# Expression of Androgen, Estrogen, and Progesterone Receptors in Salivary Gland Tumors

## Frequent Expression of Androgen Receptor in a Subset of Malignant Salivary Gland Tumors

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**Key Words:** Salivary gland; Androgen; Progesterone; Estrogen; Receptors; Hormones

DOI: 10.1309/RVTP1G0Q727WJUQD



## Abstract

*The expression of sex hormone receptors in some tumors suggests a role for these receptors in tumor pathogenesis and therapy. Previous studies of the expression of estrogen and progesterone receptors in salivary gland tumors have reported conflicting results. We evaluated the immunohistochemical expression of androgen, estrogen, and progesterone receptors (AR, ER, and PR) in a series of 78 formalin-fixed, paraffin-embedded salivary gland tumors. Immunoreactivity for AR was seen in 14 of 14 carcinoma ex pleomorphic adenomas, 6 of 6 salivary duct carcinomas, and 2 of 2 basal cell adenocarcinomas but in only 2 of 10 acinic cell carcinomas, mucoepidermoid carcinomas, and adenoid cystic carcinomas each. AR expression was distributed evenly between the sexes. ER and PR were expressed in only a few cases of salivary gland tumors. All 26 benign salivary gland tumors were negative for AR, ER, and PR. The uniform expression of AR exclusively in a subset of malignant salivary gland tumors suggests a possible role for AR in the histogenesis and possibly in the clinical management of these malignant salivary gland tumors.*

The development of sex hormone antagonists and their successful use in the treatment of patients with estrogen receptor (ER)- and progesterone receptor (PR)-positive breast carcinomas and androgen receptor (AR)-positive prostate carcinomas has prompted investigators to evaluate the expression of these receptors in a variety of other tumors, including those arising in the salivary glands. Previous studies have focused on the expression of ER and PR in various salivary gland tumors and have reported widely disparate results.[1-8] However, consistent expression of AR in salivary duct carcinoma, a highly aggressive salivary gland tumor, has been reported recently.[9-13] We evaluated AR, ER, and PR expression in 78 assorted salivary gland neoplasms. To the best of our knowledge, this represents the largest and the most diverse series of salivary gland tumors to be studied for the expression of sex hormone receptors.

## Materials and Methods

### Case Selection

A computer search of the archival surgical pathology files of the pathology departments of the Massachusetts General Hospital and the New England Medical Center, Boston, was performed for cases of salivary gland tumors excised between January 1988 and December 1999, and 78 cases were selected. These tumors, from 44 women and 34 men between 21 and 79 years of age (mean age, 58 years), arose in the parotid and the submandibular glands and consisted of 26 benign lesions (Warthin tumor, 10; pleomorphic adenoma, 10; oncocytoma, 3; basal cell adenoma, 2; and

myoepithelioma, 1) and 52 malignant tumors (carcinoma ex pleomorphic adenoma, 14; mucoepidermoid carcinoma, 10; acinic cell carcinoma, 10; adenoid cystic carcinoma, 10; salivary duct carcinoma, 6; and basal cell adenocarcinoma, 2). The malignant components of the carcinoma ex pleomorphic adenomas consisted of adenocarcinoma, not otherwise specified (11 cases), salivary duct carcinoma (2 cases), and a high-grade mucoepidermoid carcinoma (1 case).

The relevant clinical features of the cases selected for study are summarized in **Table 1**. All original slides from each of the cases were reviewed to confirm the histologic diagnosis and to select appropriate representative blocks for immunohistochemical studies of ER, PR, and AR expression.

### Immunohistochemical Technique

Serial 5-µm-thick tissue sections cut from selected paraffin blocks were mounted individually onto charged glass slides and air dried overnight in a 58°C convection oven. Sections were deparaffinized and rehydrated before staining. All immunohistochemical staining was performed after microwave antigen retrieval, for which the tissue sections were immersed in Coplin jars containing 0.01-mol citrate buffer, pH 6.0, and subjected to intermittent heating for 3 cycles of 5 minutes each in a 625-W microwave oven to maintain the temperature of the buffer at about 95°C. After the slides had cooled to room temperature, nonspecific immunoreactivity was blocked appropriately by incubation with normal guinea pig or horse serum for 20 minutes at room temperature, and endogenous peroxidase activity was blocked by incubating the slides in a 0.3% solution of hydrogen peroxide in methanol for 30 minutes.

Following a rinse for 2 minutes each in running tap water and phosphate-buffered saline at room temperature, tissue sections were stained immunohistochemically on an automated system (Ventana, Tucson, AZ) using commercially available monoclonal antibodies and diaminobenzidine as the chromogen. The primary antibodies used in the study were as follows: ER (clone 6F11, prediluted; Ventana), PR (clone 1A6, prediluted; Ventana), and AR (clone F39.4.1, 1:40 dilution; Biogenex). Positive controls (sections of known ER- and PR-positive breast carcinoma and AR-positive prostate cancer) and negative controls (omission of the primary antibody and its replacement with phosphate-buffered saline) were run simultaneously.

Immunostaining for ER, PR, or AR was identified by a dark brown stain confined exclusively to the nucleus and was scored as negative when seen in fewer than 5% of the tumor cell nuclei, weak when 5% to 20% of the tumor cell nuclei were stained, moderate when 21% to 50% of the nuclei showed staining, and strong when more than 50% of the tumor cell nuclei were positive. All staining results were evaluated independently by 2 observers (S.M.N. and Y.D.), and differences were resolved by consensus.

## Results

### AR Expression

Strong nuclear immunoreactivity for AR was demonstrated in all 14 cases of carcinoma ex pleomorphic adenoma **Image 1**, 6 of 6 salivary duct carcinomas **Image 2**, and 2 of 2 basal cell adenocarcinomas studied. In contrast, nuclear immunostaining was seen in only 2 of 10 mucoepidermoid carcinomas, acinic cell carcinomas, and adenoid cystic carcinomas each. All 26 benign tumors studied were immunonegative for AR, but nuclear staining was noted in fewer than 3% of the cells in 4 cases of pleomorphic adenoma. In each of the various tumor types in which it was detected, immunoreactivity for AR was expressed in tumors from similar numbers of men and women, and, therefore, no gender bias was observed **Table 2**.

**Table 1**
**Salivary Gland Tumors: Types and Clinical Features**

| Tumor Type | Tumor Site | Mean Age (Range), y | Sex Distribution |
|---|---|---|---|
| Benign | | | |
| Pleomorphic adenoma (n = 10) | Parotid, 10 | 35 (21-48) | F, 6; M, 4 |
| Warthin tumor (n = 10) | Parotid, 10 | 65 (51-73) | F, 4; M, 6 |
| Oncocytoma (n = 3) | Parotid, 3 | 61 (46-71) | F, 2; M, 1 |
| Basal cell adenoma* (n = 2) | Parotid, 2 | 61, 64 | F, 1; M, 1 |
| Myoepithelioma* (n = 1) | Parotid, 1 | 63 | F, 1 |
| Malignant | | | |
| Carcinoma ex pleomorphic adenoma (n = 14) | Parotid, 14 | 67 (45-79) | F, 9; M, 5 |
| Mucoepidermoid carcinoma (n = 10) | Parotid, 8; submandibular gland, 2 | 62 (31-79) | F, 6; M, 4 |
| Acinic cell carcinoma (n = 10) | Parotid, 10 | 50 (32-70) | F, 8; M, 2 |
| Adenoid cystic carcinoma (n = 10) | Parotid, 7; submandibular gland, 3 | 55 (39-76) | F, 6; M, 4 |
| Salivary duct carcinoma (n = 6) | Parotid, 5; submandibular gland, 1 | 63 (46-79) | F, 1; M, 5 |
| Basal cell adenocarcinoma* (n = 2) | Parotid, 2 | 45, 57 | M, 2 |

* Individual ages are given.




DOI: 10.1309/RVTP1G0Q727WJUQD

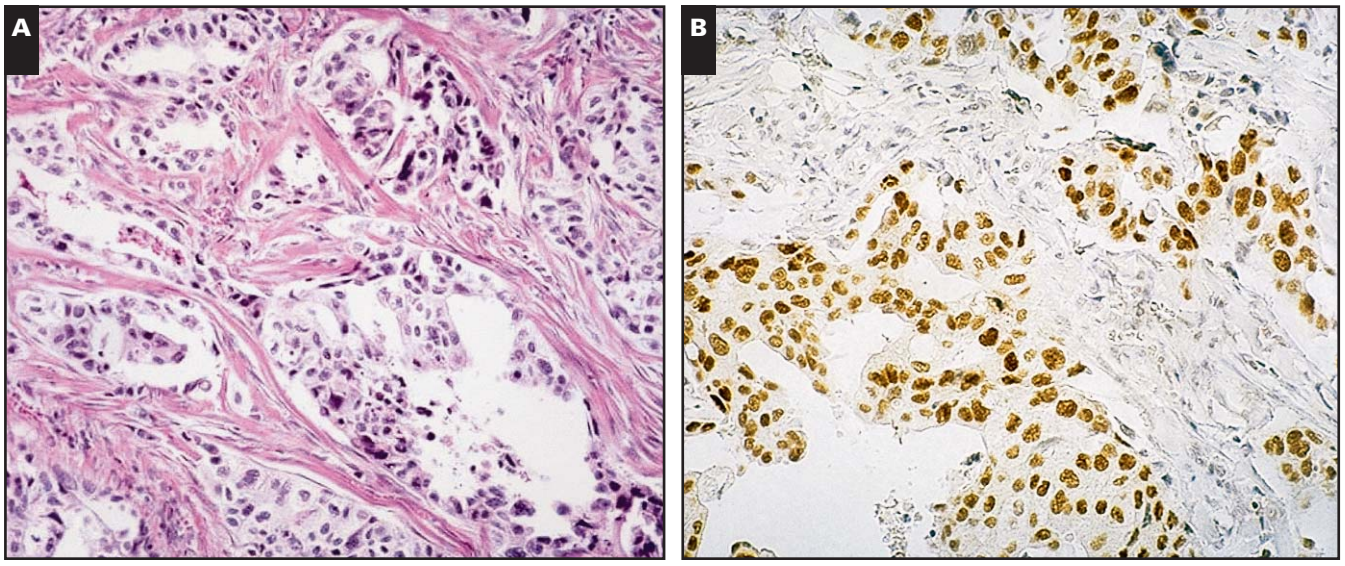


**Image 1** Carcinoma ex pleomorphic adenoma (**A**, H&E, ×10). **B**, Strong nuclear immunoreactivity (androgen receptor, hematoxylin counterstain, ×20).

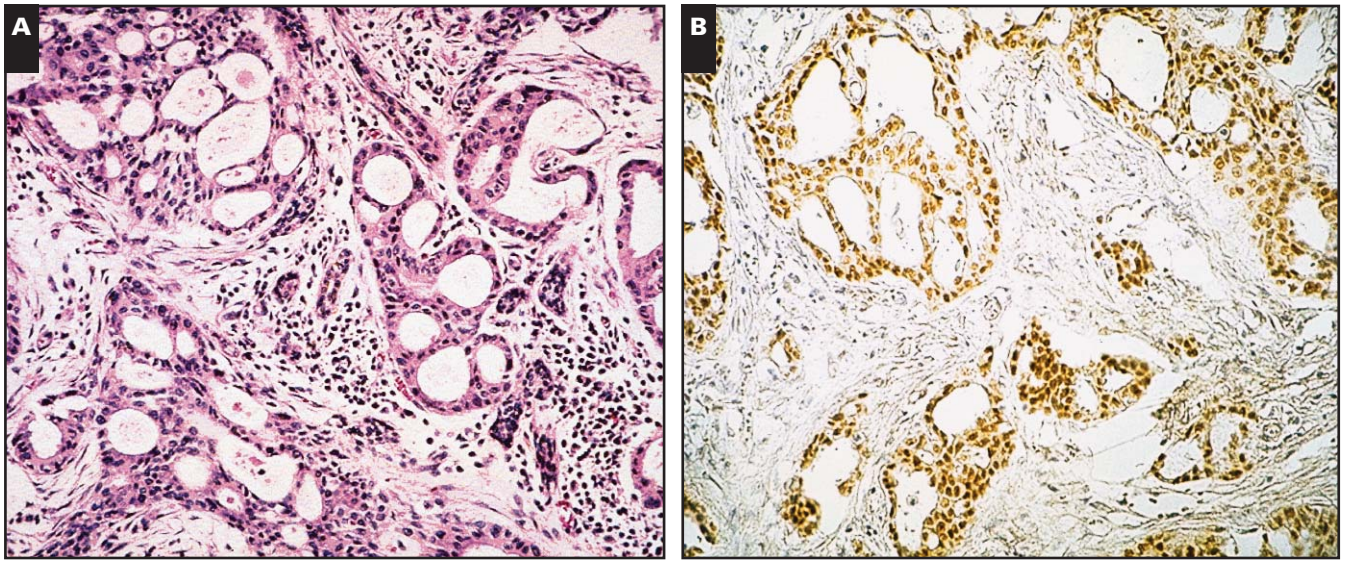


**Image 2** Salivary duct carcinoma (**A**, H&E, ×4). **B**, Strong nuclear immunoreactivity in the tumor cell nuclei (androgen receptor, hematoxylin counterstain, ×4).



### ER Expression

While each of the 26 benign tumors was nonimmunoreactive for ER, weak positivity for ER was seen in 2 of the 52 malignant tumors studied: an acinic cell carcinoma from a woman and a mucoepidermoid carcinoma from a man. Interestingly, this latter tumor was strongly immunoreactive for AR as well. All other malignant tumors were ER-negative.

### PR Expression

All 26 benign tumors studied were negative for PR. Immunoreactivity for PR was identified in 5 of 52 malignant tumors. These included 4 carcinoma ex pleomorphic adenomas (from 2 men and 2 women) and 1 mucoepidermoid carcinoma from a man. Except for the 1 carcinoma ex pleomorphic adenoma that showed moderately intense nuclear staining for PR, staining was weak.

Faint nuclear staining for AR, ER, and PR was seen occasionally in salivary gland tissues adjacent to the tumors. This was limited to a small subset of cells lining occasional salivary ducts and acini in both parotid and submandibular glands. The immunohistochemical findings are summarized in Table 2 and **Table 3**.




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**Table 2**
**Male/Female Ratio in Malignant Salivary Gland Tumors Expressing Sex Hormone Receptors**

| | M/F Ratio | | | |
|---|---|---|---|---|
| **Tumor Type** | **Overall** | **Androgen Receptor** | **Estrogen Receptor** | **Progesterone Receptor** |
| Carcinoma ex pleomorphic adenoma | 5/9 | 5/9 | — | 2/2 |
| Mucoepidermoid carcinoma | 4/6 | 1/1 | 1/0 | 1/0 |
| Acinic cell carcinoma | 2/8 | 1/1 | 0/1 | — |
| Adenoid cystic carcinoma | 4/6 | 0/2 | — | — |
| Salivary duct carcinoma | 5/1 | 5/1 | — | — |
| Basal cell adenocarcinoma | 2/0 | 2/0 | — | — |

## Discussion

Salivary gland tumors generally arise more commonly in females than in males, but this sex distribution varies with tumor type.[14] While this difference in tumor frequency in the sexes suggests a possible role for sex hormones in the histogenesis of salivary gland tumors, our results did not reveal a sex difference in the expression of sex hormone receptors. The majority (71/78 [91%]) of salivary gland tumors in our series were negative for both ER and PR. Although ER and PR expression has been studied in a wide variety of salivary gland tumors, there is substantial disparity in the reported results, with positive expression for these receptors varying from 0% to 100%.[1-8] This marked disparity in the reported results could be related to differences in tissue fixation, the sensitivity and specificity of the antibodies used, the methods used by each group, or even the criteria adopted for judging a tumor positive for the marker. In addition, some of the differences might be related to the relatively small number of cases studied. Larger studies that take into account the aforementioned factors may be necessary for a more definitive assessment of ER and PR expression in salivary gland tumors.

In our study of a relatively large number of cases of a wide spectrum of salivary gland tumors, the most salient finding was the consistent expression of AR in all 14 cases of carcinoma ex pleomorphic adenoma. AR also was expressed in all 6 cases of salivary duct carcinoma and in both of the basal cell adenocarcinomas studied. Overall, we found immunohistochemical evidence of AR expression in 28 (54%) of 52 malignant salivary gland tumors; the frequency of AR expression varied from as low as 20% in carcinomas such as mucoepidermoid, adenoid cystic, and acinic cell to 100% in carcinoma ex pleomorphic adenoma, salivary duct carcinoma, and basal cell adenocarcinoma. Our results are in agreement with those reported in previous studies documenting AR expression in salivary duct carcinoma,[9-13] but to the best of our knowledge, AR expression has not been reported in carcinoma ex pleomorphic adenoma and basal cell adenocarcinoma.

Salivary duct carcinoma occurs predominantly in men and is a highly aggressive tumor with a propensity for early local and distant dissemination.[15-17] Its morphologic similarity to breast carcinoma and its dismal prognosis have led to the evaluation of the expression of breast cancer markers and several other biologic markers such as p53, DNA content, retinoblastoma gene product, Ki-67, Her2, and others.[15,18-20] However, only 2 markers have emerged as potentially significant for prognosis and treatment: Her2-neu, which has been reported to be overexpressed in the majority of these tumors by most studies, and AR, which seems to be expressed invariably in almost all salivary duct carcinomas.

**Table 3**
**Sex Hormone Receptor Expression in Salivary Gland Tumors***

| | Receptor Positive | | |
|---|---|---|---|
| **Tumor Type** | **Androgen** | **Estrogen** | **Progesterone** |
| Benign tumors, all types (n = 26) | 0 | 0 | 0 |
| Malignant tumors | | | |
| Carcinoma ex pleomorphic adenoma (n = 14) | Weak, 2; moderate, 3; strong, 9 | 0 | Weak, 3; moderate, 1 |
| Mucoepidermoid carcinoma (n = 10) | Weak, 1; strong, 1 | Weak, 1 | Weak, 1 |
| Acinic cell carcinoma (n = 10) | Weak, 1; strong, 1 | Weak, 1 | 0 |
| Adenoid cystic carcinoma (n = 10) | Strong, 2 | 0 | 0 |
| Salivary duct carcinoma (n = 6) | Moderate, 1; strong, 5 | 0 | 0 |
| Basal cell adenocarcinoma (n = 2) | Moderate, 1; strong, 1 | 0 | 0 |

* Weak, 5%-20% stained nuclei; moderate, 21%-50% stained nuclei; strong, >50% stained nuclei.




DOI: 10.1309/RVTP1G0Q727WJUQD

Carcinoma ex pleomorphic adenoma is a rare aggressive tumor that develops in association with pleomorphic adenoma. Adenocarcinoma, not otherwise specified, and salivary duct carcinoma are the 2 most common histologic types of carcinoma ex pleomorphic adenoma.[21,22] Only a handful of studies have evaluated the expression of biologic indicators in these tumors,[23-25] and no clear-cut, clinically useful indicator has been identified. Although Her2-neu is overexpressed in fewer than 30% of these tumors, no prognostic significance has emerged for this feature.

Basal cell adenocarcinoma is a rare, predominantly low-grade carcinoma. Not infrequently, it may arise from preexisting basal cell adenoma, a tumor closely related to pleomorphic adenoma.[26,27]

The histologic interrelationship among carcinoma ex pleomorphic adenoma, salivary duct carcinoma, and, to a lesser degree, basal cell adenocarcinoma and their uniform expression of AR raise the possibility that AR may be involved in one of the carcinogenic pathways in salivary gland carcinomas.

Androgen is known to have an important role in the normal development and differentiation of a variety of cell and tissue types. This function is mediated by its binding to AR, a member of the family of steroid hormone receptors. The presence of AR in a given cell type or organ system indicates a possible role for androgen in its growth and differentiation. AR mediates the effect of androgen by binding to specific DNA sequences and influences the transcription and translation of various genes.[28,29] Fan et al[11] suggested that AR may have a role in the pathogenesis of salivary duct carcinoma through the mediation of an epidermal growth factor receptor and transforming growth factor–α autocrine pathway similar to that seen in prostatic carcinoma. In addition, experimental studies have shown that androgen influences the expression of proto-oncogenes (like c-*myc*) and apoptotic factors (like the bcl-2 family) in lacrimal, salivary, and prostatic tissues of both mice and rats, as well as in cell line models.[30-32] Whether the expression of AR in malignant salivary gland tumors indicates a role for androgen in the pathogenic process or simply represents an epiphenomenon of the malignant transformation remains to be determined.

Although partial remission of a salivary gland carcinoma following goserelin (an antiandrogen) therapy has been reported,[33] this needs to be confirmed in a larger group of patients. Our study clearly demonstrated strong and consistent expression of AR exclusively in a sharply defined subset of malignant salivary gland tumors. When considered in the light of previous studies, our results constitute an additional incentive for a more intensive and systematic evaluation of the role of AR in the pathogenesis and treatment of certain malignant salivary gland tumors.

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DOI: 10.1309/RVTP1G0Q727WJUQD

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DOI: 10.1309/RVTP1G0Q727WJUQD

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