ORIGINAL ARTICLE

# Expression of Androgen Receptor, Epidermal Growth Factor Receptor, and Transforming Growth Factor α in Salivary Duct Carcinoma

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**Background:** Salivary duct carcinoma (SDC) is a rare, highly aggressive neoplasm that primarily affects the major salivary glands. It is a distinct clinicopathological entity characterized by its morphologic resemblance to ductal carcinoma of the breast, a high incidence of regional lymph node metastasis, and distant dissemination. Frequent expression of androgen receptor (AR) but not estrogen receptor or progesterone receptor in SDCs suggests that SDC bears a close immunophenotypic homology with prostatic carcinoma. An AR-mediated autocrine growth pathway consisting of epidermal growth factor receptor (EGFR) and its ligand, transforming growth factor α (TGF-α), has been implicated in the carcinogenesis of prostatic carcinoma. Androgens, in the presence of AR, mediate their mitogenic effects on prostatic cancer cells by up-regulating the transcriptional and translational activities of EGFR and TGF-α. Through an autocrine mode of action, TGF-α produced in the tumor cells binds to its receptor, EGFR, which is also expressed by these cells, resulting in a proliferative response.

**Objective:** To investigate whether a TGF-α/EGFR autocrine pathway is present in SDCs.

**Design:** Retrospective analysis of the expression of AR, EGFR, and TGF-α in 12 SDCs.

**Setting:** An academic medical center.

**Results:** Salivary duct carcinoma expresses AR, TGF-α, and EGFR in 11 (92%), 8 (67%), and 11 (92%) of 12 cases, respectively.

**Conclusion:** An AR-mediated TGF-α/EGFR autocrine pathway may be implicated in the tumorigenesis of SDC.

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GROWTH FACTORS are thought to be involved in the formation and progression of cancer. Studies[1] have demonstrated a decreased growth factor requirement for proliferation of transformed neoplastic cells cultured in serum-free media. The loss of requirement for specific growth factors is a common finding in many types of cancer cells[2] and could be mediated by the activation of autologous growth factor synthesis (autocrine activation).

Transforming growth factor α (TGF-α) can recognize its cellular receptor, epidermal growth factor receptor (EGFR), and is produced by a variety of human tumors.[3] High levels of TGF-α and EGFR are detected in epithelial tumors, particularly renal and squamous cell carcinomas.[3] The production of TGF-α by human tumors points to the fact that it may play an important role in tumor cell growth.

It is hypothesized that TGF-α contributes to carcinogenesis via an autocrine mechanism whereby the growth factor helps sustain the transformed character of the same cell population from which it is secreted.[4] In addition to this function, it has also been shown that TGF-α is able to transform cells.[5] Androgen-mediated autocrine or paracrine mechanisms by which TGF-α and EGFR exert their mitogenic effects in prostatic cancer have been demonstrated by various groups.[6,7]

Salivary duct carcinoma (SDC) is a rare but clinically highly aggressive adenocarcinoma of salivary origin that has a striking resemblance to breast carcinoma of the ductal type, with intraductal and invasive components. However, by immunohistochemical staining, SDCs frequently express androgen receptor (AR) (92%)[8,9] and, occasionally, 2 prostatic-specific markers, prostatic acid phosphatase (58%) and prostatic-specific antigen (17%),[9] and only rarely estrogen recep-

## MATERIALS AND METHODS

### TISSUE SPECIMENS

The Medical Archival System was used to retrieve 12 cases of SDC with available microscopic slides and paraffin-embedded tissue blocks between 1990 and 1997 from the surgical pathology files of the University of Pittsburgh Medical Center, Pittsburgh, Pa. Three control samples were obtained from parotidectomy specimens for pleomorphic adenoma of the parotid gland. In all cases, the tissue sections were fixed in 10% buffered formalin, routinely processed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin-eosin. Hematoxylin-eosin–stained slides of all 12 cases were reviewed to confirm the diagnosis and to select the most representative section for immunostaining. All 12 cases were used in our previous study on immunostaining profile for AR, prostatic acid phosphatase, and prostatic-specific antigen.[9]

### IMMUNOHISTOCHEMICAL TECHNIQUES

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissues sectioned at 5 µm with antibodies against human TGF-α (clone 2; Oncogene Science, Cambridge, Mass), human EGFR (clone 1; Genosys Biotechnologies, The Woodlands, Tex), and human AR (Biogenex Laboratories, San Ramon, Calif). The positive controls were derived from skin for TGF-α, breast carcinoma for EGFR, and prostatic carcinoma for AR. Negative controls consisted of a matched primary antibody of unrelated specificity. The standard avidin-biotin-peroxidase technique was used for all antibodies in this study.[10] Antigen retrieval with microwave treatment was used in immunostaining.[11] The immunostaining was assessed semiquantitatively, with minus sign indicating negative and single to quadruple plus signs indicating increasing intensities of positive staining. All sections were counterstained with hematoxylin.

tor and progesterone receptor (1% and 6%, respectively).[9] Thus, this immunophenotypic profile suggests that SDC may be more reminiscent of prostatic carcinoma despite its morphologic resemblance to breast cancer.

The present study aims to demonstrate that a TGF-α/EGFR autocrine pathway operative in prostatic carcinoma is also present in SDC.

## RESULTS

### CLINICAL FEATURES

The patient population consisted of 7 men and 5 women who ranged in age from 36 to 90 years, with a mean age of 66.2 years. Eleven tumors arose in the parotid gland. One presented as facial nerve paralysis, and, therefore, only a facial nerve biopsy was done. Most patients presented with clinical stage IV disease. Regional lymph node metastasis was found in 7 cases (58%) at presentation. Local recurrence was found in 1 patient and distant metastasis in 2 patients. These 3 patients died of their disease at 6, 13, and 14 months, after the diagnosis. Five patients without local recurrence or distant metastasis are alive with no evidence of disease at a mean interval of 28.8 months (range, 13-44 months) after the diagnosis. Four patients were lost to follow-up. The clinical features are summarized in **Table 1**.

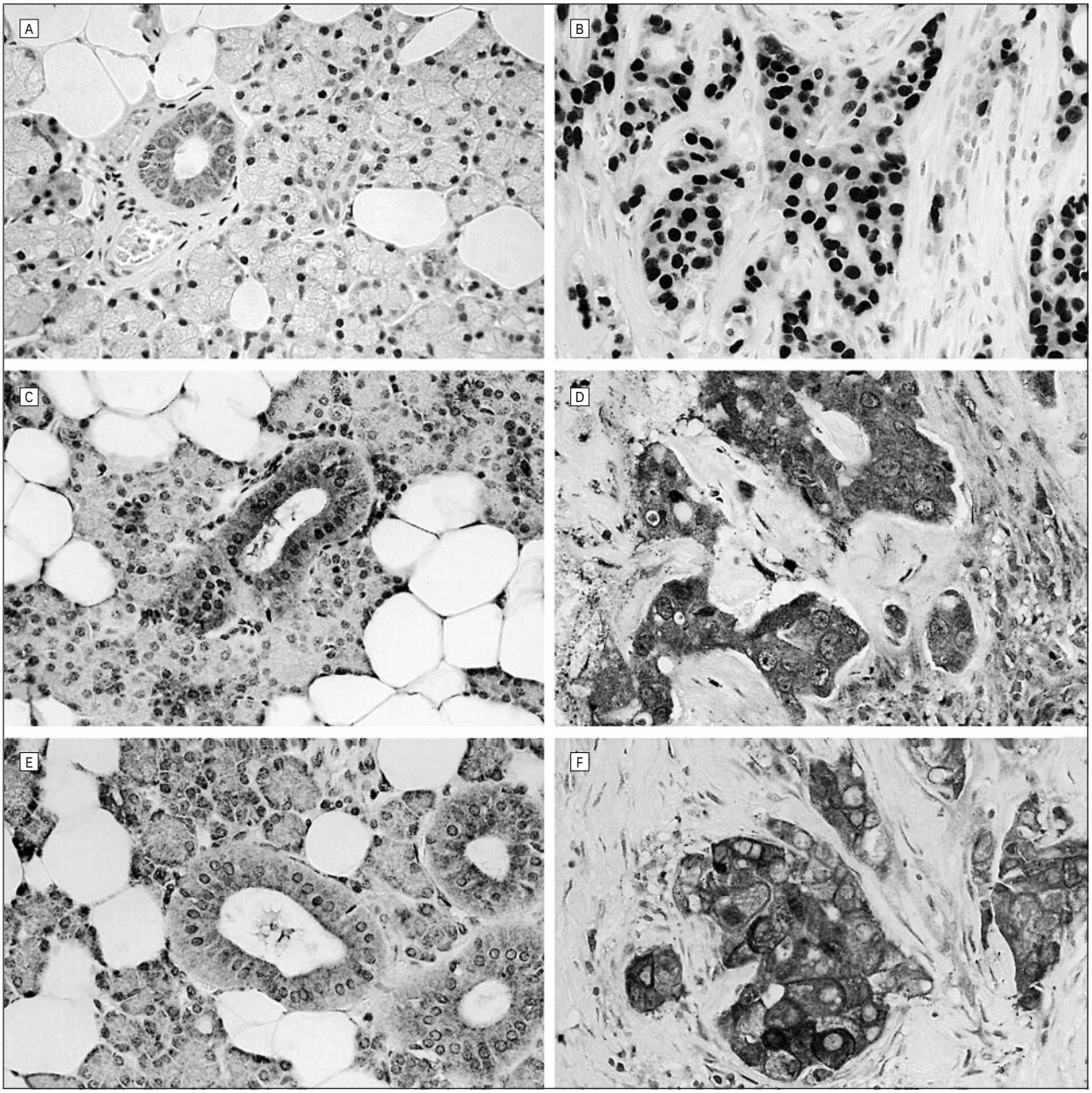
### PATHOLOGIC RESULTS

On gross examination, the tumors ranged from 1.0 to 5.5 cm, with a mean size of 2.9 cm. Most were gray-white, gritty, firm, and poorly circumscribed, with frequent invasion into the adjacent normal salivary gland tissue. There were 3 cases in which SDC arose from a preexisting pleomorphic adenoma (cases 1, 4, and 7). In these cases, the tumors appeared well circumscribed and lobulated but without a true fibrous capsule. Microscopically, both intraductal and infiltrating ductal carcinomas were seen. The intraductal components displayed a variety of growth patterns, including solid, papillary, cystic, and cribriform. Extensive central comedonecrosis was seen in association with the intraductal components. The infiltrating ductal carcinoma consisted of irregular glands

**Table 1. Clinical Features of Salivary Duct Carcinoma***

| Case No./Sex/Age, y | Cancer Stage | Primary Site | Period of Follow-up | Local Recurrence | Distant Metastasis |
|---|---|---|---|---|---|
| 1/M/90 | Unknown | Parotid gland | Lost | NA | NA |
| 2/F/86 | IV | Parotid gland | Lost | NA | NA |
| 3/F/37 | IV | Parotid gland | 44 mo | No | No |
| 4/F/67 | IV | Parotid gland | 34 mo | No | No |
| 5/F/66 | IV | Parotid gland | Lost | NA | NA |
| 6/F/49 | IV | Parotid gland | 29 mo | No | No |
| 7/M/78 | IV | Parotid gland | 6 mo | No | Yes |
| 8/M/64 | IV | Parotid gland | Lost | NA | NA |
| 9/M/77 | II | Parotid gland | 24 mo | No | No |
| 10/M/36 | IV | Parotid gland | 14 mo | Yes | NA |
| 11/M/60 | IV | Parotid gland | 13 mo | No | Yes (bone) |
| 12/M/84 | IV | Unknown | 13 mo | No | No |

*NA indicates not available.




Immunoperoxidase staining of salivary duct carcinoma (SDC) for androgen receptor (AR) (A and B), transforming growth factor α (TGF-α) (C and D), and epidermal growth factor receptor (EGFR) (E and F). A, Weak cytoplasmic staining for AR in ductal epithelium of normal parotid gland. B, Intense nuclear staining for AR in SDC. C, Weak cytoplasmic staining for TGF-α in ductal epithelium of normal parotid gland. D, Intense cytoplasmic staining for TGF-α in SDC cells. E, Moderate cytoplasmic staining for EGFR in ductal epithelium but no staining in the acini of normal parotid gland. F, Intense immunostaining for EGFR in both cytoplasm and membrane of SDC cells. All sections were counterstained with hematoxylin (original magnification ×400).

and cords of compressed cells, which were frequently associated with a prominent desmoplastic reaction. Both the intraductal and infiltrating components were reminiscent of those seen in breast carcinomas. Extensive intraneural and perineural invasion were seen in 9 (75%) of 12 cases. Angiolymphatic permeation was also very common, with cervical lymph node metastasis in 7 (58%) of 12 cases.

### DETECTION OF AR, EGFR, AND TGF-α EXPRESSION IN SDC

Expression of AR in a variety of human tissues has been previously investigated using immunohistochemical techniques.[12] In this study, AR expression was absent in the nuclei of normal parotid acini and ductal epithelium (**Figure**, A) except for focal faint cytoplasmic staining (Figure, A). In carcinoma cells, intense immunostaining for AR was found in more than 90% of the tumor cells in 11 (92%) of 12 cases, mainly localized to the nuclei (Figure, B). The immunohistochemical staining results for AR are summarized in **Table 2**. Ten cases in this study were previously analyzed for AR expression using semiquantitative scoring of the staining intensity.[8]

Transforming growth factor α is expressed in a variety of normal and neoplastic tissues.[13,14] In our study,

**Table 2. Results of Androgen Receptor (AR), Epidermal Growth Factor Receptor (EGFR), and Transforming Growth Factor α (TGF-α) Immunostaining in 12 Salivary Duct Carcinoma Cases**

| Case No./ Sex/Age, y | Intensity* | | |
|---|---|---|---|
| | AR† | EGFR | TGF-α |
| 1/M/90 | ++++ | ++ | +++ |
| 2/F/86 | ++++ | ++ | +++ |
| 3/F/37 | + | ++ | + |
| 4/F/67 | +++ | ++ | ++ |
| 5/F/66 | – | ++ | – |
| 6/F/49 | ++++ | ++ | ++ |
| 7/M/78 | +++ | – | – |
| 8/M/64 | ++++ | ++ | ++ |
| 9/M/77 | ++++ | ++ | ++ |
| 10/M/36 | ++++ | +++ | ++++ |
| 11/M/60 | +++ | +++ | ++++ |
| 12/M/84 | ++++ | ++ | + |

*Plus signs indicate increasing intensities of positive staining; minus signs, negative.

†These results have been previously reported.[9]

weak expression was seen in the cytoplasm of ductal epithelial cells, and no staining was seen in normal parotid acini (Figure, C). Moderate-to-strong staining for TGF-α was seen in the cytoplasm of more than 90% of the tumor cells in 8 (67%) of 12 cases (Figure, D). In another 4 cases, weak-to-negative staining was seen. The immunohistochemical staining results for TGF-α are summarized in Table 2.

Epidermal growth factor receptor is normally expressed in nonneoplastic adult tissues but appears to be overexpressed in carcinoma cells.[15] In this study, EGFR expression was not detected in normal parotid acini but was seen in ductal epithelial cells (Figure, E). Moderate-to-strong staining for EGFR was seen in the membrane and cytoplasm of more than 90% of the cancer cells (Figure, F) in 11 (92%) of 12 cases (Table 2). Negative staining was detected in 1 case (Table 2).

## COMMENT

It has been established that overexpression of EGFR and/or TGF-α may represent an underlying mechanism of the development and progression of human cancers. For example, expression of EGFR protein in breast carcinoma is correlated with a poor prognosis and a poor response to hormonal therapy.[16,17] Poor clinical outcome appears to be linked to elevated EGFR and TGF-α levels in patients with cancer.[18,19] In patients with head and neck squamous cell carcinoma, increased EGFR and TGF-α levels are significantly correlated with shortened disease-free survival.[20]

Human prostate and mammary glands are dependent on androgens and estrogens for the maintenance of their growth and functional integrity. It is, therefore, not surprising to find that the TGF-α/EGFR autocrine growth pathway, present in both prostatic and breast carcinomas, is also affected by these hormones.[21,22]

The expression of AR has been shown in prostate, breast, sebaceous, and sweat glands but not in salivary glands, gastrointestinal tract, thyroid, pancreas, and adrenal gland.[12] The mechanisms underlying the aberrant expression of AR in SDC of the parotid glands currently remain unknown.

Our previous study[9] established that SDCs frequently express AR (92%) and occasionally prostatic acid phosphatase (58%) and prostatic-specific antigen (17%), indicative of a close immunophenotypic homology between SDC and prostatic carcinoma. In the present study, we further establish that a TGF-α/EGFR autocrine pathway is present in SDCs, suggestive of a similar mechanism of carcinogenesis between SDC and prostatic carcinoma.

Because of a small sample size (12 cases) in the present study, it is impossible to establish a definitive link between the expression of AR and TGF-α/EGFR. Our study, however, provides the direction for future investigation in which definitive roles of AR expression in the modulation of TGF-α/EGFR autocrine pathway can be analyzed. Unequivocal supportive evidence for the roles of AR expression in the tumorigenesis of SDC is of paramount significance because it may provide a basis for the use of alternative therapies, such as antiandrogen therapy as used in prostatic carcinoma in patients with metastatic SDC in whom current conventional therapeutic modalities fail.

In summary, we have shown that SDC frequently expresses AR (92%), TGF-α (67%), and EGFR (92%), suggestive of the presence of an AR-mediated TGF-α/EGFR autocrine pathway in these neoplasms. We propose that SDC may share a mechanism of carcinogenesis similar to that of prostatic carcinoma.

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