Original Article

Comparative immunohistochemical characteristics of benign and malignant major salivary gland tumors: A retrospective study

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ABSTRACT

Objectives: This study aims to characterize the cellular components of major salivary gland tumors and to identify the differentiating markers between tumor subtypes.

Patients and Methods: Between January 2006 and December 2010, a total of 83 patients (42 males, 41 females; mean age 50.2±15.8 years; range, 21 to 82 years) with major salivary gland tumors (n=12 mucoepidermoid carcinomas, n=8 adenoid cystic carcinomas, n=3 acinic cell carcinomas, n=4 salivary duct carcinomas, n=2 myoepitheliomas, n=5 basal cell adenomas, n=31 pleomorphic adenomas, and n=18 Warthin tumors) with myoepithelial and epithelial immunohistochemical markers (smooth muscle actin [SMA], calponin, S100, CD10, GFAP, p63, GCDFP15, GLUT1, 34ßE12, CK14, CK19, CD117, and galectin-3) were evaluated using tissue microarray method.

Results: The GFAP, S100, CK14, p63, and CK5/6 expressions were significantly lower in the malignant tumors ($p<0.05$), whereas the expression of neither SMA, nor calponin was significantly different between benign and malignant tumors. The CK19 expression was significantly higher in malignant tumors ($p=0.004$). Diffuse CD117 expression favored an adenoid cystic carcinoma; GFAP expression favored a pleomorphic adenoma; 34ßE12, p63, and CK5/6 expression favored a mucoepidermoid carcinoma; and GCDFP15 favored a salivary duct carcinoma and acinic cell carcinoma.

Conclusion: Our study results showed that distinct tumor types exhibited different preferences for various markers. We, therefore, suggest that immunohistochemical characteristics of myoepithelial cells, rather than the quantity per se, show a significant difference between malignant and benign salivary gland tumors and CK19 expression may indicate the malignant nature of a salivary gland tumor in difficult-to-diagnose tumors.

Keywords: Differential diagnosis, immunohistochemistry, myoepithelial marker, salivary gland tumor.

Salivary gland tumors reveal a broad morphological spectrum, and myoepithelial (ME) cells are regarded as one of the main components of these tumors.[1] Myoepithelial cells contribute in different ways to tumor development, giving rise to a diversity of histological patterns. Despite several developments, salivary gland tumors remain as a challenging tumor group, both for pathologists and clinicians. Neoplasms of the major salivary glands, according to the World Health Organization (WHO) classification of Head and Neck Tumors,[1] are comprised of 11 benign and 22 malignant tumors. Accurate classification of these tumors is important for both

Received: November 21, 2018 **Accepted:** April 17, 2019 **Published online:** May 02, 2019

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Doi: http://dx.doi.org/10.5606/Tr-ENT.2019.21939

Citation:
Kiremitci S, Dizbay Sak S. Comparative immunohistochemical characteristics of benign and malignant major salivary gland tumors: A retrospective study. Tr-ENT 2019;29(1):9-20.

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prognostic and therapeutic approach. However, due to the striking range of morphological diversity among different tumor types and, occasionally, within an individual tumor mass, salivary gland tumors represent a considerable diagnostic challenge. Although histopathological examination is the gold standard for diagnosis, immunohistochemistry may be useful in the diagnosis according to the cell differentiation, in terms of ME and luminal cell participation.[1]

Histologically, a salivary gland comprises ducto-acinar units consisting of four cell types: ductal, acinar, myoepithelial, and basal cells. In complex tissue organization of the salivary gland, ductal and acinar cells are located at the luminal side of the duct and are called luminal cells. The ME cells and basal cells lie down between the basement membrane and the luminal cells and are called abluminal cells. The ME cells are considered to have the structural features of both epithelial and smooth muscle cells. These cells contain pinocytic vesicles, microfilaments, and dense bodies resembling smooth muscle cells, which contribute to their main function as the contractile cells.[2] Several studies have also attributed remarkable functions to these cells, related to the tumor behavior, tumor suppression, and invasion suppression.[2-4] Determination of ME cells in salivary gland tumors is important for both understanding of the tumor development and diagnostic purposes. In the differential diagnosis of salivary gland tumors, various immunohistochemical (IHC) markers including a wide spectrum of epithelial and ME indicators such as S100, smooth muscle actin (SMA), CD10, calponin, p63, caldesmon, GFAP, CK14, CK19, CK5/6, GCDFP15, GLUT1, and 34ßE12 have been used. Among these markers, SMA and calponin seem to be the most reliable indicators of ME origin.[5,6] The ME cells exhibit variable histological appearances in tumor development such as epithelioid, spindle, plasmacytoid, and clear-cell morphologies. This modified phenotype of ME cells is thought to be related with the changing IHC profile of the cells.[7,8] Hence, the identification of ME cells in salivary gland tumors becomes a more complex issue, and an ongoing debate still exists regarding to the role of ME cells in different salivary gland tumors.

In the present study, we aimed to characterize the cellular components of major salivary gland tumors by IHC indicators of epithelial and ME origin and to identify the differentiating markers between tumor types.

PATIENTS AND METHODS

Between January 2006 and December 2010, a total of 83 patients (42 males, 41 females; mean age 50.2±15.8 years; range, 21 to 82 years) with major salivary gland tumors (pleomorphic adenomas [PAs] n=31, Warthin tumors [WTs] n=18, mucoepidermoid carcinomas [MECs] n=12, adenoid cystic carcinomas [AdCCs] n=8, basal cell adenomas [BCAs] n=5, salivary duct carcinomas [SDCs] n=4, acinic cell carcinomas [ACCs] n=3, and myoepitheliomas [MyoEs] n=2) with myoepithelial and epithelial immunohistochemical markers (SMA, calponin, S100, CD10, GFAP, p63, GCDFP15, GLUT1, 34ßE12, CK14, CK19, CD117, and galectin-3) were evaluated using tissue microarray (TMA) method. Tumor samples were obtained from the archive of Ankara University, Faculty of Medicine, Department of Pathology. Medical data of the patients were retrospectively analyzed. The cases were deemed as anonymous by the Institutional Review Board. A written informed consent was obtained from each patient. The study protocol was approved by the Ankara University Medical School Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Immunohistochemistry

Tissue microarray technique,[9] involving a (semi-automatic) tissue-arraying instrument, was used for the IHC analysis. Two tissue cylinders representing different areas of each tumor were selected and punched out by a biopsy apparatus with a diameter of 2 mm from the donor paraffin-blocks and were mounted into the recipient TMA paraffin-blocks. Four-micrometer sections in thickness, mounted on to positively charged slides were incubated with the IHC indicators of epithelial and ME origin (SMA, calponin, S100, CD10, GFAP, p63, GCDFP15, GLUT1, 34βE12, CK14, CK19, CD117, galectin-3) in an automatic immunostainer (BenchMark XT Staining Module, Ventana Medical Systems Inc., AZ, USA) using streptavidin-biotin complex

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immunodetection system. Data for primary antibodies are shown in Table 1. For all markers, cytoplasmic and/or membranous staining was considered specific staining, except for nuclear staining of p63 and galectin-3. Staining was scored semi-quantitatively on a scale of 0 to 3 in each tumor core, and when the score was ≥2 at least in any of the two cores of each case, the case was considered positive. For each case, a final staining score was obtained by the average of scores of the two cores.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 11.5 software (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max) values, or number and frequency. Staining scores of the IHC markers in different tumor types were compared using the Kruskal Wallis and post-hoc analysis. To compare the significance of the IHC markers between benign and malignant tumors, the Mann-Whitney U test was used. A p value of <0.05 was considered statistically significant.

RESULTS

Of all tumors, 75 were located in the parotid gland and eight were located in the submandibular gland. In the present study, each tumor demonstrated epithelial and ME marker expressions in varying degrees. Positivity rates and staining scores of the IHC markers for each tumor type are shown in Table 2.

In MECs; 34βE12 (100%), CK19 (100%), CK5/6 (100%), and p63 (90.9%) were the markers which were diffusely positive. In MECs, 34βE12 and CK19 were expressed in mucous, intermediate, and squamous cells, whereas p63 and CK5/6 were expressed in squamous cells and basal cells of the cystic component (Figure 1a-f). The GLUT1 (41.7%) and galectin-3 (41.7%) expressions were high in some of the tumors, particularly in squamous cells and intermediate/mucinous cells, respectively (Figure 1g-h). The CD10 (25%), CD117 (9.1%), and GCDFP15 (8.3%) expressions showed a focal distribution. The S100 (16.6%) was unexpectedly positive in two cases in a diffuse manner (Figure 1i; inlet). The SMA, GFAP, and calponin were completely negative.

Acinic cell carcinomas showed only GCDFP15 (66.7%) and CK19 (66.7%) positivity in acinar tumor cells (Figure 2). Other markers were consistently negative.

Adenoid cystic carcinomas revealed diffuse CK19 (100%) and CK14 (100%) positivity. In

Table 1. Antibody panel and methodology of immunohistochemistry

Primary antibody	Clone	Dilution	Source
Smooth muscle actin	1A4	1:500	Dako
Calponin	CALP	1:500	Cell Marque
S100	4C4.9	1:200	Cell Marque
GFAP	GA-5	1:150	Neomarkers
CD10	56C6	1:60	Neomarkers
p63	BC4A4	1:200	Biocare
GCDFP15	23A3	1:50	Cell Marque
GLUT1	polyclonal	1:200	Dako
34ßE12	34ßE12	1:200	Cell Marque
CK14	LL002	1:200	Neomarkers
CK19	A53/A426	1:500	Neomarkers
CD117	polyclonal	1:400	Neomarkers
Galectin3	9C4	1:40	Neomarkers

GFAP: Glial fibrillary acidic protein; GCDFP15: Gross cystic disease fluid protein 15; GLUT 1: Anti-glucose transporter 1; CK; Cytokeratin.

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Table 2. Positivity rates and staining scores of immunohistochemical markers in tumor groups

Tumor type → / IHC markers ↓	MEC (n=12)			ACC (n=3)			AdCC (n=8)			SDC (n=4)			PA (n=31)			BCA (n=5)			MyoE (n=2)			WT (n=18)		
	n	%	Median	Min-Max	n	Median	n	%	Median	Min-Max	n	%	Median	Min-Max	n	%	Median	Min-Max	n	%	Median	Min-Max	n	%	Median	Min-Max
SMA	-	-	-	-	-	-	5	83.3	1.25	0-3	-	-	-	-	4	12.9	0.5	0-2.5	4	80	1.5	1-2	-	-	-	-	-	-	-
S100	2	16.6	-	0-3	-	-	3	37.5	1	0-2	-	-	-	-	29	93.5	3	1-3	1	20	0.5	0.5-1.5	2	100	3	3-3	-	-	-	-
Calponin	-	-	-	-	-	-	5	62.5	1	1-3	-	-	-	-	14	51.9	1	0-3	4	80	2	1-2	-	-	-	-	-	-	-
CD10	3	25	1.0	0-2	-	-	-	-	0.5	0-1	-	-	-	-	11	36.7	1	0-3	-	-	-	-	1	50	1	0-2	-	-	-
p63	10	90.9	2	1-3	-	-	5	62.5	2.5	0-3	-	-	-	-	25	83.3	2	0-3	5	100	3	2.5-3	-	-	-	-	18	100	3	2-3
GFAP	-	-	-	-	-	-	-	-	0.37	0-1	-	-	-	-	22	84.6	2.0	0-3	-	-	-	-	1	50	1.5	0-3	-	-	-
GLUT1	5	41.7	1	0-2.5	-	-	3	37.5	1	0-2	1	25	0.5	0-2	3	9.7	0	0-2	1	20	0	0-1	-	-	0.5	0-1	18	100	2.5	1.5-3
CD117	1	9.1	0	0-1.5	-	-	6	75	2	0.5-3	-	-	-	-	7	22.6	0.5	0-3	1	20	1	0-1.5	-	-	-	-	-	-	-	-
CK14	6	50	1.25	0-3	-	-	8	100	2	2-3	-	-	-	-	22	71	1.5	0-3	5	100	3	3-3	-	-	-	-	15	83.3	2	0.5-3
CK5/6	12	100	2	1-3	-	-	6	75	1.5	1-2	-	-	-	-	22	71	2	0-3	3	60	3	0-3	-	-	-	-	18	100	3	3-3
GCDFP15	1	8.3	-	0-2	2	66.7	2	0-3	-	-	2	50	1.75	0-3	12	38.7	1	0-3	-	-	-	-	-	-	-	-	-	-	-	-
Galectin3	5	41.7	1	0-2.5	-	-	5	62.5	1	0-2	1	25	0	0-2	18	62.1	2	0-3	5	100	2	1.5-3	1	50	1.5	1-2	17	94.4	2	0.5-3
34βE12	12	100	3	1.5-3	-	-	7	87.5	2	1-2.5	3	75	2	0-2.5	24	80	2	0-3	5	100	3	3-3	-	-	0.5	0-1	18	100	3	2-3
CK19	11	100	3	2.5-3	2	66.7	1	1-3	8	100	2.5	2-3	4	100	3	3-3	15	48.4	1	0-3	5	100	3	2-3	-	-	18	100	3	3-3

MEC: Mucoepidermoid carcinomas; ACC: Acinic cell carcinomas; AdCC: Adenoid cystic carcinomas; SDC: Salivary duct carcinomas; PA: Pleomorphic adenomas; BCA: Basal cell adenomas; MyoE: Myoepitheliomas; WT: Warthin tumors; Min: Minimum; Max: Maximum; SMA: Smooth muscle actin.

addition, CD117 (75%), SMA (83.3%), calponin (62.5%), p63 (62.5%), CK5/6 (75%), 34βE12 (87.5%), and galectin-3 (62.5%) were also expressed in nearly diffuse manner; except for few cases showing focal staining (Figure 3a-j). The GLUT1 (37.5%) and S100 (37.5%) were significantly positive in few cases (Figure 3k, l). The CD10, GFAP, and GCDFP15 were negative in AdCCs.

In SDCs, invasive component was consistently positive for CK19 (100%). The 34βE12 (75%) and GCDFP15 (50%) were also positive in most of the cases (Figure 4). The SMA, calponin, p63, CK5/6, and CK14 were only expressed in ME cells of *in situ* component. Galectin-3 was positive only in one case (25%) (Figure 4f).

Pleomorphic adenomas expressed most of the markers with varying degrees, in which S100 (93.5%) was the most common marker in all cell types of epithelial, ME, and stellate appearance (Figure 5). Secondly, tumors expressed 34βE12 (80%), CK5/6 (71%), and CK14 (71%) in both epithelial and ME cells in nearly diffuse manner. The p63 (83.3%) was diffusely expressed in ME cells and in epithelial cells with squamous differentiation. In most of the cases, GFAP (84.6%) and calponin (51.9%) were expressed in ME and stromal cells. Galectin-3 was positive (62.1%) with ductal predominance. In a small proportion of PAs, CK19 (48.4%) and GCDFP15 (38.7%) were expressed in ductal cells, and SMA (12.9%) in ME cells in focal distribution. The CD117 (22.6%) and CD10 (36.7%) were also focally positive.

Basal cell adenomas were diffusely positive for CK14 (100%), CK19 (100%), 34βE12 (100%), p63 (100%), and galectin-3 (100%) (Figure 6a-l). The CK5/6 expression was also diffuse, but only in 60% of the cases. The SMA (80%) and calponin (80%) were stained in most of the cases with a peripheral-basaloid cell accentuation. The S100 (20%), GLUT1 (20%), and CD117 (20%) were focally expressed. The GCDFP15, GFAP, and CD10 were consistently negative.

In addition, MyoEs were diffusely positive for S100 (Figure 6j-l). One of them was positive for GFAP and galectin-3, and the other case was positive for CD10. Other markers were negative in MyoEs.

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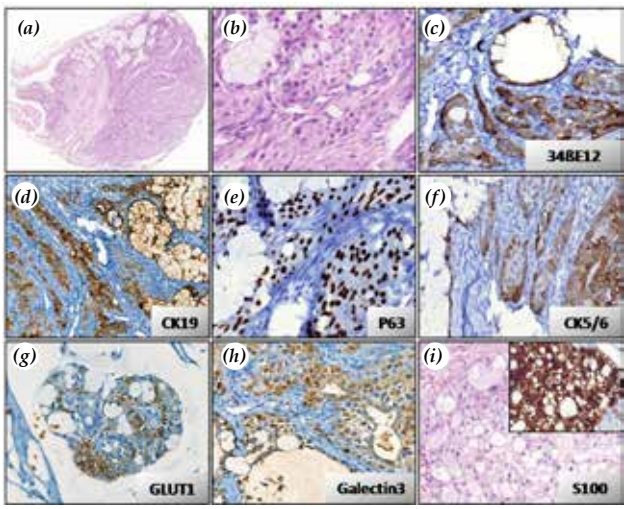

Figure 1. *The tissue section of a mucoepidermoid carcinoma represents the basic histologic pattern composed of squamous cells, mucinous cells and intermediate cells* ***(a)*** *H-E ×50,* ***(b)*** *H-E ×400, respectively. MECs revealed diffuse 34ßE12* ***(c)*** *CK19* ***(d)*** *p63* ***(e)*** *and CK5/6* ***(f)*** *positivity, with squamous and basal cell predominance for p63 and CK5/6 (×400, for all). Specific staining patterns of membranous GLUT1* ***(g)*** *and nuclear galectin-3* ***(h)*** *were seen in nearly half of the cases with more than focal distribution. Two cases of MEC demonstrating characteristic histologic pattern of three cell types revealed diffuse S100 positivity strictly different from other MECs which were completely negative,* ***(i)*** *inlet-S100; ×200.*

MEC: Mucoepidermoid carcinomas.

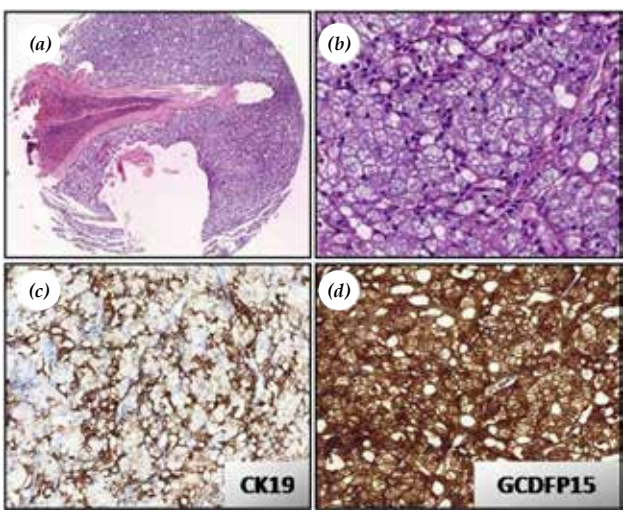

Figure 2. *The tissue section of an acinic cell carcinoma showing sheets of serous acinar like cells with vacuolated abundant cytoplasm* ***(a)*** *H-E ×50,* ***(b)*** *H-E ×400, respectively. ACCs were diffusely positive for CK19,* ***(c)*** *×400 and GCDFP15* ***(d)*** *×400).*

In WT, CK19 (100%), CK5/6 (100%), p63 (100%), CK14 (83.3%), and 34βE12 (100%) were diffusely expressed with ME predominance. Epithelial cells were also stained with CK19 diffusely and with CK14 and 34βE12 focally. Besides, galectin-3 (94.4%) was expressed in only epithelial cells.

Medium staining scores (SS) of benign and malignant tumor types were compared using the Mann-Whitney U test. Among the markers, GFAP (p=0.001), S100 (p=0.001), CK14 (p=0.016), p63 (p=0.008), CK5/6 (p=0.001), and galectin-3 (p=0.001) expressions were significantly higher in benign tumors, compared to malignant tumors, whereas CK19 was the only marker expressed in higher rates in malignant tumors [2.65±0.59, 3 (1 to 3)] compared to benign tumors [1.80±1.20, 2 (0 to 3)] (p=0.004). Staining scores for SMA (p=0.156) and calponin (p=0.056) were

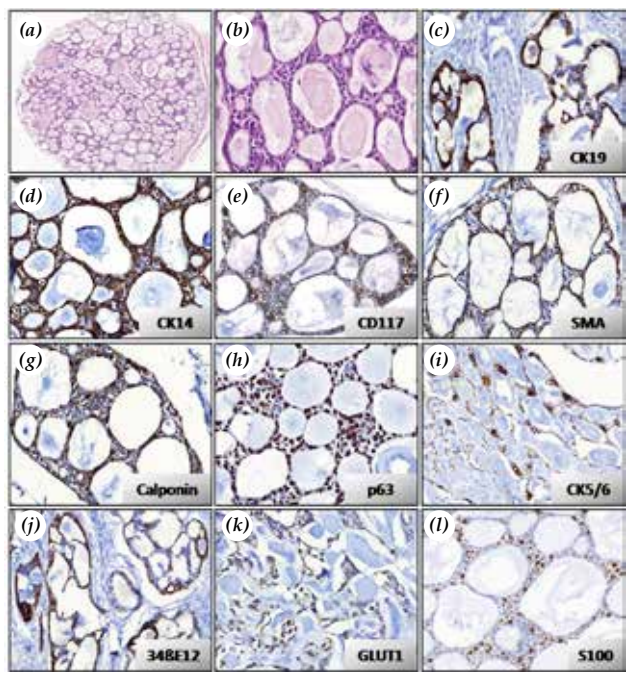

Figure 3. *The tissue cylinder of an adenoid cystic carcinoma composed of small cells with dark nuclei and scant cytoplasm in tubular pattern with gland like spaces filled with excess basement membrane* ***(a)*** *H-E ×50,* ***(b)*** *H-E ×400, respectively. AdCCs revealed diffuse CK19* ***(c)****, CK14* ***(d)****, CD117* ***(e)****, SMA* ***(f)****, Calponin* ***(g)****, p63* ***(h)****, CK5/6* ***(i)*** *and 34ßE12* ***(j)*** *(×400 for all). GLUT1* ***(k)*** *and S100* ***(l)*** *positivity was more than focal in a few cases (×400).*

SMA: Smooth muscle actin.

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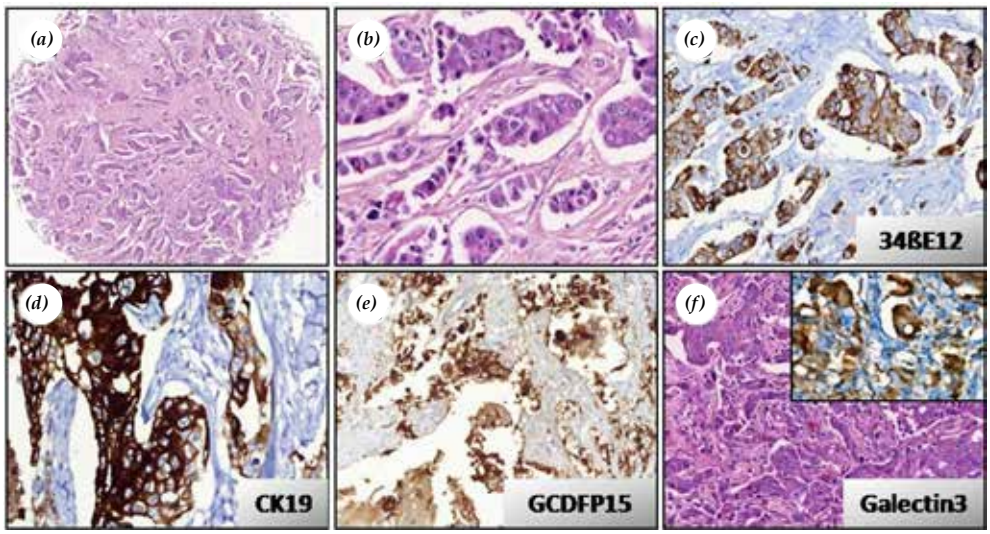

Figure 4. *The tissue section of a salivary duct carcinoma composed of squamous cells with eosinophilic cytoplasm, prominent nucleoli and vesicular nuclei in fibrous and hyalinized stroma* ***(a)*** *H-E ×50,* ***(b)*** *H-E ×400, respectively. Invasive component of SDCs were diffusely positive with 34ßE12,* ***(c)*** *CK19 and* ***(d)*** *GCDFP15 (3) (×400, for all). Only one case demonstrated galectin-3 positivity in a diffuse manner while the other 3 cases were completely negative* ***(e)*** *H-E ×400,* ***(f)*** *inlet, ×400).*

not significantly different between malignant and benign tumor types.

The IHC markers showing differential expression between tumor types were as follows (Table 3):

1. *In differentiation of PA from MEC:* GFAP (p=0.001), calponin (p=0.001) and SMA (p=0.001) positivity favored PA.

   In differentiation of PA from ACC: p63 (p=0.005), galectin-3 (p=0.004) and 34βE12 (p=0.013) positivity favored PA.

   In differentiation of PA from AdCC: GFAP (p=0.001), CD10 (p=0.015), GCDFP15 (p=0.005) positivity, and diffuse expression of S100 (p=0.001) favored PA, whereas diffuse expression of CK14 (p=0.014), CD117 (p=0.002), and CK19 (p=0.001) favored AdCC.

   In differentiation of PA from SDC: Higher expression of CK19 favored SDC. On the other hand, CD10 (p=0.001), S100 (p=0.001), CK14 (p=0.001), SMA (p=0.001), calponin (p=0.001), p63 (p=0.001), CK5/6 (p=0.001), and CD117 (p=0.001) positivity favored PA.

   In differentiation of PA from BCA: GFAP (p=0.001) and CD10 (p=0.001) positivity, and diffuse expression of CK19 (p=0.001) favored PA.

2. *In differentiation of MEC from ACC:* GLUT1 (p=0.032), p63 (p=0.004), CK5/6 (p=0.04), and 34ßE12 (p=0.001) positivity favored MEC.

   In differentiation of MEC from SDC: CD10 (p=0.001), CK14 (p=0.045), p63 (p=0.002), and CK5/6 (p=0.001) positivity favored MEC.

   Half of the SDCs and most of the ACCs were diffusely positive with GCDFP15, while MECs showed only focal GCDFP15 expression. However, this marker was not significant in differentiating these tumor groups according to the post-hoc test (p=0.075, p=0.112, respectively).

3. *In differentiation of AdCC from ACC:* CD117 positivity favored AdCC (p=0.001), whereas GCDFP15 positivity favored ACC (p=0.010).

   In differentiation of AdCC from BCA: Diffuse expression of CK5/6 (p=0.038) and galectin-3 (p=0.030) favored BCA.

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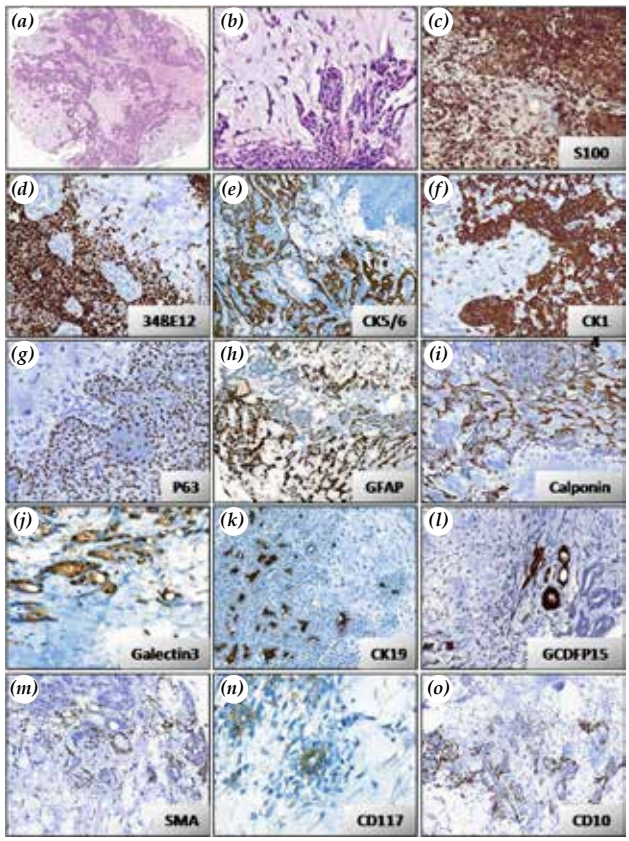

Figure 5. *A pleomorphic adenoma demonstrating epithelial/myoepithelial component dispersed within a condroid matrix* ***(a)*** *H-E ×50,* ***(b)*** *H-E ×400, respectively. S100 expression was diffuse in all components* ***(c)****. Epithelial and ME cells were positive for 34Be12* ***(d)****, CK5/6* ***(e)*** *and CK14* ***(f)****. P63 positivity was limited in ME and squamous cells* ***(g)****. ME cells and stromal cells stained with GFAP* ***(h)*** *and Calponin* ***(i)*** *in most of the cases. Galectin-3 was positive with ductal predominance* ***(j)****. CK19 expression was consistently low in PAs and limited to ductal component in focal distribution* ***(k)****. GCDFP15* ***(l)****, SMA* ***(m)****, CD117* ***(n)*** *and CD10* ***(o)*** *expressions were focal and observed only in a few cases.*

ME: Myoepithelial; PA: Pleomorphic adenomas; SMA: Smooth muscle actin.

4. *In differentiation of BCA from MyoE:* SMA (p=0.001), calponin (p=0.001), p63 (p=0.001), CK5/6 (p=0.004), 34βE12 (p=0.001), CK14 (p=0.001), and CK19 (p=0.001) positivity favored BCA, whereas GFAP (p=0.005) and S100 (p=0.001) positivity favored MyoE.

DISCUSSION

Cytokeratins are expressed in the epithelium in a tissue specific manner, which is attributed

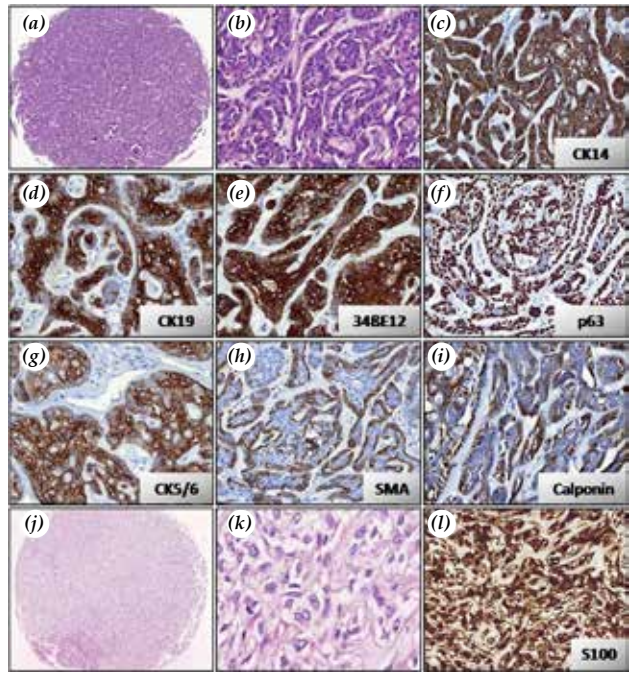

Figure 6. *The tissue section of a basal cell adenoma with basaloid cells and peripheral palisading* ***(a)*** *H-E ×50,* ***(b)*** *H-E ×400, respectively. CK14* ***(c)****, CK19* ***(d)****, 34βE12* ***(e)****, p63* ***(f)*** *and CK5/6* ***(g)*** *were diffusely positive throughout the tumor cells. SMA* ***(h)*** *and Calponin* ***(i)*** *expression were mostly significant in peripheral basaloid cells (IHC; ×400 for all). The tissue cylinder of a myoepithelioma composed of clear myoepithelial cells* ***(j)*** *×50,* ***(k)*** *×400, respectively). S100 expression was diffuse in MyoE cases* ***(l)*** *×400).*

SMA: Smooth muscle actin; MyoE: Myoepitheliomas.

to their biological functions.[10] The CK19, the smallest member of the cytokeratin family, is normally expressed mainly in the simple epithelia and in salivary glands and luminal cells are more intense for CK19. On the contrary, CK14 and CK5/6 are expressed mainly in the stratified epithelia and show intense staining for the abluminal cells with ME morphology.[11-15] Cytokeratin expression levels may be altered during tumorigenesis, and the possible role of higher CK19 expression in tumor diagnosis and tumor behavior has been stressed in different studies in distinct tumors such as thyroid carcinomas[16] and hepatocellular carcinomas.[17-19] Although it is still a controversial topic, most of these studies present high CK19 expression as a poor prognostic indicator in terms of local aggressiveness, increased rate of recurrence, and higher metastatic potential. However, there are

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Table 3. Differential expressions of immunohistochemical markers between distinct tumor subtypes

	PA	ACC	SDC	AdCC
MEC	GFAP (+), 0.001 Calponin (+), 0.001 SMA (- vs focal), 0.001	GLUT1 (+), 0.032 P63 (+), 0.004 CK5/6 (+), 0.040 34ßE12 (+), 0.001	CD10 (focal vs -), 0.001 CK14 (+), 0.045 P63 (+), 0.002 CK5/6 (+), 0.001	CK14 (focal vs ↑), 0.003 SMA(+), 0.001 CD117 (+), 0.001
ACC	P63 (+), 0.005 Galectin3 (+), 0.004 34ßE12 (+), 0.013	X	No differential expression of IHC markers	CD117 (+), 0.001 GCDFP15 (+), 0.010
AdCC	GFAP (+), 0.001 CD10 (+), 0.015 GCDFP15 (- vs focal), 0.005 S100 (focal vs ↑), 0.001 CK14 (↑ vs ↓), 0.044 CD117 (↑ vs ↓), 0.002 CK19 (↑ vs focal), 0.001	CD117 (+), 0.001 GCDFP15 (+), 0.010	GCDFP15 (+), 0.004	X
SDC	CK19 (↑ vs focal), 0.001 CD10 (+), 0.001 S100 (+), 0.001 CK14 (+), 0.001 SMA (- vs focal), 0.001 Calponin (+), 0.001 P63 (+), 0.001 CK5/6 (+), 0.001 CD117 (- vs focal), 0.013	No differential expression of IHC markers	X	GCDFP15 (+), 0.004
BCA	GFAP (+), 0.001 CD10 (+), 0.001 CK19 (↑ vs focal), 0.001	No differential expression of IHC markers	No differential expression of IHC markers	CK5/6 (↑ vs ↓), 0.038 Galectin3 (↑ vs ↓), 0.030

PA: Pleomorphic adenomas; ACC: Acinic cell carcinomas; SDC: Salivary duct carcinomas; AdCC: Adenoid cystic carcinomas; MEC: Mucoepidermoid carcinomas; BCA: Basal cell adenomas.

some contradictory reports, particularly on breast cancer, associating loss of CK19 with unfavorable prognostic factors.[20,21] Among malignant salivary gland tumors in the literature, diffuse CK19 expression in mammary analogue secretary carcinomas and in malignant component of carcinoma ex PA have been reported.[22,23] In addition, variable expression of CK19 has been reported in cribriform adenocarcinoma and polymorphous low-grade adenocarcinoma of salivary glands.[24] In this study, we observed CK19 expression in both benign and malignant salivary gland tumors. However, CK19 expression levels were significantly higher in malignant tumors (p=0.004). Thus, high CK19 expression may suggest the malignant nature of a salivary gland tumor, in consistent with the previous reports on other organ tumors. According to our results, we may suggest that, with the exception of basal cell adenoma, diffuse expression of CK19 in a salivary gland tumor, should alert the pathologist for a more careful examination of the tumor for malignancy. This feature may be particularly useful for differentiating pleomorphic adenoma from adenoid cystic carcinoma.

In early studies regarding ME cells in salivary gland morphogenesis, S100 protein was the most popular marker.[6,25] However, in following studies, the authors suggested S100 as a transient marker, rather than a consistent marker of differentiated ME cells of mammary and other glands.[6,26] Also, one study[27] attributed S100 expression in salivary gland to the unmyelinated thin nerve fibers and argued on the misinterpretation of nerve fibers as ME cells. In that period, the attention concentrated on sensitive myogenous differentiation markers, such as alpha SMA which indicate the distinct alpha isoform of smooth muscle actin protein present in ME cells of breast and salivary gland, and calponin

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which is specific for the smooth muscle.[28,29] In addition, p63 protein, a member of the p53 family of transcription factors, was used as a marker for both ME and basal cells in salivary glands.[30] However, recently p63 expression has been predominantly reported in stem cells of the epithelium in salivary gland.[6] The GFAP is another marker which was investigated as a ME marker, and GFAP positivity was most reliably related to certain type myoepithelial tumors, such as soft tissue MyoEs with cartilaginous differentiation.[31] In general, GFAP failed to be a reliable indicator of ME cells in glandular organs, such as salivary gland and breast.[6] Among ME markers, SMA and calponin are considered to be the most reliable and determinative ones.[5,6] In our study, SMA and calponin expression did not differ significantly between benign and malignant tumor types ($p>0.05$). On the other hand, S100, CK14, p63, CK5/6, and GFAP expressions were significantly lower in malignant tumors ($p<0.05$). With these findings, it may be speculated that the quantities of ME cells in benign and malignant salivary gland tumors are not very different; however, immunophenotypic and possibly biological characteristics of ME cells differ between malignant and benign salivary gland tumors. In addition to contractile function, ME cells also have a tumor suppressor effect in association with matrix synthesis and proteinase inhibition.[3] Breast carcinoma cell lines with ME participation demonstrate less invasive behavior, compared to the carcinoma cell lines without ME cell participation,[4] suggesting an increased invasiveness and metastatic capacity due to the loss of myoepithelial phenotype. In recent studies, biological behavior of distinct tumors has been also related with the evidence of epithelial cells shifting to a mesenchymal phenotype which is referred to epithelial-mesenchymal transition (EMT).[15] Epithelial mesenchymal transition defines a series of changes in cellular phenotype, in which epithelial markers (cytokeratins) are downregulated and the mesenchymal markers (vimentin, SMA) are upregulated, leading to an increased migratory behavior.[15,32-34] Loss of CK14 was interpreted as an indicator of myoepithelial-mesenchymal transition in canine mammary tumors.[15] Among salivary gland tumors, EMT-like transformation was described in AdCC, and it was related with the metastatic capacity of the tumor.[34] In this study, lower expression of CK5/6 and CK14 in the malignant tumor type may be consistent with EMT, showing the loss of (myo)epithelial phenotype of these cells in malignant tumors.

The morphological diversity among salivary gland tumor subtypes, and morphological heterogeneity within an individual tumor may be diagnostically challenging. Pleomorphic adenomas, the most common tumor of salivary glands, are biphasic benign tumors composed of both epithelial and mesenchymal elements. Due to the diverse appearance of epithelial and stromal components, distinguishing this tumor from other benign and malignant salivary gland tumors may be problematic, particularly when chondro-myxoid stroma is inconspicuous. Squamous/mucinous metaplasia, and predominant tubular, cribriform structures may be suspicious of MEC and AdCC, respectively. In our study, distinct markers proved to be useful in differentiating PA from MEC, AdCC, ACC, SDC, and BCA. In the present study, GFAP expression was limited to PA and MyoE, and appeared as a differentiating marker of PA from MEC, AdCC, and BCA. The role of GFAP in the differential diagnosis was attributed to its excellent ability to show potential myxochondromatous differentiation in a previous study.[35] All BCAs (n=5) in this study were negative for GFAP and also for CD10 and this finding may be useful in differentiating PAs from BCAs. The SMA and calponin are consistently negative in malignant salivary gland tumors, except for diffuse positivity of both in AdCCs, and are often focal positive in PAs. Thus, SMA and calponin positivity, either diffuse or focal; favors PA rather than MEC, SDC and ACC in the differential diagnosis. However, in the presence of diffuse expression of these two markers, AdCC should be also considered in the differential diagnosis.

Our study results revealed CD117 positivity in the majority of AdCCs (75%) with generally diffuse distribution and in a minority of PAs (22.6%) with focal distribution. The CD117 positivity in AdCC is a common finding in previous studies with variable positivity rates from 53 to 83%.[36-39] Some recent studies reported variable expression of CD117 in Pas, in which up to 76.5% of tumors

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showed staining, expression being diffuse in most of the tumors.[39-41] In our study, the positivity was focal and limited to the duct-like structures in PAs, and diffuse CD117 positivity was significant in differentiating AdCCs from PAs. We observed S100 positivity in the majority of PAs (93.5%), in some AdCCs (37.5%) and in a minority of MECs (16.6%), usually in a diffuse manner. In this context, although the statistical analysis showed that S100 positivity was significant for differentiation of PA from MEC (p=0.001) and AdCC (p=0.001), it cannot be considered as a reliable marker, due to same expression pattern in MEC and AdCC, albeit in a small percentage of cases.

Mucoepidermoid carcinomas and ACCs are two tumors which may create diagnostic confusion due to overlapping morphologic features, including cystic changes and mucinous secretions. The p63 staining was previously investigated in certain studies and MECs were reported as p63 positive, whereas ACCs were p63 negative with minor exceptions.[30,42,43] In accordance with the previous reports, in this study, all ACCs were p63 negative and 10 of 12 MECs (90.9%) were diffusely positive. Similarly, 34βE12 and CK5/6 expressions also differed between these two tumors: all ACCs were negative, and all MECs were diffusely positive with both markers. It is noteworthy to state that, p63, CK5/6 and 34βE12 staining in MECs is related to squamous, rather than ME differentiation. Furthermore, GLUT1 positivity significantly favored MEC (p=0.032). In the light of these findings, we may suggest a differentiating IHC panel consisting of p63, 34ßE12, CK5/6, and GLUT1 for the differential diagnosis between these two entities. Acinic cell carcinomas were positive with only CK19 and GCDFP15. As diffuse CK19 positivity was a common finding among the malignant salivary gland tumors in our study, CK19 does not seem to have a role in differential diagnosis within the malignant group. However, GCDFP15 positivity seems to be promising, since it was diffusely positive in most of the ACCs and SDCs, in contrast to AdCCs which were completely negative. Our findings are consistent with a previous report which investigated the differential diagnosis of SDC from other salivary gland carcinomas and associated GCDFP15 positivity with SDC.[44]

Galectin-3, a member of B-galactoside binding lectins family, is suggested as an indicator of malignancy, cancer aggressiveness and metastasis in certain tumors including AdCC of the salivary gland.[45-47] In our study, both benign and malignant tumors were galectin-3-positive in varying degrees. Among the malignant tumors, MECs (41.7%) and AdCCs (62.5%) were significantly galectin-3-positive, while most of the SDCs (75%) were negative. In the benign group, 100% of BCAs and 62.1% of PAs were galectin-3-positive. Our findings did not support a correlation between galectin-3 and malignancy.

In conclusion, expression of myoepithelial markers is a common finding in salivary gland tumors. However, distinct tumor types may show different preferences with regard to various myoepithelial and other markers, which may be exploited in the differential diagnosis of these tumors. In this study, SMA and calponin expression did not differ significantly between benign and malignant tumor groups, while S100, CK14, p63, CK5/6, and GFAP expressions were significantly lower in malignant tumors. It may be suggested that, IHC characteristics of ME cells, rather than the quantity per se, shows significant difference between malignant and benign tumors. According to the results of this study, diffuse CD117 expression favors AdCC, GFAP expression favors PA; 34βE12, p63, and CK5/6 expression favors MEC, while GCDFP15 favors SDC and ACC. In addition, diffuse expression of CK19 may suggest the malignant nature of a salivary gland tumor in cases with diagnostic difficulties.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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