

Immunohistochemical study of androgen, estrogen and progesterone receptors in salivary gland tumors

Fabio Augusto Ito^(a)
Kazuhiro Ito^(b)
Ricardo Della Coletta^(c)
Pablo Agustín Vargas^(c)
Márcio Ajudarte Lopes^(c)

^(a)DDS, PhD; ^(b)MD, Professor – Department of Pathology, Londrina State University, Londrina, PR, Brazil.

^(c)DDS, PhD, Professor, Department of Oral Diagnosis, Piracicaba Dental School, University of Campinas (UNICAMP), Piracicaba, SP, Brazil.

Abstract: The aim of this work was to study the immunohistochemical expression of androgen receptor, estrogen receptor and progesterone receptor in pleomorphic adenomas, Warthin's tumors, mucoepidermoid carcinomas and adenoid cystic carcinomas of salivary glands. A total of 41 pleomorphic adenomas, 30 Warthin's tumors, 30 mucoepidermoid carcinomas and 30 adenoid cystic carcinomas were analyzed, and the immunohistochemical expression of these hormone receptors were assessed. It was observed that all cases were negative for estrogen and progesterone receptors. Androgen receptor was positive in 2 cases each of pleomorphic adenoma, mucoepidermoid carcinoma and adenoid cystic carcinoma. In conclusion, the results do not support a role of estrogen and progesterone in the tumorigenesis of pleomorphic adenomas, Warthin's tumors, mucoepidermoid carcinomas and adenoid cystic carcinomas. However, androgen receptors can play a role in a small set of salivary gland tumors, and this would deserve further studies.

Descriptors: Receptors, androgen; Receptors, estrogen; Receptors, progesterone; Salivary gland neoplasms.

Corresponding author:

Márcio Ajudarte Lopes
Semiologia, Faculdade de Odontologia de
Piracicaba, UNICAMP
Av. Limeira, 901
Caixa Postal: 52
CEP: 13414-903
Piracicaba - SP - Brazil
E-mail: malopes@fop.unicamp.br

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Introduction

Androgen, estrogen and progesterone, acting through specific receptors, play an important role in the growth and development of several tumors, including breast, endometrium, and prostate carcinomas.^{1,2} Additionally, patients with breast carcinomas positive to estrogen receptor (ER) and progesterone receptor (PgR) have been treated with antagonist hormones and shown decreased recurrences and higher survival rates. Similar results have been reported in patients with prostate cancer positive to androgen receptor (AR).^{3,4}

In salivary gland tumors, ER, PgR and AR expression has been described in several studies.^{2,5-11} However, there are great disparities in the literature, possibly because of the variations in the technique employed, which includes fixation of the tissue, antigen retrieval and scoring criteria, and/or in the type of antibodies used. We sought to investigate the immunohistochemical expression of ER, PgR and AR in a large sample of pleomorphic adenomas (PA), Warthin's tumors (WT), mucoepidermoid carcinomas (MEC) and adenoid cystic carcinomas (ACC) of salivary glands, regarding particularly the tumorigenesis role of these receptors.

Methods

Files of the Department of Pathology, Londrina Cancer Institute, Paraná State, Brazil, from 1972 to 2001 were retrieved and 496 cases of salivary gland tumors were analyzed.¹² The last 41 cases of PA and the last 30 cases of WT, MEC and ACC were selected for immunohistochemical evaluation. PA were classified as stroma-rich, cell-rich and classic (balanced amount of epithelial and stromal components),¹³ WT were classified as stroma-rich, stroma-poor and classic, MEC were classified as low-grade, intermediate-grade and high-grade according to Ellis, Auclair¹⁴ (1996), and ACC were classified as cribriform, tubular and solid.

Sections from formalin-fixed and paraffin-embedded tissue were cut with 3 μ m thickness and mounted on glass slides. The tissues were deparaffinized, rehydrated through graded concentrations of alcohol to distilled water. For antigen retrieval of progesterone receptor, the slides were transferred to

a sodium citrate buffer (pH 6.0), and heated twice for 12 min in a 750 W microwave oven. For estrogen receptor and progesterone receptor, a Tris-EDTA pH 9.0 solution was used in a pressure cooker for antigen retrieval. Slides were cooled at room temperature for 20 min. Endogenous peroxidase was blocked by incubation in a 0.05% solution of hydrogen peroxide for 30 min. The sections were incubated overnight at 4°C with the following primary antibodies: anti-Androgen receptor (clone AR441, 1/50 dilution, Dako, Carpinteria, CA, USA), anti-Estrogen receptor (clone 1D5, 1/50 dilution, Dako, Carpinteria, CA, USA) and anti-Progesterone receptor (clone PgR 636, 1/50 dilution, Dako, Carpinteria, CA, USA). The slides were washed in phosphate-buffered saline (PBS) and incubated with a dextran polymer reagent conjugated with peroxidase and secondary antibody (Envision+, Dako, Carpinteria, CA, USA) for 1 h and subsequently reacted with 3,3'-diaminobenzidine and counterstained with Carazzi's hematoxylin. Positive and negative controls were included in all reactions.

Immunoreactivity was evaluated semiquantitatively under a light microscope and scored as negative (0-5% of positive cells), weak (6-50% of positive cells) and strong (> 50% positive cells). Statistical comparisons between variables were performed with Fisher's exact test and the chi-square test.

This study was approved by the ethical committee of the Piracicaba Dental School, University of Campinas.

Results

From 41 cases of PA, 25 (61%) were females and 16 (39%) males, with a mean age of 46.4 years, ranging from 16 to 75 years. Thirty cases were located in the parotid gland, 6 in the submandibular and 5 in minor salivary glands. The majority of WT were in males (24 cases, 80%), all cases were located in the parotid gland and the mean age was 56 years, ranging from 42 to 78 years. Sixteen cases (53.3%) of MEC were in males and 14 (46.7%) were in females, the mean age was 51.3 years, ranging from 5 to 81 years. Nineteen cases of MEC were located in the parotid, 3 in the submandibular and 8 in minor salivary glands. Regarding the 30 cases of ACC, 15

(50%) were in males, the mean age was 52.4 years, ranging from 28 to 88 years. Eight ACC cases were located in the parotid, 2 in the submandibular and 20 in minor salivary glands (Table 1).

According to the histopathological examination, 22 cases (55%) of PA were classified as stroma-rich, 13 (30%) as cell-rich and 6 (15%) as classic. Seventeen cases (56.7%) of WT were classified as classic, 10 (33.3%) as stroma-poor and 3 (10%) as stroma-rich. Fifteen cases (50%) of MEC were classified as low-grade, 3 (10%) as intermediate-grade and 12 (40%) as high-grade. Cribriform pattern was the most common subtype of ACC with 15 cases (50%), followed by the solid subtype with 8 cases (26.7%)

and by the tubular pattern with 7 cases (23.3%).

Immunohistochemistry for ER and PR were negative in all cases studied. AR was positive in only 2 of 41 cases of PA (Figure 1 - A,D), negative in all WT, positive in 2 of 30 cases of MEC (Figure 1 - B,E), and in 2 of 30 cases of ACC (Figure 1 - C,F). Of the 2 cases positive for AR in PA, one was classified as classic and the other as cell-rich. Both MEC positive cases were high-grade tumors and both ACC were of the cribriform type.

Discussion

It's well known that hormonal therapy is very useful in the treatment of breast and prostate can-

Table 1 - Demographic data of 41 cases of pleomorphic adenoma (PA), 30 cases of Warthin's tumor (WT), 30 cases of mucoepidermoid carcinoma (MEC) and 30 cases of adenoid cystic carcinoma (ACC).

	Age		Gender		Location		
	Mean age	Age range	Male	Female	Parotid	Submandibular	Minor
Pleomorphic Adenoma	46.4	16-75	16	25	30	6	5
Warthin's Tumor	56.0	42-78	24	6	30	0	0
Mucoepidermoid Carcinoma	51.3	5-81	16	14	19	3	8
Adenoid Cystic Carcinoma	52.4	28-88	15	15	8	2	20

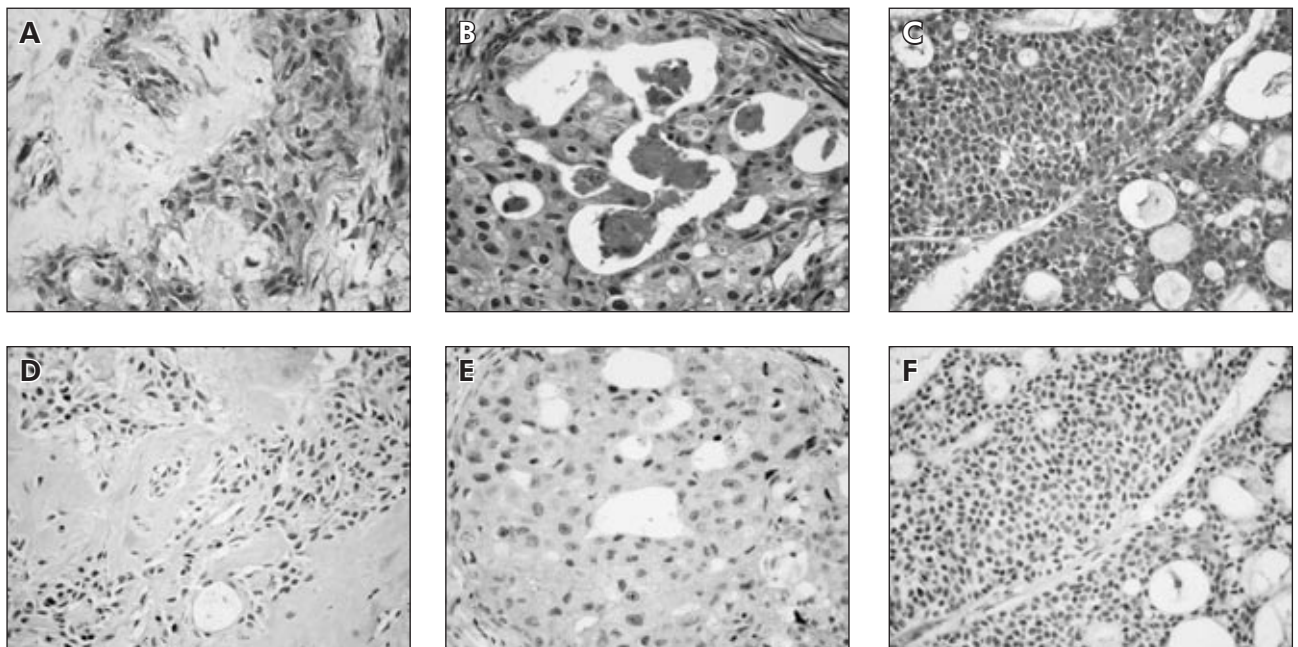


Figure 1 - Histological features of pleomorphic adenoma (A), mucoepidermoid carcinoma (B) and adenoid cystic carcinoma (C) (hematoxylin and eosin, original magnification X 200). Immunohistochemical positivity for androgen receptor (AR) in pleomorphic adenoma (D), mucoepidermoid carcinoma (E) and adenoid cystic carcinoma (F) (original magnification X 200).

cer.^{3,4} However, in spite of the confirmed expression of sex hormone receptors in other types of cancer such as endometrial carcinomas,¹⁵ carcinomas of the thyroid,¹⁶ renal cell carcinomas,¹⁷ malignant melanoma,¹⁸ and meningiomas,¹⁹ the efficacy of hormonal therapy in these tumors has not yet been established.

Studies with ER, PgR and AR in salivary gland tumors are conflicting (Tables 2 and 3). ER expression was detected by Jeannon *et al.*²⁰ (1999) in 3 of 10 MEC, 3 of 6 ACC and in 4 of 10 PA, while Nasser *et al.*² (2003) reported 1 of 10 MEC positive for ER, and, in a study by Glas *et al.*¹ (2002), 19% of PA demonstrated immunoreactivity for ER. On the other hand, several studies,^{8,9,21-24} including ours, did not reveal ER expression in the cases studied.

PgR has been reported more frequently than ER. Shick *et al.*⁹ (1995) and Dori *et al.*²² (2000), study-

ing ACC, found 50% and 6.9% of PgR positive cases, respectively. Jeannon *et al.*²⁰ (1999) related one positive case out of 10 cases of PA, but all 10 cases of MEC and 6 of ACC were negative. Nasser *et al.*² (2003) related one positive MEC, but all ACC, PA and WT were negative. Teymoortash *et al.*²⁴ (2001) found 6 cases positive for PgR in 9 cases of WT, but all 5 cases of PA were negative. Glas *et al.*¹ (2002) reported 60 positive cases of PA out of 69 studied, and also suggested that PgR could be a prognostic factor in recurrent PA of the parotid gland. In the present study, we did not find PgR expression in PA, WT, MEC and ACC.

There are few studies of AR in PA, WT, MEC and ACC. Moriki *et al.*²⁵ (2001) related that all these tumors were negative for AR. Nasser *et al.*² (2003) did not find AR expression in benign salivary gland

Table 2 - Summary of the reports of estrogen receptor (ER), progesterone receptor (PgR) and androgen receptor (AR) in pleomorphic adenoma (PA) and Warthin's tumor (WT).

Authors	Year	PA				WT			
		No. of cases	ER +	PgR +	AR +	No. of cases	ER +	PgR +	AR +
Lamey <i>et al.</i> ²³	1987	4	0	0	np	np	np	np	np
Jeannon <i>et al.</i> ²⁰	1999	10	4	1	np	np	np	np	np
Moriki <i>et al.</i> ²⁵	2001	10	np	np	0	10	np	np	0
Teymoortash <i>et al.</i> ²⁴	2001	5	0	0	np	9	0	6	np
Glas <i>et al.</i> ¹	2002	69	13	60	np	np	np	np	np
Nasser <i>et al.</i> ²	2003	10	0	0	0	10	0	0	0
Our study	2009	41	0	0	2	30	0	0	0

np: not performed.

Table 3 - Summary of the reports of estrogen receptor (ER), progesterone receptor (PgR) and androgen receptor (AR) in mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC).

Authors	Year	MEC				ACC			
		No. of cases	ER +	PgR +	AR +	No. of cases	ER +	PgR +	AR +
Miller <i>et al.</i> ²¹	1994	np	np	np	np	5	0	np	np
Shick <i>et al.</i> ⁹	1995	np	np	np	np	12	0	6	np
Jeannon <i>et al.</i> ²⁰	1999	10	3	0	np	6	3	0	np
Dori <i>et al.</i> ²²	2000	np	np	np	np	29	0	2	np
Moriki <i>et al.</i> ²⁵	2001	6	np	np	0	8	np	np	0
Nasser <i>et al.</i> ²	2003	10	1	1	2	10	0	0	2
Pires <i>et al.</i> ⁸	2004	136	0	np	np	72	0	np	np
Our study	2009	30	0	0	2	30	0	0	2

np: not performed.

tumors, but related 2 of 10 MEC and 2 of 10 ACC positive for AR. In our study, AR was seen in only 2 cases each, of 41 PA, 30 MEC and 30 ACC. In contrast to the low or absent AR expression in PA, MEC, WT and ACC, it has been described to be positive in almost all salivary duct carcinomas^{2,10,25} and therefore potentially useful for their diagnosis in biopsy or cytological samples.^{25,26} Additionally, Locati *et al.*²⁷ (2003) reported a complete remission with androgen-deprivation therapy in a recurrent adenocarcinoma of the parotid gland that expressed AR. Considering this data of remission with an androgen-deprivation therapy, some cases that are positive for AR, particularly the recurrent ones, should be better studied and considered as potential targets for treatment with anti-androgen drugs. On the other hand, although AR has been described in salivary gland tumors, particularly in malignancies, it's not known if this fact is due to a role of androgen in the pathogenic process or if it simply represents an epi-

phenomenon of the malignant transformation.²

Conclusions

Our results suggest that ER and PgR seem to be not important to the tumorigenesis of PA, WT, MEC and ACC. Nevertheless, androgen receptors may play a role in a small group of salivary gland tumors, and should be better investigated specially correlating with their clinico-pathological features.

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