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Original Article

Mammary analog secretory carcinoma of salivary glands: A report of 2 cases with expression of basal/myoepithelial markers (calponin, CD10 and p63 protein)

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ABSTRACT

Mammary analog secretory carcinoma (MASC) of salivary glands is a recently described neoplasm with favorable outcome. We describe 2 cases of MASC occurring in a 34-year-old female and a 58-year-old male, both presenting with a swelling of upper lip and right parotid gland, measuring 15 and 20 mm, respectively. Without adjuvant treatment, both patients have been free of disease for 15 months and 12 months since the operation. Microscopically, both tumors were cystic and showed tubular and cystopapillary architecture. The tumor cells had round to oval nuclei and eosinophilic cytoplasm. Presence of eosinophilic material was evident within cystic spaces. Immunohistochemically, both tumors expressed cytokeratins (CK), CK7, CK8, CK18, epithelial membrane antigen, vimentin, S-100 protein, mammaglobin, and STAT5a (signal transducer and activator of transcription 5a). Interestingly, both tumors showed variable expression of basal/myoepithelial markers. In one case, we observed diffuse expression of calponin and focal expression of p63 whereas expression of CD10 was absent. In the second case, the staining of calponin was negative, but there was focal expression of both p63 and CD10. Both neoplasms harbored the *ETV6-NTRK3* fusion transcript as proved by RT-PCR. Although previously reported only rarely, we conclude that MASC may show expression of basal/myoepithelial markers.

Introduction

Mammary analog secretory carcinoma (MASC) of salivary glands is a distinctive tumor entity that was first described by Skálová et al. in 2010 [19]. Since that time, several smaller series or case reports have been published [4,6,8,9,17]. As the name implies, there is a significant morphological overlap between MASC and secretory (juvenile) carcinoma of the breast (SCB), which is a rare tumor occurring mainly but not exclusively in young girls and carrying favorable prognosis [20]. Microscopically, both MASC and SCB are composed of cells with low-grade vesicular nuclei and eosinophilic or vacuolated cytoplasm, arranged in tubular, microcystic, and solid growth patterns. Furthermore, both tumors are S-100 protein, epithelial membrane protein and vimentin-positive and "triple negative" (ER/PR/Her2) [7,19,20]. SCB is the only tumor among breast carcinomas that harbors a recurrent balanced chromosomal translocation, t(12;15)(p13;q25), creating an *ETV6-NTRK3* fusion gene encoding a chimeric tyrosine kinase [12,21]. The same genetic alteration has been identified in all MASC cases analyzed so far, but it was not found in any of the other salivary gland tumor types, including acinic cell carcinoma [4,6,8,9,17,19].

According to the original paper on MASC by Skálová et al. [19], serous acinar differentiation of tumor cells was not seen, and expression of basal cell and/or myoepithelial markers, including calponin, cytokeratin (CK)5/6, CK14, p63 protein (p63) and smooth muscle actin (SMA), was consistently absent. However, others have noticed occasional presence of tumor cells with scant zymogen granules [9] or reported focal expression of high molecular weight-CK (HMW-CK) and CK5/6 [6,8]. These findings indicate that the morphological appearance and immunohistochemical profile of MASC may be variable to some extent, making the differential diagnosis difficult.

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The aim of our report is to describe 2 new cases of MASC, both showing variable expression of basal/myoepithelial markers.

Clinical data

Case 1

A 34-year-old female with an unremarkable personal history presented with a slow-growing painless swelling of the upper lip lasting for 2 years. Clinical examination showed a soft freely movable nodule measuring 15 mm; the overlying skin and oral mucosa displayed no pathological changes. The lesion was excised with negative margins and sent for microscopic examination with the diagnosis "mucocele". After achieving the final diagnosis, ultrasound examination of the neck was perfomed, revealing no cervical lymphadenopathy. The tumor was staged pT1 cN0. Adjuvant chemo/radiotherapy was not indicated. The patient has been free of disease for 15 months since the operation.

Case 2

A 58-year-old obese male with arterial hypertension and bronchial asthma presented with a slowly enlarging painless lesion in the right parotideomasseteric area lasting for "several years". Clinical examination showed a freely movable soft nodule measuring 20 mm; the overlying skin displayed no pathological changes, and facial paralysis was absent. Computed tomography revealed in the parotid gland a tumor measuring 20 mm in greatest diameter; cervical lymphadenopathy was absent. Examination by fine needle aspiration cytology was not performed. The lesion was removed with negative margins and sent for microscopic examination with the diagnosis "pleomorphic adenoma or acinic cell carcinoma". After microscopic examination, the tumor was staged pT1 cN0. Adjuvant chemo/radiotherapy was not indicated. The patient has been free of disease for 12 months since the operation.

Materials and methods

The tissue specimens were immediately fixed in 10% neutral formalin, routinely processed, embedded in paraffin, and stained with hematoxylin eosin.

Immunohistochemical examination

Four μm-thick sections were cut from paraffin blocks, mounted on slides coated with 3-aminopropyltriethoxy-silane, deparaffinized in xylene, and rehydrated in descending grades (100%–70%) of ethanol. The primary antibodies used are listed in Table 1. The staining for mammaglobin was done manually. Antigen retrieval was performed in a water bath for 40 min at 97 °C at pH 9.9 (buffer S3308). Endogenous peroxidase activity was inhibited by immersing the sections in 3% hydrogen peroxide. After incubation with the antibody, the sections were subjected to EnVision + Dual Link System-HRP (Dako, Glostrup, Denmark). Finally, the reaction was visualized using 3-3′-diaminobenzidine, and the slides were counterstained with hematoxylin. The staining of all remaining antibodies was performed using immunostainer BenchMark Ultra (Roche, Basel, Switzerland), with Ultra View Universal DAB Detection Kit and Bluing Reagent as visualization reagent and chromogen. Appropriate positive and negative controls were used.

Reverse transcription polymerase chain reaction (RT-PCR)

RNA from the formalin-fixed and paraffin-embedded tissue was extracted using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion, Austin, TX). cDNA was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (RNA input 1 μg) (Roche Diagnostics, Mannheim, Germany). All procedures were carried out according to the manufacturer's protocols. Amplification of a 105-bp product of the β2-microglobulin gene, of a 126-bp product of the PBGD gene, and of a 247-bp product of PGK gene was used to test the quality of the extracted RNA as described earlier [22]. A detection of 110-bp fragment of *ETV6-NTRK3* fusion transcript was carried out according to the method described by Bourgeois et al. [2].

Briefly, 2 μL of cDNA was added to reaction consisting of 12.5 μL of HotStar Taq PCR Master Mix (QIAgen, Hilden, Germany), 10 pmol of each primer (TRKC1059 complementary to NTRK3 with sequence 5′-CAGTTCTCGCTTCAGCACGATG-3′ and TEL971 complementary to ETV6 with sequence 5′-ACCACATCATGGTCTCTGTCTCCC-3′), and distilled water up to 25 μL. The amplification program comprised denaturation at 95 °C for 14 min and then 45 cycles of denaturation at 95 °C for 1 min, annealing at 65 °C for 1 min and extension at 72 °C for 1 min. The program was finished by incubation at 72 °C for 7 min.

Successfully amplified RT-PCR products of the *ETV6-NTRK3* fusion transcript were purified with Montan PCR Centrifugal Filter Devices (Millipore, Billerica, MA). Then, PCR products were sequenced using a Big Dye Terminator Sequencing Kit (Applied Biosystems, Forster City, CA), ran on an automated genetic analyzer ABI Prism 3130X1 (Applied Biosystems) at a constant voltage of 13.2 kV for 20 min and compared to the GeneBank sequence.

Fluorescence in situ hybridization (FISH)

From each tumor and a normal control, 4 μm-thick sections were cut from paraffin embedded blocks and placed on slides. Tissues were deparaffinized in xylene 3 times for 5 min and then washed twice in 100% etanol, once in 95% etanol and once in deionized water for 5 min. Then, the slides were heated in the 1× Target Retrieval Solution (pH 6.0) (Dako, Glostrup, Denmark) for 40 min at 95 °C and subsequently cooled for 20 min at room temperature (RT) in the same solution. The slides were washed in deionized water for 5 min, and tissues were covered with the Proteinase K (20 mg/mL) (SERVA, Heidelberg, Germany) for 10 min at RT. The slides were then placed into deionized water for 5 min, dehydrated

Table 1
List of primary antibodies.

Antibody	Clone	Dilution	Source
Calponin	CALP	1:500	Dako
CD10	56C6	1:10	Novocastra
CK	AE1/AE3	1:100	Dako
CK5/6	D5/16 B4	1:100	Dako
CK7	OV-TL 12/30	1:50	Dako
CK8	35βH11	1:1	Dako
CK18	DC 10	1:50	Dako
CK19	RCK 108	1:100	Dako
CK20	K_s 20.8	1:25	Dako
EMA	E29	1:800	Dako
GCDFP-15	EP1582Y	pre-diluted	Ventana
Ki-67	MIB-1	1:50	Dako
Mammaglobin	304-1A5	1:200	Dako
S-100 protein	polyclonal	1:1500	Dako
SMA	1A4	pre-diluted	Ventana
STAT5a	polyclonal	1:400	Assay Designs Inc.
p63 protein	4A4	pre-diluted	Ventana
Vimentin	V9	1:400	Dako

Abbreviations: CK = cytokeratin; EMA = epithelial membrane antigen; GCDFP-15 = gross cystic disease fluid protein-15; SMA = smooth muscle actin; STAT5a = signal transducer and activator of transcription 5a.
Sources: Assay Designs Inc. (Ann Arbor, USA), Dako (Glostrup, Denmark); Novocastra/Leica Biosystems (Newcastle Upon Tyne, UK); Ventana/Roche (Basel, Switzerland).