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 associated with 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. Predominately 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.
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.1

TGFB-1 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 TGFB-1, was used at a dilution of 1:40. (The primary antibody was purified by means of affinity chromatography using TGFB-1. As determined by sandwich enzyme-linked immunosorbent assay [ELISA] with monoclonal antibody to TGFB-1, cross-reactivity was less than 5% with TGFB-1, 1 and less than 1% with TGFB-2, TGFB-3, TGFB-4, or TGFB-5. There was no significant cross-reactivity with any other cytokine tested.)

The secondary-linking antibody, a rabbit antichicken antiserum (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 TGFB-1, 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, diaminobenzidine as chromogen for 5 minutes, and hematoxylin counterstain for 1 minute. These incubations were performed at room temperature; between incubations, sections were washed with buffer.

TGF-βRII DETECTION

The immunohistochemical technique used for the detection of TGF-βRII was identical to that used for TGFB-1. The primary antibody, a polyclonal goat antihuman antibody (R & D Systems) specific for TGFB-RII, was used at a dilution of 1:40. The antibody was purified by means of affinity chromatography using TGFB-RII. This antibody was chosen for its ability to neutralize the biological activity mediated by TGFB-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 TGFB-RII.

Two pathologists (S.M. and C.C.) independently assessed each case. Immunostained sections of TGFB-1 and TGFB-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 TGFB-1 and TGFB-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 TGFB-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>
<thead>
<tr>
<th>Location</th>
<th>Grade</th>
<th>Age, y</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid gland</td>
<td>Low</td>
<td>42</td>
<td>M</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>Low</td>
<td>32</td>
<td>F</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>Low</td>
<td>79</td>
<td>F</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>Low</td>
<td>44</td>
<td>F</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>Intermediate</td>
<td>27</td>
<td>M</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>Intermediate</td>
<td>37</td>
<td>M</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>Intermediate</td>
<td>37</td>
<td>M</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>High</td>
<td>18</td>
<td>M</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>High</td>
<td>88</td>
<td>F</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>High</td>
<td>69</td>
<td>F</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>High</td>
<td>71</td>
<td>F</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>High</td>
<td>71</td>
<td>F</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>High</td>
<td>79</td>
<td>F</td>
</tr>
<tr>
<td>Submandibular gland</td>
<td>Intermediate</td>
<td>36</td>
<td>F</td>
</tr>
<tr>
<td>Base of tongue</td>
<td>Intermediate</td>
<td>55</td>
<td>M</td>
</tr>
<tr>
<td>Hard palate</td>
<td>High</td>
<td>57</td>
<td>F</td>
</tr>
</tbody>
</table>

©2001 American Medical Association. All rights reserved.

Downloaded From: https://archotol.jamanetwork.com/ by a Non-Human Traffic (NHT) User on 08/20/2019
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 II R (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, nonneoplastic 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 cells.
etic cells and stimulates extracellular matrix protein production by mesenchymal cells. Three distinct iso-
forms of the peptide are expressed in mammalian species, with the most concentrated source of TGF-β being the type
1 isoform.2 Transforming growth factor β1 exerts control on the cell cycle through its antiproliferative effect that in-
hibits key transitions required for progression from the G1 to the S phase of the cell cycle.5,10-13 The current findings
show that TGF-β1 is strongly expressed in stromal and endo-
thelial 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.14 In our se-
rries, it is apparent that loss of differentiation in MEC cor-
relates with progressive loss of TGF-β RI expression. The
least 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 sug-
gest that TGF-β RII plays a key role in tumor suppres-
sion mediated by TGF-β1. In prostatic, gastric, and breast
cancer and in leukemia, TGF-β RII alterations are sug-
gested to be important factors in altered tumor suppress-
ion and apoptosis.10-12 Loss of TGF-β RII expression cor-
relates with poorer prognosis and more aggressive local
behavior in human prostate and thyroid cancer.18,19

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 expres-
sion of TGF-β RII may define a transition from low- to
high-grade MEC. There are, however, reports of histo-
logically low-grade MEC with aggressive biological be-
haviors.2,3 Therefore, TGF-β RII expression is not by it-
self an independent assessment of tumor grade.

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 his-
tological grades of MEC. Although our study was lim-
ited to a small number of cases, additional, larger co-
operative 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.

CONCLUSIONS

Accepted for publication January 17, 2001.
Funding provided in part by Emory University Educa-
tional Research Funds, Atlanta, Ga.

Corresponding author: Anthony A. Gal, MD, Depart-
ment of Pathology and Laboratory Medicine, 1364 Clifton
Rd NE, Atlanta, GA 30322 (e-mail: agal@emory.edu).

REFERENCES

1. Pinkston JA, Cole P. Incidence rates of salivary gland tumors: results from a popu-
2. Sprio RH, Huvos AG, Berk R, Strong EW. Mucoepidermoid carcinoma of sali-
3. Goode RK, Auclair PL, Ellis GL. Mucoepidermoid carcinoma of the major sali-
vary glands: clinical and histopathologic analysis of 234 cases with evaluation
4. Suzuki M, Ichimiya I, Matsuhashi F, Mogi H. Histological features and prognosis
of patients with mucoepidermoid carcinoma of the parotid gland. J Laryngol
Otol. 1998;112:944-947.
5. Böttiger EP, Letterio JJ, Roberts AB. Biology of TGF-beta in knockout and trans-
6. Moller A, Schwarz A, Neuner P, Schwarz T, Luger TA. Regulation of monocyte
and keratinocyte interleukin 6 production by transforming growth factor beta.
of the steps of angiogenesis by human head and neck squamous cell carci-
8. Shah M, Foreman DM, Ferguson MWJ. Neutralisation of TGF-beta1 and TGF-
beta2 or exogenous addition of TGF-beta3 to cutaneous rat wounds reduces
360:361-364.
10. Lagneaux L, Delforge A, Bernier M, Stryckmans P, Bron D. TGF-beta activity and
expression of its receptors in B-cell chronic lymphocytic leukemia. Leuk Lym-
for role of transforming growth factor-beta in RRR-alpha-tocopheryl succinate-
1997;27:267-278.
K. Transforming growth factor-beta 1 induces apoptosis in gastric cancer cells
of transforming growth factor-beta type II receptor associated with malignant
factor beta type II receptor correlates with high tumour grade in human breast
growth factor beta induces malignant transformation of nontumorigenic rat pros-
16. Guo Y, Kyprianou N. Restoration of transforming growth factor beta signalling
pathway in human prostate cancer cells suppresses tumorigenicity via induc-
by chronic TGF-beta1 treatment results in downregulation of the type II TGF-
malignant thyroid tumors displayed reduced levels of transforming growth fac-
tor beta receptor type II levels of transforming growth factor beta receptor type
beta receptors is associated with poor prognosis in prostate cancer patients. Clin
Cancer Res. 1998;4:1625-1630.