High Tumor Grade in Salivary Gland Mucoepidermoid Carcinomas and Loss of Expression of Transforming Growth Factor β Receptor Type II

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Background: Mucoepidermoid carcinoma (MEC) of salivary glands is a malignant, locally aggressive neoplasm with metastatic potential. The clinical course is usually dependent on histology; however, low-grade carcinomas can result in metastases and tumor-related death. Transforming growth factor β1 (TGF-β1) is a potent cytokine that affects growth inhibition of various cells and stimulates extracellular matrix production and angiogenesis. Loss of TGF-β receptor type II (TGF-β RII) expression has been related to resistance of TGF-β1-mediated growth control and tumor progression. In this study, we correlate MEC tumor grade with expression of TGF-β1 and TGF-β RII.

Design: Immunohistochemical staining was performed on 16 MEC specimens for activated forms of TGF-β1 and TGF-β RII. The percentage of cells in which staining yielded positive findings for activated TGF-β1 and TGF-β RII was correlated with tumor grade.

Results: Activated TGF-β1 was detected in 16 specimens (100%) of MEC and showed strong positive and diffuse staining. Predominantly cytoplasmic staining of TGF-β1 was seen in salivary gland ducts, stroma, and endothelial cells. There was an inverse correlation between tumor grade and loss of expression of TGF-β RII. All low-grade MEC tumors yielded positive staining results, whereas only one case of intermediate-grade MEC had TGF-β RII expression. No high-grade MEC showed TGF-β RII expression.

Conclusions: Loss of expression of TGF-β RII correlates with tumor grade. The localization of activated TGF-β1 within neoplastic epithelium, tumor-associated stroma, and endothelium suggests that it might play a role in the stromal proliferation and/or angiogenesis associated with MEC.


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MATERIALS AND METHODS

Sixteen acceptable cases of MEC were identified between January 1, 1985, and December 31, 1998, in the surgical pathology files at Emory University Hospital, Atlanta, Ga. Two pathologists (S.M. and A.A.G.) reviewed hematoxylin-eosin–stained slides to assess the diagnosis and grade. Grading of MEC was based on published, accepted standards for MEC.13

TGF-β1 DETECTION

Formalin-fixed, paraffin-embedded tissue blocks in sections of 5 µm were processed for immunohistochemical analysis using an avidin-biotin complex kit (LSAB 2; Dako Corporation, Carpinteria, Calif) and for steam antigen retrieval using an autostainer (Dako Corporation). The primary antibody, a polyclonal chicken antihuman antibody (R & D Systems, Minneapolis, Minn) specific for the activated TGF-β1, was used at a dilution of 1:40. (The primary antibody was purified by means of affinity chromatography using TGF-β1. As determined by sandwich enzyme-linked immunosorbent assay [ELISA] with monoclonal antibody to TGF-β1, cross-reactivity was less than 5% with TGF-β1, 2, and less than 1% with TGF-β2, TGF-β3, TGF-β4, or TGF-β5. There was no significant cross-reactivity with any other cytokine tested.) The secondary-linking antibody, a rabbit antichicken antisera (Chemicon International Incorporated, Temecula, Calif) was used at a dilution of 1:80.

Positive controls consisted of tissue sections from human myometrial blood vessels (the endothelium is known to yield staining results positive for TGF-β1, and human myometrium was chosen because of its high concentration of blood vessels). For negative control sections, buffer replaced the primary antibody. Sections were deparaffinized and rehydrated, then steamed in citrate buffer (pH, 6) for 20 minutes and cooled for 5 minutes before immunostaining. All tissues were then exposed to 3% hydrogen peroxide for 5 minutes, primary antibody for 25 minutes, biotinylated secondary-linking antibody for 25 minutes, avidin-biotinylated enzyme complex for 25 minutes, dianinobenzidine as chromogen for 5 minutes, and hematoxilyn counterstain for 1 minute. These incubations were performed at room temperature; between incubations, sections were washed with buffer.

TGF-β RII DETECTION

The immunohistochemical technique used for the detection of TGF-β RII was identical to that used for TGF-β1. The primary antibody, a polyclonal goat antihuman antibody (R & D Systems) specific for TGF-β RII, was used at a dilution of 1:40. The antibody was purified by means of affinity chromatography using TGF-β RII. This antibody was chosen for its ability to neutralize the biological activity mediated by TGF-β RII. Based on direct ELISA findings, there was no significant cross-reactivity with any other cytokine tested. Specificity was greater than 99%. Positive controls consisted of tissue sections of healthy esophageal mucosa, which has a high concentration of TGF-β RII.

Two pathologists (S.M. and C.C.) independently assessed each case. Immunostained sections of TGF-β1 and TGF-β RII were reviewed by means of semiquantitative analysis according to the percentage of positive cells (1 indicates <25%; 2, 25%-75%; and 3, >75%). Staining intensity was graded as weak, moderate, and strong by comparing staining results with the positive controls. The pathologists scoring specimens for TGF-β1 and TGF-β RII were unaware of histological grade. For statistical evaluation, 1-tailed Fisher exact test was used to correlate positive and negative results of staining for TGF-β RII with respect to histological grade. The statistical analysis compared low- and intermediate- vs high-grade MEC and low- vs intermediate- and high-grade MEC.

Clinical and Pathological Characteristics of Mucoepidermoid Carcinoma

<table>
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<th>Location</th>
<th>Grade</th>
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<td>Low</td>
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<tr>
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*Figure 1 and Figure 2.* In addition, staining of stromal fibroblasts, endothelial cells, smooth muscle cells, and epithelium was positive for TGF-β1. Staining of adjacent...
non-neoplastic salivary gland tissue also showed greater than 75% positive findings for TGF-β1. The pattern of staining was consistent throughout the tumor, both at the peripheral and central portions.

**TGF-β RII DETECTION**

All low-grade tumors showed diffuse cytoplasmic (2+ to 3+) staining for TGF-β IIR (Figure 1). The intensity of the staining pattern was moderate. In intermediate-grade MEC, there was local (<3%) cytoplasmic staining of tumor cells in 1 case with weak staining intensity. No TGF-β RII was identified in 7 high-grade MEC specimens (Figure 2). Statistical analysis demonstrated significant differences in staining between low- and intermediate-grade MEC vs high-grade MEC (P = .03) and between low-grade MEC vs intermediate- and high-grade MEC (P = .003). When present, surface epithelium, endothelial cells, non-neoplastic salivary gland ducts, and stromal fibroblasts yielded staining results positive for TGF-β RII.

**COMMENT**

Transforming growth factor β1 is a potent multifunctional cytokine that regulates growth and differentiation via a complex interaction with other cytokines, growth factors, and mediators. Transforming growth factor β1 inhibits the growth of epithelial, endothelial, and hematopoietic.
etic cells and stimulates extracellular matrix protein production by mesenchymal cells. Three distinct isoforms of the peptide are expressed in mammalian species, with the most concentrated source of TGF-β being the type I isoform. Transforming growth factor β1 exerts control on the cell cycle through its antiproliferative effect that inhibits key transitions required for progression from the G1 to the S phase of the cell cycle. The current findings show that TGF-β1 is strongly expressed in stromal and endothelial cells and in MEC as well as in normal salivary gland tissue. Expression of TGF-β1 was independent of tumor grade. This suggests other mechanisms by which tumor cells modify their response to TGF-β1.

Alterations in TGF receptors have been reported in breast, colon, and head and neck cancers. In our series, it is apparent that loss of differentiation in MEC correlates with progressive loss of TGF-βR II expression. The greatest reduction in TGF-β RII expression was in high-grade MEC, although intermediate-grade tumors showed little to no expression. There is strong evidence to suggest that TGF-β RII plays a key role in tumor suppression mediated by TGF-β1. In prostastic, gastric, and breast cancer and in leukemia, TGF-β RII alterations are suggested to be important factors in altered tumor suppression and apoptosis. Loss of TGF-β RII expression correlates with poorer prognosis and more aggressive local behavior in human prostate and thyroid cancer.

Our finding of lack of expression of TGF-β RII in high-grade MEC with concomitant high expression of TGF-β1 seems to support the prevailing opinion in the literature that alterations in TGF-β1 and TGF-β RII might play a key role in tumorigenesis. The prognosis of MEC is closely related to clinical and histological stage, age, sex, and location. Our data suggest that a loss of expression of TGF-β RII may define a transition from low- to high-grade MEC. There are, however, reports of histologically low-grade MEC with aggressive biological behaviors. Therefore, TGF-β RII expression is not by itself an independent assessment of tumor grade.

CONCLUSIONS

Our study shows the frequent loss of expression of TGF-β RII in intermediate- and high-grade MEC, whereas TGF-β1 expression was consistently present in all histological grades of MEC. Although our study was limited to a small number of cases, additional, larger cooperative studies among several centers are needed to evaluate TGF-β1 and TGF-β RII expression as potential prognostic factors and to elucidate their roles in the pathogenesis of MEC.

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REFERENCES