Expression of a Dominant-Negative Mutant Inhibitor-κBα of Nuclear Factor-κB in Human Head and Neck Squamous Cell Carcinoma Inhibits Survival, Proinflammatory Cytokine Expression, and Tumor Growth in Vivo

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ABSTRACT

We demonstrated recently that constitutive expression of proinflammatory cytokines interleukin (IL)-1α, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor in head and neck squamous cell carcinoma is correlated with activation of transcription factor nuclear factor (NF)-κB/Rel A (p50/p65), which binds the promoter region within each of the genes encoding this repertoire of cytokines. NF-κB can be activated after signal-dependent phosphorylation and degradation of inhibitor-κBα and has been reported to promote cell survival and growth. In the present study, we expressed a phosphorylation site mutant of inhibitor-κBα (IκBαM) in head and neck squamous cell carcinoma lines UM-SCC-9, -11B, and -38 to determine the effect of inhibition of NF-κB on cytokine expression, cell survival in vitro, and growth in vivo. After transfection with IκBαM, only a few UM-SCC-9 clones were obtained that stably expressed the mutant IκBα, suggesting that expression of a mutant IκBα may affect survival of the transfected UM-SCC cell lines. After cotransfection of IκBαM with a Lac-Z reporter, we found that the number of surviving β-galactosidase-positive cells in the three cell lines was reduced by 70–90% when compared with controls transfected with vector lacking the insert. In UM-SCC-9 cells that stably expressed IκBαM, inhibition of constitutive and tumor necrosis factor-α induced NF-κB activation, and production of all four cytokines was observed. Although UM-SCC-9 IκBαM-transfected cells proliferated at the same rate as vector-transfected cells in vitro, a significant reduction in growth of tumor xenografts was observed in SCID mice in vivo. The decreased growth of UM-SCC-9 IκBαM-transfected tumor cells accompanied decreased immunohistochemical detection of the activated form of NF-κB in situ. These results provide evidence that NF-κB and IκBα play an important role in survival, constitutive and inducible expression of proinflammatory cytokines, and growth of squamous cell carcinoma. NF-κB could serve as a potential target for therapeutic intervention against cytokine and other immediate-early gene responses that contribute to the survival, growth, and pathogenesis of these cancers.

INTRODUCTION

Autonomous production of proinflammatory cytokines has been detected in many different types of human and murine cancers, suggesting that their expression or the mechanism underlying their activation may provide a selective survival or growth advantage to cancer cells. We and others have observed previously that human head and neck SCCs3 autonomously produce proinflammatory cytokines IL-1α, IL-6, IL-8, and GM-CSF in vitro and in vivo (1–5), and these cytokines have also been detected in tumor specimens and cell lines from patients with SCCs arising within the lungs, cervix, and skin (6–8). We reported that transformed murine keratinocytes that express homologues of IL-1α, IL-6, IL-8, and GM-CSF at increased levels exhibit more aggressive growth and metastasis in vivo (9–11). IL-1α has been shown to promote autocrine proliferation of transformed human keratinocytes and cervical SCC cell lines (12). Elevated expression of IL-8 by human lung SCC has been shown to promote angiogenesis and tumor growth in experimental models in vivo (8). Expression of GM-CSF has been associated with homing of CD34+ granulocyte precursors that promote increased metastatic potential of human and murine SCC of the upper aerodigestive tract (13). Both IL-1 and IL-6 have been implicated in the increase in catabolism often observed in patients with these cancers (14). The molecular mechanisms that contribute to activation of this repertoire of cytokines in SCC have not been defined.

The expression of proinflammatory cytokines can be induced in response to a variety of stimuli through activation of transcription factor NF-κB/Rel A (p50/p65), which is a heterodimer formed by members of the Rel family of proteins (15). We noted that the 5′ promoter region of the IL-1α, IL-6, IL-8, and GM-CSF genes expressed by HNSCC contain binding sites for the NF-κB/Rel family of transcription factors (16–19), and we showed recently that the autonomous expression of proinflammatory cytokines in HNSCC is correlated with constitutive activation of NF-κB/Rel A p50/p65 (20). Constitutive activation of NF-κB/Rel family members has also been demonstrated recently in other laboratories in several different neoplasms, including lymphoma, melanoma, and carcinoma of the colon and breast (21–26). Members of the Rel family were originally identified as viral and cellular oncogenes (27, 28). At present, the mechanisms by which they can promote tumor growth have not been fully elucidated.

Activation of NF-κB has been shown to involve signal-induced phosphorylation and degradation of cytoplasmic IκB proteins, which release NF-κB for nuclear translocation (29–32), and for binding to the promoter sites of target genes. Studies in several laboratories have shown that mutations in the serine phosphorylation sites at S32 and/or S36 of IκBα can inhibit the signal-dependent activation of NF-κB to a variety of stimuli (29–32). Such phosphorylation mutants can therefore exert a dominant-negative effect, preventing the activation of NF-κB-dependent genes. By expression of a dominant-negative IκBα mutant, NF-κB has been shown to be important in activation of genes necessary for survival and protection of cells from injury by a variety of cytotoxic stimuli, including cytokine TNF-α, chemotherapy, and radiation therapy (33–35). In the present study, we provide evidence that the expression of a phosphorylation mutant of IκBα (IκBαM) can reduce survival, constitutive and inducible activation of NF-κB, expression of proinflammatory cytokines, and tumor growth in vivo.

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MATERIALS AND METHODS

Cell Culture and Transfection of UM-SCC Cells with IeBoM and Control Vector. HNSCC cell lines UM-SCC-9, -11B, and -38 were obtained from the well-characterized UM-SCC series maintained by Dr. T. E. Carey (University of Michigan, Ann Arbor, MI; Ref. 36). Human SCC cell line UM-SCC-9 was derived from a patient with a stage T2 SCC of the pharynx; UM-SCC11B was obtained from a patient, 65 years of age, with a T2N2a SCC of the hypopharynx; and UM-SCC-38 was cultured from a patient, 60 years of age, with a T2N2 SCC of the oropharynx. The conditions for culture, karyotype, and epithelial marker analysis of UM-SCC have been described previously (36–38). The cell lines were tested and found to be free of Mycoplasma using a Gibco MycoTest Kit (Life Technologies, Inc., Gaithersburg, MD).

The cDNA plasmid pCMX IeBoM contains a mutation at S36 of the NH2-terminus and a COOH-terminal PEST sequence mutation and was a generous gift from Dr. Inder M. Verma (Salk Institute, La Jolla, CA; Ref. 35). The plasmid containing the neomycin (neo) resistance gene used is described by Brown et al. (29). UM-SCC-9, -11B, and -38 cells were plated in 6-well plates and transfected with 0.2 μg of plasmid containing neomycin resistance gene, 2 μg of plasmid DNA of either pCMVlacZ, pCMXlBovM, or pCMX vector alone, and 20 μg of LipofectAMINE (Life Technologies, Inc.) in serum-free medium. Expression of Lac-Z by X-gal staining and IeBoM by Western blot was confirmed 72 h after transfection, and selection was carried out in medium containing G418 sulfate (Life Technologies, Inc.).

Immunoblot Analysis of Murine and Endogenous Human IeBo Expression. Expression of murine and human IeBo in transfected UM-SCC-9 cells cultured in media or media containing 10,000 units of human TNFα (Knoll Pharmaceuticals, Parsippany, NJ) was compared by Western immunoblot analysis. Cell protein was extracted from cells growing to 80% confluence of extracts was determined using the BCA protein assay (Pierce, Rockford, IL), and proteins were separated by electrophoresis on 10% denaturing SDS-polyacrylamide gels (39) and transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA). Purified human IeBo (1–317; Santa Cruz Biotechnology), along with 20 μl of MultiMark pigmented markers (Novex, San Diego, CA) were used as standards. Whole-cell extracts from the murine Pan L-1 SCC cell line were used as a positive control for murine IeBo. Chemiluminescent detection of horseradish peroxidase was obtained using the SuperSignal Substrate method (Pierce, Rockford, IL), followed by exposure on X-Omat AR film (Kodak, Rochester, NY).

Electromobility Shift Assay. EMSA were performed using standard methods (40, and nuclear extracts were obtained from UM-SCC-9 in log phase growth using the methods of Dignam et al. (43) and Lee et al. (44). Double-stranded DNA oligonucleotide probes for NF-κB and OCT-1 were purchased commercially (Promega, Madison, WI and Santa Cruz Biotechnology). The oligonucleotide sequences used were: NF-κB, 5′-AGTTAGGGGCATTTC-CCAGC-3′; and OCT-1, 5′-TGTTCAATGCGAATCTAGA-3′. The mutant oligonucleotide double-strand probe used for a specificity control for NF-κB was 5′-AGTTAGGGGCATTTCAGAGC-3′. EMSA reactions included 5 μg of nuclear extract protein incubated for 25 min at 20°C in buffer containing 20 nm HEPES (pH 7.9), 4.6 mM MgCl2, 63 mM KC1, 11% glycerol, and 1 mM DTT, 1 μg poly(deoxynucleoic-deoxycytidylic acid) (Pharmacia, Piscataway, NJ), and 60,000 cpm of 32P-labeled oligonucleotide probe. For expression of mutation probes, nuclear extracts were preincubated with a 100-fold excess of each probe before the addition of labeled probe. Binding complexes were resolved on 5%, 0.25 × TBE polyacrylamide gel, and imaged with a Packard Instant Imager (Packard Technologies, Downers Grove, IL).

Quantification of Cytokine Secretion by ELISA. UM-SCC cells (3 × 104) were cultured in 12 ml of fresh medium in 75-cm2 flasks, and culture supernatants were collected after 5 days. Standardized ELISA assay kits were used to quantify IL-1α, IL-6, IL-8, and GM-CSF (R&D Systems, Minneapolis, MN). The absorbance of the reaction product was quantitated with a multichannel plate spectrophotometer at 450 nm (Biotek 311; Biotek Systems, Winsko, VT). All samples were assayed in triplicate. Results were standardized for amount of cytokine secreted as pg/106 cells/5 days ± SD.

MTT Assay. The cell proliferation rate of UM-SCC-9 and transfected cells in vitro were compared by using the MTT reaction assay (Boehringer Mannheim, Mannheim, Germany). Replicate cultures of each of the cell lines were plated in 96-well, flat-bottomed microplates at an initial concentration of 5 × 103 cells in 100-μl medium/well. On days 1–5, 10 μl of MTT labeling agent were added, and cells were incubated for an additional 4 h. One hundred μl of solubilization solution were added to lyse cells overnight. The absorbance of the colorimetric assay was determined at a wavelength of 570 nm using a multichannel plate spectrophotometer (Biotek Instruments, Winooski, VT).

Growth of UM-SCC-9 Parental and Transfected Cell Xenografts in SCID Mice. UM-SCC-9 parental and transfected cell lines were inoculated in SCID mice at 4 weeks of age. UM-SCC-9, vector transfectants C11 and C13, and the IeBoM transfectants I11 and I13 cells in logarithmic growth phase were harvested and injected s.c. into SCID mice, at a dose of 5 × 104 or 1 × 106 cells. Three animals were injected for each cell line at each dose. Tumors were measured weekly. The data are presented as mean ± SE. Animals were sacrificed if the tumor exceeded 10% of the animal’s body weight or exceeded 2.5 cm3 in volume.

Immunohistochemical Staining of Activated NF-κB in SCC Tissue and Cultured Cells. Immunohistochemical analysis of the activated form of NF-κB p65 in tissue sections from UM-SCC-9 and transfectant tumor xenografts was performed using a modification of the protocol of Kaltshmidt et al. (46). Frozen tissue sections (10–12 μm) from tumor xenograft specimens were fixed and permeabilized in cold 1:1 methanol:acetone for 10 min, and endogenous peroxidase was quenched by incubation with 3% H2O2 in methanol for 30 min at room temperature. Sections were incubated with 10% horse serum in PBS (Vector Laboratories, Inc., Burlingame, CA) for 30 min, followed by incubation with 2 μg/ml mouse monoclonal anti-human NF-κB p65 diluted in a 1% BSA in PBS (Boehringer Mannheim, Mannheim, Germany). One μg/ml of mouse anti-human pan cytokeratin K5, K6, K8, and K18 (IgG1; Novocasta Lab, Newcastle upon Tyne, United Kingdom) or isotype antibody

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(mouse IgG1; Dako, Carpenteria, CA) diluted in 1% BSA in PBS were used as controls. The samples were incubated with horse anti-mouse antibody (1:200 in PBS containing 1.5% horse serum; Vector Lab), followed by a 30-min incubation with biotin/avidin horseradish peroxidase conjugates (Vectastain Elite ABC kit; Vector Lab), and an 8-min incubation with the chromogen diaminobenzidine tetrahydrochloride (Vector Lab). Stained sections were dehydrated and mounted with Permount (Fisher Scientific, Fair Lawn, New Jersey).

RESULTS

Expression of a Phosphorylation Mutant of IκB in UM-SCC Inhibits Survival of Transfected Cells. We transfected a phosphorylation site-deficient mutant of murine IκBα (IκBαM, Ref. 35) or a control vector lacking an insert into head and neck squamous cell carcinoma lines UM-SCC-9, -11B, and -38 to determine whether we could obtain a UM-SCC line that stably expressed a dominant-negative mutant of IκBα. After selection in G418 and cloning, we obtained UM-SCC-9 cells expressing IκBαM, but we did not detect expression of IκBαM in over 30 UM-SCC-11B or 38 clones plated from two independent transfections, despite similar transfection efficiencies and evidence of transient expression of mutant IκBα in transfected cultures by Western blot (data not shown). Because NF-κB/Rel transcription factors have been reported to regulate genes involved in cellular survival (21–25, 33–35), we examined the possibility that expression of an IκBα mutant might reduce survival of transfected UM-SCC cells. UM-SCC-9, -11B, and -38 cell lines were cotransfected with a Lac-Z reporter in the presence of a 4-fold excess of vector containing a human IκBα phosphorylation mutant (39) or control vector, and a comparison of β-gal-positive cells in cultures was made 72 h after transfection. β-gal-positive blue staining cells were detected in all three UM-SCC cell lines in the presence of control vector, at a transfection efficiency of 20–30%. However, Fig. 1 shows that after cotransfection with the IκBα mutant, the number of β-gal-positive cells was significantly reduced in the three cell lines by 70–90%. These data provide evidence that the difficulty obtaining stable IκBαM transfectants was due to decreased survival of UM-SCC cells transfected with an inhibitor of NF-κB.

Expression of IκBαM in UM-SCC-9 Cells Inhibits Constitutive and TNF-α-inducible Activation of NF-κB. Fig. 2 shows a Western blot analysis demonstrating expression of IκBαM in UM-SCC-9, vector control transfectants UM-SCC-9 C11 and C13, IκBαM transfec-

![Fig. 1. Survival of β-gal-positive UM-SCC cells cotransfected with mutant IκBα. UM-SCC-9, -11B, and -38 cells were cotransfected with Lac-Z in the presence of a 4-fold excess of human IκBα mutant or control vector, and cells were stained using X-gal 72 h after transfection. The percentage of β-gal-positive UM-SCC cells surviving cotransfection with mutant IκBα was reduced to 10–30% of that obtained with vector control lacking the insert. The transfection efficiency of UM-SCC cell lines ranged between 20 and 30%. Bars, SD.](image)

![Fig. 2. Western blot of murine IκBα phosphorylation mutant (IκBαM) and endogenous human IκBα expression in UM-SCC-9. UM-SCC-9 cells were transfected with a murine IκBαM or vector control and selected in G418 as described in “Materials and Methods.” Anti-mouse and human IκBα antibodies were used to detect IκBα species in UM-SCC-9, vector-transfected C11, C13, and IκBαM-transfected I11 and I13 cell line extracts, as described in “Materials and Methods.” Murine Pam LU-1 SCC cytoplasmic protein was used as a control for the faster migrating murine IκBα (Lane 1). UM-SCC-9 and the transfected cells were stimulated with TNF-α (+) (10,000 units/ml for 1 h) prior to extraction to demonstrate degradation of endogenous IκBα and resistance of the IκBα phosphorylation mutant protein to degradation.](image)

![Fig. 3. Inhibition of NF-κB binding activity in UM-SCC-9 IκBαM transfectants I11 and I13 by EMSA. A, the NF-κB binding activity in UM-SCC-9 (Lanes 1 and 2), vector control transfectants C11 and C13 (Lanes 3–6), and IκBαM transfectants I11 and I13 (Lanes 7–10) was compared by EMSA as described in “Materials and Methods.” Cells were incubated in the absence or presence of 10,000 units/ml of TNF-α for 1 h before isolation of nuclear extracts. Binding specificity was confirmed by competition with unlabeled probe (Lanes 11–14). Comp., competitor; NS, nonspecific. B, probe containing Oct-1 motif was used in EMSA as a control for quality and quantity of cell extracts. The specificity of Oct-1 binding activity was demonstrated by competition with unlabeled Oct-1 probe (Lane 11) but not AP-1 probe (Lane 12).](image)
observed in the UM-SCC-9 cell line and vector control transfectants C11 and C13 and is further induced by addition of TNF-α (Fig. 3A, Lanes 1–6). The level of constitutive and TNF-α-induced NF-κB activation is significantly reduced in UM-SCC-9 IxBoM transfectants I11 and I13 (Fig. 3A, Lanes 7–10). The NF-κB DNA binding activity observed in UM-SCC-9, C11, and C13 cells could be specifically inhibited by competition with unlabeled NF-κB oligonucleotide (Fig. 3A, Lanes 11–14). Fig. 3B shows that expression of IxBoM did not inhibit DNA binding activity of the unrelated transcription factor Oct-1 (Lanes 1–10), which could be blocked by unlabeled Oct-1 but not AP-1 probe competition (Lanes 11 and 12).

To determine whether the reduction in NF-κB DNA binding activity observed corresponded to a reduction in the functional activation of NF-κB, we examined NF-κB reporter activity in UM-SCC-9 vector and IxBoM transfected clones. Fig. 4 confirms that functional activation of NF-κB as determined by luciferase reporter assay is reduced ~10-fold in the IxBoM transfectant I11 and I13 cells relative to UM-SCC-9 vector control transfectants C11 and C13. The reporter activity was specific for NF-κB, because UM-SCC-9 cells transfected with a reporter containing a wild-type NF-κB sequence, but not a mutant sequence, exhibited luciferase signal (data not shown).

Expression of Proinflammatory Cytokines II-1α, IL-6, IL-8, and GM-CSF Is Inhibited in UM-SCC-9 Cells Transfected with IxBoM. We determined if expression of the IxBoM in UM-SCC-9 resulted in reduced expression of II-1α, IL-6, IL-8, and GM-CSF cytokine genes that contain NF-κB sites in their 5′ promoter regions (16–19). The quantity of each of these proinflammatory cytokines secreted by vector control and IxBoM transfectants as determined by ELISA is shown in Fig. 5. Secretion of all four of the proinflammatory cytokines was significantly reduced in IxBoM transfectants I11 and I13 relative to two vector control transfectants C11 and C13. Thus, expression of IxBoM in UM-SCC-9 inhibited activation of NF-κB and production of proinflammatory cytokines II-1α, IL-6, IL-8 and GM-CSF, indicating that NF-κB promotes constitutive expression of these proinflammatory cytokines by UM-SCC-9.

Effect of Expression of IxBoM on Growth of UM-SCC-9 Cells in Vitro and in Vivo. We compared the growth of three UM-SCC-9 IxBoM transfectants I11 and I13 and control vector transfectants C11 and C13 in vitro and as xenografts in SCID mice in vivo. The growth of the cell lines in vitro was compared in a 5-day MTT assay as described in “Materials and Methods,” and the results are shown in Fig. 6A. There was no significant difference in growth between IxBoM transfectants I11 and I13 and control vector transfectants C11 and C13 in vitro. However, when SCID mice were inoculated s.c. with 1 × 10⁷ cells of IxBoM transfectants I11 and I13 or control vector transfectants C11 and C13, the growth of the IxBoM transfectants was significantly reduced when compared with vector-transfected cell lines as determined by luciferase reporter assay (Fig. 4A) and as xenografts in SCID mice (Fig. 6B). We observed complete tumor regression of IxBoM transfectant I11 cell line when compared with vector-transfected cell lines. We observed complete tumor regression of IxBoM transfectant I13 at a tumor cell dose of 1 × 10⁷ cells, whereas the control line C13 grew progressively (Fig. 6B). Similar but smaller differences were obtained in both experiments in animals inoculated with a lower dose of 5 × 10⁶ cells (data not shown). Thus, expression of IxBoM in UM-SCC-9 cells inhibited tumor growth in vivo but did not inhibit tumor cell growth in vitro.

Inhibition of Activation of NF-κB in UM-SCC-9 IxBoM Transflectant Tumor Xenografts. To establish whether there is a relative difference in constitutive activation of NF-κB in UM-SCC-9 and IxBoM transfected tumor xenografts, we compared NF-κB activation in UM-SCC-9, C11, and I11 tumor specimens in situ by immunohistochemistry, using a monoclonal antibody specific for the nuclear localization sequence of the activated form of human NF-κB p65 (46). As shown in Fig. 7, staining for the activated form of NF-κB protein was observed in UM-SCC-9 (Fig. 7A) and vector transfectant C11 cells (Fig. 7B) but was significantly decreased in IxBoM-transfected I11 cells (Fig. 7C). All three tumor specimens stained positively with monoclonal antibody against pan cytokeratin (Fig. 7, D–F) but not with isotype-matched control antibody (Fig. 7, G–I). Thus, expression of IxBoM inhibited constitutive activation of NF-κB in vivo as well as in vitro.

DISCUSSION

In this study, we encountered difficulty in obtaining human HNSCC lines that stably express a dominant-negative IκBα phosphorylation site mutant, suggesting that expression of the mutant IκBα could affect survival of transfected UM-SCC cells. When the three UM-SCC cell lines were cotransfected with a Lac-Z reporter in the presence of a 4-fold excess of vector containing a human IκBα phosphorylation mutant or control vector, it was found that transfection of mutant IκBα markedly reduced the survival of
β-gal-positive cells by 70–90% in cultures within 72 h (Fig. 1). These results are consistent with recent studies that show that inhibition of activation or deletion of NF-κB/RelA inhibits survival of a variety of normal and neoplastic cells of different tissue origin (21–24, 33–35). The promotion of cell survival by NF-κB has recently been attributed to several mediators. NF-κB has been reported to induce TRAF1, TRAF2, c-IAP1, and c-IAP2, resulting in suppression of caspase-8 activation, thereby inhibiting apoptosis (47). A novel apoptosis inhibitor IEX-1 L has also been reported to be involved in NF-κB-mediated cell survival (48). Recently, the prosurvival Bcl-2 homologue Bfl-1/A1 has been shown to be a transcriptional target of NF-κB, which can block TNF-α-induced apoptosis (49). Although the pathways by which NF-κB promotes survival in HNSCCs remain to be defined, our results and those of others suggest that NF-κB may be an important target for therapeutic intervention against immediate-early responses that promote survival and resistance of cancer.

When we examined UM-SCC-9 cells that survive expression of IκBαM, we found that IκBαM suppressed constitutive and TNF-α-induced activation of NF-κB, production of proinflammatory cytokines, as well as growth in vitro. We confirmed that expression of IκBαM inhibited constitutive activation of NF-κB in UM-SCC-9 tumors in vivo. Whereas expression of IκBαM inhibited growth of UM-SCC-9 in vivo, there was no significant difference in growth between the IκBαM and vector control transfectants in vitro. These results provide evidence that cytokines or other genes activated by NF-κB in UM-SCC-9 promote interactions with the host that contribute to malignant growth in vivo. These data are consistent with observations made in our laboratory in an independent murine model of SCC, in which expression of the murine homologues of these cytokines was found to be associated with more aggressive growth in vivo but not in vitro (9–11). We found that low cytokine-producing clones of the murine SCC line Pam 212 grew slowly or regressed, whereas those that expressed the cytokine homologues IL-1α, IL-6, KC, and GM-CSF grew more aggressively in vivo. However, no significant differences in proliferative rate were observed in vitro.

Among the NF-κB-induced cytokines expressed by human and murine SCCs, there is evidence that the C-X-C family of chemokines to which human IL-8 and murine KC belong can induce host angiogenesis responses and thereby provide a differential growth advantage in vivo. Smith et al. (8) have shown that expression of IL-8 by human lung SCC can promote an increase in angiogenesis and growth of tumor xenografts in SCID mice. In murine SCC, we have obtained preliminary evidence that overexpression of murine cytokine KC in the low KC expressing Pam 212 SCC line promotes an increase in angiogenesis.
growth in vivo, whereas it has no effect on the rate of growth in vitro.4 The relative contribution of additional autocrine or paracrine mechanisms by which proinflammatory cytokines IL-1α, IL-6, GM-CSF, and others expressed by SCC may be found to promote growth remains to be determined. NF-κB and IκBα could serve as potential targets for therapy to inhibit expression of cytokine and other NF-κB-dependent genes that are ultimately found to contribute to the growth of SCCs in vivo.

Although NF-κB/Rel A and IκBα appear to be important in the regulation of expression of cytokines and other genes that promote growth in UM-SCC cell lines, it is possible that activation of other pathways contribute to expression of these cytokines and other NF-κB-dependent genes. The inhibition of activation of cytokine expression and growth by IκBα in UM-SCC-9 that we observed was less than complete. The promoter region of the cytokine genes expressed by SCC contains AP-1 and NF-IL6 as well as NF-κB binding sequences (16–19), and coactivation of the promoter region of proinflammatory cytokine genes by NF-κB, AP-1, and NF-IL6 has been reported to have synergistic effects. Consistent with this possibility, both NF-κB and AP-1 have been reported to be activated and have transforming activity in experimental murine tumors derived from squamous epithelia (50–52). Studies are under way to define the activation of protective pathways, or inactivation of molecules that induce apoptosis, determine the requirement for activation of NF-κB. In addition to cytokine-induced activation, NF-κB and alternative pathways may be activated by alterations in epidermal growth factor receptor expression, Ras activation, or through cell damage and changes in oxidation-reduction state (26, 28, 53–55). Baldwin and colleagues have shown previously that Bcr-Abl and Ras promote activation of NF-κB in leukemia, and an IκBα super-repressor could inhibit Bcr-Abl-mediated tumors in nude mice (56). Thus, F-κB could serve as a common pathway for oncogenesis involving altered expression or function of a variety of components of these pathways.

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10. Dong, G., Loukinova, E., Smith, C. W., Chen, Z., and Van Waes, C. Genes differentially expressed with malignant transformation of the human head and neck squamous cell carcinoma cell line UM-SCC-9 that we observed was less than complete. The promoter region of the cytokine genes expressed by SCC contains AP-1 and NF-IL6 as well as NF-κB binding sequences (16–19), and coactivation of the promoter region of proinflammatory cytokine genes by NF-κB, AP-1, and NF-IL6 has been reported to have synergistic effects. Consistent with this possibility, both NF-κB and AP-1 have been reported to be activated and have transforming activity in experimental murine tumors derived from squamous epithelia (50–52). Studies are under way to define the activation, composition, and contribution of other immediate-early transcription factors that may contribute to the constitutive expression of these cytokine genes in head and neck SCC. These studies should in turn facilitate the identification of additional genes that may promote growth and malignant behavior of SCC.

The mechanism of constitutive activation of NF-κB/Rel A and IκBα in SCC and in other neoplasms remains to be determined. It will be interesting to determine whether changes in expression or activation of other signal transduction molecules upstream that lead to activation of protective pathways, or inactivation of molecules that induce apoptosis, determine the requirement for activation of NF-κB. In addition to cytokine-induced activation, NF-κB and alternative pathways may be activated by alterations in epidermal growth factor receptor expression, Ras activation, or through cell damage and changes in oxidation-reduction state (26, 28, 53–55). Baldwin and colleagues have shown previously that Bcr-Abl and Ras promote activation of NF-κB in leukemia, and an IκBα super-repressor could inhibit Bcr-Abl-mediated tumors in nude mice (56). Thus, F-κB could serve as a common pathway for oncogenesis involving altered expression or function of a variety of components of these pathways.

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