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ORIGINAL PAPER

Expression of Membrane-Bound Mucins and p63 in Distinguishing Mucoepidermoid Carcinoma from Papillary Cystadenoma

Emily A. Lanzel[1] · Ali Pourian[2] · Saulo L. Sousa Melo[1] · Kim A. Brogden[3] · John W. Hellstein[1]

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Abstract The aim of this study was to compare the immunoexpression of epithelial mucins (MUCs) in salivary duct cysts, papillary cystadenomas, and mucoepidermoid carcinomas and to evaluate if any of these markers could be useful for differentiating between mucoepidermoid carcinoma and papillary cystadenoma. We also sought to validate the p63 expression pattern found to differentiate between mucoepidermoid carcinoma and papillary cystadenoma. Immunoexpression of MUC1, MUC2, MUC4, MUC7, and p63 was studied and quantified in 22 mucoepidermoid carcinomas, 12 papillary cystadenomas, and 3 salivary duct cysts. The immunohistochemical evaluation was collectively performed by 3 oral pathologists. Scores and trends in proportions were assessed using the nonparametric Wilcoxon–Mann–Whitney rank sum test. Mucoepidermoid carcinomas, papillary cystadenomas, and salivary duct cysts demonstrated variable MUC expression patterns. All tumors were positive for p63 immunoexpression with p63 labeling in salivary duct cysts and papillary cystadenomas (15/15) limited to the basal layers of the cystic spaces, whereas in mucoepidermoid carcinomas (22/22) the p63 labeling extended throughout the suprabasal layers ($p < 0.001$). This study adds more confirmatory data to validate that the reactivity pattern of p63 protein can be used in distinguishing between papillary cystadenoma and low-grade mucoepidermoid carcinoma. Although positive reactivity in a tumor with MUC1 and MUC4 was inconclusive, negative reactivity suggests the diagnosis of a benign PC or SDC.

Keywords Mucoepidermoid carcinoma · Papillary cystadenoma · Immunohistochemistry · Mucins

Introduction

Mucoepidermoid carcinoma (MEC) is the most common malignant neoplasm of salivary glands—comprising 30 % of all malignant tumors in major and minor salivary glands [1]. MEC is a glandular epithelial tumor composed of varying proportions of mucous, epidermoid, intermediate, columnar, clear, and occasionally, oncocytic cells and is thought to arise from pluripotent reserve cells of excretory ducts [2]. Many tumors have a cystic component as well as solid cords, islands and/or sheets of tumor cells [1]. In fact, MEC is classified into low-, intermediate-, and high-grade tumors according to its amount of cystic and solid components. Low-grade tumors are often characterized by an extensive cystic component whereas high-grade tumors present more solid and cytomorphologic variability. Intermediate grade tumors are of course between these two extremes [3–5].

Papillary cystadenoma (PC) of the salivary glands is a rare benign epithelial tumor characterized by primarily multicystic growth, intraluminal papillary proliferations, and duct-like structures [1]. Histologically, PC can resemble low-grade MECs given that it presents a similar, predominately cystic pattern, which makes the distinction between the two challenging.

✉ Emily A. Lanzel
emily-lanzel@uiowa.edu

[1] Department of Oral Pathology, Radiology and Medicine, The University of Iowa, 801 Newton Rd. S357 DSB, Iowa City, IA 52246, USA

[2] Roseman University, South Jordan, UT, USA

[3] Department of Periodontics, The University of Iowa, Iowa City, IA, USA

Epithelial mucins (MUCs) are multifunctional high molecular weight glycoproteins consisting of a linear polypeptide chain with numerous oligosaccharide-glycosylated carbohydrate chains of variable length that are different for each type of MUC, with 19 MUC-encoding genes currently identified [7]. MUCs have served as molecular markers of malignant transformation in several organs and tissues and their expression is related to the prognosis of some neoplasms, including mucoepidermoid carcinoma [7–14]. Although MUC expression has been previously studied in MEC, its immunoexpression in PC has not been reported.

In 2013, the immunohistochemical expression pattern of p63 was reported to distinguish characteristic low-grade MEC from PC. Fonseca et al. [6] found that p63 expression in PC was limited to the basal layers of the tumor's cystic spaces, whereas in MEC, expression was also seen throughout the suprabasilar layers. However, after applying this to a low-grade tumor encountered in our biopsy service, the results were inconclusive due to minimal cystic epithelial layers present and those that were present showing focal areas of reactivity and nonreactivity.

The aim of the present study was to compare the immunoexpression of MUCs (MUC1, MUC2, MUC4, and MUC7) in PCs and MECs and to evaluate if any of these markers could be useful for differentiating between MEC and PC. We also sought to validate the p63 expression pattern found by Fonseca et al. [6].

Materials and Methods

Fifteen low-grade MECs, 5 intermediate-grade MECs, 2 high-grade MECs, 12 PCs, and 3 salivary duct cysts (SDCs) derived from minor salivary glands of the oral cavity were retrieved from the surgical pathology archives of the University of Iowa College of Dentistry Surgical Oral Pathology Laboratory under the University of Iowa Institutional Review Board (201407720). These cases were used to determine immunohistochemically the expression of p63 protein, MUC1, MUC2, MUC4, and MUC7. All samples were fixed in 10 % formalin and embedded in paraffin. The original diagnoses were reviewed by 2 independent oral pathologists (EL, AP), who also screened the hematoxylin and eosin-stained slides for representative tumor samples, which included adjacent salivary gland tissue in all cases.

Immunohistochemistry (IHC) was performed in the Histology Research Laboratory of the University of Iowa Hospitals and Clinics, Department of Pathology, using the following commercially available antibodies: p63 (Dako, Carpinteria, CA), MUC1 (Abcam, Cambridge, UK), MUC2 (Abcam, Cambridge, UK), MUC4 (Abcam, Cambridge, UK), and MUC7 (Abcam, Cambridge, UK). The antibody catalog number/clone, type, dilution, and vendor information is summarized in Table 1. Briefly, IHC was performed on 4 μm sections that were first deparaffinized, rehydrated, and subjected to heat-induced epitope retrieval in citrate buffer (pH 6.0 or pH 8.0) in a pressure cooker. After incubation with the primary antibody, the Dako EnVision Kit was used for detection. The reaction was visualized with the chromogen 3,3′-diaminobenzidine (DAB) (Dako Carpinteria, CA), followed by DAB Enhancer (Dako Carpinteria, CA). Slides were counterstained with Leica/Surgipath (Buffalo Grove, IL) hematoxylin.

The immunohistochemical evaluation was collectively performed by 3 oral pathologists (EL, AP, JH). For each antibody, the positivity and reactivity pattern were recorded for tumor tissue. Reactivity was considered to be positive when the reactivity intensity was equal to or stronger than the control and greater than 5 % of tumor cells reacted. In case of divergence among the oral pathologists, consensus was enforced.

Scores and trends in proportions were assessed using the nonparametric Wilcoxon–Mann–Whitney rank sum test. Data analyses were performed using SigmaStat (version 3.5; Systat Software Inc, Erkrath, Germany). The a priori level of significance was set at $p < 0.05$.

Results

The clinical features are summarized in Table 2. Examples of reactivity expression are shown in Fig. 1. The distribution of the immunohistochemical expressions is shown in Table 3.

p63 Expression

Regardless of grade, 21/22 MECs demonstrated positive reactivity for p63. In all positive cases, sheets of intermediate or squamous cells were diffusely positive. For cells within the cystic spaces, the expression of p63 was seen within the basilar layer and throughout the suprabasilar layers, near the lumen, as well. All PCs and SDCs (15/15), demonstrated positive reactivity. In all but one case of PC, the expression pattern was seen exclusively in the basal cells of the cystic structures. The difference in expression patterns between malignant MECs and benign PCs/SDCs was statistically significant ($p < 0.001$).

MUC1 Expression

All MECs (22/22) were positive for MUC1. MUC1 demonstrated reactivity of the cellular membranes and the cytoplasm of epidermoid, intermediate, clear, and mucous

Table 1 Summary of the antibodies evaluated in this study

Antibody	Catalog number	Vendor	Type	Dilution	Positive control tissue
MUC1	ab#45167	Abcam (Cambridge, UK)	Rabbit Monoclonal Antibody	1:100	Normal submandibular gland
MUC2	ab#134119	Abcam (Cambridge, UK)	Rabbit Monoclonal Antibody	1:10,000	Normal colon
MUC4	ab#150381	Abcam (Cambridge, UK)	Rabbit Monoclonal Antibody	1:100	Normal colon
MUC7	ab#55542	Abcam (Cambridge, UK)	Rabbit Monoclonal Antibody	1:500	Normal submandibular gland
P63	4A4	Dako (Carpinteria, CA)	Mouse monoclonal	1:25	Normal skin

Table 2 Clinicopathologic features of papillary cystadenomas, salivary duct cysts and mucoepidermoid carcinomas (MEC)

Case	Diagnosis	Age	Sex	Site	Clinical presentation
1	Papillary cystadenoma	77	F	Buccal Mucosa	Nodule
2	Papillary cystadenoma	59	M	Buccal Mucosa	Hard nodule
3	Papillary cystadenoma	66	F	Palate	Ulcer
4	Papillary cystadenoma	73	M	Vestibule	Swelling
5	Papillary cystadenoma	60	F	Buccal Mucosa	Nodule
6	Papillary cystadenoma	62	F	Hard Palate	Nodule
7	Papillary cystadenoma	74	F	Floor of mouth	Swelling
8	Papillary cystadenoma	52	F	Lower lip	Nodule
9	Papillary cystadenoma	72	F	Buccal Mucosa	Nodule
10	Papillary cystadenoma	55	M	Lower lip	Fluctuant nodule
11	Papillary cystadenoma	70	F	Floor of mouth	Movable nodule
12	Papillary cystadenoma	37	F	Hard Palate	Blue Papule
13	Salivary duct cyst	45	F	Floor of mouth	Swelling
14	Salivary duct cyst	57	F	Mandibular vestibule	Swelling
15	Salivary duct cyst	71	F	Buccal Mucosa	Ulcer
16	Low-grade MEC	47	M	Palate	Swelling
17	Low-grade MEC	25	M	Retromolar pad	Mass
18	Low-grade MEC	66	M	Retromolar pad	Firm nodule
19	Low-grade MEC	77	M	Mandibular vestibule	Firm nodule
20	Low-grade MEC	45	F	Buccal Mucosa	Firm nodule
21	Low-grade MEC	36	F	Buccal Mucosa	Nodule
22	Low-grade MEC	16	M	Soft Palate	Swelling
23	Low-grade MEC	71	F	Palate	Fluctuant nodule
24	Low-grade MEC	83	F	Buccal Mucosa	Hard nodule
25	Low-grade MEC	78	M	Upper lip	Hard nodule
26	Low-grade MEC	68	F	Hard Palate	Mass
27	Low-grade MEC	35	F	Soft Palate	Nodule
28	Low-grade MEC	48	M	Hard Palate	Fluctuant nodule
29	Low-grade MEC	45	M	Hard Palate	Mass
30	Low-grade MEC	42	M	Lower lip	Nodule
31	Intermediate-grade MEC	49	F	Tuberosity	Nodule
32	Intermediate-grade, MEC	64	F	Hard Palate	Swelling
33	Intermediate-grade, MEC	58	F	Upper lip	Firm nodule
34	Intermediate-grade, MEC	64	M	Retromolar pad	“Boggy” mucosa
35	Intermediate-grade, MEC	38	F	Hard Palate	Swelling
36	High-grade, MEC	26	M	Pterygoid notch	Pedunculated lesion
37	High-grade, MEC	58	M	Tongue	Ulcer

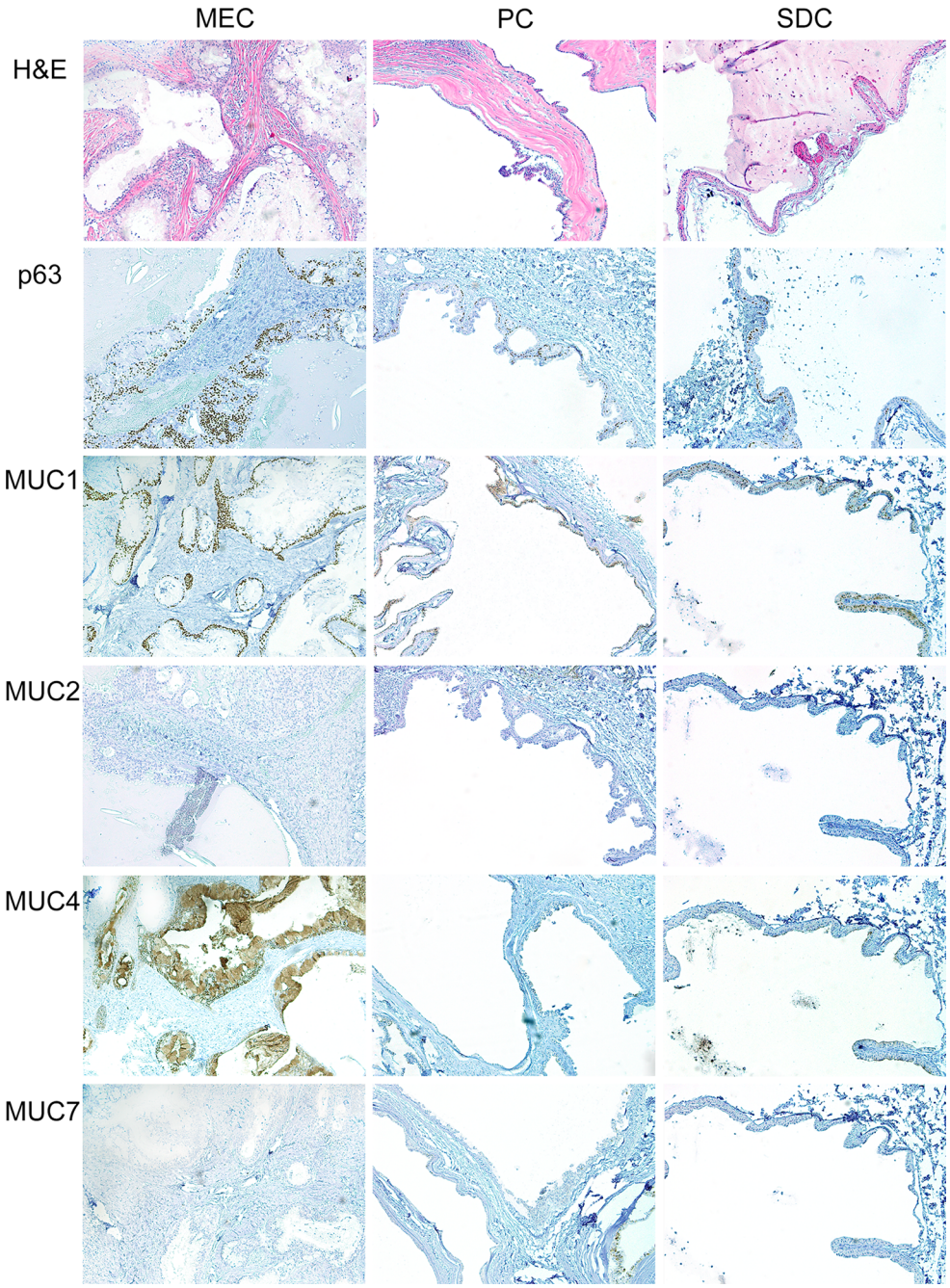

Fig. 1 Representative examples of mucoepidermoid carcinoma (MEC), papillary cystadenoma (PC), and salivary duct cyst (SDC) reactivity expressions with p63, MUC1, MUC2, MUC4, and MUC7 antibodies

cells. All SDCs (3/3) were also positive for MUC1 showing cytoplasmic reactivity. PCs demonstrated reactivity in 9/12 cases, showing expression in the cellular membranes and cytoplasm of epidermoid cells. This difference in expression patterns between malignant MECs and benign PCs/SDCs was statistically significant ($p = 0.012$).

MUC2 Expression

One MEC showed reactivity for MUC2 focally in mucous cells. All other MECs (21/22) were negative for reactivity. All PCs (12/12) and SDCs (3/3) were negative for MUC2 reactivity.

Table 3 Distribution *n* (%) of the immunohistochemical expression according to diagnosis

	p63		p63 pattern			MUC1		MUC2		MUC4		MUC7	
	−	+	Basal and suprabasal layers	Basal layer only	None	−	+	−	+	−	+	−	+
MEC	1 (4.55)	21 (95.45)	21 (95.45)	0 (0.00)	1 (4.55)	0 (0.00)	22 (100)	21 (95.45)	1 (4.55)	0 (0.00)	22 (100)	22 (100)	0 (0.00)
PC/SDC	0 (0.00)	15 (100)	0 (0.00)	15 (100)	0 (0.00)	4 (26.67)	11 (73.33)	15 (100)	0 (0.00)	3 (21.43)	11 (78.57)	15 (100)	0 (0.00)
p value	0.441		<0.001*			0.012*		0.441		0.028*		1.000	
Mann–Whitney *U* Statistic	157.500		0.000			209.000		172.500		187.000		165.000	

MEC mucoepidermoid carcinoma, *PC* papillary cystadenoma, *SDC* salivary duct cyst

* The difference between the two groups is greater than would be expected by chance; there is a statistically significant difference

MUC4 Expression

All MECs (22/22) were positive for MUC4. There was reactivity of the cellular membranes, nuclei and the cytoplasm of epidermoid, intermediate, clear, and mucous cells. In all SDCs and PCs, if mucous cells were present, MUC4 cytoplasmic reactivity was present in these cells. All SDCs (3/3) showed reactivity with MUC4, showing an expression pattern localized to the apical membrane of epidermoid cells. Positive reactivity for MUC4 was seen in 9/12 PCs. In 5 of these positive tumors, the reactivity pattern was seen in the apical membrane only of epidermoid cells. In 4 of the positive tumors, the expression pattern was localized to the apical membrane and cytoplasm of epidermoid cells. This difference in expression patterns between malignant MECs and benign PCs/SDCs was statistically significant ($p = 0.028$).

MUC7 Expression

No reactivity for MUC7 was seen in any tumor type.

Discussion

In our study we sought to compare the immunoexpression of MUCs (MUC1, MUC2, MUC4, MUC7) in salivary duct cysts (SDCs), PCs, and MECs and to evaluate if any of these markers could be useful for differentiating between MEC and PC. No variable expression pattern was found between MECs, PCs, and SDCs with MUC2 or MUC7 antibodies. Although positive reactivity in a tumor with MUC1 and MUC4 is inconclusive, negative reactivity suggests the diagnosis of a benign PC or SDC ($p = 0.012$ and 0.028, respectively).

Histologically, PC can demonstrate a close resemblance to those low-grade MECs with a predominately cystic pattern making the distinction between the two lesions challenging. The present study confirmed the findings of Fonseca et al. [6] that p63 protein is expressed in both tumors, but with distinctive expression patterns. In 95.5 % (21/22) of MEC cases here, the distinct expression pattern showed reactivity of cystic epithelial cells within the basilar layer and throughout the suprabasilar layers, near the lumen. However, in all cases of PCs and SDCs (15/15), the p63 reactivity was isolated to the basal cells of the cystic structures ($p < 0.001$).

MUCs are proteins consisting of a linear polypeptide chain with numerous oligosaccharide-glycosylated carbohydrates chains of variable length. The role of MUCs includes acting as a molecular barrier at the epithelial surface, facilitating glandular secretory processes, and participating in signal transduction. The expression of each

MUC gene is specific to the organ, tissue, and cell type [7–9]. Alterations in the structure and expression of MUCs have been reported in various neoplasms. In several organs and tissues, MUCs serve as molecular markers of malignant transformation such as breast and pancreatic cancers. In various tumor types, MUC expression has also been shown to correlate with prognosis [7, 10–14]. This is true in several salivary gland tumor types as well. For instance, Soares et al. [15] assessed MUC1 expression in pleomorphic adenomas, recurrent pleomorphic adenomas, and carcinomas ex-pleomorphic adenoma and found that MUC1 reactivity in recurrent pleomorphic adenomas was stronger than in pleomorphic adenomas and the reactivity in carcinomas was significantly higher than in either recurrent pleomorphic adenomas or pleomorphic adenomas. Alos et al. [16] found that MUC1 and MUC4 are overexpressed in MEC cells and their expression pattern correlated with tumor differentiation. An inverse relationship was seen with high MUC1 and low MUC4 expression associated with tumor progression and worse prognosis, whereas low MUC1 expression and high MUC4 expression was associated with a better prognosis.

Recent studies have shown that p40 (ΔNp63), a major isoform of the p63 gene, appears to be a more specific marker for squamous differentiation with great diagnostic utility in salivary gland pathology [17–20]. As p63 has been previously validated in marking MECs and was used in the previous study by Fonseca et al. [21, 22] we chose this antibody, though a future similar study with use of p40 may prove interesting.

In conclusion, this study adds more confirmatory data to validate that the reactivity pattern of p63 protein can be used in distinguishing between papillary cystadenoma and low-grade mucoepidermoid carcinoma as originally reported by Fonseca et al. [6].

Compliance with Ethical Standards

Conflict of interest None.

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