Significance of C-KIT and SOX10 in the Diagnosis of Salivary Gland Neoplasms: An Immunohistochemical Study

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Abstract

The histopathological features of salivary gland tumors on routine H & E are the gold standard for diagnosis. Tumors with somewhat equivocal histological features may need additional immunohistochemical markers for establishing final diagnosis.

Objective: To study the immunohistochemical pattern of SOX10 and CD 117 (c-KIT) in human samples of normal and neoplastic salivary gland tissues, trying to characterize and distinguish the various types of salivary gland tumors and to determine their origin from epithelial (luminal) and myoepithelial/basal (abluminal) cells of salivary glands.

Material and Methods: Forty malignant salivary gland tumors [ten cases of Mucoepidermoid Carcinoma (MEC), 10 cases of Adenoid Cystic Carcinoma (AdCC), 8 cases of Acinic Carcinoma (AcC), 6 cases of Polymorphous Low Grade Adenocarcinoma (PLGA), 3 cases of Carcinoma Ex-pleomorphic Adenoma (CXPA) and 3 cases of Epithelial Myoepithelial Carcinoma (EMC)] and 22 benign tumors [12 cases of Pleomorphic Adenoma (PA) and 10 cases of Warthin Tumor (WT)], in addition to 10 control cases of chronic sialadenitis were included in this study. Histopathological diagnosis was made on freshly prepared H & E sections followed by immunohistochemical application and analysis of c-KIT (CD117) and SOX10 markers.

Results: The mean age of the patients with salivary gland tumors was 48±12 (mean SD) (range, 18-84) years. There were 33 male and 29 female patients. Eight cases of PA, 9 cases of AdCC, 6 cases of AcC and 4 cases of PLGA were positive for c-KIT. No significant difference was found between the positivity of c-KIT expression in these four types of salivary gland tumors. Although the percent of positive tumor cells was different between them, however this difference was still insignificant. Other salivary gland tumors examined showed weak and focal c-KIT expression.

In normal human salivary gland tissue, SOX10 expression was specific to the nuclei of acini and both luminal and abluminal cells of intercalated ducts but not in other sites. SOX10 expression can differentiate salivary tumors into two subgroups in which acinic cell carcinomas, adenoid cystic carcinomas, epithelial-myoepithelial carcinomas, and pleomorphic adenomas, including the pleomorphic adenoma component of carcinoma (CXPA), were strong SOX10 positive, while mucoepidermoid carcinomas, PLGA and Warthin tumors were weak or negative for SOX10.

Conclusion: These results suggested that c-kit of no use in differential diagnosis between AdCC, AcC, PLGA and PA. Using of SOX10 may be helpful in diagnosis of some neoplastic lesions of salivary gland and may help in understanding the histogenesis of salivary gland tumors. SOX10 expression pattern of salivary gland tumors mirrors those of normal tissues, showing acinus and intercalated duct differentiation in a biphasic manner.

Key Words: Salivary gland neoplasms – SOX10 – CD117 (c-KIT) – Immunohistochemistry.

Introduction

THE incidence of salivary gland tumors worldwide is increasing, irrespective of the etiologic factor [1]. There are 34 benign and malignant salivary gland epithelial tumors according to the 2005 third histologic classification of the World Health Organization [2]. These tumors can show diverse morphology and overlapping histologic features with a wide variety of prognostically important histological subtypes. Although pathologists have investigated this diverse group of tumors, their diagnosis and treatment remain a complex and challenging problem [3,4]. Diagnoses of most salivary gland tumors can be made on the basis of hematoxylin-eosin sections, however immunohistochemistry can provide a powerful adjunct tool for diagnosis and determining the cell type of origin for that heterogeneous group of tumors particularly in situation of loss of characteristic histopathological features as in an incisionally biopsied or fragmented biopsy sample [5].

C-KIT (CD117) maps to the long arm of chromosome 4 and encodes a transmembrane receptor-type tyrosine kinase, KIT, that is structurally and functionally related to platelet-derived growth
factor and colony stimulating factor-1 receptors and contributes to the regulation of cell growth and differentiation [6]. Overexpression of c-KIT has been reported in a subset of malignant neoplasms such as gastrointestinal stromal tumors, myeloid leukemia, testicular germ cell tumors, endometrial carcinomas, papillary and follicular thyroid carcinomas, renal and hepatic angiomylipoma, synovial sarcoma, osteosarcoma, and Ewing’s sarcoma, suggesting a role for c-KIT and its mutant forms in carcinogenesis [6-8]. In human salivary gland neoplasms, reports of c-kit expression have been contradictory. Currently, there is little information on the altered expression of c-kit in salivary gland tumors, which is mainly limited to AdCC and PLGA. Furthermore, recent studies about using c-kit for distinguishing other salivary gland tumors are controversial.

The SRY-related HMG-box 10 (SOX 10) protein is a transcription factor of particular interest because of its role as a marker of Neural Crest Stem Cells (NCSCs) and in the maintenance and migration of NCSCs [9,10]. NTRK3, NTF3, and SOX10 were identified as independent drivers of Hirschsprung disease [11]; a genetic condition linked to the inability of NCSCs to migrate, differentiate, and develop into the enteric nervous system [12,13]. SOX10 is a more sensitive and specific marker for melanocytic and schwannian tumors than S 100 [13,14].

In the development of mouse submandibular glands, SOX10 expression has been associated with the presence of epithelial stem/progenitor cells from embryonic day 13.5 (E13.5) to adulthood. In addition, SOX10 has been noted to be expressed more frequently in buds (acinii) than in ducts with high levels of endogenous expression in the luminal (epithelial) cells of acinic E 14.5 embryos [15]. Recently, SOX10 was found to be expressed in both luminal and abluminal cells of intercalated ducts of normal human major salivary glands and positive expression has also been noted in myoepithelial cells of the bronchial submucosa, and mammary glands [3].

The objective of this study was to find whether c-KIT and SOX10 have the potential to serve as pathological markers in benign and malignant salivary gland tumors.

**Material and Methods**

This is a retrospective controlled study on formalin fixed, paraffin embedded salivary gland specimens collected from the Department of Pathology of Benha University, Pathology Department of National Cancer Institute, Cairo University and from private labs, from April 2004 to June 2014. These specimens included 22 benign salivary gland tumors: 12 cases of PA and 10 cases of Warthin tumor, and 40 malignant tumors: Ten cases of mucoepidermoid carcinoma, 10 cases of AdCC, 8 cases of acinic carcinoma, 6 cases of PLGA, 3 cases of carcinoma ex-pleomorphic adenoma, 3 cases of epithelial myoepithelial carcinoma, in addition to 10 control cases of chronic sialadenitis. Histological features of all the selected cases were reviewed from freshly prepared H & E. Diagnosis and classification were based on the criteria of the World Health Organization for salivary gland tumors [2]. The data of gender, age and the site of involvement were extracted from the archival files.

From each block three sections, 4 gm thick, were cut. Deparaffinized sections were subjected to hematoxylin and eosin in addition to immuno-histochemical staining. For c-KIT immunostaining, antigen retrieval in a pressure cooker of 56C for 24h followed by microwave heat induced epitope retrieval (595 W microwave for 10min). Then an Envision-HP, ChemMateTM/TechMateTM Detection System (Dako Cytomation A/S, Glostrup, Denmark) was performed, using a primary polyclonal rabbit anti-CD117 antibody (A4502, Dako- Cytomation A/S, Glostrup, Denmark), diluted 1:50, for 30min, a 3min application of 3% H2O2, followed by EnvisionTM incubation for 25min and diaminobenzidine (DAB) for 10min. The slides then washed in water and counterstained with aqueous hematoxylin. Sections incubated in buffer Tris (hydroxymethyl) methylamine Analar (BDH Lab. Supplies, Poole, England) instead of the primary antibody were used as negative controls, whereas skin tissue was used as positive control.

Staining with Sox10 antibodies (goat polyclonal, 1:200, N-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed as described by Nonaka et al., [14].

Incubations were done in the humidity chamber. Appropriate positive and negative controls were stained as advised by the manufacturer. ChemMate EnVision (Dako) methods were used for detection.

Immunoreactivity of c-KIT was semiquantitatively assessed and considered to be positive if greater than 10% of tumor cells were stained. The membrane and/or cytoplasmic positive staining of cells were countered as positive.

Positivity was graded according to the percentage of tumor cells stained as weak (10-25%), moderate (26-50%) and strong (50-100%) [16,17].
For SOX10, nuclear staining of >10% tumor cells was considered positive [3].

**Statistical analysis:**

Statistical analysis was performed using the SPSS (statistical program for social science) software version 18.0 (SPSS Inc., Chicago, Illinois, USA).

**Results**

The mean age of the patients was 48 ± 12 (mean SD) (range, 18-84) years. There were 33 male and 29 female patients.

**C-KIT results:**

The results of c-KIT expression of all neoplastic salivary samples are summarised in (Table 1). In sialadenitis cases C-KIT was strongly expressed in the cytoplasm and the membrane of serous cells, intercalated ducts with less intensity in the striated and interlobular ducts, whereas mucous and myoepithelial cells were negative Fig. (1). On the other hand normal salivary tissues adjacent to tumor tissue, showed no immunoreactivity of acinar cells and very weak in the cytoplasm of intercalated and striated ductal cells of major salivary glands. Six cases (50%) of pleomorphic adenomas showed moderate (25-50%), and two cases (16.7%) show strong positivity (>50%). The positivity mainly of the luminal neoplastic cells of the duct-like structures, whereas the tumor cells in solid, trabecular or reticular areas, myoepithelial cells, were negative Fig. (2). Two cases (20%) of Warthin's tumors showed c-KIT positivity, one was moderately stained and the other showed weak positivity Fig. (3). One case (1/10) of mucoepidermoid carcinoma showed focal moderate C-KIT positivity Fig. (4). Most adenoid cystic carcinoma cases (80%) showed strong and diffuse expression of c-KIT (mean value: 68.2%) and expression is predominantly located in the inner ductal cells of the tubular and cribriform patterns and was homogeneously expressed in the solid form. Four cases (66.6%) of polymorphous low-grade adenocarcinomas showed immunoreactivity for c-KIT in luminal and occasional in non-luminal cells. In all positive cases 25-50% of neoplastic cells were positive (mean value: 40.6%) Fig. (5). Acinic cell carcinoma showed moderate positivity in 62.5% (5/8) of cases, mainly as a diffuse, cytoplasmic pattern and one case showed weak staining. No significant difference between the positivity of c-kit expression in the four types of salivary gland tumors: PA, AdCC, PLGA and AcC was found. Although the percent of positive tumor cells was different between them, however this difference was still insignificant. One case (33.3%) of epithelial-myoepithelial carcinoma showed weak positivity (<25%) of neoplastic cells of the inner layer of bi or multilayered ductal structures.

Immunopositivity of c-Kit was found in two cases (66.6%) of carcinoma expleomorphic adenoma in the pleomorphic adenoma component, whereas the carcinoma components of them lacked c-KIT expression.

**SOX10 results:**

The results of SOX10 expression of all neoplastic salivary samples are summarised in (Table 2). In normal salivary gland tissue adjacent to benign tumor and in sialadenitis cases, SOX10 was expressed in almost all (>90%) of the abluminal (myoepithelial) and luminal (epithelial) cells in acini and intercalated ducts. SOX10 expression in mucinous acini was weaker than in serous acini; however, it was not expressed in the abluminal (basal) and luminal cells of striated and excretory ducts. Benign salivary gland tumors examined showing different pattern of SOX10 expression. While all cases (12/12) of pleomorphic adenoma were positive (in 70-90% of tumor cells) Fig. (6); all Warthin's tumor cases were negative (0/10). SOX10 was expressed in 9 out of 10 AdCC specimens (90%). SOX 10 staining in AdCC tumour cells was intense in the nuclei and was also detectable in the cytoplasm in the majority of cells (70-90% of cells in all tumours examined) Fig. (7). SOX10 expression in primary AdCC specimens was markedly higher than in normal salivary tissue. Of ten MEC specimens examined, only one case (10%) was SOX10 positive, but, unlike AdCC, staining of this tumour revealed only moderate nuclear/cytoplasmic expression. Sox10 staining was observed in >80% of cancer cells of the three cases of epithelial-myoepithelial carcinoma examined Fig. (8). All acinic cell carcinoma cases (8/8), were diffusely (>90% of cells) positive for SOX10. Also all cases of carcinoma expleomorphic adenoma (3/3) were positive in more than 70% of tumor cells of pleomorphic adenoma component, however in one case of them, 30-40% of malignant cells showed SOX10 positivity. Two cases out of 6 of PLGA cases (33.3%) were SOX10 positive in 20-50% of malignant cells.

No significant difference between the expression of SOX10 in acinic cell carcinomas, adenoid cystic carcinomas, epithelial-myoepithelial carcinomas, and pleomorphic adenomas, including the pleomorphic adenoma component of carcinoma (CXPA) was found.
## Table (1): C-KIT expression in different salivary gland tumors examined.

<table>
<thead>
<tr>
<th>Type of the tumor</th>
<th>c-KIT expression</th>
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<tr>
<td>PA (n=12)</td>
<td>4 (33.3%)</td>
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<td>WT (n=10)</td>
<td>8 (80%)</td>
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<td>MEC (n=10)</td>
<td>9 (90%)</td>
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<td>AdCC (n=10)</td>
<td>1 (10%)</td>
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<td>PLGA (n=6)</td>
<td>2 (33.3%)</td>
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<td>AcC (n=8)</td>
<td>2 (25%)</td>
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<td>EMC (n=3)</td>
<td>2 (66.7%)</td>
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<td>CXPA (n=3)</td>
<td>3 (100%)</td>
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PA : Pleomorphic Adenoma.
WT : Warthin Tumor.
MEC : Mucoepidermoid Carcinoma.
AdCC : Adenoidcystic Carcinoma.
PLGA : Polymorphous Low Grade Adenocarcinoma.
AcC : Acinic Carcinoma.
EMC : Epithelial-Myoepithelial Carcinoma.
CXPA : Carcinoma Expleomorphic Adenoma.

## Table (2): SOX10 expression in different salivary gland tumors examined.

<table>
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<td>4 (66.7%)</td>
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<td>AcC (n=8)</td>
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Fig. (1): A case of chronic sialadenitis showing strong diffuse cytoplasmic expression of c-KIT (streptavidin/biotin DAB X100).

Fig. (2): A case of pleomorphic adenoma with strong diffuse cytoplasmic expression of c-KIT of luminal neoplastic cells of the duct-like structures (streptavidin/biotin DAB X100).

Fig. (3): Moderate cytoplasmic expression of c-KIT in a case of WT (streptavidin/biotin DAB X100).

Fig. (4): A case of MEC showing cytoplasmic and membranous expression of c-KIT: Adjacent acini and duct showed c-KIT positivity of sialadenitis (streptavidin/biotin DAB X100).
Discussion

The interaction of c-KIT transmembrane tyrosine kinase receptor with its ligand, stem cell factor, promotes phosphorylation and the activation of intracytoplasmic signal cascades, which are essential in embryogenesis, hematopoiesis, development, proliferation, and migration of germ cells. There is only limited information on the immunoreactions of c-KIT in salivary gland tumors. Few studies examined the c-KIT immunoreactivity in certain subtypes of salivary gland carcinomas \[6,17\] and focused in the expression of c-KIT in adenoid cystic carcinomas \[18\] to differentiate them from polymorphous low grade adenocarcinomas \[19\], from monomorphic adenomas \[7\] and from pleomorphic adenomas \[20\].

In the present study, except focal weak staining of striated ductal epithelial cells, non neoplastic salivary gland tissue adjacent to tumor tissues examined was negative for c-KIT. In contrast it was found that in cases of sialadenitis, the serous-acinar and intercalated ductal cells showed strong expression of c-KIT in both cytoplasmic and membrane pattern instead of weak cytoplasmic positivity of striated ducts, indicating a possible participation in their pathogenesis. Somatic mutations of c-KIT gene are known to be present in over half of GISTs most commonly in exon 11 which encodes juxtamembrane domain and less commonly in exons 9 and 13 of extracellular and kinase domains respectively \[21\]. On the other hand mutations have not been occurred in breast tissue tumor \[22\] as well as salivary gland neoplasms \[17,18\]. Other probable mechanisms including gain of function mutations of c-KIT outside of the examined domains and overexpression of c-KIT secondary to C-KIT gene amplification. In addition, dysregulated c-KIT signalling may also occur by autocrine activation of its ligand stem cell factor \[18\]. Similarly to neuronal stem cells that activate c-KIT in cases of injury \[23\], reserve cells of intercalated ducts (and some of striated) and serous-acinar cells in cases of sialadenitis (type of injury) may have...
a capability for autocrine activation [15]. Expression of c-KIT was reported in normal salivary gland tissue in one study [24], whereas Holst et al., [18] found absence of expression. Edwards et al., [7], while Jeng et al., [17] and Chandan et al., [20] revealed striated ductal cell weak positivity, and Mino et al., [6] found positivity in luminal epithelia of both intercalated and striated ducts.

In the present work, c-KIT expression was examined in certain types of neoplastic salivary gland specimens. In benign tumors c-KIT immunoreactivity was observed in 66.7% of pleomorphic adenomas cases. However in the studies of Chandan et al., [20] and Andreadis et al., [15], all pleomorphic adenoma were positive for c-KIT while Mino et al., [6] found positivity only in (19% of cases). In contrast to Mino et al., [6], positive c-KIT immunoreactions was found in the present study in two cases (20%) of Warthin tumor which was also in accordance to results of Andreadis et al., [15].

In malignant neoplasms, the results of the current study showed that most adenoid cystic carcinoma cases showed strong and diffuse expression of c-KIT (80% of cases, up to 50% of cells). c-KIT expression was predominantly located in the inner ductal cells of the tubular and cribriform patterns and was homogeneously expressed in the solid form. Similar results were achieved by Penner et al., [19], Mino et al., [6], Andreadis et al., [15] and Zhu et al., [5].

The majority of Polymorphous Low Grade Adenocarcinoma (PLGA) cases examined was positive (4/6) with at least 25% of the tumour cells being immunoreactive, however overall intensity of staining weaker than in adenoid cystic carcinomas. The pattern of c-KIT expression specific for AdCC was not appreciated by PLGA, probably because of its diverse histological morphology [4].

Previous results of the expression of c-KIT in PLGA is controversial. While some researchers reported that PLGA rarely expressed c-KIT or had low expression [17,19], others have reported frequent expression of c-KIT in PLGA [7,15,25].

In the current research, 10% (1/10) of mucoepidermoid cases showed weak and focal c-KIT immunoreactivity, however, 75% (6/8 cases) of acinic carcinoma examined were positive for c-KIT.

Mino et al., [6] revealed only a variable weak positive staining in luminal epithelia of the intercalated and striated ducts of normal salivary glands and qualitatively/quantitatively similar to the above studies staining for adenoid cystic carcinomas, however pleomorphic adenoma, mucoepidermoid carcinoma, acinic cell carcinoma and Warthin tumor were only rarely expressed c-KIT. Similar to Mino et al., [6] results, Tsuura et al., [26], Miettinan and Lasota, [27] found that except for adenoid cystic carcinomas, lymphoepithelial and myoepithelial carcinomas were consistently c-KIT positive, all other salivary gland carcinomas were negative.

In a study carried out by Chandan et al., [20] in order to distinguish adenoid cystic carcinomas from pleomorphic adenomas in cell block materials obtained during Fine-Needle Aspiration Biopsy (FNAB), they found positive c-KIT in all cases of pleomorphic adenomas with up to 50% immunoreactive cells in the 60-66.7% of cases. Similarly, all adenoid cystic carcinomas were positive with up to 50% immunoreactive cells in the 80% of cases. No obvious expression of c-KIT was observed in nonneoplastic salivary gland tissue specimens. Study of Jeng et al., [17] showed immunoreactivity only in striated ducts (weak) of normal salivary gland and in adenoid cystic carcinoma types (75% of tubular, 76.5% of cribriform, 100% of solid variants). In contrast, polymorphous low grade adenocarcinomas (25% of the cases), mucoepidermoid carcinoma (11%), acinic cell carcinomas (13% of the cases), pleomorphic adenomas (19%), showed little c-KIT positivity, whereas all cases of Warthin tumours were completely negative.

According to the present results, c-KIT couldn't significantly differentiate between AdCC, AcC, PLGA and PA. Although it was found that the major difference in c-KIT expression between these types lies in the number of positive tumor cells, in all positive cases of AdCC more than 50% and in all other tumor types positive cases less than 50% of the tumor cells were positive except two cases of PA that showed more than 50%.

However, according to Jeng et al., [17] Penner et al., [19] but not to Edwards et al., [7] c-KIT could consider as a useful marker for distinguishing adenoid cystic carcinoma from PLGA. Also, although they both used the same antibodies, dilutions and methods Chandan et al., [20] but not Mino et al., [6] revealed that this molecule does not restricted to adenoid cystic carcinomas comparing to polymorphic adenomas. These contradictory findings may be due to using of different protocol (include deparaffinization and immunohistochemical method), antibodies and dilutions performed.

Present results suggest that c-KIT expression, thus of no use practically in differential diagnosis between adenoid cystic carcinoma, PLGA, AciC
and pleomorphic adenoma (p > 0.05). Similarly, Zaub et al., [4] found no difference in the percentage of positive cases which can differentiate between adenoid cystic carcinoma and PLGA. Epivatianos et al., [28] also found that the major difference in c-KIT expression between these two types lies in the number of positive tumor cells, in all positive cases of AdCC more than 50% and in all positive PLGA cases less than 50% of the tumor cells were positive.

Concerning the differential diagnosis between AdCC and PA, Chancvfdan et al., [29] found that all cases of both tumors were positive for CD 117 but here again, the difference between them was the extent of staining.

Moreover, differences of results worldwide may be attributed to the technique sensitivity of IHC leading to false positive and false negative results.

Interestingly it was observed in the present study that the luminal cells of bi or multilayered structures of 1/3 (33.3%) of epithelial-myoepithelial Carcinoma (EMC) cases were positive for c-KIT that was similar to a bronchial case of a study carried by Ru et al., [30]. Accordingly, in a study carried out by Kim et al., [31], c-KIT was expressed only in the inner luminal cells of an EMC.

In the current work it was found that the malignant cells of the 3 cases Carcinoma Ex Pleomorphic Adenoma (CXPA) examined were negative for c-KIT, however the pleomorphic adenoma components of two cases were positive.

Only limited information on the c-KIT expression in benign and malignant components of CXPA has been reported [16,31,32].

Similarly, Andreadis et al., [15] and Kim et al., [31] observed the loss of c-KIT expression in the invasive and noninvasive carcinoma components but not in the benign component of CXPA; this suggests that the loss of c-KIT is associated with the malignant transformation of CXPA and c-KIT may serve as a useful ancillary diagnostic marker for CXPA. In CXPA cases, c-KIT was expressed in the most residual PAs (83.3%). These expression patterns also suggest that c-KIT may play a role in the differentiation of common precursor cells of myoepithelial and ductal cells.

The transcriptional factor SOX10 appears to support stem-like properties in normal tissues and cancer cells. In normal tissue, it maintains stem cells in their undifferentiated state and controls differentiation [9,33], whereas in melanoma it serves as a marker of the stem-like CD271-positive cells [34].

SOX10 was expressed in the current study in almost all acini and intercalated ducts of normal salivary glands in both luminal and abluminal cells. By examining of SOX10 expression in salivary gland neoplasms, the present results suggest that SOX10 is a useful marker to differentiate two distinct subgroups of tumors; tumors with frequent and high SOX10 expression were acinic cell carcinoma (8/8 of cases), adenoid cystic carcinoma (9/10 of cases), epithelial-myoepithelial carcinoma (3/3 of cases) and pleomorphic adenoma (12/12 of cases). These tumors show similarities to acini and intercalated ducts. The tumors with no or low SOX10 expression were Warthin tumor (0/10), mucoepidermoid carcinoma (1/10), PLGA (2/6 of cases) and carcinoma component of CXPA (1/3 of cases), which are usually thought to resemble striated and excretory ducts. These results were agreed with that of Ohtomo et al., [3]. Similar results were achieved by Schmitt et al., [35] in their study to evaluate SOX10 in differentiating acinic cell carcinoma (AcicCC) from other salivary gland neoplasms with oncocytic features on fine-needle aspiration cell blocks (FNA CB).

Adenoid cystic carcinoma is a malignant biphasic epithelial tumor composed of modified myoepithelial and ductal cells. Acinic cell carcinoma demonstrates both serous acinar and intercalated ductal epithelial differentiation Ohtomo et al., [3].

When compared salivary gland malignant tumors, AdCC overexpressed a large cluster of neuronal genes grouped around TrkC/NTRK3, a tyrosine kinase neurotrophic receptor associated with neurogenesis and cancer [36]. This observation suggested that AdCC aberrantly expresses genes involved in neural stem cell differentiation [37].

In AdCC, Ivanov et al., [36] demonstrated previously that SOX10 expression correlates with the neural stem markers TrkC, MAP2, SALL2, and SLITRK6. In the present study it was demonstrated that SOX10 expressed normally during salivary gland differentiation and markedly upregulated in a great majority of AdCC cells and other malignant tumor such as acinic cell carcinoma and epithelial myoepithelial carcinoma that can be used as a sensitive marker for their diagnosis and similar to AdCC, these tumors may express genes involved in neural stem cell differentiation.

Unlike previously described TrkC, which is highly specific for the myoepithelial cells/cancers
of salivary gland and myoepithelial cells of breast tissue [13], in the current work SOX10 expression was not restricted to the myoepithelial cells and tumours that show myoepithelial differentiation but is also seen in acinar cells, acinic tumours, and, occasionally, in the basal cells of the intercalated duct. Thus, Sox10 shows a broader specificity than TrKC and may be helpful for the diagnosis of salivary cancers that originate from the acinar and intercalated duct areas of the salivary gland.

Previous reports established SOX10 as a marker of myoepithelial tumors other than Schwann and melanocytic tumors, and confirmed SOX10 negativity in neoplastic epithelial cells of multiple organs; lung, breast, esophagus, stomach, colon, liver, kidney, prostate gland, uterine cervix, ovary, and skin [38,39]. The findings of the present study suggest that SOX10 is not limited to neoplastic myoepithelial cells but include some neoplastic epithelial cells, which are commensurate with SOX10 distribution in the normal salivary glands examined. SOX10 was highly expressed in tumors exhibiting similarities to acini and intercalated ducts (e.g., acinic cell carcinoma, adenoid cystic carcinoma, pleomorphic adenoma and epithelial-myoeplithelial carcinoma), and not in tumors resembling striated and excretory ducts (eg, mucopeidermoid carcinoma and warthin tumor). Some reports have indicated that intercalated duct hyperplasia and adenoma might be precursor lesions to epithelial-myoeplithelial carcinoma or other biphasic tumors [40]. It is also known that intercalated ducts exhibit high proliferation activity under conditions of postradiation and chronic sialadenitis [41]. Intercalated ducts are considered to have high potential for entering the neoplastic process, and several tumors might have originated from intercalated ducts [40,42].

Conclusions:

In conclusion, the expression of c-KIT marker was not restricted to any specific salivary gland tumor type. Further study is necessary to compare the expression of c-KIT in large number of different types of salivary gland tumors.

SOX10 expression appears to be a part of a highly coordinated transcriptional programme characteristic for cancers with basal/myoepithelial features. In addition, SOX10 appears to be a potential marker for acinar and intercalated duct differentiation in the diagnosis of salivary gland tumors. SOX10 can be useful in the differential diagnosis of some salivary gland tumors and understanding their histogenesis, SOX10 is valuable in ruling out benign lesions such as WT; however, negative results for SOX10 do not favor a benign diagnosis since MEC is often negative for SOX10. Identification of conserved elements of the SOX10 signatures may help in better understanding of SOX10-related signaling and development of novel diagnostic and therapeutic tools.

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