

# P63 Expression Can Be Used in Differential Diagnosis of Salivary Gland Acinic Cell and Mucoepidermoid Carcinomas

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**Abstract** Differentiation of salivary gland acinic cell carcinoma from mucoepidermoid carcinoma can be diagnostically challenging as both may have prominent mucin production. P63 is a p53 homologue required for limb and epidermal morphogenesis. It is expressed in basal and myoepithelial cells of normal salivary gland tissues. In this immunohistochemical study, we examined the expression of p63 in salivary gland acinic cell and mucoepidermoid carcinomas (MEC) and its use in differentiating these two entities. A search was performed and appropriate cases were selected from Lifespan Hospital System archives as well as the consult archives of one author (DRG). 31 salivary gland acinic cell carcinomas (ACC) and 24 MEC were examined for p63 expression by immunohistochemistry. The nuclear immunoreactivity was examined by both authors and was graded semi-quantitatively with negative being less than 10 % of cells staining. Positive staining was graded as follows: 10–25 % of tumor cells staining was weakly positive, 26–75 % of tumor cells staining was moderately positive, and 76–100 % of tumor cells staining was strongly positive. Negative nuclear staining of the tumor cells was seen in 30/31 (96 %) of salivary gland ACC while 1/31 (3 %) showed diffuse nuclear staining of the tumor cells. This latter case was later reclassified as mammary analogue secretory carcinoma following confirmatory molecular testing for the ETV6-NTRK3 fusion gene. Strong positive nuclear staining of the tumor cells was seen in 24 (100 %) of salivary gland MEC cases. P63

is an immunohistochemical stain that can potentially aid in differentiating unusual ACC with prominent mucin production from MEC of the salivary gland. According to this study, acinic cell carcinoma is always negative for p63 immunoreactivity while mucoepidermoid carcinoma is always positive.

**Keywords** Acinic cell carcinoma · Mucoepidermoid carcinoma · Mammary analogue secretory carcinoma · p63 · Salivary gland

## Introduction

Salivary gland acinic cell carcinoma is a malignant epithelial neoplasm that frequently demonstrates variable microscopic morphologies, which can make definitive diagnosis quite challenging. One particularly difficult task is differentiating between the papillary cystic and microcystic acinic cell carcinomas (ACC) and MEC due to the significant histomorphological overlap as both have prominent cystic changes and mucinous secretions. The authors report the use of the p63 basal and myoepithelial immunohistochemical stain to aid with this differential diagnosis. This is the first report to use this marker in this differential diagnosis. Other tissue markers have been investigated for the differentiation of these tumor types previously but have not been very helpful.

P63 is a p53 homologue required for limb and epidermal morphogenesis. Having been mapped to 3q27-29, it is essential for epidermal-mesenchymal interactions during embryonic development as demonstrated by the lack of development of teeth, hair follicles, and mammary gland in experiments utilizing knock-out mice lacking p63 [1]. Basal cells lacking p63 expression undergo terminal

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differentiation leading to the depletion of stem cells [2]. The role of p63 in tumorigenesis is not fully understood at this time. The elucidation of its role has been difficult because p63 encodes six different proteins with distinct and even opposing functions including inhibiting as well as inducing apoptosis [3]. P63 is expressed in basal and myoepithelial cells of human normal and tumor salivary gland tissues [4, 5]. Bilal et al. [5] demonstrated that normal salivary gland tissue expressed strong p63 staining along with strong positive cytokeratin 14 staining of myoepithelial and basal duct cells.

The p63 staining characteristics of acinic cell carcinoma and mucoepidermoid carcinoma have been separately touched upon in other reports, but have never been reported together as an approach to assist in distinguishing these tumors. Weiler et al. [6] reported 19 salivary gland ACC which demonstrated a complete lack of basal cell component manifested by negative p63 expression. Bilal et al. [5] have reported 9 MEC that stained with p63.

## Materials and Methods

After Institutional Review Board review and approval, specimens in the archives of Lifespan Health System and the consult files of one of the authors (DRG) were examined to identify all salivary gland ACC and MEC. Formalin-fixed paraffin-embedded sections from all salivary gland acinic cell and MEC were retrieved for evaluation with accession dates ranging from 1989 to 2010. Cases with sufficient histological material were selected with all variants included. Primary tumors, recurrent tumors, and metastatic tumors were all evaluated; however, in cases of multiple specimens for a single patient, only the specimen with the greatest amount of tumor material was chosen to be included in the study. All slides were reviewed by both authors for appropriateness of diagnosis according to the recent WHO salivary tumor classification [7, 8]. If sufficient histologic material was available, the specimen was chosen for p63 immunohistochemical staining. The specimens were pre-treated by heat-induced epitope retrieval methods using target retrieval solution (DakoCytomation, Carpinteria, CA, USA). Monoclonal mouse anti-human p63 protein kit was used in a 1:800 dilution, which was found to be optimal in our laboratory. (DakoCytomation, Carpinteria, CA, USA) Visualization was performed using the DAKO LSAB +/HRP kit (Dako Cytomation, Carpinteria, CA, USA). Tonsil was used for a positive control, and all methods were performed according to manufacturer specifications. The staining expression was assessed by both study participants and agreement was reached on all specimens. Immunostaining was considered negative if less than 10 % of tumor cells stained and

positive if 10 % or greater of tumor cells stained. Positive results were sub-classified and graded as follows: weakly positive (10–25 % of tumor cells staining), moderately positive (26–75 %), or strongly positive (76–100 % of tumor cells staining).

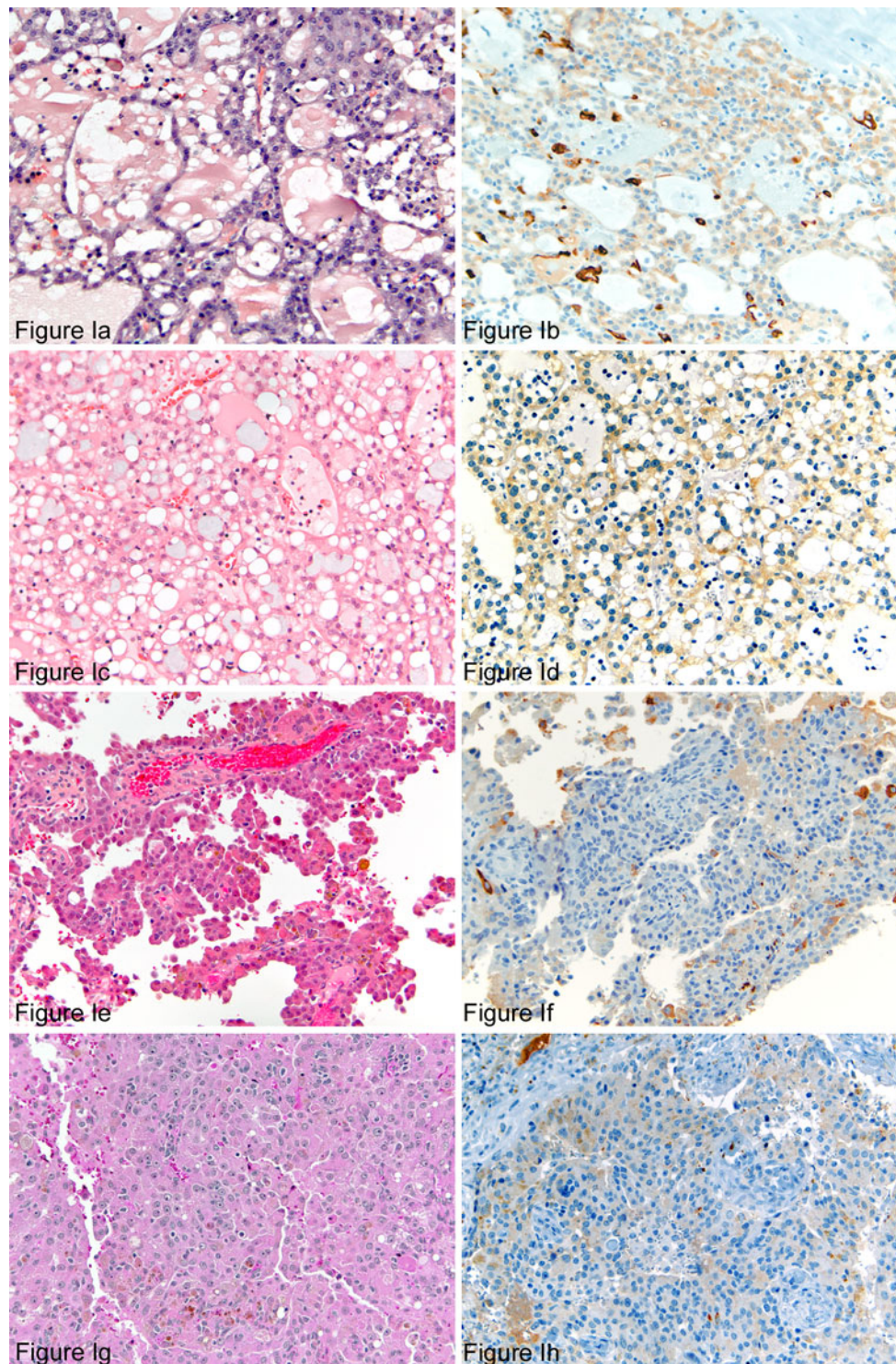
## Results

Thirty-one ( $n = 31$ ) salivary gland acinic cell carcinoma specimens and twenty-four ( $n = 24$ ) salivary gland mucoepidermoid carcinoma specimens were found to have appropriate diagnoses and sufficient histologic material to be included in the study. They all were stained with p63. Of the 31 acinic cell carcinomas, 97 % (30) were negative for p63 (Fig. 1); one showed strong positive staining. All four variants of ACC included in our study (follicular, microcystic, papillary cystic, and solid types) were negative. The one ACC that showed positive p63 staining was originally diagnosed as the microcystic variant. This case was later reclassified as mammary analogue secretory carcinoma (MASC) of the salivary gland after publication of the first paper describing this new tumor (Fig. 2; see Discussion) [9]. All 24 salivary gland MEC (100 %) showed strong positive staining (76–100 % reactivity) with p63. (Fig. 3) The MEC cases included low, moderate, and high grade tumors.

## Discussion

Acinic cell carcinoma (ACC) comprises 7–17.5 % of malignant salivary gland tumors, involving the parotid gland in as many as 90 % of cases. Mucoepidermoid carcinoma (MEC) comprises 12–29 % of malignant salivary gland tumors with 85–88 % of tumors occurring in the parotid gland [10]. Acinic cell and mucoepidermoid carcinoma of the salivary glands may have significant histomorphological overlap among the variants as well as highly variable antigen expression which can make diagnosis difficult. MEC cellular morphology is characterized by epidermoid cells, mucus-secreting cells, as well as cells displaying features varying between these two types which are termed intermediate cells. The ratio of these cells present in MEC varies greatly and, frequently, cystic spaces contain mucicarmine positive material.

In the majority of the ACC at least some of the cells typically show acinar or serous differentiation with well defined small intracytoplasmic bluish-purple small granules noted on hematoxylin and eosin (H&E) staining which are PAS-diacetate (PAS-D) positive allowing proper classification. However, occasional tumors with the follicular, microcystic and papillary cystic patterns contain only very

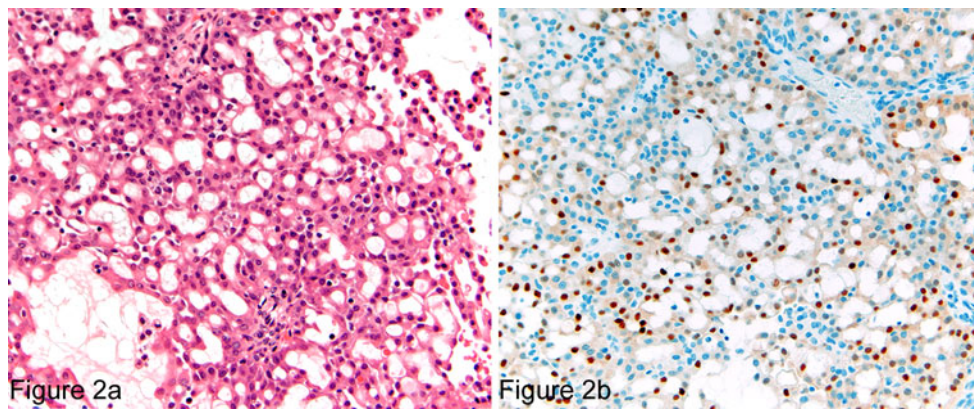


**Fig. 1** Acinic Cell Carcinoma, follicular type stained with H&E (a) and p63 (b), no nuclear staining was seen in tumors cells in b. Rare nuclear staining of dendritic cells is present. Acinic Cell Carcinoma, microcystic

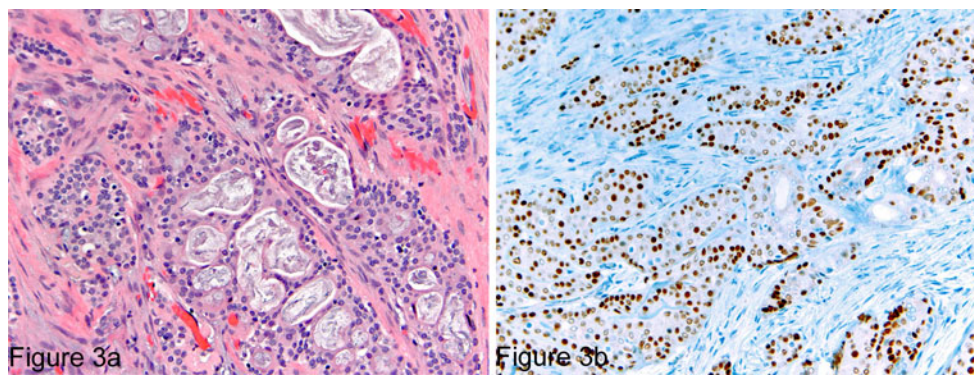
area stained with H&E (c) and p63 (d). Acinic Cell Carcinoma, papillary cystic type stained with H&E (e) and with p63 (f). Acinic Cell Carcinoma, solid type stained with H&E (g) and with p63 (h).  $\times 20$

occasional zymogen granules and may also contain intraluminal/intracystic mucin and rare intracytoplasmic mucinous material leading to difficulty in separation from

MEC which frequently has both intracytoplasmic and intracystic or intraluminal small mucinous material which will also stain with PAS-D. Also further complicating this



**Fig. 2** Mammary analogue secretory carcinoma. This tumor was initially diagnosed as acinic cell carcinoma, microcystic variant. The p63 positivity prompted molecular analysis which confirmed this diagnosis.  $\times 20$



**Fig. 3** Mucoepidermoid carcinoma stained with H&E (a) and p63 (b).  $\times 20$

problem are occasional ACC with zymogen granules that aberrantly stain with a mucicarmine stain (personal experience of one author, DRG).

ACC has seven recognized variants: solid, follicular, microcystic, papillary cystic, oncocytic, undifferentiated, and the well differentiated variant with lymphoid stroma. The microcystic and follicular variants of ACC are most notable for being mistaken for MEC. These frequently have intercellular cystic change with lattice-like or fenestrated characteristics or small follicles which often contain secretory material. Both of these variants can be very zymogen granule poor and finding and/or demonstrating intracytoplasmic zymogen granules may be particularly challenging with only focal presence evident after careful review using H&E and PAS-D staining. The PAS staining in these variants frequently overlaps with intracellular mucin noted in both ACC and MEC. Due to the variable morphology, alternative methods are necessary to aid in diagnosis, especially in the consultation service of one of the authors (DRG).

Immunohistochemical stains have been thoroughly studied in the characterization of these tumors. Meer et al. [11] report that Cytokeratin 7 positivity and cytokeratin 20

negativity is characteristic of salivary gland neoplasia in general. Weiler et al. [6] reported 100 % p63 negativity in ACC (19 of 19 cases) but did not include cases of MEC in their study. Positive p63 staining is well-known in MEC. Weinreb et al. [12] reported 100 % p63 staining in 6 MEC. Bilal et al. [5] reported nine MEC that all stained strongly and diffusely with p63. Their study included four low-grade, three intermediate-grade, and two-high grade cases according to the Armed Forces Institute of Pathology (AFIP) criteria [5]. Sparse data exist comparing the immunohistochemical characterization in head-to-head evaluations of ACC and MEC.

Hamper et al. [13] compared the expression of multiple immunohistochemical markers in ACC and MEC, but did not include p63 in their study. To our knowledge, this is the first study to evaluate p63 expression to aid in the differentiation of salivary gland acinic cell carcinoma from mucoepidermoid carcinoma.

P63, a p53 homologue is well known and commonly used in breast and prostate pathology due to its expression in basal and myoepithelial cells. P63 has also been reported to stain basal and myoepithelial cells of normal and neoplastic salivary gland tissue [5]. In our study, we found

ACC and MEC to have distinctly different immunophenotypes when evaluated with p63. Salivary gland MEC was found to exhibit strong positive staining for p63 in 100 % of tumors that were evaluated (24/24). No tumors that were evaluated exhibited moderate, weak, or negative staining.

ACC was found to have negative (null) staining in all our cases, including all four variants examined in this study. The microcystic variant of ACC is known to be particularly difficult to differentiate from MEC as both may have abundant mucin secretions. One case in our study was initially signed out as an acinic cell carcinoma, microcystic variant. This case was found to have strong and diffuse positive p63 staining and was the only originally diagnosed ACC found to have positive p63 staining. After re-evaluation, the case was reclassified as a mammary analogue secretory carcinoma (MASC) of the salivary gland, when the recent series by Skalova et al. [9] was published and it was found to have the ETV6-NTRK3 fusion gene. Although this is the first case of MASC reported with prominent p63 staining, other observers have occasionally found focal to strong p63 staining in this neoplasm (personal communication). Morphologically, the microcystic variant of ACC and MASC have a great deal of overlap. Enough, in fact, that the only way at this time to distinguish between these two entities in occasional tumors is through testing for the presence of the ETV6-NTRK3 fusion gene using molecular techniques. This gene fusion has been shown to be negative in 100 % of ACC and positive in 100 % of MASC [9]. The ETV6-NTRK3 fusion gene can be evaluated through real-time polymerase chain reaction (RT-PCR) as well as fluorescence in situ hybridization (FISH) methods. Our tumor was found to be positive for the fusion gene by both methods.

In conclusion, p63 immunohistochemical staining can be useful in the differential diagnosis of acinic cell and MEC of the salivary glands. MEC is always strongly positive for p63. In mucin rich MEC, the mucin containing goblet cells frequently will not stain with p63. However, the adjacent basal and intermediate cells are always positive. ACC is typically negative, although occasional cases may demonstrate focal staining, always less than 10 %. When one gets more experience with the MASC, it may not be necessary to confirm the diagnosis by molecular studies or using FISH. Until one feels comfortable with this tumor morphology, all cases of zymogen granule poor ACC with microcystic, follicular and secretory characteristics should be further evaluated using RT-PCR or FISH

for the ETV6-NTRK3 fusion gene to rule out mammary analogue secretory carcinoma.

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