Abrogation of Head and Neck Squamous Cell Carcinoma Growth by Epidermal Growth Factor Receptor Ligand Fused to Pseudomonas Exotoxin Transforming Growth Factor α–PE38

Sufi M. Thomas,1 Qing Zeng,1 Michael W. Epperly,3 William E. Gooding,4 Ira Pastan,5 Qing Cheng Wang,5 Joel Greenberger,3 and Jennifer Rubin Grandis1,2

Departments of 1Otolaryngology, 2Pharmacology, 3Radiation Oncology, and 4Biostatistics, University of Pittsburgh and the University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania; and 3Laboratory of Molecular Biology, Division of Basic Sciences, National Cancer Institute, NIH, Bethesda, Maryland

ABSTRACT

Purpose: This study was undertaken to determine whether low intratumoral doses of the epidermal growth factor receptor ligand-transforming growth factor α (TGF-α) fused to Pseudomonas exotoxin (TGF-α–PE38)-abrogated head and neck squamous cell carcinoma (HNSCC) tumor growth in vitro and in vivo.

Experimental Design: In vitro cytotoxicity assays were carried out to determine the sensitivity of HNSCC cells to TGF-α–PE38. TGF-α–PE38-treated HNSCC cells were examined by immunoblotting for cleaved poly(ADP-ribose) polymerase to evaluate apoptosis. Nude mice bearing established HNSCC xenografts were treated with several doses of TGF-α–PE38 to evaluate the antitumor efficacy in vivo. Tumor sections were stained with terminal deoxynucleotidyl transferase-mediated nick end labeling for apoptosis. To determine the effect of oral administration of TGF-α–PE38, gavage injections of TGF-α–PE38 were administered, and the esophagus and surrounding soft tissue were then stained for apoptotic cells.

Results: HNSCC cell lines examined were sensitive to low doses of TGF-α–PE38 (EC50 in the range of 1.6 to 10 ng/mL). HNSCC cells treated with TGF-α–PE38 undergo apoptosis. Antitumor effects were observed using 0.1 and 0.03 μg of TGF-α–PE38 administered intratumorally. At these doses, the treatment was well tolerated. Tumors treated with the toxin had a higher number of apoptotic cells compared with the control tumors. No apoptotic cells were observed in the pharyngoesophageal tissues of the mice after gavage administration of the toxin suggesting that the toxin could be orally administered without toxicity.

Conclusions: These results indicate that topical or intratumoral administration of low doses of TGF-α–PE38 may demonstrate antitumor effects in HNSCC without associated systemic toxicity.

INTRODUCTION

The development of cytotoxic drugs that can specifically target tumors with minimal toxicity to normal tissue continues to be a challenge in cancer therapy. Head and neck squamous cell carcinoma (HNSCC) are aggressive tumors that are difficult to treat with conventional therapies, including chemotherapy, radiation, and surgery. Increased understanding of the biology of this tumor type has facilitated the development of tumor-targeting approaches. Others and we have previously shown that >95% of HNSCC tumors express high levels of the epidermal growth factor receptor (EGFR; ref. 1). The increase in EGFR expression in the primary tumors has been shown to correlate with decreased survival (2). Down-modulation of EGFR or its ligand, transforming growth factor α (TGF-α), has been shown to inhibit tumor growth in vitro and in vivo (3). Several strategies have been developed to target EGFR, including small molecule tyrosine kinase-specific inhibitors, monoclonal antibodies, and antisense approaches (4). These EGFR-targeting strategies are currently in clinical trials or have been approved for clinical administration by the Food and Drug Administration (e.g., Iressa/ZD1839). Another strategy that has proven effective in the treatment of malignant brain tumors is the use of EGFR ligand fused to a cytotoxin (5, 6).

Pseudomonas exotoxin, derived from the bacteria Pseudomonas aeruginosa, exerts its toxic effects by inactivating protein synthesis in mammalian cells. The exotoxin has three functional domains, including a cell binding domain, a domain responsible for cytosol translocation, and a cytotoxic domain. The exotoxin has been engineered to remove the cell-binding domain, and site-specific mutations have been introduced to increase its chemical stability (7). The active cytosolic form of Pseudomonas exotoxin A (PE38) has been fused to the EGFR ligand TGF-α to generate a targeted toxin known as TGF-α–PE38 (8). Thus, only cells that express EGFR will internalize the fusion protein with minimal toxicity to normal cells that don’t express or demonstrate low levels of EGFR. TGF-α–PE38 is currently under clinical investigation for the treatment of malignant brain tumors.

In the present study, we tested the antitumor efficacy of...
TGF-α–PE38 in HNSCC preclinical models. Four HNSCC cell lines tested in vitro for their sensitivity to TGF-α–PE38 and were demonstrated to undergo apoptotic cell death. The in vivo antitumor efficacy of TGF-α–PE38 was tested in HNSCC tumors expressing high levels of EGFR. Antitumor effects without apparent toxicity were demonstrated with intratumoral administration of TGF-α–PE38. These results suggest that TGF-α–PE38 may be used to prevent or treat HNSCC.

MATERIALS AND METHODS

Cell Lines and Reagents. Four well-characterized human HNSCC lines 1483, OSC-19, PCI-15b, and PCI-37a were used in the study. HNSCC line 1483 was derived from a tumor of the retromolar trigone. OSC-19 and PCI-15b were derived from metastatic lymph nodes (9, 10). Cells were maintained in DMEM (1483) or MEM (OSC-19) with 10% heat-inactivated FCS at 37°C in a humidified 5% CO2 incubator.

In vitro Cytotoxicity Assay. HNSCC cells were plated at a density of 5 × 10⁴ cells/mL in a 24-well plate. The next day cells were treated with increasing doses of TGF-α–PE38 (0 to 10 ng/mL) in serum containing media. After 24 hours, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was carried out to measure the number of metabolically active cells. The IC₅₀, at which 50% of the cells were dead at 24 hours, was calculated from the dose-response curve generated using GraphPad PRISM software (version 3).

Immunoblotting. HNSCC cells were plated at a density of 5 × 10⁴ cells/mL in 100-mm dishes. The next day, cells were treated with TGF-α–PE38 (0 to 8 ng/mL) in serum containing media. To demonstrate specificity of TGF-α–PE38 to EGFR, cells were incubated with EGFR-specific antibody C225 (Immunclone Systems, New York, NY) at a concentration of 6 µg/mL for 2 hours before TGF-α–PE38 treatment. Cisplatin (80 µM/L; Bedford Laboratories, Bedford, OH) was used to induce apoptosis in HNSCC cells as a positive control for poly(ADP-ribose) polymerase (PARP) cleavage. After 24 hours, cells were harvested as described previously (11). Protein quantitation was performed on the supernatant using Protein Assay Reagent (Bio-Rad Laboratories, Hercules, CA). Forty micrograms of protein were loaded on an 8% PAGE gel and electrophoresed along with 10 µL of prestained broad range protein marker (Cell Signaling Technology, Beverly, MA). After electrophoresis, proteins were transferred onto a Protran membrane in a semi-dry transfer apparatus (Bio-Rad Laboratories) and subjected to immunoblotting for EGFR (Transduction Laboratories, Lexington, KY), PARP (Santa Cruz Biotechnology, Santa Cruz, CA), or β-Actin (Oncogene Research Products, Boston, MA) to demonstrate equal loading.

In vivo Studies. HNSCC 1483 cells were trypsinized, washed with 1X HBSS, counted using a hemocytometer, and 1 × 10⁶ cells were injected s.c. in female nude mice 5 to 6 months of age obtained from Harlan-Sprague Dawley (Indianapolis, IN). There were five mice per group. When tumors reached a diameter of 2 to 3 mm, they were injected once every alternate day for a week with 0.625, 0.1, or 0.03 µg of TGF-α–PE38 in 50 µL of 0.2% BSA for a total of three injections. Control mice received injections of 0.2% BSA in saline. Tumors were measured three times a week with a vernier caliper in three dimensions. Tumor volumes were determined using the formula 4/3 π (d1 × d2 × d3), where d1, d2, and d3 are the diameters in three dimensions (12). Mice were sacrificed when control tumors reached a volume of 1 cm³. Blood and serum from mice treated with 0.1 and 0.03 µg of TGF-α–PE38 were collected for analysis via cardiac puncture. Blood was collected in 0.5 mol/L EDTA. Serum was collected from 0.5 mL of the blood. Hematologic and serum chemistry analysis was conducted by Antech Diagnostics (Farmingdale, NY). Tumors were snap frozen, sectioned, and fixed in 4% paraformaldehyde solution for 20 minutes, followed by staining for apoptotic cells using TUNEL apoptotic cell detection kit (Roche, Penzberg, Germany) using the manufacturer’s protocol.

To assess the toxicity of TGF-α–PE38 to the esophagus, male C3H/HeNsd (Harlan-Sprague Dawley) mice were administered 200 µL of water followed by 100 µL of water containing 0.4 mg of TGF-α–PE38 by placing a feeding tube at the mouth of the esophageal inlet and injecting the toxin. The mice were allowed to swallow the toxin. Subgroups of mice were sacrificed 24 or 48 hours after injection, and the remaining mice were followed over a 30-day period for survival. The esophagus was removed from the mice sacrificed at 24 or 48 hours, frozen in Ornithine carbamyl transferase, sectioned, and stained for apoptotic cells using a Promega Apoptosis Detection kit (Promega, Madison, WI). The slides were fixed in 4% methyl-methacrylate formaldehyde solution in PBS for 15 minutes at room temperature, washed three times in PBS for 5 minutes at room temperature, and treated with protease K (20 µg/mL) solution for 8 to 10 minutes at room temperature. The slides were rinsed in PBS, fixed in 4% methanol-free formaldehyde solution for 5 minutes at room temperature, rinsed, and placed in equilibration buffer for 5 minutes. Fifty microliters of a reaction mixture containing equilibration buffer, terminal deoxynucleotidyltransferase enzymes, and a nucleotide mix was added followed by incubation at 37°C for 60 minutes. The sections were washed three times in 2X SSC for 15 minutes at room temperature, followed by addition of propidium iodide (1 µg/mL) for 15 minutes at room temperature, washed three times in PBS at room temperature, mounted in antifade and observed under a fluorescent microscope. Animal use and care was in strict compliance with institutional guidelines established by the University of Pittsburgh, Institutional Animal Care and Use Committee.

Statistical Methods. To determine whether blood parameter values of the toxin-treated mice differed from the control group and whether any differences from the control group were associated with the delivered dose, three exact permutation tests were performed. We first determined if there was a dose-response effect, i.e., a linear trend. The Jonckheere-Terpstra test was used to assess a linear trend among treatment groups. If this test was found to be nonsignificant, the Kruskal-Wallis test was used to determine whether lab values among the treatment groups differed from each other. If the Kruskal-Wallis test was significant, a combination of the Wilcoxon rank-sum test and plots was used to determine which treatment groups differed.

RESULTS

TGF-α–PE38 Is Cytotoxic to HNSCC Cells. The EGFR levels of four HNSCC cell lines 1483, OSC-19, PCI-15b,
or PCI-37a were examined by immunoblotting (Fig. 1). HNSCC cell line PCI-15b had the highest level and PCI-37a had the lowest level of EGFR among the four cell lines tested. To determine the antitumor efficacy of TGF-α–PE38 in vitro, the four HNSCC cell lines were treated with increasing concentrations of TGF-α–PE38 for 24 hours. The number of metabolically active cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and a dose-response curve was generated. 1483 and OSC-19 were sensitive to TGF-α–PE38 with IC_{50} of 3 ng/mL and IC_{50} of 4.78 ng/mL, respectively (Fig. 2A). HNSCC line PCI-15b (containing the highest level of EGFR expression) was the most sensitive to TGF-α–PE38 with an IC_{50} of 1.6 ng/mL, and PCI-37a (expressing the lowest EGFR levels) was the least sensitive (IC_{50} of 10 ng/mL; Fig. 2B).

Having determined that TGF-α–PE38 is cytotoxic to HNSCC cells in vitro, we investigated the mechanism whereby it causes tumor cell death. HNSCC cell lines 1483 and OSC-19 were treated with TGF-α–PE38 (4 or 8 ng/mL) for 24 hours. Both adherent as well as cells in the supernatant were lysed and analyzed by immunoblotting for cleaved PARP. Treatment of HNSCC cells with TGF-α–PE38 induced cleavage of PARP at both doses of TGF-α–PE38, indicating that the cells were undergoing apoptosis (Fig. 3, A and B). Cisplatin treatment was used as a positive control for apoptosis. These data suggest that HNSCC cells treated with TGF-α–PE38 undergo apoptotic cell death after 24 hours of treatment.

TGF-α–PE38 Specifically Targets Cells Expressing EGFR. To demonstrate the specificity of TGF-α–PE38 in targeting HNSCC cells via EGFR, HNSCC cells (1483 and OSC-19) were pretreated with the EGFR-specific monoclonal antibody C225 for 2 hours. C225 binds to the extracellular domain of EGFR preventing ligand binding to the receptor. Subsequent treatment of C225 pretreated cells with TGF-α–PE38 resulted in an abrogation of TGF-α–PE38-induced PARP cleavage (Fig. 4). Cells treated with TGF-α–PE38 (4 ng/mL) alone exhibited PARP cleavage, although those treated with either vehicle control, C225 alone, or with C225 did not. These results indicate that TGF-α–PE38 exerts its effects on cells by specifically binding to EGFR followed by internalization into the cytosol.

TGF-α–PE38 Inhibits HNSCC Tumor Growth In vivo. Several studies have examined the antitumor effects of various truncated forms of *Pseudomonas* exotoxin A in xenograft models. Truncated *Pseudomonas* exotoxin A (PE38KDEL) fused to interleukin 4 was used to treat HNSCC xenografts via intratumoral or intraperitoneal administration (13). In that study, mice administered intratumoral injections demonstrated better treatment responses compared with those given intraperitoneal injections. Hence, in the present study, we chose to use an intratumoral route of administration. Mice bearing HNSCC xenografts received injections once every alternate day for 1 week intratumorally of 0.625 (32 µg/kg), 0.1 (5 µg/kg), or 0.03 (0.15 µg/kg) µg of TGF-α–PE38 for a total of three injections. Inhibition of tumor growth was seen at all doses of TGF-α–PE38 (Fig. 5, A and B). Although five mice treated with 0.625 µg of TGF-α–PE38 had a dramatic reduction in tumor burden (Fig. 5A), this dose was associated with systemic toxicity as demonstrated by cachexia and mortality in some of the treated animals. The lower doses of TGF-α–PE38 (0.1 and 0.03 µg) were well tolerated (the experiment was repeated twice with similar results). The lower doses (0.1 and 0.03 µg) are comparable with those used in mouse models of glioma where TGF-α–PE38 was inoculated into the caudate nuclei of mice (14). TGF-α–PE38-treated HNSCC tumors demonstrated lower growth rates compared with control tumors (Fig. 4B), indicating that TGF-α–PE38 could effectively be used to treat HNSCC tumors in vivo.

Frozen sections (7 µm) of control tumors and tumors treated with 0.625 µg of TGF-α–PE38 were analyzed for apoptotic cells by terminal deoxynucleotidyl transferase-mediated nick end labeling. The treated tumors had four times the number of apoptotic cells compared with the control cells as evidenced by the nuclear staining of fragmented DNA (Fig. 6).

Lack of Toxicity of Intratumoral Administration of TGF-α–PE38. Truncated *Pseudomonas* exotoxin PE38 cannot bind to cellular receptors recognized by its full-length counterpart, leading to low systemic toxicity when administered to mice. However, PE38 fused to the Fv portion of erbB2-specific antibodies has been reported to elicit hepatotoxicity in patients on a phase I clinical trial (15). We examined TGF-α–PE38-treated mice for evidence of systemic toxicity. Blood parameters of mice treated with 0.1 and 0.03 µg of TGF-α–PE38 were examined for levels of hepatic enzymes aspartate aminotransferase and alkaline phosphatase. In addition, we examined other hematologic indices, including hemoglobin, hematocrit, creatinine, albumin, absolute lymphocytes, and absolute polymorphonuclear leukocytes, because the information is required for future clinical applications. Blood parameters from the treated and control mice were compared (Table 1). The Jonckhere-Terpstra test was nonsignificant for all of the laboratory end points. There was no dose-response effect for any of the laboratory values. The Kruskal-Wallis test was significant for hemoglobin values of mice treated with 0.03 µg of TGF-α–PE38 compared with the untreated control mice and mice treated with the higher dose of TGF-α–PE38 (0.1 µg; *P* = 0.0041; Fig. 7A). However, the lack of an apparent dose-response association suggests that the lower hemoglobin values detected in the group receiving 0.03 µg of TGF-α–PE38 were not due to TGF-α–PE38 therapy. There was no significant difference in hematocrit levels across groups, thereby providing additional evidence that the changes in hemoglobin levels in the 0.03 µg group are not due to TGF-α–PE38 (Fig. 7B). No differences between the
control animals and the TGF-α–PE38 treatment groups were found for the other laboratory end points. In summary, there was no indication that intratumoral administration of TGF-α–PE38 caused abnormal hematology or serum chemistry laboratory values when compared with untreated control mice at the doses tested.

**Effect of Oral Gavage of Toxin in Mice.** In addition to intratumoral inoculation, HNSCC lesions that arise on the mucosal surfaces of the oral cavity and pharynx can theoretically be treated by a topical preparation. In the absence of a suitable animal model of mucosal disease, we investigated the potential toxicity of oral administration of TGF-α–PE38 in immunocompetent mice. C3H/HeNsd mice were treated with a single dose of TGF-α–PE38 (0.4 mg) via oral gavage administration. All mice appeared healthy with no deaths, weight loss, decreased activity, or difficulty in swallowing. Examination of the mice sacrificed 24 or 48 hours (Fig. 8) after injection for the appearance of apoptotic cells in the esophagus revealed no apoptotic cells. The esophagus not only demonstrated lack of apoptotic cells but also showed no inflammatory pathological changes after gavage administration of the toxin. Five mice administered either saline or TGF-α–PE38 (0.4 mg) and followed for a period of 30 days demonstrated no indications of toxicity as manifest by stable weight and activity levels.

**DISCUSSION**

The EGFR confers a growth advantage upon cells when expressed at high levels. Several phase III clinical trials have been conducted with EGFR-specific antibody (C225/Erbitux/ Cetuximab) and small molecule inhibitors (ZD1829/Iressa and OSI-774/Tarceva/Erlotinib). Results from two phase III clinical trials evaluating small molecule EGFR inhibitor ZD1839 in combination with chemotherapy in non–small-cell carcinoma patients failed to demonstrate an advantage of ZD1839 administration on survival (reviewed in ref. 16). Targeting EGFR in HNSCC tumors is theoretically a rational therapeutic strategy considering that 90% of these tumors overexpress the receptor (1). Although two EGFR inhibitors used in phase III clinical trials have been approved by the Food and Drug Administration (small molecule inhibitor ZD1839 and antibody C225), novel strategies for effectively targeting EGFR without causing severe adverse effects are still being investigated. In this study, we examined the antitumor efficacy of a truncated form of Pseudomonas exotoxin A fused to the EGFR ligand TGF-α.

Pseudomonas exotoxin A is a protein secreted by the bacteria Pseudomonas aeruginosa. Over the last decade, the protein has been engineered to minimize its size while retaining its potency at ADP ribosylation of elongation factor 2, which
arrests protein synthesis (17, 18). To efficiently enter cells it requires a targeting moiety and then can translocate to the cytosol where it exerts its cytotoxicity. PE38 has been fused to several ligands such as the Fv portion of erb B2 antibodies (18) and cytokines, including interleukin 4 (13) and interleukin 13 (19). PE38 has also been fused to the EGFR ligand TGF-α (14), enabling its use in the treatment of tumors expressing high levels of EGFR. HNSCC cells demonstrate elevated EGFR levels compared with normal epithelia (1). To date, there are no reports demonstrating the efficacy of TGF-α–PE38 in the treatment of HNSCC.

In the present study, HNSCC cell lines were sensitive to TGF-α–PE38 at IC50 values ranging from 1.6 to 10 ng/mL. TGF-α–PE38 has been previously reported to be cytotoxic to human glioma cell lines with a reported ID50 ranging from 0.1 to 35 ng/mL (14). To determine whether HNSCC cells treated with TGF-α–PE38 undergo apoptosis, we analyzed levels of cleaved PARP. Cleavage of PARP is one of the last steps in the

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**Fig. 3** TGF-α–PE38 induces apoptosis in HNSCC cells. HNSCC cell lines (A) OSC-19, (B) 1483, (C) PCI-15b, and (D) PCI-37a were treated with TGF-α–PE38 (4 or 8 ng/mL) were lysed and analyzed by immunoblotting for PARP cleavage. Cisplatin (80 μmol/L) was used as a positive control. β-Actin levels were determined to demonstrate equal loading of protein in all lanes.

**Fig. 4** TGF-α–PE38 specifically targets cells expressing EGFR. The ligand-binding domain of EGFR was blocked with anti-EGFR antibody C225 before TGF-α–PE38 treatment. C225 prevented TGF-α–PE38-induced PARP cleavage in HNSCC cells.
apoptotic pathway downstream of the mitochondria. Cells undergoing apoptosis demonstrate increased levels of cleaved PARP. Although there are reports on the type of apoptotic cell death observed after TGF-α-PE38 treatment in HNSCC, our results corroborate findings in a previous study where HNSCC cells treated with interleukin 13 fused to PE38 underwent apoptotic cell death with cleaved PARP and caspases 3, 8, and 9 accumulation (20). To investigate the antitumor efficacy of TGF-α-PE38 in vivo, we treated nude mice bearing s.c. HNSCC tumors with the agent.

Established tumors were treated intratumorally with varying concentrations of TGF-α-PE38. Although the highest dose of TGF-α-PE38 (0.625 μg) was associated with tumor eradication, there was also substantial toxicity as demonstrated by the death of two thirds of the mice. The maximum-tolerated dose of TGF-α-PE38 in athymic nude mice has been reported to be at 0.3 μg when stereotactically injected into the caudate nuclei (14). In that study, 0.1 μg of TGF-α-PE38 were safe and efficacious for the treatment of intracranial tumors in athymic nude mice (14). Our results corroborate this finding in that mice treated with lower doses of (0.1 and 0.03 μg) demonstrated tumor growth inhibition compared with the control. Furthermore, on staining the tumor sections with terminal deoxynucleo-

![Graph](image1.png)

Fig. 5 HNSCC tumor growth inhibition in vivo after TGF-α-PE38 treatment. A. HNSCC cells (1483) were injected subcutaneously in both flanks of nude mice. The left tumor was injected intratumorally with the vehicle (0.1% BSA in sterile PBS). The right tumor was injected intratumorally with 0.625 μg of TGF-α-PE38. Tumors were treated once a day every other day for 1 week. Tumor volumes were measured, and fractional tumor volume was calculated by normalizing tumor volumes to the volume measured at the first time point. B. In another experiment, lower doses of TGF-α-PE38 were administered intratumorally (0.1 and 0.03 μg) using the same treatment regimen (the experiment was repeated twice with similar results).

![Graph](image2.png)

Fig. 6 TGF-α-PE38 treatment induces apoptosis in HNSCC tumors. Frozen HNSCC tumors (1483) from (A) control and (B) TGF-α-PE38-treated mice were sectioned (7 μm), fixed, and stained by terminal deoxynucleotidyl transferase-mediated nick end labeling for apoptotic cells. The apoptotic cells demonstrated a dark brown stain in the nucleus, indicating DNA fragmentation. C. The number of apoptotic cells from five separated fields were counted at ×400 magnification and plotted. Error bars indicate ± SE.
otidyl transferase-mediated nick end labeling, we observed that the cells in the TGF-α–PE38-treated tumors were undergoing apoptosis. TGF-α–PE38 did not demonstrate any adverse effects in the mice at low doses. No significant difference was found between the blood parameters from treated and control mice, indicating that there was no drug-related toxicity. Although there are no reports of systemic administration of TGF-α–PE38 in clinical trials, the enzymatically active domain of *Pseudomonas* exotoxin fused to EGF (DAB389,EGF) has been administered i.v. in cancer patients without reaching dose-limiting toxicities (21). Although no hematologic toxicities were observed, transaminase and creatinine elevations were reported. Our data corroborates the hematologic findings reported in the study; however, we did not see any significant elevations in the creatinine levels.

Intratumoral or topical administration of TGF-α–PE38 in patients suffering from easily accessible HNSCC tumors may result in exposure of normal esophageal tissue to the drug. Dose-limiting toxicity was observed at the highest dose of 100 ng/mL (4 μg) when injected stereotactically into glioblastoma tumors (14). This is the only dose-limiting toxicity reported in clinical trials with TGF-α–PE38. To test the toxicity of TGF-α–PE38 on normal esophageal tissue, we used a dose of 0.4 mg for oral gavage administration in immunocompetent mice. Higher doses may be toxic if administered intraesophageally. When tissues were examined for drug-related toxicity, there were no adverse effects observed in the TGF-α–PE38-treated mice compared with the control animals, indicating that the drug can be injected into tumors of the oral cavity with little or no effect on normal regional tissues. The lack of toxicity may be due to low levels of EGFR expression in normal esophageal mucosa. Alternatively, the lack of toxicity could be attributed to low affinity of the human TGF-α for the mouse EGFR. However, our in vitro data demonstrate that TGF-α–PE38 is cytotoxic to murine fibroblasts expressing EGFR, implying that human TGF-α can bind to murine EGFR (data not shown). To date, there are no reports of topical administration of TGF-α–PE38. Most studies examining the antitumor effects of PE38 in vivo deliver the drug via intratumoral or intravenous administration (20). Although we did not specifically investigate the antitumor efficacy of an oral route of administration, our data indicate a lack of toxicity to normal epithelia and support additional investigations of the topical administration of the drug in patients with accessible oral tumors.

TGF-α fused to *Pseudomonas* exotoxin has been previously demonstrated to have antitumor effects in several cancers, including brain (8), bladder (22), breast (23), and non–small-cell lung cancer (24). This is the first report demonstrating its effects in head and neck tumors. HNSCC cells expressing EGFR are susceptible to TGF-α–PE38 at low concentrations and mice administered the drug do not suffer adverse effects. Because HNSCC tumors express high levels of EGFR compared with the normal adjacent mucosa, targeted therapy may prove efficacious in this tumor type. Increased EGFR expression in precancerous dysplastic lesions compared with normal adjacent mucosa may justify the use of TGF-α–PE38 in prevention of tumor progression (25). Direct intratumoral injections of TGF-α–PE38 are possible in patients with easily accessible head and neck tumors. The dose and treatment regimen of TGF-α–PE38 could be

![Image](https://example.com/image.png)

**Table 1** Permutation test results comparing serum chemistries of TGF-α–PE38-treated and control mice

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* Test of equality across dose groups.
† Test of dose response.

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**Fig. 7** TGF-α–PE38 does not have any apparent toxicity to normal tissues in vivo. A, hemoglobin values by treatment group. The Kruskal-Wallis test was used to compare the group of control untreated mice and those receiving TGF-α–PE38 at two doses. The group of mice receiving 0.05 μg of TGF-α–PE38 had lower hemoglobin values than the other treatment groups (P = 0.0041). There was no significant difference between the hemoglobin values of the control mice and treated mice. B, hematocrit values by treatment group. The Kruskal-Wallis test was used to compare the group of control mice receiving and mice treated with the toxin conjugate. There was no significant difference between the groups.
extrapolated for clinical application from in vivo data presented in this study. These finding indicate that TGF-α–PE38 may be used as a therapeutic agent for the treatment of HNSCC tumors.

REFERENCES


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