Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

Research Article

Attenuated Transforming Growth Factor β Signaling Promotes Nuclear Factor-κB Activation in Head and Neck Cancer

Jonah Cohen,[1,2] Zhong Chen,[2] Shi-Long Lu,[3] Xin Ping Yang,[2] Pattatheyil Arun,[2] Reza Ehsanian,[1,2] Matthew S. Brown,[2] Hai Lu,[2] Bin Yan,[2] Oumou Diallo,[2] Xiao-Jing Wang,[3] and Carter Van Waes[2]

[1]Howard Hughes Medical Institute-NIH Research Scholars Program, [2]Head and Neck Surgery Branch, National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, Maryland; and [3]Department of Otolaryngology, Oregon Health and Science University, Portland, Oregon

Abstract

Although constitutively activated nuclear factor-κB (NF-κB), attenuated transforming growth factor β (TGFβ) signaling, and *TP53* mutations frequently occur in human cancers, how these pathways interact and together contribute to malignancy remains uncertain. Here, we found an association between overexpression of NF-κB–related genes, reduced expression of TGFβ receptor (TβR) subunits and downstream targets, and *TP53* genotype in head and neck squamous cell carcinoma (HNSCC). In response to recombinant TGFβ1, both growth inhibition and TGFβ target gene modulation were attenuated or absent in a panel of human HNSCC lines. However, in HNSCC cells that retained residual TGFβ signaling, TGFβ1 inhibited both constitutive and tumor necrosis factor α–stimulated NF-κB activity. Furthermore, HNSCC lines overexpressing mutant (mt) TP53 and human tumor specimens with positive TP53 nuclear staining exhibited reduced TβRII and knocking down mt*TP53* induced *TβRII*, increasing TGFβ downstream gene expression while inhibiting proinflammatory NF-κB target gene expression. Transfection of ectopic TβRII directly restored TGFβ signaling while inhibiting inhibitor κBα degradation and suppressing serine-536 phosphorylation of NF-κB p65 and NF-κB transcriptional activation, linking these alterations. Finally, experiments with *TβRII* conditional knockout mice show that abrogation of TGFβ signaling promotes the sustained induction of NF-κB and its proinflammatory target genes during HNSCC tumorigenesis and progression. Together, these findings elucidate a regulatory framework in which attenuated TGFβ signaling promotes NF-κB activation and squamous epithelial malignancy in the setting of altered TP53 status. [Cancer Res 2009;69(8):3415–24]

Introduction

The development and progression of cancer are the result of sequential genetic and biological events, including loss of tumor suppressor genes and gain of function in proto-oncogenes. These multiple defects lead to self-sufficient proliferation with limitless replicative potential, evasion of apoptosis, tissue invasion and metastasis, and sustained angiogenesis (1). Mounting evidence indicates that nuclear factor-κB (NF-κB) is a critical mediator in many of these processes through transcriptional regulation of several hundred downstream genes, the products of which promote the malignant phenotype (2).

In regulating this broad genetic program, NF-κB transcription factors are assembled through dimerization of five subunits: RelA(p65), p50(NF-κB1), p52(NF-κB2), c-Rel, and RelB. Each dimer is bound in the cytoplasm by an inhibitor κB (IκB), which prevents its nuclear translocation (3). Cell stimulation transiently activates the IκB kinase (IKK) complex, which is composed of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ). Activated IKK phosphorylates IκB proteins, resulting in their proteosomal degradation and subsequent translocation of NF-κB dimers to the nucleus. NF-κB is constitutively activated in diverse human malignancies, including head and neck squamous cell carcinoma (HNSCC), wherein numerous activating signals have been elucidated, including proinflammatory cytokines tumor necrosis factor α (TNFα) and interleukin-1 (IL-1); growth factors epidermal growth factor (EGF) and hepatocyte growth factor; and intermediate kinases phosphoinositide 3-kinase (PI3K), casein kinase 2, and IKK (4). Whereas previous studies have focused on the positive signals contributing to NF-κB activation in HNSCC, signals that negatively regulate NF-κB activity remain poorly understood (5).

The transforming growth factor β (TGFβ) signaling pathway performs an essential regulatory role in maintaining normal epithelial homeostasis (6). TGFβ signals through three TGFβ receptor (TβR) subunits (TβRI, TβRII, and TβRIII), resulting in phosphorylation of Smad2 and Smad3, which, with Smad4, enter the nucleus and regulate transcription. TGFβ potently inhibits epithelial proliferation by up-regulation of cyclin-dependent kinase inhibitor genes $p15^{INK4b}$, $p21^{Cip1}$, and $p57^{Kip2}$ and down-regulation of *c-MYC* and *ID1* expression (6). Thus, TGFβ signaling shows potent tumor suppression, and transcriptional inactivation or mutations of *TβRI* and *TβRII* have been reported in human epithelial malignancies (7). Whereas mutation of *TβRII* is uncommon in HNSCC development, decreased TβRII expression occurs frequently and leads to a less differentiated, more aggressive phenotype (8, 9). However, the relationship between alterations in TGFβ signaling and NF-κB activation, and the mechanisms contributing to reduced *TβRII* expression in HNSCC have not been elucidated.

The TP53 tumor suppressor represents another commonly altered target underlying the development of cancer, including HNSCC. Defects in TP53 function contribute to loss of cell cycle regulation, genomic instability, and therapeutic resistance, promoting the survival of malignant cells. Mutation of *TP53* in HNSCC occurs with a frequency of ~50%, resulting in altered *TP53* expression and function (10). In HNSCCs that retain a wild-type

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Z. Chen and C. Van Waes contributed equally to this work.

Requests for reprints: Carter Van Waes, National Institute on Deafness and Other Communication Disorders, NIH, Building 10, CRC Room 4-2732, 10 Center Drive, Rockville, MD 20892. Phone: 301-402-4216; Fax: 301-402-1140; E-mail: vanwaesc@nidcd.nih.gov and Zhong Chen, Phone: 301-435-2073; Fax: 301-402-1140; E-mail: chenz@nidcd.nih.gov.

doi:10.1158/0008-5472.CAN-08-3704

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

(wt) *TP53* genotype, approximately one-half exhibit deficient *TP53* expression by immunohistochemistry (11, 12). Finally, *TP53* mutations are not restricted to neoplastic cells but frequently affect adjacent, normal-appearing keratinocytes, implicating *TP53* mutation in the initiation of HNSCC (13).

We recently identified distinct molecular signatures in HNSCCs differing in *TP53* status, wherein genome-wide microarray and bioinformatic analysis revealed activation of NF-κB target gene expression with concomitant down-regulation of TGFβ pathway genes (14, 15). These observations prompted us to hypothesize that a potential relationship exists among TGFβ signaling, NF-κB activation, and *TP53* status, which we define here using HNSCC-derived cell lines, human tissue specimens, and an animal model of HNSCC generated by *TβRII* deletion from murine head and neck epithelia (16).

Materials and Methods

Cell lines and mice. HNSCC cell lines from University of Michigan squamous cell carcinoma (UM-SCC) series were obtained from Dr. T.E. Carey (University of Michigan). UM-SCC cell lines were previously characterized and found to possess molecular and phenotypic alterations expressed *in situ* and important in the pathogenicity of HNSCC (15). The tumor and outcome characteristics of patients providing UM-SCC cell lines are shown in Supplementary Table S1. The *TP53* mutation status of these cell lines was analyzed by bidirectional DNA sequencing of exons 2 to 9 (Supplementary Table S2). UM-SCC lines and primary human epidermal keratinocytes (HeKa) were cultured as previously described (12). All animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee at the Oregon Health and Science University. *TβRII*-floxed mice and *K5.CrePR1* transgenic mice were established and maintained as described (16).

Reagents and transfection. Recombinant TNFα and TGFβ1 were from R&D Systems. Primary antibodies against the following proteins were used: TβRII (Santa Cruz), p-Smad2 (Cell Signaling), Smad2/3 (BD Biosciences), TP53 (Santa Cruz), serine-536 and serine-276 phosphorylated p65 (Cell Signaling), p65 (Santa Cruz), p50 (Santa Cruz), Oct-1 (Cell Signaling), and β-actin (Cell Signaling). All transfections were performed using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). Transient transfection was performed using constructs for TβRII and pcDNA3 control vector. The expression vector containing the human TP53 open reading frame (pORF-hTP53) and the empty control vector (pORF-mcs) were obtained from InvivoGen. Control small interfering RNA (siRNA) was from Qiagen and *TP53* siRNA was from Dharmacon.

Real-time quantitative reverse transcription-PCR. RNA isolation and cDNA synthesis were performed as previously described (12, 16). cDNA products were subjected to real-time quantitative reverse transcription-PCR (QRT-PCR) using TaqMan Assays-on-Demand probes (Applied Biosystems). An 18S rRNA probe was used as an internal control. Each sample was examined in triplicate. The relative mRNA expression levels were determined by normalizing with the 18S transcripts, the values of which were calculated using the comparative C_t method. Further details are available in the Supplementary Materials and Methods.

Immunohistochemistry and immunofluorescence. Formalin-fixed and paraffin-embedded HNSCC tissue arrays were obtained from Cybrdi as previously described (17). Each array contained HNSCC tumor tissues from 20 individuals spotted in triplicate plus normal mucosa tissues from six normal subjects spotted in duplicate. Immunohistochemistry and immunofluorescence analysis are described in Supplementary Materials and Methods.

Results

Aberrant expression of TGFβ and activated NF-κB genes in HNSCC cells with distinct TP53 status. Previous microarray profiling and bioinformatic analyses of gene expression in a panel of nine HNSCC cell lines (Supplementary Table S1; ref. 18) and primary HeKa cells predicted potential cross talk among NF-κB, TGFβ, and TP53 signaling pathways (14). We performed real-time QRT-PCR to assess the expression of critical components of these pathways and downstream effector genes generating a cluster-based heatmap normalized to the expression pattern in primary keratinocytes (HeKa; Fig. 1*A*).

Increased basal expression of an NF-κB activating kinase, subunit (*IKKA* and *C-REL*) and target proto-oncogenes and proinflammatory genes (*CCND1, cIAP1, IL-6*, and *IL-8*) were detected (Fig. 1*A*). Conversely, attenuated TGFβ signaling was indicated by down-regulation of at least one of three essential TGFβ receptor subunits and other downstream targets (Fig. 1*A* and *B*). Strikingly, reduction in receptor expression clustered closely with the TP53 genotype and expression signature (Supplementary Table S2; Fig. 1*A*, *bottom row*). HNSCC cells deficient in wt*TP53* transcript and protein (UM-SCC-1, UM-SCC-6, UM-SCC-9, and UM-SCC-11A) underexpressed *TβRI* and *TβRIII*, whereas most lines expressing mutant (mt) *TP53* (UM-SCC-11B, UM-SCC-22A, UM-SCC-22B, UM-SCC-38, and UM-SCC-46) showed reduced *TβRII* levels. Expression of downstream signaling components *SMAD2, SMAD3*, and *SMAD4* varied but was not markedly reduced. Additionally, TGFβ target genes $p15^{INK4b}$, $p21^{Cip1}$, *SMAD7, HPGD, PAI1*, and *MMP2* were strongly down-regulated in most UM-SCC lines. By contrast, *ID1*, previously implicated in metastatic aggressiveness of human SCC, was up-regulated in all UM-SCC lines (19). *KRAS* or *HRAS* overexpression was also detected in this panel of human HNSCC cell lines, as previously observed in ~80% of human HNSCC tissue samples (16). Together, these data suggest that HNSCC exhibit aberrant NF-κB gene signatures in the context of multiple deficiencies in the TGFβ signaling network, which are linked to deficient wtTP53 or mtTP53 status.

HNSCC cells exhibit deficient TGFβ-induced growth inhibition and transcriptional activity. To determine if reduced expression of *TGFβ* receptor subunits affects TGFβ signal-induced growth arrest in HNSCC cells, we measured cell proliferation by a 5-day 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using five UM-SCC lines of differing TP53 status (UM-SCC-6, UM-SCC-11B, UM-SCC-22A, UM-SCC-22B, and UM-SCC-46) and HeKa control cells under varying concentrations of rTGFβ1. TGFβ1 significantly inhibited HeKa but not HNSCC cell proliferation (Fig. 2*A* and Supplementary Fig. S1*A* for UM-SCC-11B and UM-SCC-22A). UM-SCC-11B, UM-SCC-22A, and UM-SCC-46 exhibited complete resistance to TGFβ-induced cytostatic effects. UM-SCC-6 and UM-SCC-22B showed a partial reduction in cell proliferation, suggesting attenuated TGFβ signaling and cytostatic activity.

We next examined whether a reduction in cellular response to TGFβ1 affects expression of TGFβ downstream target genes important in growth arrest and other biological functions. In HeKa, TGFβ1 exposure strongly induced mRNA expression of *PAI1, MMP2,* $p15^{INK4b}$, $p57^{Kip2}$, and *SMAD7* and suppressed expression of *c-MYC*, an NF-κB target gene (ref. 20; Fig. 2*B* and Supplementary Fig. S1*B* for UM-SCC-11B and UM-SCC-22A; data not shown for $p57^{Kip2}$). By contrast, all HNSCC lines exhibited diminished sensitivity to TGFβ-modulated gene expression. Collectively, these findings show that HNSCC cells with deficient expression of component(s) of the TGFβ signaling pathway exhibit functional attenuation of TGFβ signaling through loss of TGFβ-mediated growth arrest and transcriptional activity.

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

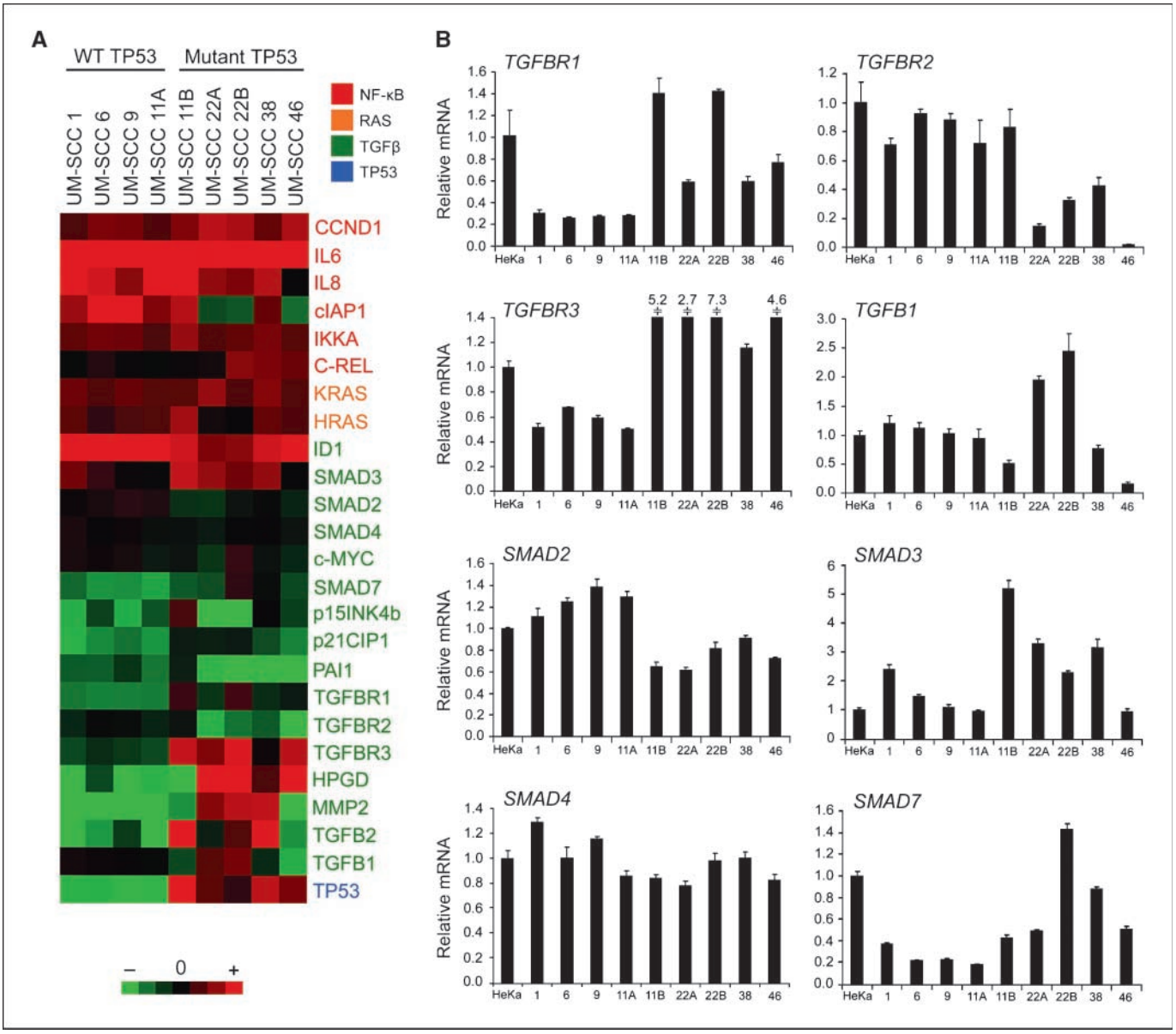

Figure 1. Aberrant NF-κB and deficient TGFβ pathway gene expression signatures in HNSCCs with distinct TP53 status. *A,* gene expression heatmap of important genes related to TGFβ, NF-κB, TP53, and RAS signaling pathways in nine HNSCC (UM-SCC) cell lines, normalized to normal primary HeKa. *Red,* increased expression; *green,* decreased expression. Clustering was associated with TP53 genotype and mRNA levels of wt*TP53* and mt*TP53. B,* reduced TGFβ pathway component expression in HNSCC cells. Quantitative comparison of relative mRNA levels for eight TGFβ signal pathway genes. Expression levels of individual genes in HeKa cells were set to the value of 1 arbitrary unit. *Columns,* mean between triplicate samples; *bars,* SD.

Induction of residual TGFβ signaling inhibits NF-κB activation. As our previous bioinformatic analysis (14) and results in Fig. 1 suggested potential cross talk and an inverse relationship between the TGFβ and NF-κB gene expression, we examined if TGFβ could inhibit NF-κB activation. We selected the two HNSCC lines (UM-SCC-6 and UM-SCC-22B) that exhibited residual TGFβ-induced growth inhibition and UM-SCC-46 as a negative control line because of its deficient TGFβ signaling (Fig. 2). In Fig. 3*A*, UM-SCC-6 and UM-SCC-22B exhibited significant detectable TGFβ1-induced TGFβ luciferase reporter gene at 12 hours, whereas UM-SCC-46 was not responsive to TGFβ1 treatment. UM-SCC-6, UM-SCC-22B, and UM-SCC-46 were transfected with a NF-κB reporter plasmid and treated ±TGFβ1 and ±TNFα. TGFβ1 inhibited constitutive and/or TNFα-induced NF-κB transcriptional activity in UM-SCC-6 and 22B, whereas UM-SCC-46 was unresponsive to TGFβ1 treatment (Fig. 3*B*). Taken together, these findings show that TGFβ is able to suppress NF-κB activation in some UM-SCC cells.

Linkage between diminished TβRII expression and signaling, mtTP53 accumulation, and NF-κB target gene activation in a subset of HNSCC. Given that loss of *TβRII* gene expression and signaling was observed in a subset of HNSCC cell lines expressing mtTP53 (Fig. 1; Supplementary Table S1), we examined if a similar subset is found in HNSCC tumors *in situ*. Immunostaining for TβRII, phosphorylated (activated) Smad2, and TP53 protein expression was performed using a tissue array with 20 HNSCC

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

specimens and six normal oral mucosa specimens (Fig. 4*A*). Sixteen of the 20 HNSCC samples (80%) displayed absent (−) or low TβRII protein, with 11 of these 16 showing absent (−) or low phospho-Smad2. Of nine scored completely negative for TβRII, eight showed decreased p-Smad2 staining (Supplementary Fig. S2*A*). Conversely, all normal oral mucosa samples exhibited both strong TβRII and activated p-Smad2 staining. Together, these observations confirm that deficient TβRII expression and p-Smad2 signaling is prevalent in human HNSCC (16).

As TP53 mutations often result in proteins highly resistant to degradation, detection of TP53 nuclear accumulation by immunohistochemistry is strongly associated with mutations in the *TP53* gene (21). Consistent with an expected mutation rate of ~50% in HNSCC (10), 11 of the 20 tumors (55%) showed nuclear staining for TP53. Nine of these 11 (82%) exhibited concomitantly reduced or absent TβRII staining (Supplementary Fig. S2*A*). Of the six HNSCC samples that were most strongly positive for TP53 nuclear staining, five (83%) showed a corresponding loss of TβRII staining within the tumor tissue. None of the normal mucosa specimens exhibited significant TP53 staining. Thus, decreased TβRII expression is seen in a subset of HNSCCs exhibiting increased accumulation of TP53 protein, as well as in a subset without TP53 staining.

We next investigated whether mtTP53 may have a suppressive role in TβRII expression by comparing TP53 and TβRII expression in HeKa with wtTP53, two cell lines with a defect in wtTP53 expression (UM-SCC-9 and UM-SCC-11A), and four cell lines expressing missense mtTP53s (UM-SCC-11B, UM-SCC-22A/B, and

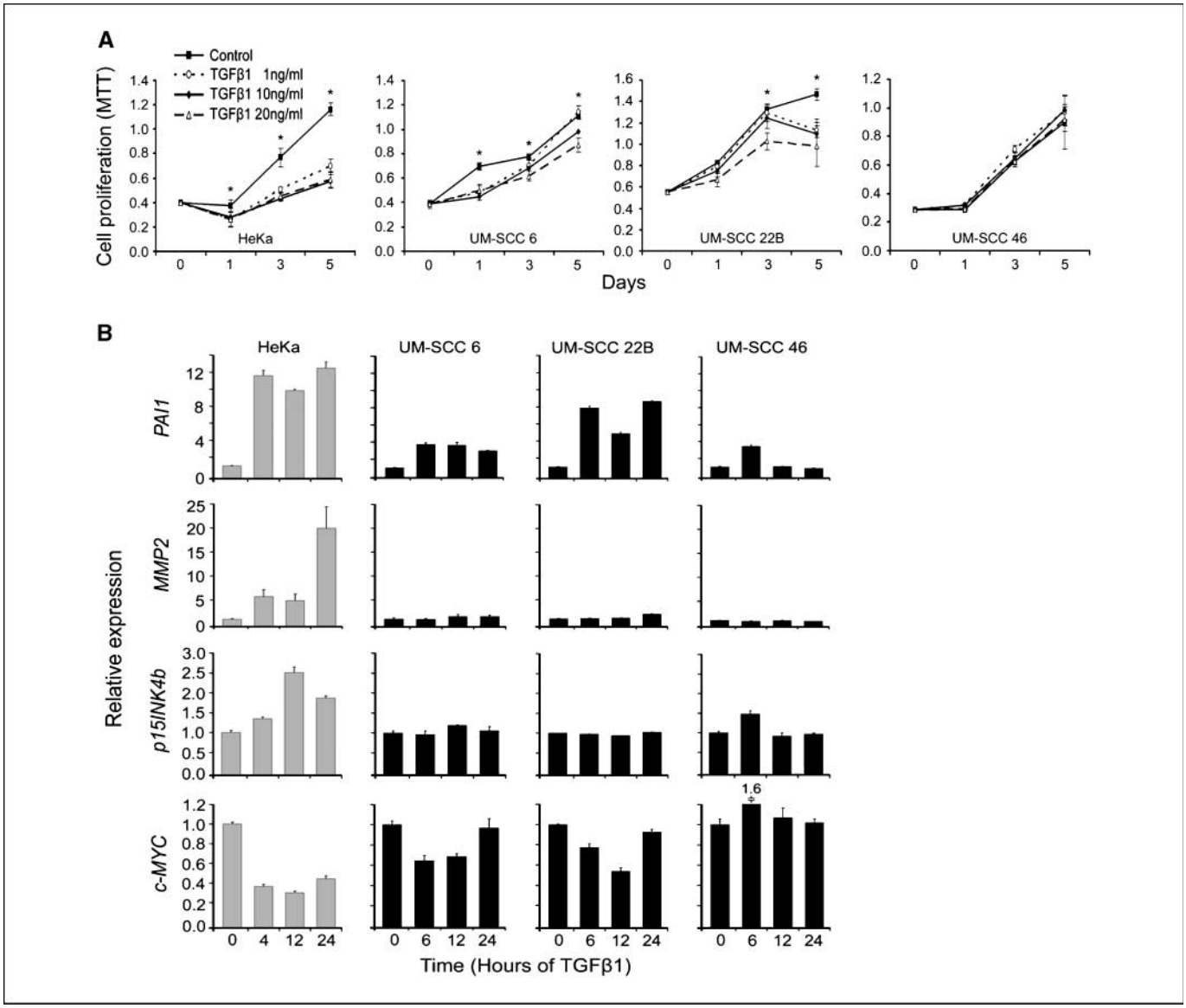

Figure 2. Attenuated TGFβ-induced growth inhibition and target gene expression in HNSCC cells. *A,* functional attenuation of TGFβ-mediated growth arrest in UM-SCC lines. Cell proliferation was measured in a 5-d MTT assay in HeKa cells and UM-SCC lines of differing TP53 status. Cells were treated with 1, 10, or 20 ng/mL of rTGFβ1. Cell growth rates were analyzed in quadruplicate. *Points,* mean; *bars,* SD. *, $P < 0.05$. *B,* attenuated TGFβ-induced activation of *PAI1, MMP2*, and *p15INK4b* and suppression of *c-MYC* in UM-SCC. UM-SCC lines and HeKa cells were stimulated with 10 ng/mL for indicated time points, and total RNA was isolated and assayed for TGFβ target gene mRNA levels by QRT-PCR. Expression of individual genes at 0 h was set to the value of 1 arbitrary unit. *Columns,* means between triplicate samples; *bars,* SD.

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

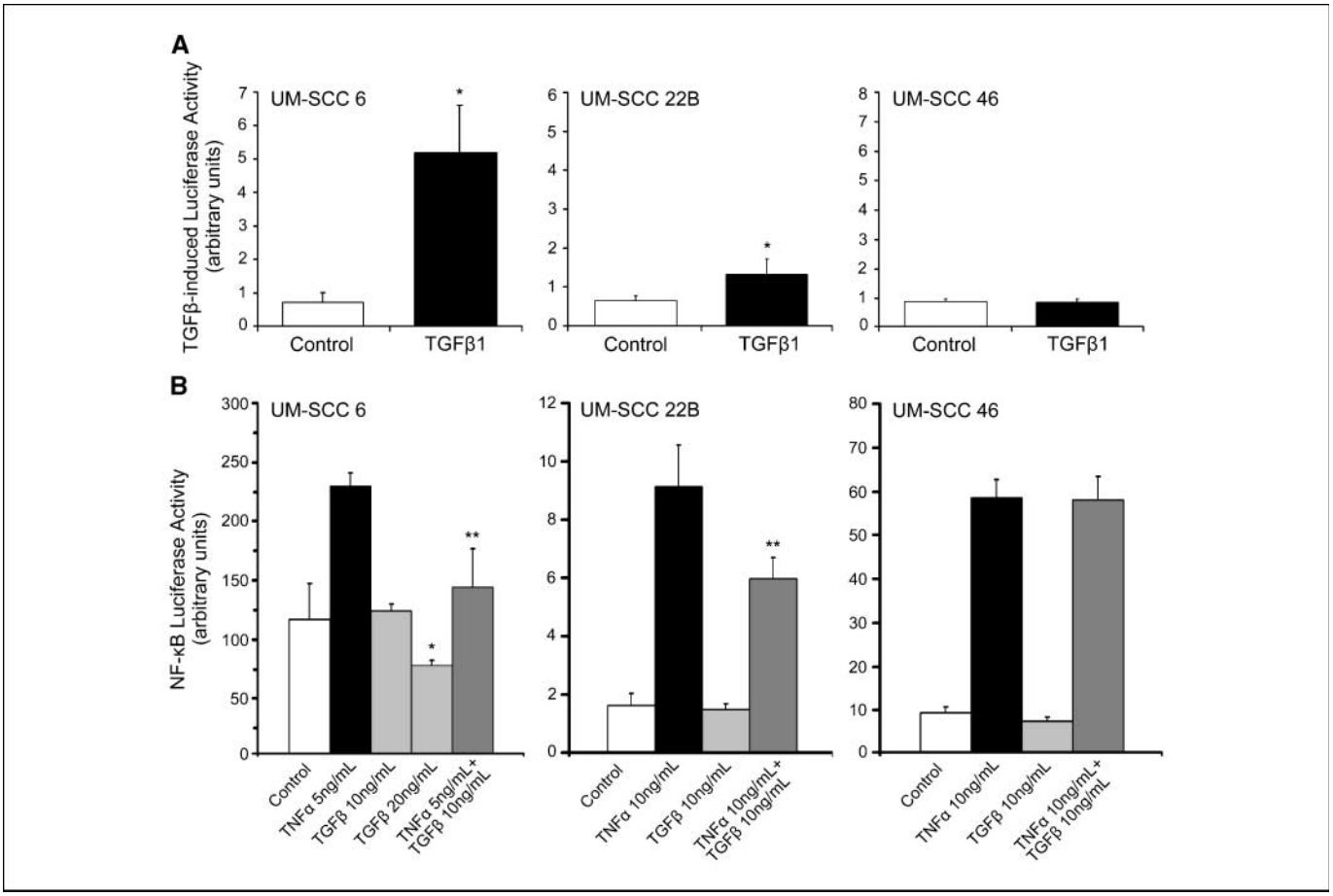

Figure 3. Stimulation of residual TGFβ signaling suppresses NF-κB activation in UM-SCC cells. *A,* TGFβ reporter gene activity in UM-SCC-6, UM-SCC-22B, and UM-SCC-46 cotransfected with a TGFβ-induced reporter plasmid and β-galactosidase reporter plasmid and cultured ±TGFβ1 (10 ng/mL) for 12 h. *B,* NF-κB reporter gene activity in the same three lines cotransfected with NF-κB and β-galactosidase reporter plasmids and treated with different concentrations of TNFα, TGFβ1, or the combination for 12 h. Luciferase values are normalized to β-galactosidase activity. *Columns,* means between triplicate samples; *bars,* SD. *, $P < 0.05$ versus untreated control cells; **, $P < 0.05$ versus TNFα-stimulated cells.

UM-SCC-46; Fig. 4*B*; Supplementary Table S2). Whereas similar levels of TβRII mRNA and protein expression were detected in either HeKa expressing or UM-SCC deficient for wtTP53 protein (Figs. 1*B* and 4*B*), diminished *TβRII* mRNA and protein levels were seen in most UM-SCC lines expressing mtTP53, consistent with the pattern observed in a subset of HNSCC tumors above (Figs. 1*B* and 4*A* and *B*). Transfection of wt*TP53* into UM-SCC-11A cells deficient in endogenous wtTP53 promoted *TβRII* and target gene expression (Supplementary Fig. S3), suggesting that expression of wtTP53 has an enhancing effect on *TβRII* expression whereas endogenous mtTP53 in the other subset may have a dominant-negative effect.

To further establish if the mtTP53 in UM-SCC lines contributes to deficient TβRII expression, we tested the effect of TP53 siRNA to inhibit *TP53* mRNA expression in UM-SCC lines with mtTP53. TP53 siRNA efficiently inhibited endogenous *TP53* expression in UM-SCC-11B and UM-SCC-22A over 96 hours (Fig. 4*C*; Supplementary Fig. S2*B*). Knockdown of mt*TP53* resulted in a progressive increase in *TβRII* gene expression relative to control siRNA (Fig. 4*C* and Supplementary Fig. S2*B*). Similar results were seen in UM-SCC-22B and UM-SCC-46 (Fig. 4*D*; data not shown). Concomitant with enhanced *TβRII* expression, restoration of TGFβ signaling was evident by increased transcription of TGFβ downstream genes, *SMAD7* and *MMP2*, at 72 hours (Fig. 4*C*). We further compared effects of TP53 siRNA on the expression of *TP53, TβRII*, and NF-κB REL(p65)-dependent proinflammatory genes *IL-6* and *IL-8* (14). Figure 4*D* shows that, after *TP53* siRNA knockdown and increase in *TβRII*, there is a significant reduction in both *IL-8* and *IL-6* expression in the mtTP53 line UM-SCC-22B. Collectively, the results above suggest that mtTP53 may be a mechanism contributing to attenuated *TβRII* gene expression, TGFβ signaling, and enhanced NF-κB inflammatory gene expression in HNSCCs.

Ectopic TβRII expression restores TGFβ signaling and suppresses NF-κB activation in HNSCC cells. To directly examine the role of TβRII expression and signaling in inhibition of NF-κB, we determined if transient transfection of a plasmid expressing exogenous TβRII protein could restore TGFβ activity and suppress NF-κB signaling in HNSCC lines that showed deficient TβRII. We selected UM-SCC-46 because it had previously exhibited marked attenuation of TGFβ signaling (Fig. 2). Upon transfection, ectopic TβRII was successfully transiently expressed between 48 and 96 hours (Fig. 5*A*). Expression of TβRII dramatically induced TGFβ signaling in UM-SCC-46, illustrated by enhanced levels of activated phosphorylated Smad2 at 48 hours (Fig. 5*B*). This implicated TβRII

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

expression as a critical defect in TGFβ signaling in these HNSCC cells.

We further examined the effect of restored TβRII signaling on established mechanisms required for NF-κB target gene transactivation. IKK-dependent signal phosphorylation of p65(RelA) serine-536 and PKA-inducible phosphorylation of serine-276 have been shown to enhance NF-κB gene transactivation after stimulation with TNFα (22). Increased expression of TβRII strongly suppressed TNFα-induced p65(RELA) serine-536 phosphorylation, but not serine-276 phosphorylation, or expression of total nuclear p65 or Oct-1, an unrelated control (Fig. 5*B* and Supplementary Fig. S4*A*). Together, these data are consistent with the hypothesis that TβRII in these HNSCC inhibits NF-κB transactivation by a signal-dependent mechanism at or above IKK. To further examine if TβRII modulates IKK-dependent IκBα degradation involved in upstream signal activation and nuclear translocation of NF-κB (2, 23), we used a plasmid expressing an IκB-luciferase fusion protein (24), which can serve as a nontranscriptional reporter of

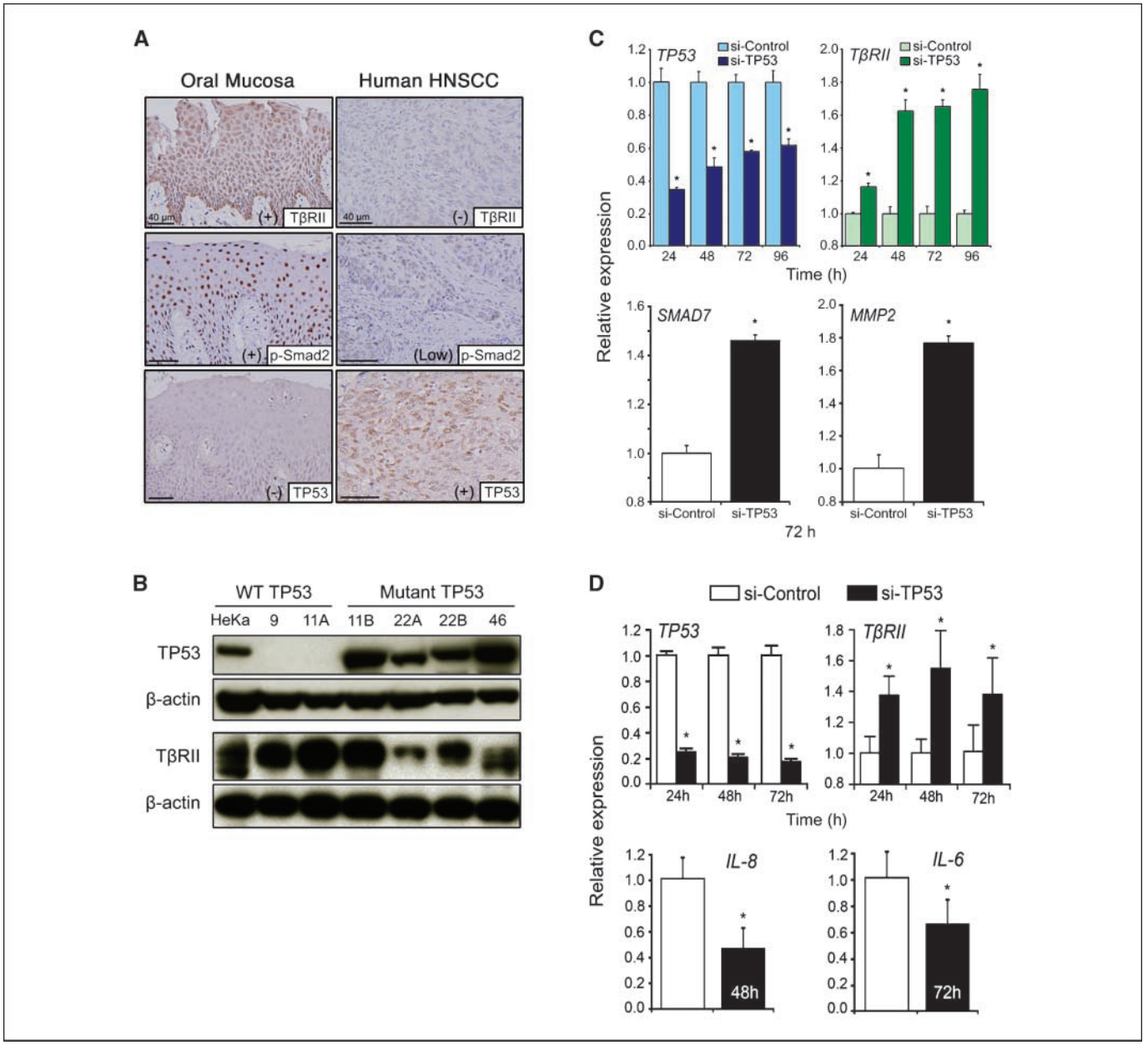

Figure 4. Linkage between diminished TβRII expression and signaling, mtTP53 accumulation, and NF-κB target gene activation in a subset of HNSCC. *A,* photomicrographs show diminished TβRII and phosphorylated (activated) Smad2 with TP53 accumulation in a representative HNSCC tumor compared with the opposite pattern in normal oral mucosa. Semiquantitative scoring and classification of TβRII, p-Smad2, and TP53 protein staining of 20 tumors is described in Supplementary Materials and Methods and Supplementary Fig. S2: −, negative; *Low*, reduced; +, strong staining. *B,* diminished TβRII expression is detected in a subset of UM-SCC lines expressing mtTP53. Western blot analysis of TβRII, TP53, and β-actin in HeKa, wt*TP53*, and mt*TP53* UM-SCC cell lines. *C,* TP53 siRNA knockdown enhances *TβRII* and target genes *SMAD7* and *MMP2* mRNA expression in mt*TP53* UM-SCC-22A. *D,* TP53 siRNA knockdown enhances *TβRII* and inhibits NF-κB target gene *IL-8* and *IL-6* mRNA expression in UM-SCC-22B. UM-SCC lines were transfected with either control or TP53 siRNA. Quantitation of mtTP53 inhibition and target genes was assessed using QRT-PCR at the indicated time points. The expression level in each control siRNA-treated sample is designated as 1.0. *Columns,* mean between triplicate samples; *bars,* SD. *, $P < 0.05$.

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

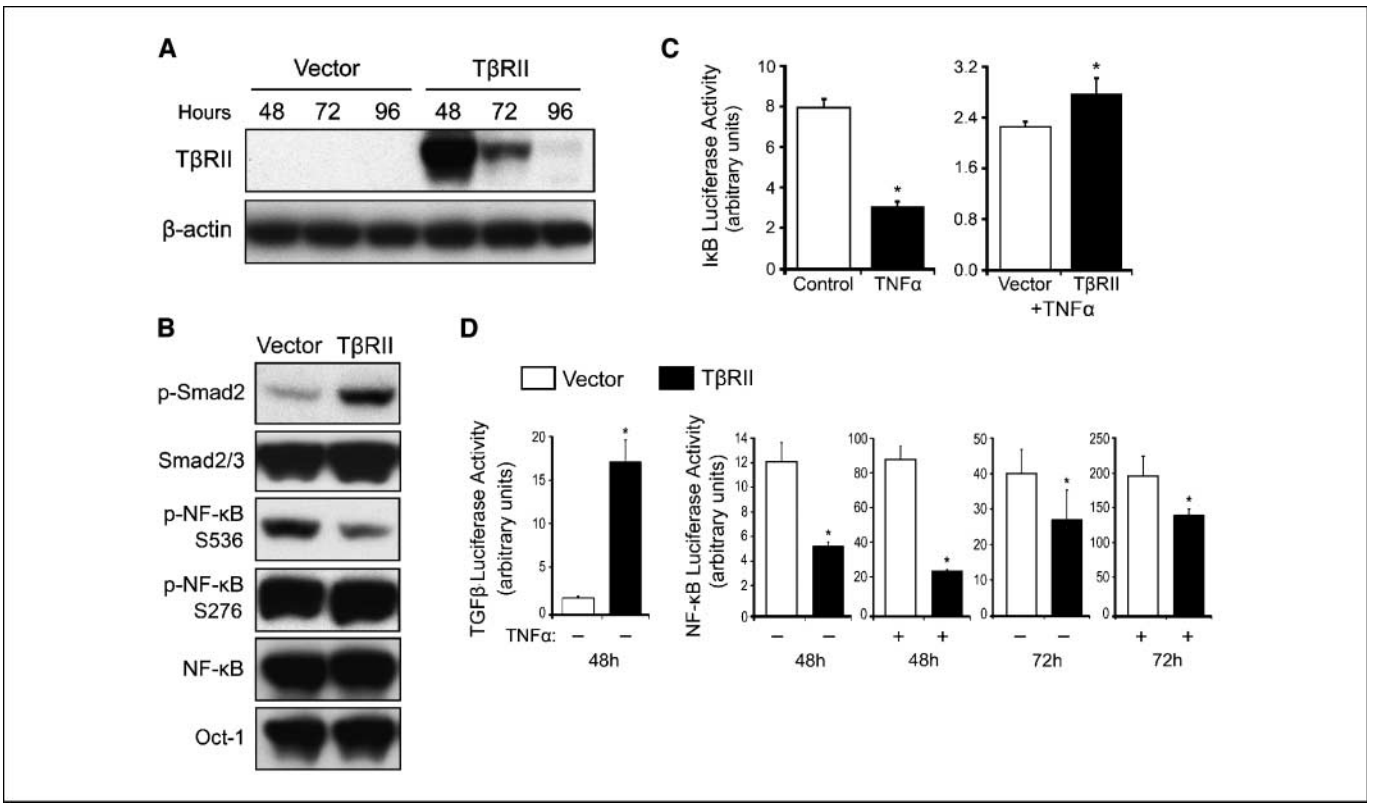

Figure 5. TβRII transfection restores TGFβ signaling and inhibits NF-κB activation in HNSCC. *A,* transient transfection with *TβRII* expression vector enhances expression of TβRII protein. Western blots were performed using whole-cell lysates from UM-SCC-46 cells at the indicated times. *B,* Western blot analysis of nuclear extracts from UM-SCC-46 showing reexpression of TβRII restores TGFβ phosphorylated Smad2 signaling and suppresses IKK-dependent serine-536 but not PKA-mediated serine-276 phosphorylation of RELA(p65) NF-κB subunit after TNFα (10 ng/mL). UM-SCC-46 cells were transfected with a *TβRII* expression plasmid or control vector, and nuclear extracts were harvested after 48 h for immunoblotting. *Oct-1,* loading control. *C,* TβRII attenuates TNFα-induced degradation of IκBα-luciferase fusion protein. UM-SCC-46 cells transfected with an IκB-luciferase fusion protein ±control or *TβRII* expression vector for 24 h and ±TNFα (50 ng/mL) for 30 min. *D,* TβRII induces TGFβ and attenuates NF-κB reporter gene transactivation in UM-SCC-46. UM-SCC-46 line was cotransfected with the respective reporter plasmid plus a *TβRII* expression or control vector and cultured ±TNFα (10 ng/mL) for 12 h before the indicated time points. All luciferase values are normalized to β-galactosidase. *Columns,* mean of triplicate samples; *bars,* SD. *, $P < 0.05$.

kinase activation and degradation. As expected, TNFα-enhanced degradation of the IκBα-luciferase fusion protein (Fig. 5*C*). Upon transfection of ectopic TβRII, restoration of TGFβ signaling resulted in a significant attenuation of TNFα-induced IκB-luciferase protein degradation (~25%; $P < 0.05$), suggesting that a mechanism upstream of IKK-mediated IκB degradation could partially contribute to the attenuating effect of TGFβ on NF-κB signaling.

Once we showed that exogenous TβRII expression could inhibit NF-κB phosphorylation and IκB degradation, we sought to confirm if transient TβRII expression and signaling suppressed aberrant basal and TNFα-enhanced NF-κB transcriptional activity, using three UM-SCC lines expressing mtTP53. UM-SCC-46 exhibited the strongest restoration of TGFβ-induced luciferase activity at 48 hours upon ectopic expression of TβRII, followed by UM-SCC-22B and UM-SCC-22A (Fig. 5*D* and Supplementary Fig. S4*B*). Restoration of TβRII signaling inhibited both constitutive and TNFα-induced NF-κB transcriptional activity in UM-SCC-46 (Fig. 5*D*). UM-SCC-22A and UM-SCC-22B also exhibited a significant reduction in NF-κB transactivation, but this was most clearly shown after exposure to TNFα (Supplementary Fig. S4*B*). These findings show that restoration of TβRII signaling not only reduces activated RELA(p65) serine-536 phosphorylation but can also suppress NF-κB reporter gene transactivation in HNSCC lines expressing different mtTP53s.

Loss of TGFβ signaling promotes NF-κB activation in murine HNSCCs with conditional deletion of *TβRII*. To further examine the role of attenuated TGFβ signaling in the aberrant activation of NF-κB, we investigated mucosa and tumors from a murine model in which K5-targeted genetic deletion of *TβRII* from mucosal stratified epithelia, in combination with 7,12-dimethylbenz(*a*)anthracene (DMBA) initiation caused the development of primary and metastatic HNSCCs histologically and molecularly similar to human HNSCC (16). Tissue samples were collected from buccal mucosa of five $T\beta RII^{+/+}$, $T\beta RII^{-/-}$, and HNSCCs from five $T\beta RII^{-/-}$ mice and compared. Representative H&E–stained tumor sections showed atypical hyperproliferative epithelia by 3 months and HNSCC by 6 months in $T\beta RII^{-/-}$ mice when compared with their wt littermates (ref. 16; Supplementary Fig. S5). Immunostaining of $T\beta RII^{-/-}$ mucosa and HNSCCs confirmed the loss of TβRII protein and the absence of phosphorylated Smad2, showing abrogation of TGFβ signaling compared with wt controls (Supplementary Fig. S5). As classical NF-κB signal activation involves the nuclear translocation of p65 and p50, we examined the aforementioned tissues for the presence and localization of these subunits using immunofluorescence microscopy (Fig. 6*A*). As in human HNSCC lines above, concomitant with loss of TGFβ signaling, a corresponding increase was seen in detectable nuclear RELA(p65) phosphorylated serine-536, as well as p50 staining in five of five

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

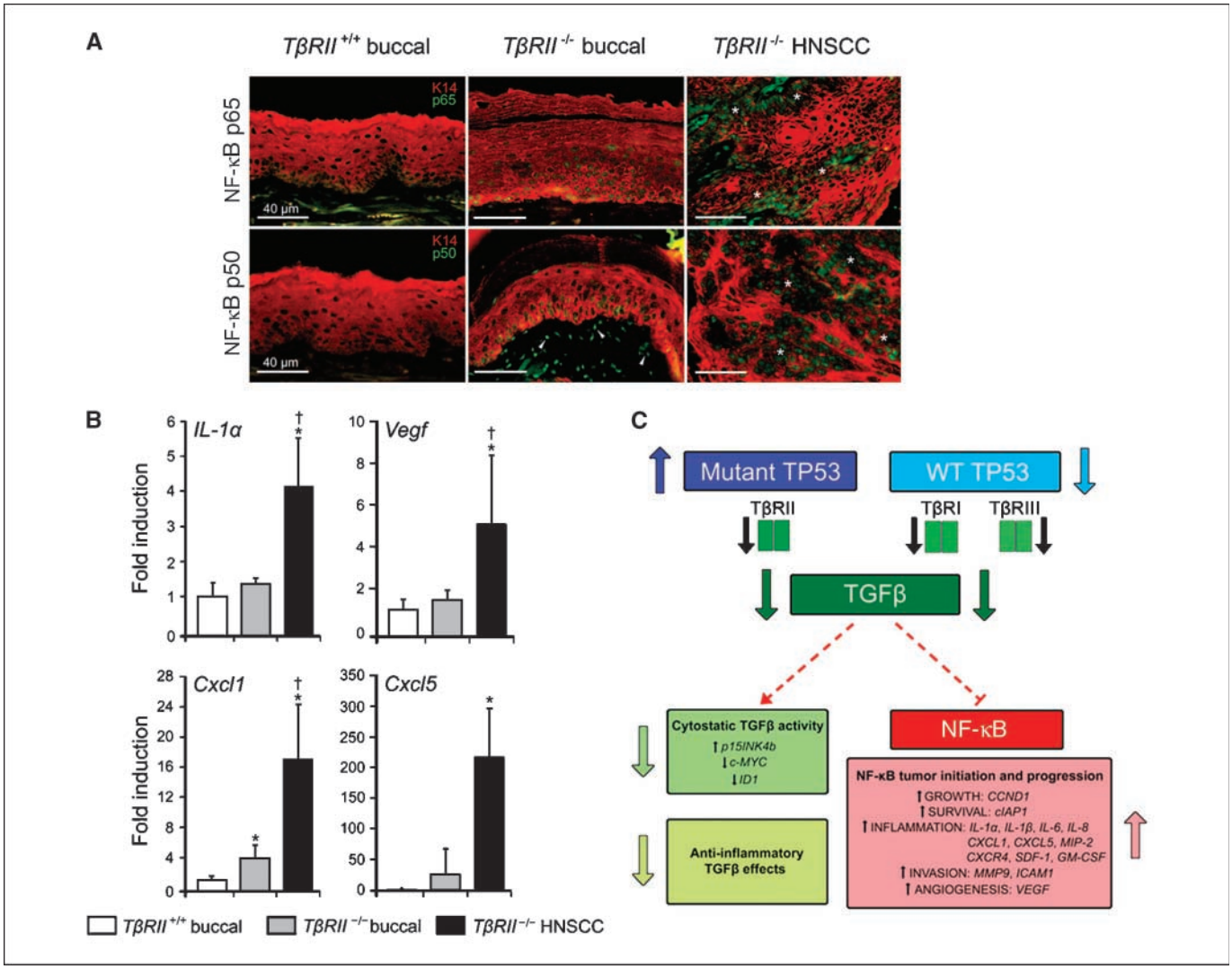

Figure 6. Abrogation of TGFβ signaling promotes NF-κB activation and proinflammatory target gene expression in HNSCCs of $T\beta RII^{-/-}$ mice. *A,* NF-κB p65 and p50 subunit immunofluorescence staining of buccal squamous mucosa from $T\beta RII^{+/+}$, $T\beta RII^{-/-}$, and HNSCC from $T\beta RII^{-/-}$ mice. Buccal tissues representative of five $T\beta RII^{+/+}$ and $T\beta RII^{-/-}$ mice at 3 mo and HNSCC from five $T\beta RII^{-/-}$ mice at 6 mo after DMBA initiation. *Green,* nuclear phosphorylated p65 and p50; *red,* epithelial K14; *arrowheads,* p50+ nuclei in $T\beta RII^{-/-}$ buccal stroma; *asterisks,* NF-κB in regions with reduced K14. *B,* QRT-PCR analysis of NF-κB proinflammatory gene mRNA expression from $T\beta RII^{+/+}$ and $T\beta RII^{-/-}$ buccal and $T\beta RII^{-/-}$ HNSCC tissue samples. Expression of genes in $T\beta RII^{+/+}$ buccal tissue was set to 1 arbitrary unit. *Columns,* mean on triplicate samples of mRNA from five mice per group; *bars,* SE. *, $P < 0.05$ versus DMBA-initiated $T\beta RII^{+/+}$ buccal tissue; †, $P < 0.05$ versus DMBA-initiated $T\beta RII^{-/-}$ preneoplastic buccal tissue. *C,* proposed model for enhanced tumorigenesis and malignant progression resulting from loss of TGFβ signaling and resultant NF-κB and gene activation. mtTP53 represses *TβRII* expression, whereas deficient wtTP53 is associated with diminished *TβRI* and *TβRIII* expression, attenuating TGFβ signaling in HNSCC subsets. Deficient TGFβ signaling renders epithelia resistant to TGFβ cytostatic and antiinflammatory programs while enhancing aberrant activation of NF-κB target prosurvival and inflammatory gene programs.

$T\beta RII^{-/-}$ buccal mucosa and five of five $T\beta RII^{-/-}$ basilar or HNSCC specimens. In contrast, normal $T\beta RII^{+/+}$ mucosa revealed basilar or no NF-κB activation in the five specimens studied. Interestingly, regions in HNSCCs with the strongest NF-κB activation exhibited reduced K14 expression, consistent with previous studies implicating aberrant NF-κB activity and down-regulation of K14 in the epithelial-to-mesenchymal transition of high-risk HNSCCs (25).

Given the critical role of NF-κB in inflammation and our findings in Fig. 6*A*, we investigated the expression of NF-κB–regulated proinflammatory and angiogenic genes in these tissues by QRT-PCR (Fig. 6*B*). $T\beta RII^{-/-}$ HNSCCs exhibited a significant induction of NF-κB–inducible genes *IL-1α, Vegf, Cxcl1/Gro1*, and *Cxcl5/Ena-78* when compared with both $T\beta RII^{-/-}$ buccal mucosa and normal $T\beta RII^{+/+}$ mucosa ($P < 0.05$). Increased expression levels of *Cxcl1* and *Cxcl5* was also detected in $T\beta RII^{-/-}$ preneoplastic lesions compared with $T\beta RII^{+/+}$ buccal tissues (Fig. 6*B*). Potentially further reflective of an inflammatory state, we observed previously (16) and here in the $T\beta RII^{-/-}$ underlying stroma the presence of numerous infiltrating cells, which are strongly positive for nuclear NF-κB (p50) immunofluorescence (Fig. 6*A*). Together, these data strongly support a model where abrogation of TGFβ signaling in stratified epithelia promotes the aberrant activation of NF-κB, target cytokines, enhanced inflammation, and development and malignant progression of HNSCC (Fig. 6*C*).

Discussion

Herein, we show that altered expression of TGFβ receptor subunits and deficient TGFβ signaling are common in human HNSCC lines and tissues, which exhibit enhanced activation of

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

NF-κB. We show that restoration of TGFβ signaling inhibits such aberrant NF-κB signaling and transcriptional activity, indicating that deficient TGFβ signaling is a critical event leading to the activation of NF-κB in HNSCC. Our data further suggest that TP53 mutation may be one mechanism that contributes to attenuated TGFβ signaling in a subset of HNSCCs through its ability to suppress *TβRII* expression. Finally, $T\beta RII^{-/-}$ conditional knockout mice that develop HNSCC with enhanced inflammation exhibited increased NF-κB activation and proinflammatory gene expression with tumor development, marking a significant connection between deficient TGFβ signaling and NF-κB activation in tumorigenesis. Whereas we have previously shown that sustained induction of NF-κB plays a critical role in the pathogenesis of HNSCC, we now implicate loss of a negative regulatory signaling pathway, TGFβ, as an integral step in this activation.

We previously reported that NF-κB–regulated gene signatures are differentially up-regulated in HNSCCs with distinct TP53 status and that overexpression of either *K-RAS* or *H-RAS* at the transcriptional level frequently occurs in human HNSCC tissues with concomitant reduction of *TβRII* mRNA levels (14, 16, 17). Here, we found reduced *TβRII* gene expression with concurrent *RAS* overexpression in a panel of human HNSCC cell lines and report additional TGFβ receptor and biological alterations, including those involving the NF-κB and TP53 pathways (Fig. 1*A* and *B*). Notably, expression of certain genes clustered according to TP53 status, including the TGFβ receptor subunits, *SMAD3, SMAD7, TGFB1, TGFB2, HPGD,* $p21^{Cip1}$, and *MMP2*, as well as the NF-κB–related genes *C-REL, IL-8*, and *cIAP1*, suggesting that differential regulatory mechanisms that exist are directly or indirectly affected by TP53 genotype. Furthermore, the aforementioned genes serve as critical effectors commonly involved in the progression of aggressive HNSCCs (4, 16, 26, 27).

Although it has been suggested that inactivation of *TβRI* and *TβRII* expression occurs more frequently than genetic disruption in human HNSCC tissues (16, 28), the mechanisms by which this inhibition takes place in HNSCC have not been well defined. Our findings provide evidence that overexpression of mtTP53 can contribute to decreased expression of *TβRII* and signaling in a subset of HNSCC. Other mechanisms independent of mtTP53 accumulation may contribute to the deficient expression of *TβRII* in other HNSCC, such as those detected in tumor tissue arrays that lack TP53 staining. Here, we also detected reduced *TβRI* or *TβRIII* expression in a subset of HNSCC tumor lines deficient in wtTP53, representing other potential defects that merit further investigation. Down-regulation of *TβRIII* transcript levels has been recently reported in breast and prostate cancers corresponding to more aggressive phenotypes and metastatic dissemination (29).

A connection between TP53 and TGFβ signaling was suggested in thyroid epithelial cells, wherein inactivation of wtTP53 induced a loss of responsiveness to TGFβ1 treatment, accompanied by a partial reduction in TGFβ receptor expression (30). Subsequent studies revealed that ectopic overexpression of mtTP53 proteins induced resistance of squamous carcinoma cells to TGFβ-mediated growth inhibition (31, 32). Here, we provide further evidence for these relationships in distinct subsets of the same tumor type. Although TβRII expression is not solely dependent on nor significantly attenuated in the UM-SCC cell lines deficient in endogenous wtTP53, transfection of wt*TP53* into these cells further enhanced *TβRII* expression and TGFβ downstream signaling (Supplementary Fig. S3) whereas, in UM-SCC lines with mtTP53, RNA interference–mediated TP53 inhibition enhanced *TβRII* and downstream *SMAD7* gene expression, supporting a dominant-negative role of mtTP53 in TβRII signaling (Fig. 4*C*).

Mutant TP53 knockdown and enhanced *TβRII* expression was also followed by attenuation in expression of prototypical NF-κB target genes *IL-6* and *IL-8* (Fig. 4*D*). In a murine squamous cell carcinoma model, we previously observed increased expression of mtTP53 protein accompanied by a corresponding reduction in *TβRII* expression, TGFβ1 responsiveness, and increased activation of NF-κB and target genes as tumors progressed to a metastatic phenotype (33, 34). Furthermore, in a TP53-null cell system, a recent study showed that ectopic mtTP53 can interact with the promoter of *TβRII*, contributing to diminished *TβRII* gene expression (35). Together, these observations suggest that mtTP53 represents one mechanism that can reduce the expression of *TβRII* and TGFβ signaling in a subset of SCCs and other cancers. However, as there are likely genetic and epigenetic mechanisms contributing to reduced or absent TP53 and TβRII expression in HNSCCs, it is not surprising that certain HNSCC specimens summarized in Supplementary Fig. S2, which exhibit reduced TβRII expression, do not also exhibit nuclear TP53 staining.

Although this is the first demonstration of a relationship between TGFβ and NF-κB signaling in HNSCC, these findings are supported by other studies showing cross talk. In human intestinal mononuclear cells, pretreatment with TGFβ1 diminished TNFα-induced activation of p65 (36), whereas in a transgenic mouse model of renal inflammation, TGFβ target gene *Smad7* promoted induction of *IκBα*, inhibiting an NF-κB–driven inflammatory response (37). Here, we show that restored TGFβ signaling partially reduces degradation of IκBα and strongly inhibits IKKβ-dependent serine-536 phosphorylation of p65, localizing such cross talk at or above the level of IKK. In HNSCC, IL-1–IL-1R, TNF-TNFR1, TGFα-EGFR, and PI3K-CK2-IKK signaling pathways have been shown to promote IKK and NF-κB activation (38–40), and these pathways have been reported to include components that may be modulated by TGFβ signaling, such as TGFβ activating kinase (TAK1), TAK1 binding kinase, CK2, and ID1-mediated PI3K-Akt activation (23, 41, 42). Studies are underway to determine if these or other mechanisms enhance cytokine and growth factor activation of NF-κB and inflammation in HNSCC.

The potential relationship between reduced expression of *TβRII* and NF-κB activation was of particular interest because of our previous demonstration that conditional targeting of *TβRII* in murine aerodigestive epithelia results in HNSCCs exhibiting dramatic inflammation (16). Here, we show that both $T\beta RII^{-/-}$ preneoplastic mucosa and HNSCCs show sustained induction of nuclear activation of classic NF-κB1/RelA (p50/p65) and its proinflammatory target genes (Fig. 6*A* and *B*). Most of these factors are significant mediators of the human NF-κB–regulated genetic program, critical in inflammation, angiogenesis, tumorigenesis, and progression of human cancers (2, 26, 43–46). Together, our findings suggest a novel regulatory framework whereby abrogation of TGFβ signaling in epithelial cells enhances NF-κB activation, tumorigenesis, and a deleterious inflammatory signaling circuit through recruitment of infiltrating immune and angiogenic cells. Furthermore, we show that mtTP53 may serve as an upstream repressor of *TβRII* expression, contributing to TGFβ inhibition (Fig. 6*C*). It is notable that alterations in TP53, abrogated TGFβ signaling, and activation of NF-κB are facets of many cancers, raising the possibility that the mechanisms we have uncovered may extend beyond HNSCCs. Indeed, agents such as heat shock protein 90 inhibitors, which simultaneously inhibit both

Published OnlineFirst April 7, 2009; DOI: 10.1158/0008-5472.CAN-08-3704

NF-κB and mtTP53, are under investigation and show promise in numerous preclinical models (47). Thus, NF-κB and mtTP53 may represent concomitant pharmacologic targets in tumors wherein compromised TGFβ signaling leads to enhanced NF-κB activation and resultant malignant progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 9/24/08; revised 2/2/09; accepted 2/16/09; published OnlineFirst 4/7/09.

Grant support: NIDCD intramural projects Z01-DC-00073-01 and 74-01 (C. Van Waes) and grants DE15953 and CA79998 (X-J. Wang).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Lalage Wakefield, Stuart Yuspa, and David Gius for their critical review and comments on the manuscript; Ning Yeh, Liesl Nottingham, and Jay Friedman for technical assistance; Anjali Shukla and Paul Albert for their valuable discussions and advice; and Melissa Stauffer for editorial assistance.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
2. Karin M. Nuclear factor-κB in cancer development and progression. Nature 2006;441:431–6.
3. Perkins ND. Integrating cell-signalling pathways with NF-κB and IKK function. Nat Rev Mol Cell Biol 2007;8:49–62.
4. Van Waes C. Nuclear factor-κB in development, prevention, and therapy of cancer. Clin Cancer Res 2007;13:1076–82.
5. Brown M, Cohen J, Arun P, Chen Z, Van Waes C. NF-κB in carcinoma therapy and prevention. Expert Opin Ther Targets 2008;12:1109–22.
6. Derynck R, Akhurst RJ, Balmain A. TGF-β signaling in tumor suppression and cancer progression. Nat Genet 2001;29:117–29.
7. Markowitz SD, Roberts AB. Tumor suppressor activity of the TGF-β pathway in human cancers. Cytokine Growth Factor Rev 1996;7:93–102.
8. Wang D, Song H, Evans JA, Lang JC, Schuller DE, Weghorst CM. Mutation and down-regulation of the transforming growth factor β type II receptor gene in primary squamous cell carcinomas of the head and neck. Carcinogenesis 1997;18:2285–90.
9. Huntley SP, Davies M, Matthews JB, et al. Attenuated type II TGF-β receptor signalling in human malignant oral keratinocytes induces a less differentiated and more aggressive phenotype that is associated with metastatic dissemination. Int J Cancer 2004;110:170–6.
10. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. N Engl J Med 2001;345:1890–900.
11. Taylor D, Koch WM, Zahurak M, Shah K, Sidransky D, Westra WH. Immunohistochemical detection of p53 protein accumulation in head and neck cancer: correlation with p53 gene alterations. Hum Pathol 1999;30:1221–5.
12. Friedman J, Nottingham L, Duggal P, et al. Deficient TP53 expression, function, and cisplatin sensitivity are restored by quinacrine in head and neck cancer. Clin Cancer Res 2007;13:6568–78.
13. Boyle JO, Hakim J, Koch W, et al. The incidence of p53 mutations increases with progression of head and neck cancer. Cancer Res 1993;53:4477–80.
14. Yan B, Chen G, Saigal K, et al. Systems biology-defined NF-κB regulons, interacting signal pathways and networks are implicated in the malignant phenotype of head and neck cancer cell lines differing in p53 status. Genome Biol 2008;9:R53.
15. Yan B, Yang X, Lee TL, et al. Genome-wide identification of novel expression signatures reveal distinct patterns and prevalence of binding motifs for p53, nuclear factor-κB and other signal transcription factors in head and neck squamous cell carcinoma. Genome Biol 2007;8:R78.
16. Lu SL, Herrington H, Reh D, et al. Loss of transforming growth factor-β type II receptor promotes metastatic head-and-neck squamous cell carcinoma. Genes Dev 2006;20:1331–42.
17. Lee TL, Yeh J, Friedman J, et al. A signal network involving coactivated NF-κB and STAT3 and altered p53 modulates BAX/BCL-XL expression and promotes cell survival of head and neck squamous cell carcinomas. Int J Cancer 2008;122:1987–98.
18. Richtsmeier WJ, Carey TE. Rationalized nomenclature for head and neck carcinomas. Arch Otolaryngol Head Neck Surg 1987;113:1339–40.
19. Yuen HF, Chan YP, Chan KK, et al. Id-1 and Id-2 are markers for metastasis and prognosis in oesophageal squamous cell carcinoma. Br J Cancer 2007;97:1409–15.
20. Lee H, Arsura M, Wu M, Duyao M, Buckler AJ, Sonenshein GE. Role of Rel-related factors in control of c-myc gene transcription in receptor-mediated apoptosis of the murine B cell WEHI 231 line. J Exp Med 1995;181:1169–77.
21. Esrig D, Spruck CH III, Nichols PW, et al. p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. Am J Pathol 1993;143:1389–97.
22. Campbell KJ, Perkins ND. Reprogramming RelA. Cell Cycle 2004;3:869–72.
23. Cavin LG, Romieu-Mourez R, Panta GR, et al. Inhibition of CK2 activity by TGF-β1 promotes IκB-α protein stabilization and apoptosis of immortalized hepatocytes. Hepatology 2003;38:1540–51.
24. Lam LT, Davis RE, Pierce J, et al. Small molecule inhibitors of IkB kinase are selectively toxic for subgroups of diffuse large B-cell lymphomas defined by gene expression profiling. Clin Cancer Res 2003;11:28–40.
25. Chung CH, Parker JS, Ely K, et al. Gene expression profiles identify epithelial-to-mesenchymal transition and activation of nuclear factor-κB signaling as characteristics of a high-risk head and neck squamous cell carcinoma. Cancer Res 2006;66:8210–8.
26. Loukinova E, Dong G, Enamorado-Ayalya I, et al. Growth regulated oncogene-α expression by murine squamous cell carcinoma promotes tumor growth, metastasis, leukocyte infiltration and angiogenesis by a host CXC receptor-2 dependent mechanism. Oncogene 2000;19:3477–86.
27. Allen C, Duffy S, Teknos T, et al. Nuclear factor-κB-related serum factors as longitudinal biomarkers of response and survival in advanced oropharyngeal carcinoma. Clin Cancer Res 2007;13:3182–90.
28. Fukai Y, Fukuchi M, Masuda N, et al. Reduced expression of transforming growth factor-β receptors is an unfavorable prognostic factor in human esophageal squamous cell carcinoma. Int J Cancer 2003;104:161–6.
29. Turley RS, Finger EC, Hempel N, How T, Fields TA, Blobe GC. The type III transforming growth factor-β receptor as a novel tumor suppressor gene in prostate cancer. Cancer Res 2007;67:1090–8.
30. Wyllie FS, Dawson T, Bond JA, et al. Correlated abnormalities of transforming growth factor-β1 response and p53 expression in thyroid epithelial cell transformation. Mol Cell Endocrinol 1991;76:13–21.
31. Reiss M, Munoz-Antonia T, Cowan JM, Wilkins PC, Zhou ZL, Vellucci VF. Resistance of human squamous carcinoma cells to transforming growth factor β1 is a recessive trait. Proc Natl Acad Sci U S A 1993;90:6280–4.
32. Reiss M, Vellucci VF, Zhou ZL. Mutant p53 tumor suppressor gene causes resistance to transforming growth factor β1 in murine keratinocytes. Cancer Res 1993;53:899–904.
33. Loukinova E, Chen Z, Van Waes C, Dong G. Expression of proangiogenic chemokine Gro 1 in low and high metastatic variants of Pam murine squamous cell carcinoma is differentially regulated by IL-1α, EGF and TGF-β1 through NF-κB dependent and independent mechanisms. Int J Cancer 2001;94:637–44.
34. Loercher A, Lee TL, Ricker JL, et al. Nuclear factor-κB is an important modulator of the altered gene expression profile and malignant phenotype in squamous cell carcinoma. Cancer Res 2004;64:6511–23.
35. Kalo E, Buganim Y, Shapira KE, et al. Mutant p53 attenuates the SMAD-dependent transforming growth factor β1 (TGF-β1) signaling pathway by repressing the expression of TGF-β receptor type II. Mol Cell Biol 2007;27:8228–42.
36. Monteleone G, Mann J, Monteleone I, et al. A failure of transforming growth factor-β1 negative regulation maintains sustained NF-κB activation in gut inflammation. J Biol Chem 2004;279:3925–32.
37. Wang W, Huang XR, Li AG, et al. Signaling mechanism of TGF-β1 in prevention of renal inflammation: role of Smad7. J Am Soc Nephrol 2005;16:1371–83.
38. Yu M, Yeh J, Van Waes C. Protein kinase casein kinase 2 mediates inhibitor-κB kinase and aberrant nuclear factor-κB activation by serum factor(s) in head and neck squamous carcinoma cells. Cancer Res 2006;66:6722–31.
39. Duffey DC, Crowl-Bancroft CV, Chen Z, et al. Inhibition of transcription factor nuclear factor-κB by a mutant inhibitor-κBα attenuates resistance of human head and neck squamous cell carcinoma to TNF-α caspase-mediated cell death. Br J Cancer 2000;83:1367–74.
40. Bancroft CC, Chen Z, Yeh J, et al. Effects of pharmacologic antagonists of epidermal growth factor receptor, PI3K and MEK signal kinases on NF-κB and AP-1 activation and IL-8 and VEGF expression in human head and neck squamous cell carcinoma lines. Int J Cancer 2002;99:538–48.
41. Yeung KC, Rose DW, Dhillon AS, et al. Raf kinase inhibitor protein interacts with NF-κB-inducing kinase and TAK1 and inhibits NF-κB activation. Mol Cell Biol 2001;21:7207–17.
42. Li B, Cheung PY, Wang X, et al. Id-1 activation of PI3K/Akt/NFκB signaling pathway and its significance in promoting survival of esophageal cancer cells. Carcinogenesis 2007;28:2313–20.
43. Helbig G, Christopherson KW II, Bhat-Nakshatri P, et al. NF-κB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. J Biol Chem 2003;278:21631–8.
44. Young MR, Lathers DM. Myeloid progenitor cells mediate immune suppression in patients with head and neck cancers. Int J Immunopharmacol 1999;21:241–52.
45. Young MR, Petruzzelli GJ, Kolesiak K, Achille N, Lathers DM, Gabrilovich DI. Human squamous cell carcinomas of the head and neck chemoattract immune suppressive CD34(+) progenitor cells. Hum Immunol 2001;62:332–41.
46. Yang L, Huang J, Ren X, et al. Abrogation of TGFβ signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis. Cancer Cell 2008;13:23–35.
47. Xu W, Neckers L. Targeting the molecular chaperone heat shock protein 90 provides a multifaceted effect on diverse cell signaling pathways of cancer cells. Clin Cancer Res 2007;13:1625–9.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Attenuated Transforming Growth Factor β Signaling Promotes Nuclear Factor-κB Activation in Head and Neck Cancer

Jonah Cohen, Zhong Chen, Shi-Long Lu, et al.

Cancer Res 2009;69:3415-3424. Published OnlineFirst April 7, 2009.