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ORIGINAL ARTICLE

DOG1, p63, and S100 protein: a novel immunohistochemical panel in the differential diagnosis of oncocytic salivary gland neoplasms in fine-needle aspiration cell blocks

Alessandra C. Schmitt, MD*, Ryan McCormick, MD, Cynthia Cohen, MD, Momin T. Siddiqui, MD

Emory University School of Medicine, Pathology, Atlanta, Georgia

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Introduction DOG1 is a calcium-activated chloride channel protein that may have a potential role in secretory cells of salivary glands and tumors derived from them. Its role in cytologic specimens is not well documented. This study was performed to evaluate its utility in separating acinic cell carcinoma (AciCC) from other closely related differential diagnoses on cytologic samples. In addition, an immunohistochemical panel consisting of DOG1, p63, and S100 protein to assist in the subclassification of these salivary gland neoplasms with oncocytic differentiation was also investigated.

Materials and methods Thirty-one fine-needle aspiration cell blocks (CBs) of oncocytic salivary gland neoplasms (16 Warthin tumors [WTs], 10 AciCCs, 3 mucoepidermoid carcinomas [MECs], and 2 oncocytomas [ONCs]), and 75 salivary gland resections (7 WTs, 27 AciCCs, 36 MECs, 2 high-grade adenocarcinomas, 2 ONCs, 1 papillary cystadenoma) were immunostained for DOG1, p63, and S100.

Results DOG1 and p63 were very useful in distinguishing AciCC from WT on CB, because 100% of WTs were DOG1-negative and 87.5% were p63-positive, whereas 70% of AciCCs were DOG1-positive and 100% were p63-negative. The resection results correlated with those on CBs: 100% of WTs were DOG1-negative and 86% were p63-positive, whereas 93% of AciCCs were DOG1-positive and 89% were p63-negative. S100 and DOG1 were negative in both WTs and ONCs, with <10% S100 positivity in AciCCs.

Conclusions DOG1 was very helpful in separating AciCC from WT, MEC, and ONC. In summary, an immunohistochemical panel including DOG1, p63, and S100 can significantly improve the accuracy of diagnosing oncocytic salivary gland neoplasms on CBs.

*Corresponding author: Alessandra C. Schmitt, MD, Grady Memorial Hospital, 80 Jesse Hill Jr Drive, Room 1C065, Atlanta, GA 30303. Tel.: 404.616.7436; Fax: 404.616.9084.

E-mail address: alessandra.schmitt@emory.edu (A.C. Schmitt).

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Introduction

Fine-needle aspiration (FNA) of salivary gland tumors are very commonly performed and frequently present significant diagnostic challenges. Given the differences in clinical management, it is important to determine on FNA whether the lesion is benign or malignant; however, in many salivary gland FNAs a specific diagnosis is not possible.

Salivary gland neoplasms with oncocytic features are commonly identified and include lesions such as Warthin tumor (WT), acinic cell carcinoma (AciCC), mucoepidermoid carcinoma (MEC), and oncocytoma (ONC) in the differential diagnosis.

WT and ONC are common causes of a false-positive diagnosis in salivary gland FNAs, with 8% and 18% false-positive diagnoses, respectively.[1] WT is occasionally misinterpreted as lymphoma, AciCC, and MEC, whereas ONC is often misdiagnosed as AciCC.[1] Such high false-positive rates can lead to more aggressive surgical treatment in patients that otherwise could have been managed clinically.

AciCC and MEC are among the most common causes of a false-negative diagnosis in salivary gland FNA, 43% and 49% respectively.[1] AciCC is most often misdiagnosed as benign salivary gland, sialadenosis, and WT,[1] but it can also be mistaken for MEC. Low-grade MECs are often misdiagnosed as benign cysts, and high-grade MECs can be misdiagnosed as ONCs.

DOG1 is a calcium-activated chloride channel protein, which may have a potential role in secretory cells of salivary glands and tumors derived from them.[2] Its role in cytologic specimens is not well documented. This study was performed to evaluate its role in detecting AciCCs, which are derived from acinic secretory cells, and to determine whether it has any utility in separating AciCC from other closely related differential diagnoses on cytology samples. Previous studies have shown that AciCCs are DOG1-positive in 50% to 100% of cases, whereas only rare mammary analog secretory carcinomas (MASCs) are DOG1-positive.[2-5]

The opposite staining pattern is usually observed with S100 protein, with nearly all conventional AciCCs staining negative and 100% of MASCs staining positive.[3] In order to flag possible MASC cases, S100 antibody was added to our study.

Frequently, p63, a p53 homologue required for limb and epidermal morphogenesis, is used as a basal and myoepithelial marker. It is expressed in basal and myoepithelial cells of normal and tumor salivary gland tissues.[6] Bilal et al[6] and Weinreb et al[7] noted it to be strongly positive in 100% of MECs, and recently, p63 has been described as a useful marker in the differential diagnosis of AciCC (p63-negative) and MEC (p63-positive). Additionally, p63 is described as positive in MASC, WT, and ONC,[8,9] further assisting in the differential with AciCC (p63-negative).

To our knowledge, this is the first study to investigate the role of an immunohistochemical (IHC) panel consisting of DOG1, p63, and S100 in cell blocks in an attempt to increase the diagnostic accuracy of oncocytic neoplasms on FNA. Surgical specimens were also immunostained to confirm cytologic findings.

Materials and methods

Patients and specimens

Investigational Review Board permission was obtained, and the cytopathology and surgical pathology files of the Emory University Hospitals were searched for salivary gland tumors with oncocytic differentiation. A total of 31 FNA cell blocks (CBs) of oncocytic salivary gland neoplasms (16 WTs, 10 AciCCs, 3 MECs, and 2 ONCs), and 75 salivary gland resections (7 WTs, 27 AciCCs, 36 MECs, 2 high-grade adenocarcinomas, 2 ONCs, 1 papillary cystadenoma), were identified.

Immunohistochemistry

Immunohistochemistry for DOG1, p63, and S100 was performed on 31 paraffin-embedded CB sections and 75 salivary gland resections.

DOG1 IHC

For cytology CB specimens and surgical resections, 5-μm sections of formalin-fixed paraffin-embedded tissue were tested for the presence of primary antibody using DAKO Envision+ dual link system, which is a horseradish peroxidase−labeled polymer (DAKO, Carpinteria, Calif.), with heat-induced antigen retrieval.

The sections were deparaffinized and rehydrated to deionized water. They were then heated in citrate buffer (pH 6.0), using an electric pressure cooker for 3 minutes at 12 to 15 pounds per square inch (approximately 120°C), and cooled for 10 minutes prior to immunostaining.

All slides were loaded on an automated system (DAKO AutoStainer plus) and exposed to 3% hydrogen peroxide for 5 minutes, incubated with primary antibody rabbit monoclonal (SP31) (DOG1; Cell Marque, Rocklin, Calif.) (1:40) for 30 minutes, with labeled polymer (Envision+ dual link) for 30 minutes, 3,3'-diaminobenzidine (DAB) as chromogen for 5 minutes, and hematoxylin as counterstain for 5 minutes. These incubations were performed at room temperature; between incubations, sections were washed with tris-buffered saline (TBS). Cover-slipping was performed using the Tissue-Tek SCA (Sakura Finetek USA, Inc, Torrance, Calif.) coverslipper. Positive control samples of known positive tissues (gastrointestinal stromal tumors) and negative control samples with primary antibody replaced with TBS were run with the patient/study slides.

p63 and S100 IHC

Sections (μm) of formalin-fixed, paraffin-embedded tissue were tested for the presence of each antigen using Bond Polymer Refine Detection Kit (DAB chromogen; Leica Microsystems, Bannockburn, Ill.). The detection system avoids the use of streptavidin and biotin and therefore eliminates nonspecific staining as a result of endogenous biotin.

All steps were performed on the Leica Bond Maxx III automated system. Specimens were deparaffinized and those for p63 only were antigen-retrieved on the instrument. All slides were incubated with p63 antibody (p63; Biocare Medical, Concord, Calif.) (1:100), or S100 (DAKO) (1:12,000) for 15 minutes, with postprimary polymer for 8 minutes, blocked with 3% hydrogen peroxide for 5 minutes DAB (brown chromogen) for 10 minutes, and hematoxylin as counterstain for 5 minutes. These incubations were performed at room temperature; between incubations, sections were washed with TBS (Bond wash solution). Cover-slipping was performed using the Tissue-Tek SCA coverslipper. Positive control samples of known positive tissues (colorectal adenocarcinoma and melanoma, respectively) and negative control samples with primary antibody replaced with TBS were run with the patient/study slides.

Scoring

Cytology and resection cases were scored as positive for DOG1 (membranous and/or cytoplasmic), p63 (nuclear), and S100 (nuclear and cytoplasmic), when staining was present in ≥5% of cells. Immunostaining was graded as weak (1+), moderate (2+), and intense (3+).

Results

Table 1 presents the IHC results of DOG1, p63, and S100 protein in CBs of oncocytic salivary gland neoplasms, and Table 2 shows the results in resections.

Fig. 1 shows DOG1 staining in AciCC and p63 staining in WT and MEC.

DOG1 and p63 were very useful in distinguishing AciCC from WTs on CBs, because 100% of WT were DOG1-negative and 87.5% were p63-positive, whereas 70% of AciCCs were DOG1-positive and 100% were p63-negative.

The results on resections correlated with those on CBs: 100% WTs were DOG1-negative and 86% were p63-positive, whereas 93% of AciCCs were DOG1-positive and 89% were p63-negative. S100 and DOG1 were negative in both WTs and ONCs, with <10% S100 positivity in AciCCs.

Strong nuclear p63 was present in 100% of CB MECs and in 97% of surgically resected MECs, which aided in the differential with AciCC, because all AciCCs were p63-negative, both on CBs and surgical resections.

The pitfalls of DOG1 staining identified in this study when trying to diagnose AciCC include focal faint apical-luminal membranous staining in normal salivary gland acini (Figs. 2A and 3A) and focal faint membranous staining in 53% of surgically resected MECs (Fig. 2B). All CB samples of MECs were DOG1-negative. In our study, DOG1-positive MECs were more likely to be of intermediate or higher grades.

In contrast to the weak membranous staining in benign salivary gland acini (Figs. 2A and 3A), DOG1 staining in AciCC was moderate to strong, diffuse, crisp, membranous, and often cytoplasmic in distribution (Figs. 1A and 3B).

Table 1 Immunohistochemical results of DOG1, p63, and S100 protein in CBs of oncocytic salivary gland neoplasms.

Result (*n*) *N* = 31	DOG1-positive	p63-positive	S100-positive
AciCC (10)	7/10 (70)	0/10 (0)	0/10 (0)
WT (16)	0/16 (0)	14/16 (87.5)	0/16 (0)
MEC (3)	0/3 (0)	3/3 (100)	0/3 (0)
ONC (2)	0/2 (0)	1/2 (50)	0/2 (0)

Abbreviations: AciCC, acinic cell carcinoma; CB, cell block; MEC, mucoepidermoid carcinoma; ONC, oncocytoma; WT, Warthin tumor.
Values are *n/n* (%).

Discussion

Salivary glands are very common sites for superficial FNAs and the differential diagnosis usually includes benign and malignant lesions. Sensitivity and specificity for salivary gland FNAs range from 64% to 95% and 86% to 99.5%, respectively.[10] High positive predictive value for benign tumors and negative predictive value for malignancy is very helpful in the management of patients that may not be good surgical candidates.[10]

Oncocytic features are commonly identified in salivary gland neoplasms, which include WT, AciCC, MEC, and ONC.

WT cytomorphology is usually straightforward; however, occasional findings can cause diagnostic challenges, such as cellular degeneration, necrotic background, squamous metaplasia, and lack of the most classic WT morphology.[11]

Table 2 IHC results of DOG1, p63, and S100 protein in surgical resections of oncocytic salivary gland neoplasms

Results (*n*) *N* = 75	DOG1-positive	p63-positive	S100-positive
AciCC (27)	25/27 (93)	3/27 (11)	2/27 (7)
WT (7)	0/7 (0)	6/7 (86)	0/7 (0)
MEC (36)	19/36 (53)	35/36 (97)	0/36 (0)
ONC (2)	0/2 (0)	2/2 (100)	0/2 (0)
ADC (2)	0/2 (0)	1/2 (50)	0/2 (0)
PC (1)	1/1 (100)	1/1 (100)	0/1 (0)

Abbreviations: ADC, adenocarcinoma; IHC, immunohistochemical; PC, papillary cystadenoma; other abbreviations as in Table 1.
Values are *n/n* (%).

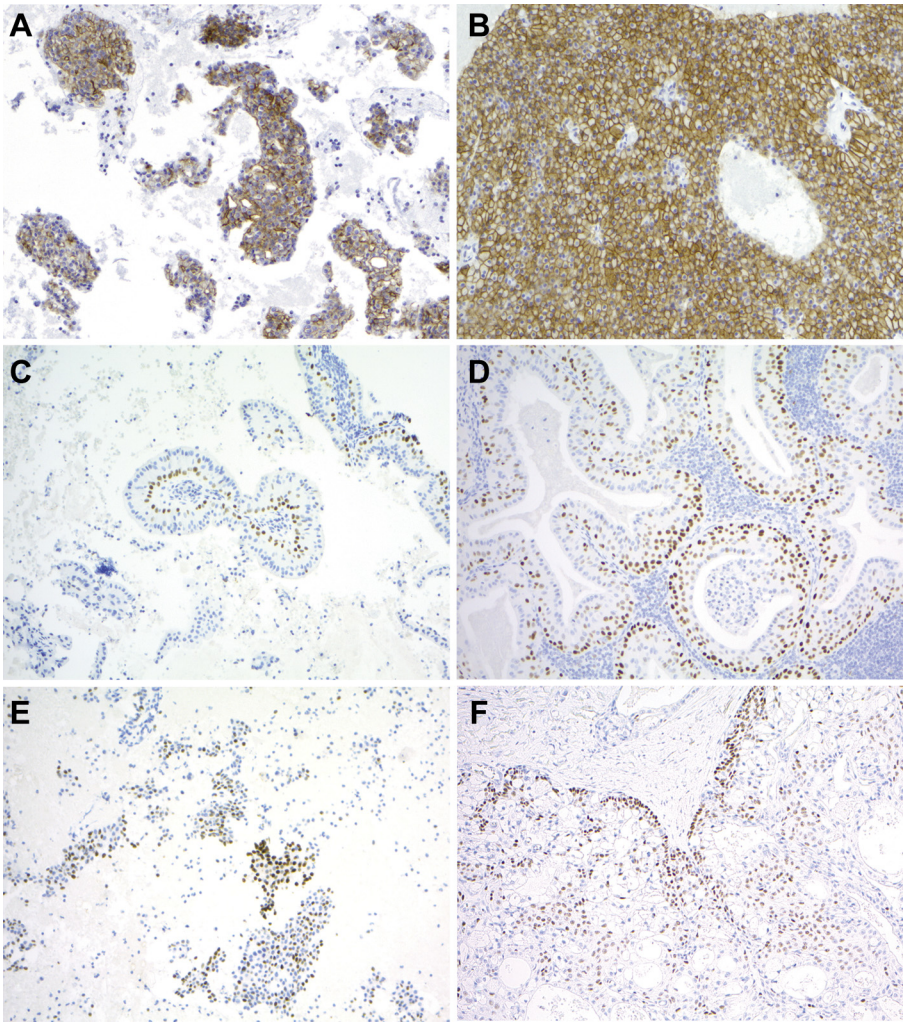

Figure 1 DOG1 and p63 immunohistochemical staining are shown. A, Acinic cell carcinoma DOG1-positive staining cell blocks (CBs) (×100). B, Acinic cell carcinoma DOG1-positive staining surgical resection (×100). C, Warthin tumor p63-positive staining CBs (×100). D, Warthin tumor p63-positive staining surgical resection (×100). E, Mucoepidermoid carcinoma p63-positive staining CBs (×100). F, Mucoepidermoid carcinoma p63positive staining surgical resection (×100).

AciCC can be mistaken for WT when prominent lymphoid stroma is present. Rarely, AciCC may contain secretory material and may show aberrant mucin production and cystic change mimicking MEC and/ or MASC.

This study used an IHC panel consisting of DOG1, p63, and S100 protein to more accurately subclassify salivary gland neoplasms with oncocytic differentiation on FNAs.

Strong DOG1-positive staining ruled out most of the benign entities in the oncocytic salivary gland neoplasm group. In contrast to the weak membranous apical-luminal staining in benign salivary gland acini, DOG1 staining in AciCC was moderate to strong, diffuse, crisp, membranous, and often cytoplasmic. A similar pattern of DOG1 staining was also noted by the study of Chênevert et al (DOG1 Clone 1.1; Zeta Co, Sierra Madre, Calif.) (1:50).[2] These staining pattern results differ from those of Hemminger et al,[4] in which a pure luminal pattern of staining was observed (DOG1, Leica catalogue number NCL-L-DOG1; mouse monoclonal clone K9). The differences in DOG1-staining patterns could be explained by the use of different DOG1 clones.[2,4] We used a rabbit monoclonal antibody (SP31; Cell Marque).

The distinct pattern of staining for DOG1 in normal acini (weak membranous, apical luminal) compared with AciCC (moderate to strong, diffuse membranous to cytoplasmic) is helpful in the differential diagnosis; however, caution is always advised in the interpretation of paucicellular CBs and IHC results should always be interpreted in conjunction with morphology (Figs. 3A and 3B).

Negative DOG1 staining in all WTs and all ONCs and focal staining in MECs noted in our study was also observed by other studies.[2]

Negative p63 nuclear staining was noted in 100% of AciCCs on CBs and in 89% of AciCCs on surgical resections; these findings correlate with the literature, which indicates that up to 100% of AciCCs are p63-negative.[8] Strong nuclear p63 was present in 100% of CB MECs and

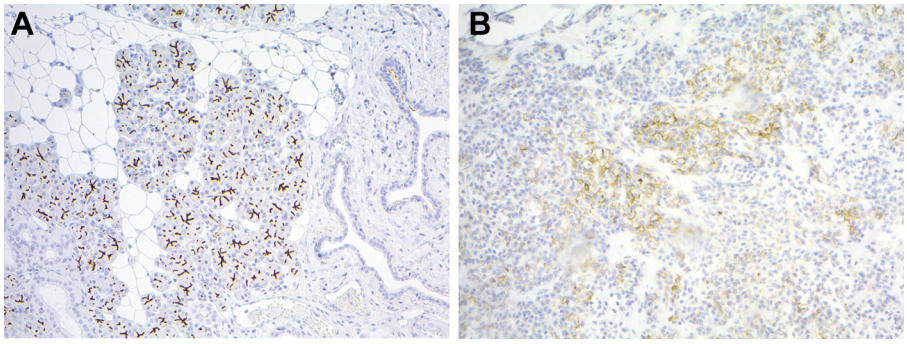

Figure 2 Pitfalls of DOG1 immunohistochemical staining are shown. A, Focal faint apical-luminal membranous DOG1 staining in normal salivary gland surgical resection (×100). B, Focal faint membranous DOG1 staining in surgically resected mucoepidermoid carcinoma (×100).

in 97% of surgically resected MECs, which also correlated with the study of Sams et al[8] that demonstrated 100% nuclear positive staining.

None of the benign oncocytic neoplasms were immunoreactive for S100. On the other hand, an S100+ DOG1-negative oncocytic neoplasm should raise the possibility of MASC.[5] The lack of DOG1 staining in MASCs is attributed to the lack of true serous acinar differentiation in these tumors.[2]

In our study, the only 2 DOG1-negative AciCC resection cases were also noted to be S100-positive and p63-positive, which raised suspicion of misdiagnosed MASCs. On the second review, these 2 tumors also lacked overt cytoplasmic basophilic zymogen granules noted in classic AciCC and were also morphologically suspicious for MASC. Additional IHC testing was performed on these 2 cases; however, in 1 of the cases, due to the small tumor size, no residual tumor was available for further studies. The second DOG1-negative, S100-positive, p63-positive AciCC morphologically suspicious for MASC was mammaglobin and MUC4-positive and GCDFP-15-negative. Therefore, this tumor should be best interpreted as MASC. Although testing positive for the *ETV6-NTRK3* translocation by fluorescence in situ hybridization is the most reliable way to subclassify these tumors as MASC, there are no significant prognostic differences between AciCC and MASC, and many experts currently believe that a suspicious morphology and a consistent IHC profile are sufficient evidence to subclassify such tumors as MASCs.[12-14]

The role of DOG1 in other groups of salivary gland neoplasms has been investigated in previous studies; however, among the basaloid salivary gland neoplasms, for example, it may not have the same impact in differentiating among benign and malignant lesions, because it is positive in most adenoid cystic carcinomas and in epimyoepithelial carcinomas, most cases of basal cell adenomas, and in a minority (8%) of pleomorphic adenomas.[2,15]

Conclusions

DOG1 and p63 were very helpful in separating AciCC from WT, MEC, and ONC on CBs and the results on surgical resections supported these findings. DOG1 is strongly positive in AciCC and is negative in WT and ONC; the opposite staining pattern is noted with p63.

Strong and diffuse DOG1 positivity in oncocytic salivary gland neoplasms virtually excludes the diagnosis of a benign lesion such as WT and ONC; however, negative DOG1 results do not ensure a benign diagnosis in this setting, because MEC

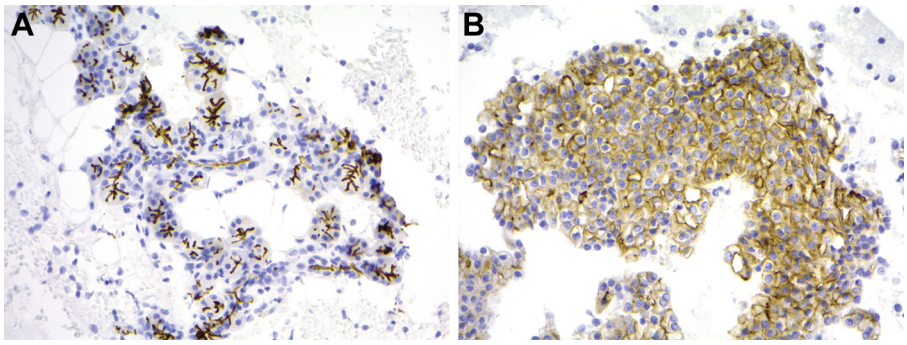

Figure 3 Comparison between DOG1 staining in normal salivary gland cell blocks (CBs) and acinic cell carcinoma CBs. A, Focal faint apical-luminal membranous DOG1 staining in normal salivary gland CBs (×200). B, Diffuse membranous and cytoplasmic DOG1 staining in acinic cell carcinoma CBs (×200).

can be DOG1-negative in approximately one-half of cases, and MASC is usually DOG1-negative.

The pitfalls to be aware of when using DOG1 to subclassify oncocytic salivary gland neoplasms include weak DOG1 staining in normal acini, negative or focal staining in one-half of MEC cases, and negative staining in MASC. Testing with S100 can be helpful to avoid misdiagnosing MASCs as benign oncocytic neoplasms as they share the DOG1-negative immunoprofile; however, none of the benign oncocytic neoplasms should be immunoreactive for S100.

In summary, an IHC panel consisting of DOG1, p63, and S100 appears to be useful to differentiate several benign from malignant oncocytic salivary gland neoplasms on CBs.

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Conflict of interest disclosures

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References

1. Hughes JH, Volk EE, Wilbur DC, for the Cytopathology Resource Committee, College of American Pathologists. Pitfalls in salivary gland fine-needle aspiration cytology: lessons from the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology. *Arch Pathol Lab Med*. 2005;129:26–31.
2. Chênevert J, Duvvuri U, Chiosea S, et al. DOG1: a novel marker of salivary acinar and intercalated duct differentiation. *Mod Pathol*. 2012;25:919–929.
3. Jung MJ, Song JS, Kim SY, et al. Finding and characterizing mammary analogue secretory carcinoma of the salivary gland. *Korean J Pathol*. 2013;47:36–43.
4. Hemminger J, Iwenofu OH. Discovered on gastrointestinal stromal tumours 1 (DOG1) expression in non-gastrointestinal stromal tumour (GIST) neoplasms. *Histopathology*. 2012;61:170–177.
5. Pinto A, Nosé V, Rojas C, Fan YS, Gomez-Fernandez C. Searching for mammary analogue [corrected] secretory carcinoma of salivary glands among its mimics. *Mod Pathol*. 2014;27:30–37.
6. Bilal H, Handra-Luca A, Bertrand JC, Fouret PJ. p63 is expressed in basal and myoepithelial cells of human normal and tumor salivary gland tissues. *J Histochem Cytochem*. 2003;51:133–139.
7. Weinreb H, Seethala RR, Perez-Ordoñez B, Chetty R, Hoschar AP, Hunt JL. Oncocytic mucoepidermoid carcinoma: clinicopathologic description in a series of 12 cases. *Am J Surg Pathol*. 2009;33:409–416.
8. Sams RN, Gnepp DR. P63 expression can be used in the differential diagnosis of salivary gland acinic cell and mucoepidermoid carcinomas. *Head Neck Pathol*. 2013;7:64–68.
9. Weiler C, Reu S, Zengel P, Kirchner T, Ihrier S. Obligate basal cell component in salivary oncocytoma facilitates distinction from acinic cell carcinoma. *Pathol Res Pract*. 2009;205:838–842.
10. Tryggvason G, Gailey MP, Hulstein SL, et al. Accuracy of fine-needle aspiration and imaging in the preoperative workup of salivary gland mass lesions treated surgically. *Laryngoscope*. 2013;123:158–163.
11. Kim JY, Yoo YS, Kwon JE, Kim HJ, Park K. Fine-Needle Aspiration Cytology with c-kit Immunohistochemical Staining in the diagnosis of Warthin's tumor. *Acta Cytol*. 2012;56:474–480.
12. Chiosea SI, Griffith C, Assaad A, Seethala RR. Clinicopathological characterization of mammary analogue secretory carcinoma of salivary glands. *Histopathology*. 2012;61:387–394.
13. Bishop JA. Unmasking MASC: bringing to light the unique morphologic, immunohistochemical and genetic features of the newly recognized mammary analogue secretory carcinoma of salivary glands. *Head Neck Pathol*. 2013;7:35–39.
14. Skálová A, Vanecek T, Sima R, et al. Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3fusion gene: a hitherto undescribed salivary gland tumor entity. *Am J Surg Pathol*. 2010;34:599–608.
15. Montalli VA, Martinez E, Tincani A, et al. Tubular variant of basal cell adenoma shares immunophenotypical features with normal intercalated ducts and is closely related to intercalated duct lesions of salivary gland. *Histopathology*. 2014;64:880–889.