Head and Neck Pathol (2015) 9:47–50
DOI 10.1007/s12105-014-0546-4

ORIGINAL PAPER

Evaluation of PAX2 and PAX8 Expression in Salivary Gland Neoplasms

Randall T. Butler · Megan A. Alderman · Lester D. R. Thompson · Jonathan B. McHugh

Received: 3 March 2014 / Accepted: 16 April 2014 / Published online: 26 April 2014
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Abstract PAX2 and PAX8 are transcription factors involved in embryogenesis that have been utilized as immunohistochemical indicators of tumor origin. Specifically, PAX2 is a marker of neoplasms of renal and müllerian origin, while PAX8 is expressed by renal, müllerian, and thyroid tumors. While studies examining these transcription factors in a variety of tumors have been published, data regarding their expression in salivary gland neoplasms are limited. The goal of this study was to assess expression of PAX2 and PAX8 in a large cohort of salivary gland tumors. Utilizing tissue microarrays, samples of normal salivary glands (n = 68) and benign and malignant salivary gland neoplasms (n = 442) were evaluated for nuclear immunoreactivity with PAX2 and PAX8. No expression was observed with either marker in the normal salivary glands, and PAX8 was negative in all neoplasms. Focal expression of PAX2 was observed in one example each of oncocytoma and acinic cell carcinoma. These results indicate that evaluation of PAX2 and/or PAX8 expression would be valuable in differentiating primary salivary gland tumors from metastases known to express PAX2 and/or PAX8.

Keywords Salivary gland neoplasms · PAX2 · PAX8

R. T. Butler · M. A. Alderman · J. B. McHugh (✉)
Department of Pathology, University of Michigan, Ann Arbor, MI, USA
e-mail: jonamch@umich.edu

L. D. R. Thompson
Department of Pathology, Woodland Hills Medical Center, Woodland Hills, CA, USA

Introduction

The *PAX* genes encode a family of nine transcription factors, defined by the presence of a 128-amino acid DNA-binding sequence called the "paired domain," that have been shown to play integral roles in development and maintenance of pluripotent stem cells [1]. PAX2 and PAX8 are members of the same subfamily and are involved in organogenesis of the central nervous, genitourinary, and müllerian systems, with PAX8 additionally involved in development of the thyroid gland [1]. Correspondingly, immunohistochemical detection of PAX2 and/or PAX8 expression has emerged as an important diagnostic tool in the diagnosis of primary benign and malignant as well as metastatic renal and müllerian neoplasms; PAX8 is also expressed by thyroid tumors [2–12]. Additionally, PAX8 immunoreactivity has been detected in thymomas and thymic carcinomas [2, 13], and, more recently, depending on the antibody employed, Merkel cell carcinoma [14]. Immunohistochemical detection of PAX8 may also serve as a "positive" marker of tumors of the male genital tract, rare tumors that are traditionally diagnoses of exclusion [15]. Reports of PAX8 expression in B cells, B cell lymphomas, and pancreatic neuroendocrine tumors, however, appear to represent cross-reactivity of polyclonal anti-PAX8 antibodies with other PAX proteins [12, 16, 17].

While a number of studies have examined immunohistochemical expression of PAX2 and PAX8 in a variety of benign and malignant neoplasms, investigation of these transcription factors in salivary gland tumors has been limited. Specifically, to our knowledge, only two previous studies have evaluated PAX8 immunostaining in salivary gland neoplasms; in these investigations, the total number of salivary gland tumors was relatively limited, and only limited histologic subtypes were included [2, 3]. Even

fewer salivary gland neoplasms appear to have been evaluated for expression of PAX2 [9]. The aim of the present study, therefore, was to assess expression of PAX2 and PAX8 in a greater number and diversity of benign and malignant salivary gland neoplasms.

Methods

Representative samples of normal salivary glands (n = 68) and benign and malignant salivary gland neoplasms (n = 442) were incorporated into several tissue microarrays (TMAs) after approval by the respective institutional review boards. The histologic subtypes of neoplasms were diagnosed according to World Health Organization criteria and numbered as follows: pleomorphic adenoma (n = 19), myoepithelioma (n = 7), oncocytoma (n = 54), Warthin tumor (n = 17), basal cell adenoma (n = 18), canalicular adenoma (n = 19), acinic cell carcinoma (n = 38), adenoid cystic carcinoma (n = 41), mucoepidermoid carcinoma (n = 148), salivary duct carcinoma (n = 47), basal cell adenocarcinoma (n = 4), polymorphous low-grade adenocarcinoma (n = 18), myoepithelial carcinoma (n = 6), and epithelial-myoepithelial carcinoma (n = 6).

In preparation for immunohistochemical staining, formalin-fixed and paraffin-embedded sections of the TMAs were first subjected to pretreatment on Ventana Benchmark Ultra stainers using proprietary buffers. The sections were then incubated with a rabbit polyclonal anti-PAX2 antibody (Invitrogen, catalog #187-0483, 60 min) or a rabbit polyclonal anti-PAX8 antibody (Cell Marque, catalog #363A-18, 32 min). For both antibodies, reaction was detected with the Ultraview diaminobenzidine kit (Ventana Medical Systems). Appropriate positive (samples of non-neoplastic kidney and thyroid) and negative controls were incorporated into the TMAs and stained in parallel with the normal and neoplastic salivary gland tissue for each antibody.

PAX2 and PAX8 expression was assessed on the basis of extent of nuclear positivity as follows: 0 for no or only cytoplasmic staining, 1+ for 1–10 % positive nuclei, 2+ for 11–50 % positive nuclei, and 3+ for ≥51 % positive nuclei. Scoring for all tumor and normal samples was performed by the senior author (J.B.M.), and cases with equivocal or positive staining were reviewed by another author (R.T.B.) to confirm their classification and extent of reactivity.

Results

Positive and negative controls stained appropriately; specifically, renal tubular epithelium exhibited reactivity with both PAX2 and PAX8, and thyroid follicular cells demonstrated expression of PAX8. Results of immunohistochemical staining for the normal salivary gland samples are shown in Table 1 and those for the neoplasms are displayed in Table 2. Note that the number of evaluable samples differed for some of the tumors due to cores that were absent and/or consisted only of non-neoplastic tissue on the stained TMA sections. A single example each of oncocytoma and acinic cell carcinoma exhibited focal (1+) reactivity with PAX2, as illustrated in Fig. 1. All benign and malignant tumors were negative for PAX8.

Table 1 Expression of PAX2 and PAX8 in normal salivary gland tissues

Tissue	PAX2	PAX8
Parotid gland	0/49	0/49
Submandibular gland	0/3	0/3
Sublingual gland	0/1	0/1
Minor salivary gland	0/15	0/15

Table 2 Expression of PAX2 and PAX8 in salivary gland neoplasms

Tumor	PAX2	PAX8
Pleomorphic adenoma	0/19	0/19
Myoepithelioma	0/7	0/7
Oncocytoma	1/53 (1.9 %)	0/53
Warthin tumor	0/16	0/16
Basal cell adenoma	0/18	0/18
Canalicular adenoma	0/19	0/19
Acinic cell carcinoma	1/32 (3.1 %)	0/33
Adenoid cystic carcinoma	0/37	0/39
Mucoepidermoid carcinoma	0/131	0/131
Salivary duct carcinoma	0/47	0/46
Basal cell adenocarcinoma	0/4	0/4
Polymorphous low-grade adenocarcinoma	0/18	0/18
Myoepithelial carcinoma	0/6	0/6
Epithelial-myoepithelial carcinoma	0/6	0/6

Discussion

PAX2 and PAX8 are transcription factors that play integral roles in the embryogenesis of the kidney, müllerian system, and thyroid gland. These proteins may also be involved in tumorigenesis, and immunohistochemical detection of PAX2 and PAX8 expression has consequently emerged as an important part of diagnosis of kidney, müllerian, and thyroid neoplasms.

A number of previous studies have evaluated expression of PAX2 and PAX8 in a wide variety of normal and

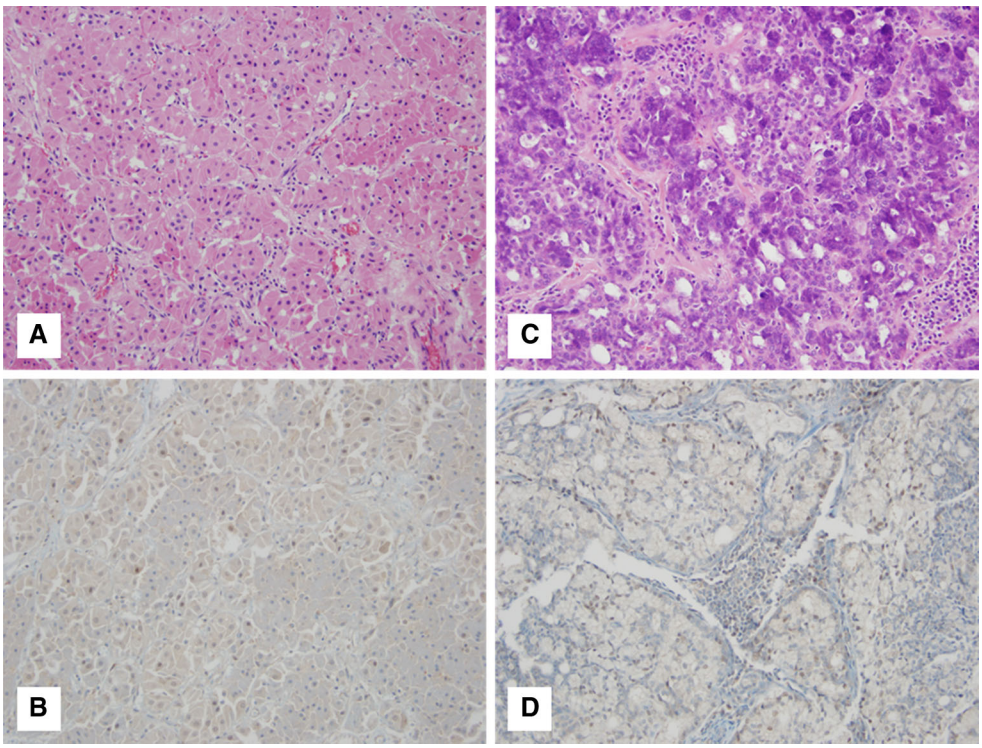

Fig. 1 Focal immunoreactivity for PAX2 in oncocytoma (**a**, **b**) and acinic cell carcinoma (**c**, **d**)

neoplastic tissues, but the number and histologic subtype of salivary gland tumors included in these investigations has been relatively limited. To our knowledge, PAX2 immunoreactivity has previously been evaluated in only four cases of pleomorphic adenoma, all of which lacked expression [9]. Similarly, one study has shown four pleomorphic adenomas to be negative for PAX8 [3], and a single example of acinic cell carcinoma demonstrated PAX8 expression among 59 salivary gland tumors examined in another study [2].

In this investigation, we sought to expand the number and type of salivary gland tumors evaluated for expression of PAX2 and PAX8 by including 442 benign and malignant primary salivary gland neoplasms and 68 normal salivary gland samples in TMAs. All normal samples lacked expression of both transcription factors (Table 1), all neoplasms were negative for PAX8, and only a single case each of acinic cell carcinoma and oncocytoma were focally positive for PAX2 (Table 2). Notably, these results include the first evaluation of any salivary gland tumor other than pleomorphic adenoma for PAX2 expression and the first assessment of PAX8 expression in oncocytoma, Warthin tumor, canalicular adenoma, and epithelial-myoepithelial carcinoma. It should be noted that a subset of the lymphocytes within the Warthin tumors demonstrated immunoreactivity with PAX8. Although the polyclonal anti-PAX8 antibody utilized in this study is different from that previously confirmed to exhibit immunoreactivity with lymphocytes due to cross-reactivity with PAX5 [12, 16], such cross-reactivity is the likely explanation for the positive staining of lymphocytes within Warthin tumors observed here [12, 16]. With acknowledgment of this potential pitfall and in conjunction with prior data [2, 3], our results confirm that PAX8 expression is not seen in a wide array of histologic subtypes of salivary gland tumors, and PAX2 expression is distinctly uncommon.

Among tumors occurring in the salivary glands, metastases from non-cutaneous primaries are far less common than primary neoplasms, comprising less than one percent of such tumors [18]. Such metastases are most likely to occur from nearby sites, including thyroid tumors [18], but infraclavicular neoplasms may also metastasize to the head and neck [19]. Among infraclavicular malignancies that metastasize to the head and neck, renal cell carcinoma is one of the most frequent and may be confused with primary salivary tumors with clear cell or oncocytic features [19, 20]. On the basis of the results of this study, PAX2 and PAX8 would have value in those admittedly rare instances in which a primary salivary gland tumor must be distinguished from metastasis from a thyroid, renal, or other neoplasm.

In summary, the current study greatly expands the total number and diversity of salivary gland neoplasms that have been evaluated for expression of the transcription factors

PAX2 and PAX8. On the basis of these data and those from previous studies, immunoreactivity for these transcription factors is very rare among benign and malignant salivary gland tumors. Immunohistochemical evaluation of PAX2 and/or PAX8 expression would, therefore, be useful in distinguishing salivary gland neoplasms from metastasis of morphologically similar tumors such as renal or thyroid carcinomas.

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