3 gene show overlapping green and red signal

None of the patients except the one described in this paper died in the course of CAMSG.

Most patients with positive surgical margins and/or cervical lymph node metastases received complementary radiotherapy [4, 7, 8, 15]. Only two patients received adjuvant chemotherapy with cisplatin [3, 8]. The treatment of CAMSG should aim toward complete surgical excision, supplemented by lymphadenectomy if dissemination is evident.

Differential diagnosis of CAMSG includes papillary thyroid carcinoma (PTC, either metastatic or developing within the lingual thyroid), polymorphous low grade adenocarcinoma (PLGA) and adenoid cystic carcinoma [1, 3, 4, 6, 9].

CAMSG was initially described as a variant of polymorphous low-grade adenocarcinoma (PLGA) [1, 2]. Not surprisingly, both neoplasms share morphological similarities [2, 11, 17]. However, PLGA has more diverse histology than CAMSG and lacks the nuclear features described above. In addition, the prominent targetoid pattern of growth is typical for PLGA, not for CAMSG. Finally, early involvement of the cervical lymph nodes is not a common feature in PLGA, whereas they are frequently observed in CAMSG, often at presentation. Nevertheless, in a recent multicenter study the authors found a number of cases with overlapping or unusual morphology [11]. *PRKD1* activating hot-spot mutation encoding p.Glu710Asp was found to be specific for PLGA and might be useful as an ancillary diagnostic marker to differentiate PLGA from other salivary gland tumors [18]. Moreover, Weinreb *et al.* found that 75% of cases (15/20) of CAMSGs harbor recurrent translocations in the PRKD gene family [11]. Unfortunately, this translocation cannot be treated as specific for CAMSGs, as nearly half of tumors showing morphological overlap between CAT and PLGA also had such translocations. In contrast, only one of 18 typical PLGAs, with unusual localization in the

parotid gland, revealed the translocation in *PRKD2*. Thus, the association of CAMSG morphology with the presence of *PRKD* gene rearrangements will require more studies.

Our case harbored *PRKD3* translocation confirmed by FISH. The PRKD gene family has not been implicated in translocation/fusion previously, and very little is known of its role in oncogenesis [11]. When activated, the pro-oncogenic role of protein kinase D family members may be in signal transduction, apoptosis, migration, cell adhesion, differentiation and proliferation [19], and thus, they may potentially serve as targets for future drug therapies.

The histological picture of CAMSG is quite characteristic, with cribriform, microcribriform, papillary and solid areas divided into lobular or cystic spaces by fibrous septa [1]. In some cases, a clear cell change, peripheral palisading or mucinous myofibroblastic stromal septa are found [9]. The most prominent feature of the tumor is the characteristic appearance of nuclei [10]. The tumor is rather monomorphous. The cells have pale to eosinophilic cytoplasm, with clear "Orphan Annie eye-like" nuclei that overlap each other. The cystic spaces of CAMSG may also contain pale proteinaceous material that may simulate colloid. Psammoma bodies have also been described. All these features result in close resemblance to PTC [20]. Moreover, PTC may develop early cervical lymph node metastases. Thus, PTC is an important entity that should be distinguished from CAMSG. The most important differentiating feature is the lack of thyroglobulin and thyroid transcription factor 1 (TTF1) protein expression in CAMSG. Ultrastructural studies may also provide evidence of myoepithelial differentiation in CAMSG, which is not present in PTC [1, 9, 20].

Another lesion requiring distinction from CAMSG is adenoid cystic carcinoma (AdCC). In contrast to "PTC-like nuclei" typical for CAMSG, AdCC has hyperchromatic and angulated nuclei [17]. AdCC generally reveals diffuse and intense CD117 immunohistochemical staining. Moreover, the number of mitotic figures is higher and the proliferative index Ki-67 is greater than 20% in AdCC, whereas in CAMSG mitotic figures are rare and Ki67 index is usually low [4].

The initial diagnosis in this case was pleomorphic adenoma. Michal *et al*. in their recent review described mucinous spindle cell myofibroblastic stromal septa, found in one third of cases [1]. The initial biopsy of our case showed edema of the tissue and copious mucinous matrix with rare spindle myofibroblasts (see also Fig. 2B). This partly explains the misdiagnosis. Consequently, cellular pleomorphic adenoma should be excluded in the differential diagnosis of CAMSG, especially in small surgical biopsies.

CAMSG is a rare entity, the number of published cases is low, and the case series mostly rely on multi-institutional collaboration. According to Laco *et al*., the relative frequency of this malignancy is 0.5-1% [8]. We found a similar incidence in our series (0.5%). We therefore think it is valuable to report every case of this rare tumor to support its separation as a distinct entity.

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The authors declare no conflict of interests.

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