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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

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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.

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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.