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Advances in Brief

Overexpression of Transforming Growth Factor β1 in Head and Neck Epithelia Results in Inflammation, Angiogenesis, and Epithelial Hyperproliferation

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Abstract

In the present study, we show that transforming growth factor β1 (TGF-β1) was frequently overexpressed in human head and neck squamous cell carcinomas (HNSCCs) and adjacent tissues in comparison with normal head and neck tissues. To determine the role of TGF-β1 overexpression in HNSCC carcinogenesis, we generated transgenic mice in which TGF-β1 transgene expression can be induced in head and neck epithelia. TGF-β1 transgene induction in head and neck epithelia, at levels similar to those in human HNSCCs, caused severe inflammation and angiogenesis. Consequently, TGF-β1-transgenic epithelia exhibited hyperproliferation. These phenotypes correlated with enhanced Smad signaling in transgenic epithelia and stroma. Our study suggests that TGF-β1 overexpression at early stages of HNSCC formation provides a tumor promoting microenvironment.

Introduction

The major and common etiological risk factors for head and neck squamous cell carcinomas (HNSCCs) are continuous exposure of head and neck epithelia to environmental carcinogens and promoters, *e.g.*, tobacco and alcohol (1). These risk factors often cause gene mutations and/or chronic inflammation, which serve as an initiation and/or a constitutive tumor promotion event.

Transforming growth factor β1 (TGF-β1) is overexpressed in many types of cancers and correlates to tumor invasion (2). However, there are only a few contradictory reports about TGF-β1 expression levels in HNSCCs (3, 4). Moreover, it is not clear at which stage of HNSCC development TGF-β1 begins to be overexpressed, and the role of TGF-β1 in HNSCC carcinogenesis remains to be determined. Because TGF-β1 is a potent growth inhibitor for epithelial cells (5), it is expected that TGF-β1 inhibits proliferation of head and neck epithelia at an early stage of HNSCC development. On the other hand, TGF-β1 has been suggested to promote tumor invasion via its paracrine effects on tumor stroma (5). TGF-β1 exerts its effects via a heteromeric receptor complex of TGF-βRI and TGF-βRII. When TGF-β binds to a TGF-βRI–TGF-βRII complex, the classic TGF-βRI, also known as activin receptor-like kinase 5 (ALK5), phosphorylates signaling mediators Smad2 and Smad3. Phosphorylated Smad2 and Smad3 form heteromeric complexes with Smad4 and translocate into the nucleus to regulate TGF-β-responsive genes (6). Smad2/Smad3 activation mediates TGF-β1-induced growth inhibition in epithelial and endothelial cells (7). Another type I TGF-βR, ALK1, is preferentially expressed in endothelial cells. Activated ALK1 phosphorylates and activates Smad1 and Smad5, which promote angiogenesis (7).

To determine the role of TGF-β1 in HNSCC development, in the present study, we first examined TGF-β1 expression patterns in human head and neck tissue and HNSCCs. We then modified our gene-switch TGF-β1-transgenic system (8) to allow inducing TGF-β1 expression in head and neck epithelia at levels similar to those in human HNSCCs. Our current study suggests that TGF-β1 may provide a tumor promotion effect even at early stages of HNSCC development.

Materials and Methods

Patients. Thirty-two HNSCC tumors and case-matched tissue samples adjacent to resected tumors, as well as normal oropharyngeal tissues (excluding tonsils) from five sleep apnea patients, were surgically resected. These tissue samples were obtained from consenting patients at the Oregon Health & Science University, under an Institutional Review Board-approved protocol. The tissue samples were frozen and stored in liquid nitrogen immediately after removal until the assays were performed. Among the tissues assayed were 13 tongue squamous cell carcinomas (SCCs), 7 oral SCCs, 2 pharyngeal SCCs, 2 tonsil SCCs, and 8 larynx SCCs. The tumors did not contain large necrotic or stromal areas. H&E-stained sections were prepared from all tissue samples and were reviewed by at least two pathologists to ensure the diagnosis.

TGF-β1 ELISA. Protein extraction and TGF-β1 ELISA were performed as described previously (9).

Generation of Gene-Switch-TGF-β1 Mice and Treatment. The gene-switch-TGF-β1 mice were generated as previously described (10) and modified to achieve transgene induction in head and neck epithelia. Briefly, the GLp65 transactivator, which is activated by RU486 (10), was inserted into a keratin 5 (K5) expression vector. The K5 vector targets transgene expression to the basal layer of stratified epithelia such as head and neck epithelia (11). The target line, tata.TGF-β1, consists of the full-length wild-type human TGF-β1 cDNA under the control of a tata minimal promoter containing GAL4 binding sites in lieu of the previously used tk promoter (12). These two transgenic lines were cross-bred to generate bigenic TGF-β1 (K5.GLp65/tata.TGF-β1) mice that contain both transgenes. Mice were genotyped by PCR analysis of tail DNA using primers specific for GLp65 (10) and for human TGF-β1 (forward, 5′-TCTGCTGAGGAGGCTCAAGTT-3′; reverse, 5′-ACCTCGGCGGCCGGTAG-3′). RU486, dissolved in sesame oil, was applied in the oral cavity of 3-month-old mice to induce acute or sustained TGF-β1 transgene expression. Throughout this study, mouse samples were analyzed for each assay from at least three mice in each group.

Histological Analysis. The tongue, buccal, and esophagus from each mouse were dissected, fixed in 10% neutral buffered formalin at 4°C overnight, embedded in paraffin, sectioned to 5-μm thickness, and stained with H&E. For dissecting the buccal tissue, mouse cheek was removed from the mandible and maxillary bone. From each cheek, a 5–7-mm strip of skin and underlying mucosa was trimmed in an anterior-posterior plane (parallel to the teeth). The cheek strip was embedded with the skin and mucosal sides parallel to each other and sectioned with skin present on one side and buccal mucosa

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Note: S-L. Lu and D. Reh contributed equally to this publication.

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on the other. Care was taken to avoid tangential embedding, as judged by the orientation of the hair follicles in the final section. Normal morphology of the epidermis and hair follicles in the section served as a negative control for tissue-specific effect of TGF-β1 transgene induction.

Immunofluorescence and Immunohistochemistry. Immunofluorescence and immunohistochemistry analyses, *in vivo* bromodeoxyuridine labeling, and detection and quantitative measurement of microscopy images were performed as described previously (9).

RNA Isolation and Quantitative Reverse Transcription-PCR. RNA isolation and quantitative reverse transcription-PCR were performed as described previously (9). An 18S probe was included to normalize RNA loading. All experiments were performed in triplicate, and the results from three samples in each group were averaged. The relative RNA expression levels were calculated using the comparative C_T Method.

Statistical Analysis. Statistical differences between two groups of data were analyzed using Student's *t* test with the exception of the case-matched analysis, which was analyzed by a paired *t* test.

Results

TGF-β1 Was Overexpressed in HNSCCs and Adjacent Tissues in Humans. To determine the expression levels of TGF-β1 in human HNSCCs, TGF-β1 protein levels were examined by ELISA in 32 HNSCCs and adjacent tissues, as well as 5 oropharyngeal samples from sleep apnea patients. The average level of TGF-β1 protein in oropharyngeal tissues of sleep apnea patients was 118.2 ± 35.6 pg/mg protein, which represented the normal control level of TGF-β1 protein in human head and neck tissues. As shown in Fig. 1*A*, of 32 samples, 14 (43.8%) tissue samples adjacent to HNSCCs exhibited a 1.5–5.3-fold increase in TGF-β1 protein levels (195.6 ± 112.6 pg/mg protein) in comparison with normal control tissues ($P < 0.01$). This result suggests that TGF-β1 overexpression may be an early event during HNSCC development. In 32 HNSCC samples, TGF-β1 protein levels (301.4 ± 186.7 pg/mg protein) were additionally elevated in compar-

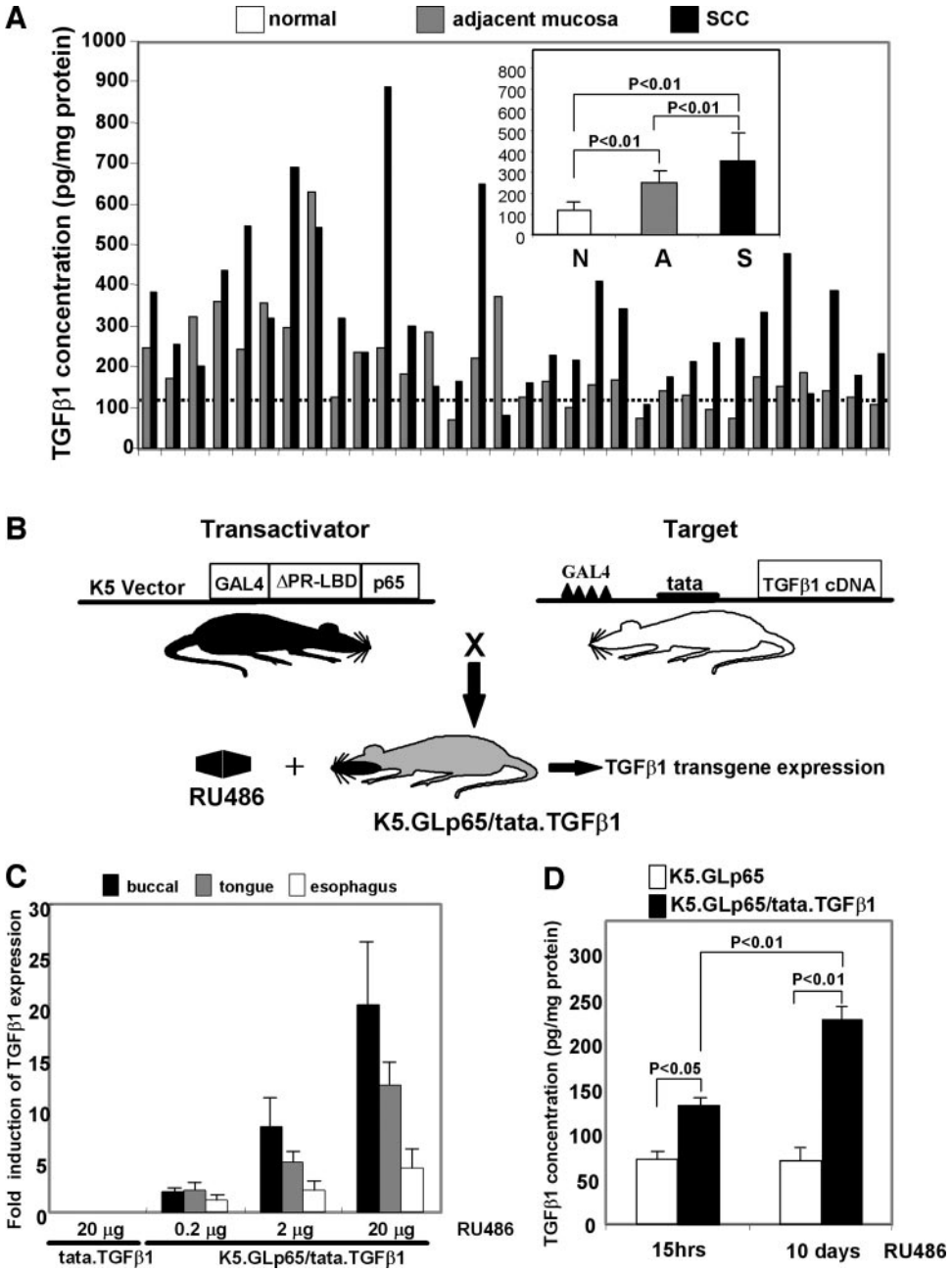

Fig. 1. *A*, quantitation of transforming growth factor β1 (TGF-β1) protein levels in human samples by ELISA. Data from head and neck squamous cell carcinomas (HNSCCs) and case-matched adjacent tissues from 32 HNSCC patients is presented. Each pair of bars represents an adjacent tissue and tumor tissue from a single case. The average level of TGF-β1 protein from each group is shown in the *inset*. N, normal oropharyngeal samples from sleep apnea patients, $n = 5$. A, tissue samples adjacent to HNSCCs, $n = 32$; H, HNSCC tumors, $n = 32$. The *dotted horizontal line* in the main graph represents the average level of TGF-β1 from 5 normal tissues. *B*, schematics of the gene-switch-TGF-β1-transgenic system in head and neck epithelia. The GLp65 transactivator was inserted into the K5 expression vector. The GLp65 comprises the GAL4 DNA binding domain, the ligand binding domain of the truncated progesterone receptor (ΔPR-LBD), and the nuclear factor-κB p65 transactivation domain. The target gene consists of a wild-type human TGF-β1 cDNA under the control of a tata promoter containing four copies of the GAL4 binding sequence upstream of the promoter. A transactivator line is cross-bred with a target line to generate bigenic mice containing both transgenes. RU486 is applied orally to induce TGF-β1 expression in head and neck epithelia. *C*, fold inductions of TGF-β1 expression quantified by quantitative reverse transcription-PCR 15 h after different doses of RU486 application. *D*, ELISA quantitation of TGF-β1 protein levels in buccal tissues of gene-switch-TGF-β1 mice 15 h after single RU486 treatment or 10 days after initiating daily RU486 treatments.

ison with the adjacent tissue samples ($P < 0.01$). Among them, 25 (78.1%) HNSCC samples exhibited 1.5–7.5-fold increase in TGF-β1 protein in comparison with normal control, and 19 cases (59.4%) showed an additional increase of 1.5–3.8-fold in TGF-β1 expression in comparison with their adjacent tissues.

Induction of TGF-β1 Transgene Expression in Head and Neck Epithelia. To induce TGF-β1 transgene expression at various levels, different dosages of RU486 (0.2, 2, and 20 μg/mouse) were applied to the oral cavities of K5.GLp65/tata.TGF-β1-bigenic mice (Fig. 1*B*). Monogenic control mice (K5.GLp65 or tata.TGF-β1) were also treated with the same doses of RU486. Induction of TGF-β1 transgene expression in head and neck epithelia was quantified by quantitative reverse transcription-PCR 15 h after RU486 application. There were no detectable TGF-β1 transgene expression levels in the buccal, tongue, and esophagus of monogenic mice when treated with the highest dose of 20 μg of RU486. However, induction of TGF-β1 transgene expression was detectable at an RU486 dosage of 0.2 μg/mouse. We set the level of TGF-β1 transgene expression in an esophageal sample after 0.2 μg/mouse of RU486 treatment as "1" arbitrary unit and used this as a baseline to compare TGF-β1 transgene expression levels in response to different dosages of RU486 treatment. At 0.2 μg/mouse of RU486, TGF-β1 transgene expression was induced 2.0 ± 0.3-, 2.2 ± 0.9-, and 1.2 ± 0.3-fold in the buccal, tongue, and esophagus, respectively ($n = 3$). TGF-β1 transgene expression was increased 8.4 ± 2.6-fold in the buccal, 4.9 ± 0.9-fold in the tongue, and 2.1 ± 0.8-fold in the esophagus after treatment with 2 μg of RU486 ($n = 3$). An additional increase of 20.2 ± 6.2-, 12.4 ± 2.2-, and 4.3 ± 1.8-fold in the buccal, tongue, and esophagus, respectively ($n = 3$), was observed after treatment with 20 μg of RU486 (Fig. 1*C*). Sustained TGF-β1 transgene induction resulted in pathological alterations (see below). The severity of these alterations correlated with transgene induction levels, *i.e.*, the most severe in the buccal and the mildest in the esophagus after the same regime of RU486 treatment to induce TGF-β1 transgene expression. Hereafter, we present the data from our observations of the buccal.

Because TGF-β1 has a short half-life (13), we first verified whether TGF-β1 protein could accumulate in the tissue after TGF-β1 transgene induction. ELISA was performed on protein extracts from buccal tissues of the bigenic and monogenic mice. Mice were treated with 20 μg/mouse RU486 once, with tissue dissected 15 h later (acute induc-

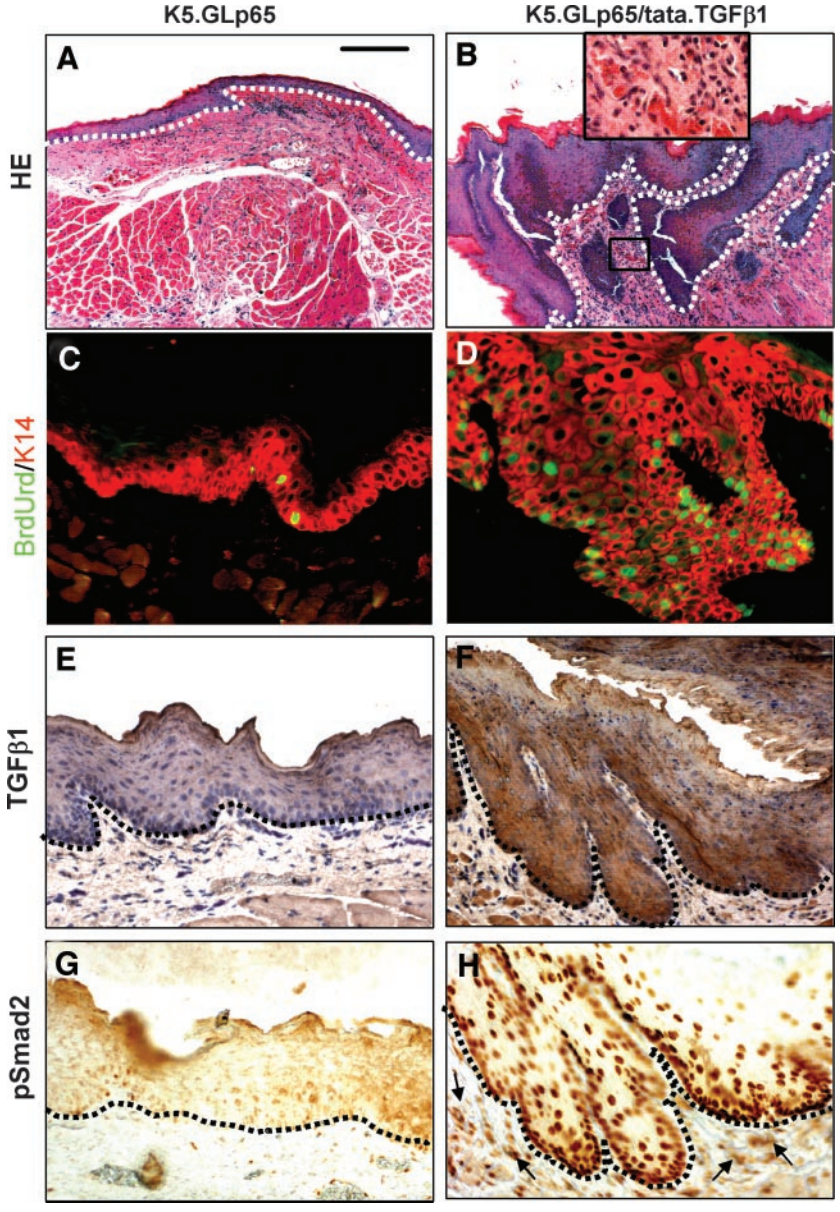

Fig. 2. Sustained induction of transforming growth factor β1 (TGF-β1) resulted in severe epithelia hyperplasia in bigenic TGF-β1 buccal tissue. Bigenic mice (*B*, *D*, *F*, and *H*) and K5.GLp65-monogenic littermates (*A*, *C*, *E*, and *G*) were treated orally with 20 μg of RU486 daily for 10 days. *A* and *B*, H&E staining of bigenic and monogenic buccal tissues. Note that bigenic tissue exhibited significant epithelial hyperplasia and increased cellular content in the stroma. The *inset* in *B* shows RBCs and infiltrated leukocytes. *C* and *D*, *in vivo* bromodeoxyuridine (BrdUrd) labeling in bigenic and monogenic mice. Bigenic buccal mucosa exhibited markedly increased BrdUrd labeling (*green*) as compared with monogenic controls. K14 (*red*), which stains the epithelial compartment, was used as a counterstain. *E–H*, immunohistochemistry of active TGF-β1 (*E* and *F*) and pSmad2 (*G* and *H*). Note that endogenous TGF-β1 expression was predominantly in suprabasal layers of the epithelia, whereas TGF-β1 was stained throughout the epithelia of bigenic buccal. *Arrows* in *H* point to examples of positive cells in the stroma. The *dotted line* in each panel highlights the epithelial-stromal boundary. The *bar* in *A* represents 166 μm for *A* and *B*, 40 μm for *C* and *D*, and 100 μm for *E–H*.

tion) or daily for 10 days, with tissue dissected 15 h after the last treatment (sustained induction). After acute transgene induction, TGF-β1 protein in bigenic tissues was increased to 131.6 ± 10.6 pg/mg protein ($n = 3$) as compared with 72.7 ± 9.1 pg/mg endogenous TGF-β1 protein in the monogenic controls ($n = 3$, $P < 0.05$). After sustained transgene induction, TGF-β1 protein in bigenic tissues was increased to 227.7 ± 13.6 pg/mg protein ($n = 3$), whereas the total amount of endogenous TGF-β1 protein was 70.7 ± 12.1 pg/mg protein in the monogenic controls ($n = 3$, $P < 0.01$, Fig. 1*D*). The increase in TGF-β1 protein by sustained induction was also significant in comparison with that after acute TGF-β1 transgene induction ($P < 0.01$).

Sustained TGF-β1 Induction in the Oral Cavity Resulted in Epithelial Hyperplasia, Inflammation, and Increased Angiogenesis. Because TGF-β1 protein level after sustained TGF-β1 transgene induction was similar to that in human HNSCCs (Fig. 1), we chose to continue our experiments using the protocol of sustained TGF-β1 transgene induction for 10 days. As shown in Fig. 2*B*, sustained expression of TGF-β1 resulted in dramatic epithelial hyperplasia in the buccal of bigenic mice compared with monogenic controls (Fig. 2*A*), with a marked increase in the number and size of blood vessels in the underlying stroma and severe inflammation, including a massive infiltration of leukocytes in the stroma. TGF-β1 transgenic buccal epithelium exhibited a 6.5-fold increase in bromodeoxyuridine labeling index (Fig. 2*D*; 20.8 ± 2.7 nuclei/mm basement membrane; $n = 3$) in comparison with the monogenic controls (Fig. 2*C*; 3.2 ± 1.4 nuclei/mm basement membrane; $n = 3$; $P < 0.01$).

Consistent with pathological alterations in both buccal epithelium and stroma of TGF-β1-transgenic mice, we observed increased immunostaining for active TGF-β1 in transgenic buccal epithelium and stroma (Fig. 2*F*) compared with nontransgenic buccal tissue (Fig. 2*E*). This result suggests that the latent TGF-β1 can be secreted into and activated in both the transgenic epithelium and stroma. Accordingly, nuclear staining of phosphorylated Smad2 was prominent in transgenic epithelium and stroma (Fig. 2*H*), whereas control tissues showed a few nuclear staining for phosphorylated Smad2 in the epithelium (Fig. 2*G*).

To further identify the subtypes of the massive infiltrating leukocytes in TGF-β1-transgenic buccal tissues, we performed a CD45 antibody staining, which confirmed the presence of leukocytes mostly in the transgenic stroma (Fig. 3*A*). Among these leukocytes, most of them were granulocytes that were stained by a Ly-6G antibody (Fig. 3*A*). Staining with BM8 antibody indicated that TGF-β1-transgenic buccal stroma and epithelium also contained macrophages (Fig. 3*A*).

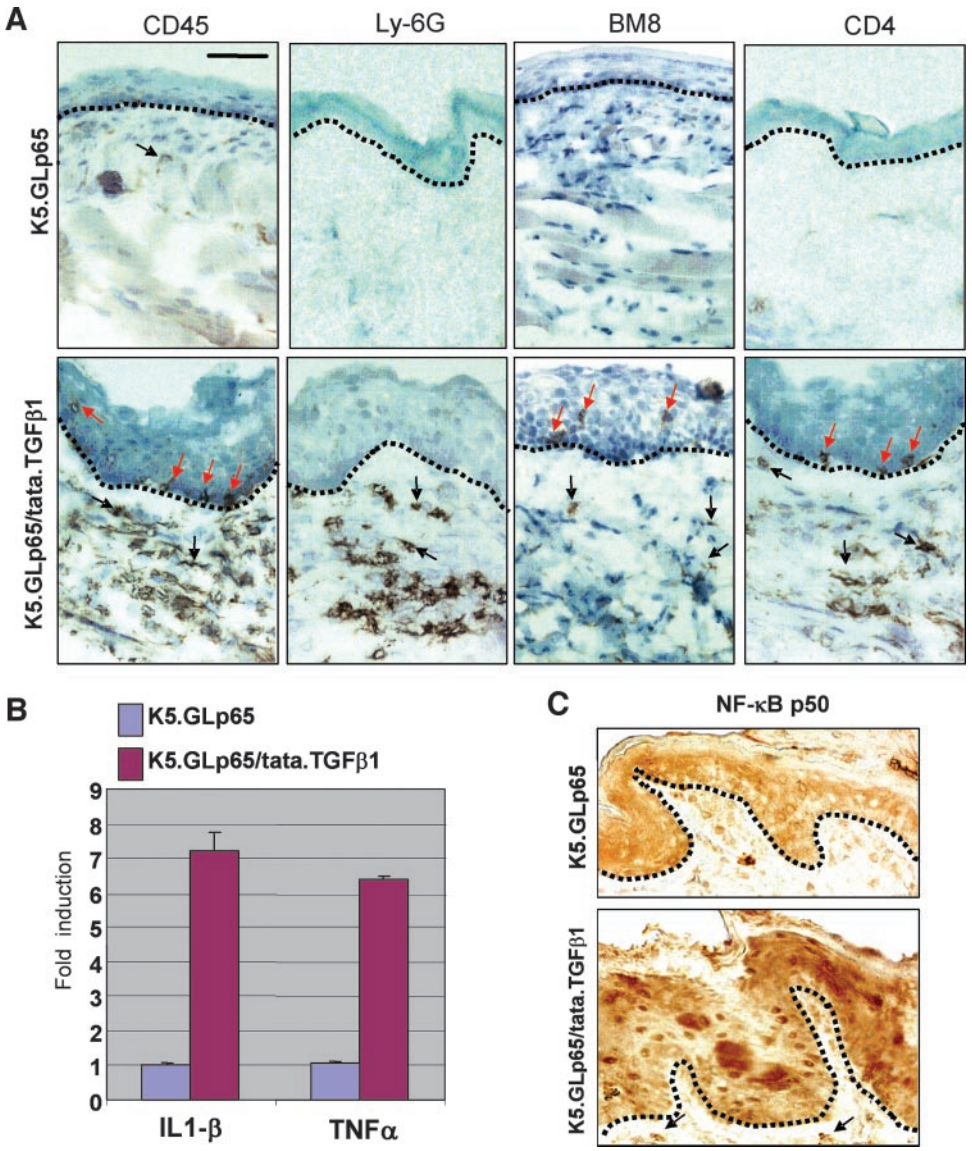

Fig. 3. Sustained induction of transforming growth factor β1 (TGF-β1) resulted in increased inflammation. *A*, immunohistochemical staining of subtypes of leukocytes. CD45, total leukocytes; Ly-6G, granulocytes; BM8, monocytes/macrophages; CD4, T lymphocytes. Note that bigenic buccal tissue contained numerous leukocytes of all subtypes, whereas monogenic buccal tissue contained relatively few leukocytes of each subtype. *Black arrows* point to examples of positive cells in stroma, and *red arrows* point to positive cells in the epithelia. The *dotted line* in each panel highlights the epithelial-stromal boundary. *B*, fold induction of interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) mRNA quantified by quantitative reverse transcription-PCR. RNA was extracted from buccal tissues and subjected to quantitative reverse transcription-PCR. *C*, immunohistochemistry staining of nuclear factor-κB p50 in buccal tissues. *Arrows* point to examples of positive cells in the stroma. The *dotted line* in each panel highlights the epithelial-stromal boundary. The *bar* in the first panel of *A* represents 40 μm for all sections in *A* and *C*.

CD4+ T cells were also present in TGF-β1-transgenic stroma and epithelium (Fig. 3*A*).

Consistent with the inflammation phenotype, two proinflammatory cytokines, interleukin 1β and tumor necrosis factor α, were increased >7- to 6-fold, respectively, in TGF-β1-transgenic buccal tissue in comparison with monogenic controls (Fig. 3*B*). Furthermore, we also examined nuclear translocation of the nuclear factor-κB subunit p50, an end point of the inflammation cascade. Immunohistochemical staining demonstrated a significant increase in nuclear translocation of p50 in TGF-β1-transgenic buccal in comparison with monogenic controls (Fig. 3*C*).

Increased angiogenesis in bigenic TGF-β1 mice was additionally confirmed by immunofluorescence staining with an endothelial marker, CD31 (Fig. 4*A*). The number of vessels was determined to be $6.7 \pm 2.1/mm^2$ stroma in monogenic mice ($n = 3$) but was increased to $28.6 \pm 3.7/mm^2$ stroma in bigenic TGF-β1 mice ($n = 3$, $P < 0.01$). The percentage of the buccal stroma area covered by vessels in monogenic controls was $4.5 \pm 1.2\%$ but was increased to $20.1 \pm 2.8\%$ in the TGF-β1-bigenic mice ($n = 3$, $P < 0.01$).

Recent studies demonstrated that TGF-β1 directly promotes angiogenesis during embryogenesis via the ALK1 receptor (7, 14). To determine whether ALK1 was activated in the TGF-β1-transgenic buccal tissue, we examined the expression pattern of ALK1 and its downstream signal mediators Smad1 and Smad5 in the buccal tissue of bigenic TGF-β1 mice after sustained TGF-β1 induction. In the monogenic buccal stroma, ALK1 expression was not observed in blood vessels. Because ALK1 is only expressed during the active phase of angiogenesis (7, 14), this result suggests that blood vessels in normal buccal tissues were quiescent. However, >50% of the vessels in TGF-β1-transgenic buccal stroma stained positive for ALK1 (Fig. 4*B*). Accordingly, an antibody that recognizes the ALK1-signaling mediators, phosphorylated Smad1, Smad5, and Smad8 (anti-pSmad1/5/8), also stained >50% of the vessels in TGF-β1-transgenic buccal stroma but did not stain vessels in the monogenic buccal stroma (Fig. 4*C*).

Discussion

Inflammation plays an important role in tumor promotion, particularly in HNSCC development (15). Our results show that TGF-β1 overexpression, at levels similar to those that occur during HNSCC development in humans, elicited a potent inflammation response in oral tissue. TGF-β1 is one of the most potent chemotactic cytokines for leukocytes (16). Thus, leukocyte infiltration may be a result of the paracrine effect of TGF-β1. In contrast to its chemotactic effect, TGF-β1 has also been shown to have an anti-inflammatory effect (17). However, in TGF-β1-transgenic oral tissue, leukocytes, together with keratinocytes, continuously produced inflammatory cytokines such as interleukin 1 and tumor necrosis factor α. These inflammatory cytokines may augment the inflammatory response, which was evidenced by increased nuclear translocation of nuclear factor-κB p50 in TGF-β1-transgenic oral tissue. Nuclear factor-κB activation subsequently induces expression of many proinflammatory molecules that additionally facilitate the inflammatory response (18). Under these circumstances, even if TGF-β1 has an anti-inflammatory effect, accumulation of inflammation inducing molecules would override this

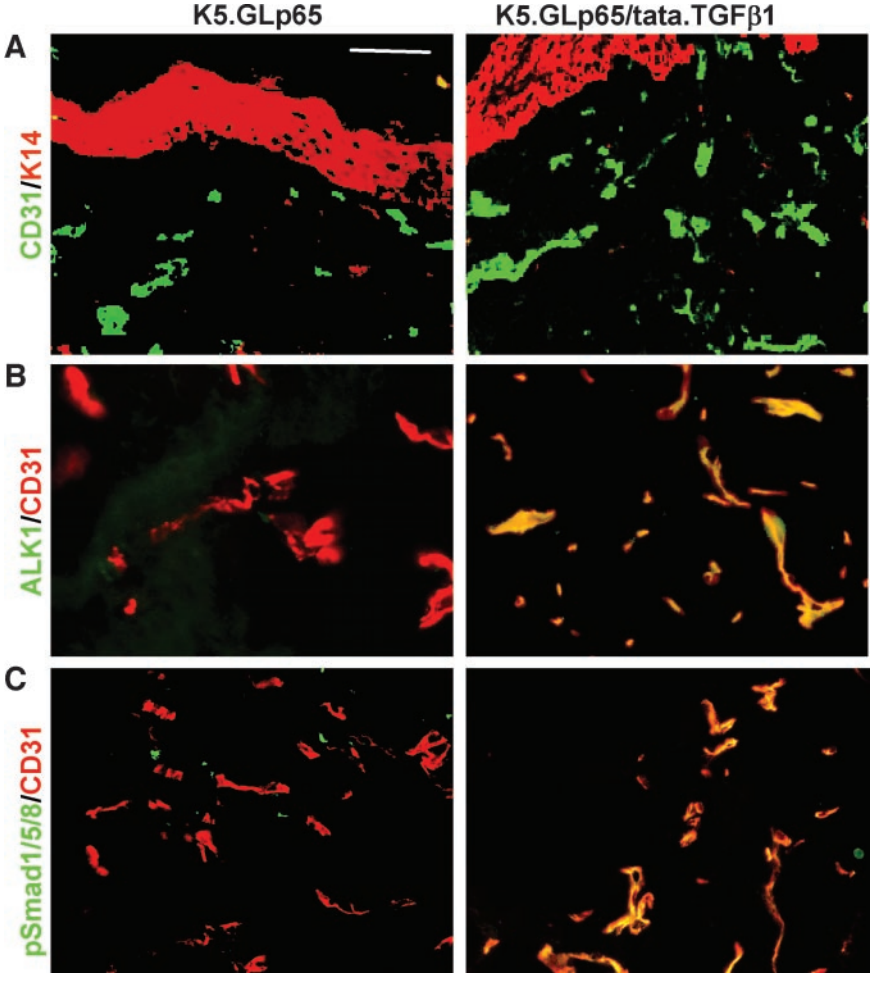

Fig. 4. Sustained induction of transforming growth factor β1 (TGF-β1) resulted in increased angiogenesis. *A*, immunofluorescence staining for CD31 (*green*) highlights increased angiogenesis in K5.GLp65/tata.TGF-β1 buccal stroma. K14 (*red*), which stains the epithelial compartment, was used as a counterstain. *B* and *C*, immunofluorescence staining for ALK1 (*B*, *green*) and pSmad1/5/8 (*C*, *green*) failed to stain cells in blood vessels highlighted by CD31 (*red*) in K5.GLp65 stroma but stained cells in >50% of the blood vessels (*yellow* indicates double fluorescence) in K5.GLp65/tata.TGF-β1 stroma. The *bar* in *A* represents 40 μm for all sections.

effect. Because the majority of oral cancer patients have a chronic inflammation history (1), our data suggest that TGF-β1 overexpression in head and neck epithelia may be an important molecular mechanism that elicits chronic inflammation, thus promoting HNSCC formation.

Similar to our previous study showing that short-term TGF-β1 transgene induction in the epidermis induces angiogenesis in the skin (8), we found that TGF-β1 transgene induction in oral epithelia also induces angiogenesis. Consistent with recent studies suggesting that TGF-β1 positively regulates angiogenesis via ALK1 signaling (7, 14), we observed that TGF-β1 transgene induction in head and neck epithelia resulted in positive staining for ALK1/pSmad1/5/8 in vessels in the stroma. This result indicates that TGF-β1 transgene expression in epithelial cells is sufficient to provide a paracrine effect on endothelial cells. As a result of angiogenesis, inflammation and epithelial hyperproliferation may also be facilitated. In return, angiogenesis factors produced by leukocytes and keratinocytes may additionally contribute to angiogenesis. For instance, interleukin 1, which was significantly overexpressed in TGF-β1-transgenic epithelia, has been suggested to play an important role in angiogenesis (19).

It has been always assumed that cancer cells at a certain stage escape from TGF-β1-induced growth inhibition to undergo autonomous growth. However, we provide *in vivo* evidence here that induction of TGF-β1 expression in head and neck epithelia resulted in keratinocyte hyperproliferation. Our previous study shows that acute TGF-β1 induction inhibits keratinocyte proliferation *in vivo* (8). Thus, increased keratinocyte proliferation seen in this study is likely a secondary effect, possibly the result of inflammation and angiogenesis. Leukocytes, which infiltrated the stroma and other stromal cells, may provide inflammatory cytokines and growth factors that override the growth inhibition effect of TGF-β1. Supporting this notion, increased interleukin 1 expression has been documented to stimulate keratinocyte proliferation (20).

In summary, here, we report that TGF-β1 is frequently overexpressed in HNSCCs and adjacent head and neck tissues in humans. Inducible expression of TGF-β1 in transgenic oral epithelia at levels similar to those seen in human HNSCC patients resulted in inflammation, angiogenesis, and epithelial hyperproliferation. Thus, our study suggests a tumor promotion role for TGF-β1 in HNSCC development, possibly beginning at early stages. Our study lays a foundation for future studies into the role of TGF-β1 in HNSCC development and progression using an inducible transgenic mouse model.

Acknowledgments

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References

1. Neville BW, Day TA. Oral cancer and precancerous lesions. CA - Cancer J Clin 2002;52:195–215.
2. Reiss M. TGF-beta and cancer. Microbes Infect 1999;1:1327–47.
3. Fukai Y, Fukuchi M, Masuda N, et al. Reduced expression of transforming growth factor-beta receptors is an unfavorable prognostic factor in human esophageal squamous cell carcinoma. Int J Cancer 2003;104:161–6.
4. Logullo AF, Nonogaki S, Miguel RE, et al. Transforming growth factor beta1 (TGF-beta1) expression in head and neck squamous cell carcinoma patients as related to prognosis. J Oral Pathol Med 2003;32:139–45.
5. Weeks BH, He W, Olson KL, Wang XJ. Inducible expression of transforming growth factor beta1 in papillomas causes rapid metastasis. Cancer Res 2001;61:7435–43.
6. Miyazono K, Kusanagi K, Inoue H. Divergence and convergence of TGF-beta/BMP signaling. J Cell Physiol 2001;187:265–76.
7. Goumans MJ, Valdimarsdottir G, Itoh S, et al. Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. EMBO J 2002;21:1743–53.
8. Wang XJ, Liefer KM, Tsai S, O'Malley BW, Roop DR. Development of gene-switch transgenic mice that inducibly express transforming growth factor beta1 in the epidermis. Proc Natl Acad Sci USA 1999;96:8483–8.
9. Li AG, Wang D, Feng XH, Wang XJ. Latent TGF-beta1 overexpression in keratinocytes results in a severe psoriasis-like skin disorder. EMBO J 2004;23:1770–81.
10. Cao T, He W, Roop DR, Wang XJ. K14-GLp65 transactivator induces transgene expression in embryonic epidermis. Genesis 2002;32:189–90.
11. He W, Li AG, Wang D, et al. Overexpression of Smad7 results in severe pathological alterations in multiple epithelial tissues. EMBO J 2002;21:2580–90.
12. Wang Y, O'Malley BW Jr, Tsai SY, O'Malley BW. A regulatory system for use in gene transfer. Proc Natl Acad Sci USA 1994;91:8180–4.
13. Wakefield LM, Winokur TS, Hollands RS, et al. Recombinant latent transforming growth factor beta 1 has a longer plasma half-life in rats than active transforming growth factor beta 1, and a different tissue distribution. J Clin Investig 1990;86:1976–84.
14. Goumans MJ, Valdimarsdottir G, Itoh S, et al. Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGFbeta/ALK5 signaling. Mol Cell 2003;12:817–28.
15. Gleich LL, Salamone FN. Molecular genetics of head and neck cancer. Cancer Control 2002;9:369–78.
16. Wahl SM. TGF-beta in the evolution and resolution of inflammatory and immune processes. Introduction. Microbes Infect 1999;1:1247–9.
17. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol 1998;16:137–61.
18. Tak PP, Firestein GS. NF-kappaB: a key role in inflammatory diseases. J Clin Investig 2001;107:7–11.
19. Voronov E, Shouval DS, Krelin Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci USA 2003;100:2645–50.
20. Bonifati C, Ameglio F. Cytokines in psoriasis. Int J Dermatol 1999;38:241–51.