INTRODUCTION

Accurate diagnoses and prognostication of salivary gland neoplasms constitute an important activity in diagnostic histopathology. Although various classification systems have been proposed over the past years, Ellis and Auclair (1990) [1] introduced the principle of classifying malignant salivary gland neoplasms according to biological behaviour into low-, intermediate- and high grade categories. Unlike carcinoma of the prostate, where the roles of p63, p504S, androgen receptor status and the growth pattern of the neoplasm in the diagnosis and prognostication have been established [2], inconsistent immunostaining patterns are reported for salivary gland malignancies for these markers. The most elaborately studied marker in salivary gland neoplasia is p63. In normal salivary glands p63 stains myoepithelial- and basal cells [3]. It therefore comes as no surprise that malignant salivary neoplasms with myoepithelial- or basal cell differentiation like myoepithelial carcinomas [4-6], myoepithelial carcinomas [7], and basaloid squamous cell carcinomas [8] show strong p63 positivity. This marker is particularly helpful in distinguishing malignant salivary neoplasms with a clear cell component from metastatic renal carcinoma, the latter which is reported to be negative for p63 [9]. Literature on the p63 staining characteristics of adenoid cystic carcinomas, polymorphous low grade adenocarcinomas [10], oncocytic carcinomas [9], carcinoma ex pleomorphic adenoma [11], reports inconsistent findings. Alpha-methylacyl CoA racemase, also known as p504S is expressed in normal kidney, liver and salivary gland tissue. p504S positivity of 250 cases of adenocarcinomas from amongst others the salivary glands were found to be rare [12]. In a study on androgen receptor status of [10] carcinomas ex pleomorphic adenoma, the majority were reported as positive [13]. whereas for salivary duct carcinomas, significantly more tumours were reported positive in men than in women [14]. No androgen receptor expression was reported in a case of oncocytic mucoepidermoid carcinoma in a male patient [15].

The purpose of this study was to investigate p63, p504S and the androgen receptor status of salivary gland malignancies.

MATERIALS AND METHODS

Wax blocks of 32 malignant salivary gland neoplasms were retrieved from the files of the Oral Pathology Unit which serves mainly a rural and peri-urban black population cohort. Four- 3 micron sections were prepared of each block and stained by the H&E method and the immunoperoxidase techniques for p63 (Dako N1604), p504S (Dako N1609) and androgen receptors (Dako M3562) according to the instructions of the manufacturers. A case of adenocarcinoma of the prostate which was known in a case of oncocytic mucoepidermoid carcinoma in a male patient [15].

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RESULTS

The results of the study are summarized in Table 1. Of the 7 polymorphous low-grade adenocarcinomas (PLA) only two stained 3+ for p63. One PLA (in a male patient) stained 1+ for androgen receptors and all cases for p504S stained negative. Of the adenoid cystic carcinomas (ACC) 4 stained positive for p63 (except one case with a solid growth pattern) and all 5 stained negative for p504S and androgen receptors. The number of cells which stained positive for p63 varied between the different growth pat-
terns of the ACC cases. The most positive cells (3+) were found in those with a cribriform growth pattern (Fig. 1a) followed by 2+ for the tubular growth pattern (Fig. 1b). No positive staining for p63 was found in the ACC with a solid growth pattern. Cells exhibiting high grade transformation in 2 of the 5 ACC cases stained negative for p63. Three of the 5 cases of salivary duct carcinoma (SDC) were 3+ positive for p63 (Fig. 2) and no cases stained positive for androgen receptors. Faint P504S staining (1+) was present in the 3 cases that were also p63 positive. All mucoepidermoid carcinomas (MEC) demonstrated 2+ staining for p63 with only the basal- and intermediate cells staining positive and the epidermoid- and mucous cells negative (Fig. 3a). Four of the 5 MEC’s were 2+ positive for p504S however staining was restricted to the cytoplasm of the epidermoid component (Fig. 3b). All acinic cell adenocarcinomas (ACA) were p63 negative, 2 showed 2+ cytoplasmic staining for p504S and one case stained 1+ positive for androgen receptors. The latter patient was male. Two of the 3 epimyoepithelial carcinomas- and both clear cell carcinomas (Fig 4) stained 3+ for p63 and one clear cell carcinoma 1+ for p504S. One of the 2 adenocarcinomas stained 2+ for p63. The negative adenocarcinoma was of the mucous producing type.

**DISCUSSION**

The diagnosis of neoplasms of salivary gland origin on morphological basis requires a great deal of expertise and experience. Except for S100 positivity of several salivary gland neoplasms, no unequivocal marker has been reported as an aid to the diagnosis- and prognostication of salivary gland tumours. This study was aimed at exploiting the potential roles of immunoperoxidase stains for p63, p504S and androgen receptors, used in the diagnosis- and prognostication of carcinomas of the prostate, on a selection of salivary gland carcinomas.

Inconsistent p63 staining of polymorphous low grade adenocarcinomas (PLAs) studied by our group support the results of Prasad et al 2008 [10] and questions the use of this marker in differentiating PLA from adenoid cystic carcinoma (ACC). Furthermore the PLAs in our study failed to stain for p504S and only one case, in a male patient, stained positive for androgen receptors. All the ACC's stained positive for p63, except a single case which exhibited a solid growth pattern. The latter growth pattern has previously been reported to be associated with a higher tendency to metastasize and a lower survival rate [16]. Pleomorphic cells indicating foci of high grade transformation observed in two of our ACC samples were negative for p63. In our study the cribriform growth pattern was generally found to be more uniformly positive than the tubular growth pattern. The growth patterns of ACC correlate with the prognosis and 52.3 percent 5 year survival rate for the cribriform pattern and 33.3 percent for the tubular pattern which have been reported [17]. The ACC with a solid growth pattern and those with areas of high grade transformation stained p63 negative and unlike the study of Ramer et al 2010 [18] our findings seem to indicate a decreasing prognosis with decreasing p63 immunostaining of ACC’s. It is suggested that p63 may be helpful to distinguish between

<table>
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<th>Tumor type</th>
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<th>P63</th>
<th>P504S</th>
<th>Androgen</th>
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**Figure 1a**: 3+ staining for p63 in an ACC with a cribriform growth pattern (immunoperoxidase technique, original magnification x 200).

**Figure 1b**: 2+ p63 staining (arrows) in an ACC with a tubular growth pattern (immunoperoxidase technique, original magnification x 400).
the solid type of ACC, which is negative (or at the most focally positive) for p63 and basaloid squamous cell carcinoma, which is reported to be uniformly p63 positive. p63 is of no value in distinguishing between ACC and salivary duct carcinoma (SDC) as most of both tumour types stain positive for the marker. Although basal cell adenomas of the parotid strongly express p63, canalicul adenomas do not demonstrate p63 positivity, consistent with the tumours’ luminal ductal differentiation.

Recent literature however appears to be in agreement that immunoreactivity for p63 indicate basal cell- and myoepithelial cell differentiation in benign and malignant salivary gland neoplasms.

Positive staining of a case of high grade mucoepidermoid carcinoma (MEC) for p63 was reported by Kwon et al 2010. Our study showed positivity of selected cell populations for p63 in all MEC’s, with prominent staining of basal- and intermediate cells and negative staining of epidermoid- and mucous cells. It is recommended that p63 be used to identify intermediate cells in MEC’s, as the verification of this cell type in routine histological sections is often difficult. All our acinic cell adenocarcinomas (ACAs) were negative for p63. The significance of cytoplasmic staining of ACAs and the epidermoid cells in MEC for p504S is speculative. In a study of 250 adenocarcinomas of various organs including the salivary glands, p504S were rarely found to be positive. Two of our three epimyoepithelial carcinomas and both clear cell carcinomas stained 3+ for p63. McHugh et al 2007 proposed p63 positivity as an important feature distinguishing clear cell malignancies of salivary gland origin from clear cell type metastatic renal cell carcinoma.

In conclusion, this study indicates that immune reactivity for p504S and androgen receptors in malignant salivary gland neoplasms are rare and follow no pattern, accept for a higher rate of positivity in SDC’s, MEC’s and ACAs. Only two of our 32 cases studied reacted positive for androgen receptors. Both cases, a PLA and an ACA, occurred in males and indicate sporadic rather than frequent occurrence of a positive androgen receptor status in malignant salivary gland neoplasms. Our findings contradict those of Williams et al 2007 who reported positive androgen receptors in a significant proportion of their SDC’s. Although we had no cases of carcinoma ex pleomorphic adenoma in our series, Nakajima et al 2009 found androgen receptor expression in 9 of 10 cases of carcinoma ex pleomorphic adenoma and 7 out of 23 pleomorphic adenomas. Further studies in this regard are indicated. Our results suggest a potential role for p63 in distinguishing different growth patterns within the group of
ACC’s, the identification of basal cells and intermediate cells in MEC and immunohistochemical confirmation of clear cells of the myoepithelial lineage in salivary gland malignancies.

REFERENCES


