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TGF-α and EGFR in Head and Neck Cancer

Jennifer R. Grandis, MD[1] and David J. Tweardy, MD[2]

[1] Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213
[2] Departments of Medicine and Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Abstract The upper aerodigestive tract mucosa of patients who develop squamous cell carcinoma of the head and neck (SCCHN) is predisposed to abnormally regulated growth, evidenced by the high incidence of synchronous and metachronous malignancies. We conducted a series of experiments to show that aberrant regulation of the epithelial growth factor, transforming growth factor alpha (TGF-α) and its cell surface receptor, the epidermal growth factor receptor (EGFR), contribute to this predisposition. Using molecular biological techniques, we compared the incidence and mechanism of TGF-α and EGFR over-production in fresh tumors and histologically normal mucosal specimens from patients with SCCHN and normal, control patients without cancer. In patients with SCCHN, TGF-α and EGFR mRNA levels were significantly elevated in both tumor and normal mucosal specimens as compared to levels in control mucosa from non-cancer patients. Neither an enhancement of message stability nor an increase in gene copy number alone accounted for the elevation of EGFR mRNA. Increased production of TGF-α and EGFR mRNAs in the histologically "normal" mucosa of patients at risk for a primary or secondary head and neck cancer may serve both as a marker for malignant transformation and as a target for chemoprevention.

Key words: Transforming growth factor alpha, epidermal growth factor receptor, squamous cell carcinoma of the head and neck

Squamous cell carcinoma of the head and neck (SCCHN) is frequently fatal with few available therapeutic modalities. Information concerning the growth factors involved in the development and maintenance of SCCHN may improve treatment options; such understanding has resulted in new therapies for some cancers, including anti-epidermal growth factor receptor (EGFR) antibodies to treat squamous cell carcinoma of the lung [1]. The impact of this type of therapy on SCCHN is unknown since the role of growth factors has yet to be systematically studied.

Head and neck cancer provides an ideal model for chemoprevention studies due to the high incidence of second primary tumors of the upper aerodigestive tract in individuals who survive their initial malignancy. Squamous cell carcinoma (SCC), the most common cancer, involves the oral cavity, oropharynx, hypopharynx, and larynx. Worldwide, SCC of the oral cavity and oropharynx alone represents the sixth most frequent site of cancer in both sexes combined [2]. Despite advances in diagnostic

Address correspondence to Jennifer R. Grandis, MD, Department of Otolaryngology, University of Pittsburgh School of Medicine, The Eye & Ear Institute, 203 Lothrop Street, Suite 500, Pittsburgh, PA 15213.

and therapeutic techniques, there is no evidence that survival rates have improved. Greater understanding of the pathogenesis of these tumors would enhance efforts to prevent SCCHN in patients at risk.

GROWTH FACTORS AND SCCHN

The combined use of alcohol and tobacco has consistently been identified as a predisposing risk factor for developing SCCHN. The mechanism(s) by which these agents affect malignant transformation remains unknown. Furthermore, only a minority of people who smoke and drink go on to develop a head and neck malignancy.

One growth factor system that may participate in the malignant transformation of upper respiratory tract squamous epithelial cells is EGFR and its ligand, transforming growth factor alpha (TGF-α). TGF-α is a cytokine produced by numerous malignant cells, yet its role in the establishment and maintenance of the transformed state is unclear. A variety of human tumor cell lines over-produce mRNA for both TGF-α and EGFR. This increase in message correlates well with increased TGF-α and EGFR protein in renal cell carcinoma [3]. The significance of this up-regulation is unclear. However, in a number of tumor systems, increased EGFR mRNA has been associated with larger tumor size and advanced stage, and hence, a worse prognosis [4–6]. For example, fresh oral cavity tumors demonstrated mRNA expression for TGF-α and EGFR; however, data on mRNA levels in normal mucosa are conflicting and appear to depend on the technique employed (*in situ* hybridization versus Northern blotting) [7,8].

Amplification of the EGFR gene has been detected in a number of cell lines derived from patients with SCCHN [9–12], although no amplification or structural rearrangement of the EGFR gene has been found in fresh head and neck tumors by Southern blot analysis [10].

We hypothesized that fresh tissues and cell lines from patients with head and neck cancer would show aberrant peptide growth factor regulation. Fresh tumor and histologically normal mucosal samples from patients with SCCHN, as well as SCCHN cell lines were used to determine the frequency and mechanism of EGFR and TGF-α over-expression.

TGF-α AND EGFR mRNA EXPRESSION IN SCCHN PATIENT TISSUES

Intact RNA was isolated from samples of tumor and histologically normal mucosa from 24 patients with SCCHN undergoing surgical resection for their disease. RNA was also isolated from normal mucosa excised from 7 age- and sex-matched control patients who had neither SCCHN nor a significant history of alcohol and tobacco exposure. The isolated RNA was analyzed on Northern blots, and up-regulation of EGFR and TGF-α was determined by comparing mRNA levels in SCCHN tissues with those in the control tissues. Levels of mRNA were quantified by two-dimensional densitometry; an 18s ribosomal cDNA probe was used to control for loading.

Normal mucosa from control patients consistently demonstrated low levels of TGF-α mRNA and little or no detectable EGFR mRNA. The vast majority of tumor specimens and histologically normal mucosal samples from SCCHN patients showed increased levels of TGF-α and EGFR mRNA when compared to mRNA levels in the controls [13]. Overall, TGF-α mRNA was up-regulated in 95% of histologically normal mucosal samples and 87.5% of tumors. TGF-α was elevated approximately 5-fold in both tumor and histologically normal mucosal samples from SCCHN patients compared with controls. EGFR mRNA was increased 69-fold in tumor samples and 29-fold in histologically normal mucosal samples from SCCHN patients compared with levels in controls. EGFR mRNA levels were greater than those found in normal control mucosa in 92% of tumors and 91% of histologically normal mucosal samples from SCCHN patients.

TGF-α AND EGFR EXPRESSION IN SCCHN CELL LINES

Since Northern blot analysis of fresh tissue cannot precisely identify which cells (*e.g.*, tumor, connective tissue, *etc.*) are responsible for the message detected, we examined TGF-α and EGFR mRNA production in 10 SCCHN cell lines. All ten cell lines (both primary and meta-

static) showed increased levels of both TGF-α and EGFR mRNAs compared with levels in control mucosa. TGF-α mRNA was elevated by a mean of 10-fold (range: 1.7–36.3) and EGFR mRNA was elevated by a mean of 77-fold (range: 9.3–362), compared with the control.

MECHANISM OF TGF-α AND EGFR UP-REGULATION

Increased steady-state mRNA levels may result from a variety of mechanisms, including increased gene copy number (DNA), enhanced mRNA stability, or an elevated rate of message transcription. Southern blot analysis of SCCHN cell lines and selected fresh tumors failed to reveal a significant increase in gene dosage when compared to the SCC cell line A431, which is known to amplify EGFR DNA sequences 15- to 30-fold [14,15].

To determine whether a prolonged message half-life could account for the increase in mRNA levels detected, Actinomycin D chase experiments were conducted on selected SCCHN cell lines. Densitometric analysis of the Northern blots revealed that the half-life of EGFR mRNA was 5.5 hours in the control cell line and 7.5 hours in the SCCHN cell line. The 36% prolongation of EGFR mRNA half-life in the SCCHN cell line could not account for the 88% increase in EGFR mRNA production, suggesting that transcriptional activation of the EGFR gene also contributes to elevated levels of EGFR mRNA. In the absence of a suitable, non-transformed negative control cell line for TGF-α mRNA production, we have not been able to draw conclusions regarding TGF-α mRNA half-life.

IMPLICATIONS FOR CHEMOPREVENTION

Differentiation agents, such as retinoic acid, have shown in randomized clinical trials to resolve premalignant lesions such as oral leukoplakia [16], as well as reduce the incidence of second primary tumors in patients with SCCHN [17]. A possible explanation for these findings at the molecular level was provided by studies in other tumor systems showing that retinoic acid down-regulates both EGFR [18] and TGF-α [19] mRNA production. Preliminary data from our lab and others confirm that SCCHN cell lines, when treated with retinoic acid, produce less TGF-α and EGFR mRNA [20]. Our findings provide an enticing explanation for the effects of retinoic acid on mucosal head and neck lesions. The over-production of TGF-α and EGFR mRNA by tumors and mucosa at risk in patients with SCCHN may be interrupted by treatment with agents such as retinoic acid, which down-regulate TGF-α and EGFR mRNA production at the level of gene transcription.

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