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Regulation of Adhesion and Growth of Fibrosarcoma Cells by NF-κB RelA Involves Transforming Growth Factor β

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The NF-κB transcription factor is a pleiotropic activator that participates in the induction of a wide variety of cellular genes. Antisense oligomer inhibition of the RelA subunit of NF-κB results in a block of cellular adhesion and inhibition of tumor cell growth. Investigation of the molecular basis for these effects showed that in vitro inhibition of the growth of transformed fibroblasts by *relA* antisense oligonucleotides can be reversed by the parental-cell-conditioned medium. Cytokine profile analysis of these cells treated with *relA* antisense oligonucleotides revealed inhibition of transforming growth factor β1 (TGF-β1) and granulocyte-macrophage colony-stimulating factor mRNA expression. Exogenous addition of purified TGF-β1 to the transformed fibroblasts reversed the inhibitory effects of *relA* antisense oligomers on soft agar colony formation and cell adhesion to the substratum. Direct inhibition of TGF-β1 expression by antisense phosphorothioates to TGF-β1 mimicked the in vitro effects of blocking cell adhesion that are elicited by antisense *relA* oligomers. These results may explain the in vitro effects of *relA* antisense oligomers on fibrosarcoma cell growth and adhesion.

NF-κB is an inducible transcription factor that was originally identified as a heterodimeric complex consisting of a 50-kDa subunit (originally called p50 and now designated NFKB1) and a 65-kDa subunit (originally called p65 and now designated RelA) (2, 6, 18). The individual subunits of the NF-κB complex can regulate a distinct set of genes by exhibiting distinct binding preferences (17). Transfection studies demonstrate that the RelA subunit can function as a homodimeric transcriptional activator (3, 28). In our efforts to identify the molecular basis for the inhibition of tumor cell adhesion and growth by *relA* antisense oligomers (9, 24, 30), we reasoned that perhaps a soluble mediator (cytokine) is affected by inhibition of RelA NF-κB activity; several cytokines and their receptors have been shown to have NF-κB binding sites in their promoter regions (7). We report here that transforming growth factor β1 (TGF-β1) mRNA expression is downregulated in fibroblast-derived tumor cells treated with *relA* antisense oligomers. The addition of conditioned medium from these tumor cells or purified TGF-β1 abrogates both the growth inhibitory and the adhesion blocking effects of *relA* antisense oligomers. Directly inhibiting TGF-β1 expression by antisense oligomer techniques mimics the adhesion-blocking effect of *relA* antisense oligomers. Our results identify NF-κB as a regulator of TGF-β function, albeit indirectly, in cellular adhesion and growth of fibroblast-derived tumor cells.

MATERIALS AND METHODS

Antisense oligonucleotides. The phosphorothioate oligomers used in the study (Table 1) were synthesized as previously described (9, 24). Each experiment was performed with three independent preparations of oligonucleotides.

Cell lines and growth assays. The K-BALB and B-16 cell lines were obtained from the American Type Culture Collection, Rockville, Md. Stable K-BALB cell lines expressing dexamethasone-inducible antisense RNA to *relA* have been described (9). For integrin-mediated adhesion assays, cells were trypsinized, mixed with oligomers (20 μM), plated onto tissue culture dishes, and incubated for 24 to 72 h. Soft-agar colony formation was measured by pretreating the adherent cells with oligomers for 48 to 72 h prior to seeding in 0.33% soft agar (10^3 cells per well) in quadruplicate wells in the presence of 20 μM oligomers. K-BALB-cell-conditioned medium was collected from confluent cultures by washing with phosphate-buffered saline (PBS) and incubating with serum-less Dulbecco modified Eagle medium for 48 h as described previously (1). The serum-free conditioned medium (SFCM) was filtered and kept frozen, and various amounts of the medium were used without acid treatment (1). The growth factors were obtained from R & D Systems, Minneapolis, Minn.

PCR analysis. A semiquantitative reverse transcriptase PCR (RT-PCR) was performed as previously described (24, 29), and the PCR products were analyzed at various cycles. The *relA* and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers have been described (24). The TGF-β primers included 5′ GCC CTG GAC ACC AAC TAT TGC 3′ (sense) and 5′ GGA GCG CAC GAT CAT GTT GGA 3′ (antisense) and detected an amplicon of 316 bp in murine and human cells. The other murine cytokine primers have been described elsewhere (29). The authenticity of the amplified products was confirmed by hybridization to respective cDNAs.

Nuclear extracts and electrophoretic mobility shift assays. Nuclear extracts were prepared as described previously (9, 24). An oligonucleotide containing the sequence 5′ GTA GGG GAC TTT CC GAG CTC GAG ATC CTA TG 3′ (with NF-κB-binding sites underlined) was labelled with [^{32}P]dCTP as previously described (17) and used as an NF-κB probe. Nuclear extracts (10 μg) and a ^{32}P-labelled probe (1 ng; 50,000 cpm) were used in the binding reactions. Complexes were resolved on a 4% nondenaturing polyacrylamide gel; this process was followed by autoradiography. Supershifts were performed with the p65 (RelA) or p50 (NFKB1) antibody from Santa Cruz Biotechnology, Inc., Santa Cruz, Calif. The antibody (1 or 2 μl) was added to the binding reaction mixture 10 min after the addition of the NF-κB probe. The reaction

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TABLE 1. Phosphorothioate oligomers used in this study

Gene[a]	Species[b]	5′ to 3′ sequence[c]	Reference
relA S	M	ACC ATG GAC GAT CTG TTT CCC CTG	24
relA AS	M	GAG GGG AAA CAG ATC GTC CAT GGT	24
TGF-β1 S	M and H	TCC CCC ATG CCG CCC TCC GGG	8
TGF-β1 AS	M and H	CCC GGA GGG CGG CAT GGG GGA	8
TGF-β1 AS-SC	M and H	GGG GAG CGA GTG AGC GCG CGG	8

[a] S, sense; AS, antisense; AS-SC, scrambled antisense.
[b] M, mouse; H, human.
[c] The TGF-β1 antisense sequence differed from the sequences of TGF-β2 and TGF-β3 at the region chosen to design the antisense oligomers (5′ end encompassing the AUG initiation codon).

mixture was incubated for an additional 20 min, and the complexes were resolved as described above.

RESULTS

Tumor cell growth and adhesion require functional RelA NF-κB activity. A k-*ras*-transformed murine fibroblast cell line, K-BALB, was used to test the growth effects of *relA* antisense oligomers (Fig. 1). Plating of K-BALB cells in the presence of *relA* antisense oligomers (20 μM) resulted in a pronounced block of cell adhesion to the substratum (Fig. 1A). These effects on adhesion were not observed with several control antisense oligomers, including NFKB1, *junD*, GAPDH, IκB, c-*rel*, and human *relA* (data not shown). Since adhesion plays an important role in diverse aspects of cell growth and differentiation, we next investigated the in vitro growth of K-BALB cells by a soft-agar colony-forming assay (Fig. 1B). The K-BALB cells failed to form colonies when treated with *relA* antisense oligomers, whereas colony formation was not inhibited by the antisense oligomers to NFKB1, GAPDH, or human RelA (not shown). We used these two assays to address the molecular mechanism involved in the RelA-mediated inhibition of cell growth.

Reversal of the effects of *relA* antisense oligomers on cell growth by conditioned medium. To understand the basis of the effects of *relA* antisense oligomers on tumor cell growth, we collected SFCM from the K-BALB cells and tested its effect on the growth of K-BALB cells in soft agar in the presence of *relA* sense or antisense oligomers (Fig. 2). The block of colony formation by the *relA* antisense oligomers was completely reversed in the presence of 40% SFCM. The reversal was dose dependent (0 to 40% [vol/vol]) and was not seen with heat-inactivated SFCM (not shown). These results suggested that treatment of K-BALB cells with *relA* antisense oligomers interferes with one or more autocrine growth factors.

***relA* antisense oligomers inhibit expression of specific cytokines in tumor cells.** Since several cytokines, growth factors, and growth factor receptors have putative NF-κB binding sites at their promoter regions, we next investigated the cytokine profile of K-BALB cells treated with *relA* antisense oligomers. Among the cytokines tested (interleukin-6 [IL-6], *kit* ligand (KL), granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor [GM-CSF], macrophage–colony-stimulating factor, alpha interferon, beta interferon, tumor necrosis factor alpha, tumor necrosis factor beta, TGF-α, and TGF-β1), significant inhibition of TGF-β1 and GM-CSF expression was seen by a semiquantitative RT-PCR in K-BALB cells treated with *relA* antisense oligomers (Fig. 3A). The inhibition of TGF-β1 mRNA was also seen in K-BALB cells expressing dexamethasone-inducible antisense RNA to *relA* (9), which correlated with dexamethasone-dependent inhibition of *relA* mRNA expression (Fig. 3B). A dexamethasone-dependent inhibition of GM-CSF mRNA expression was also seen in these transfectants (data not shown). Inhibition of TGF-β1 and GM-CSF mRNAs was also seen in B-16

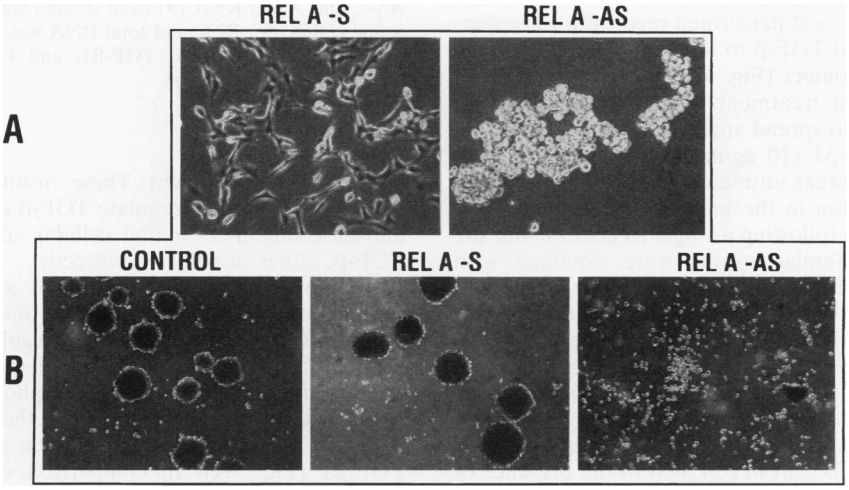

FIG. 1. Inhibition of fibrosarcoma cell adhesion and growth by *relA* antisense oligomers. (A) K-BALB cells were trypsinized and plated in the presence of 20 μM *relA* sense (-S) or *relA* antisense (-AS) oligomers in six-well dishes and photographed after 48 h in culture. Magnification, ×37. (B) K-BALB cells were treated for 72 h with PBS (control) or 20 μM *relA* sense or antisense oligomers, plated onto soft agar in the presence of oligomers, and photographed at day 10. Magnification, ×37.

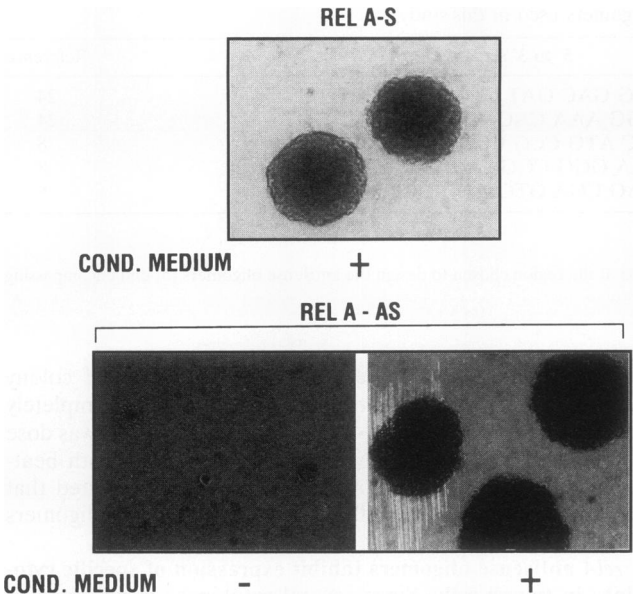

FIG. 2. SFCM from K-BALB cells reverses growth inhibition by *relA* antisense oligomers. K-BALB cells were treated with 20 μM *relA* sense (-S) or antisense (-AS) oligomers for 72 h, plated onto soft agar in the presence of oligomers and various concentrations of SFCM (0 to 40% [vol/vol]), and photographed at day 10. Magnification, ×37. Results obtained with 40% SFCM are shown.

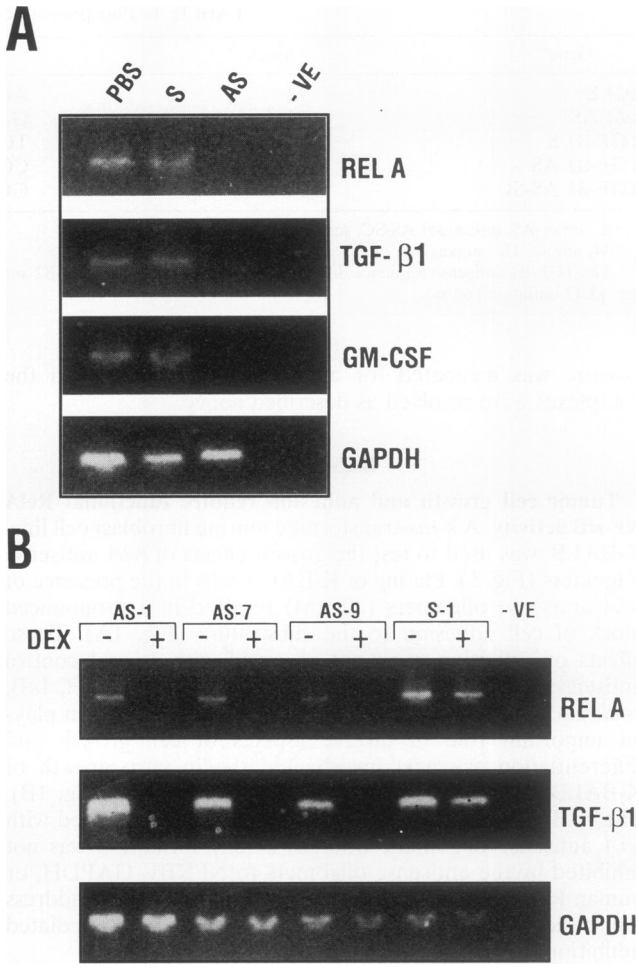

FIG. 3. Inhibition of cytokine expression in K-BALB cells in response to antisense oligomer-mediated inhibition of RelA expression. (A) K-BALB cells were treated with PBS or 20 μM *relA* sense (S) or antisense (AS) oligomers for 72 h, and total RNA was analyzed by semiquantitative PCR for RelA, TGF-β1, GM-CSF, and GAPDH expression. -VE, template-negative control. (B) K-BALB cells expressing dexamethasone-inducible RelA sense (S-1) or antisense (AS-1, AS-7, and AS-9) RNA (9) were treated with 5×10^{-6} M dexamethasone (DEX) for 72 h, and total RNA was analyzed by semiquantitative RT-PCR for RelA, TGF-β1, and GAPDH expression. -VE, template-negative control.

murine melanoma cells in response to *relA* antisense phosphorothioate treatment (not shown). The constitutive expression of IL-6, KL, granulocyte colony-stimulating factor, and macrophage colony-stimulating factor by K-BALB cells was not inhibited by *relA* antisense oligomers. The K-BALB cells had no detectable levels of IL-1α, IL-2 to IL-5, IL-7 to IL-12, tumor necrosis factor alpha, or tumor necrosis factor beta, but the basal levels of alpha interferon and beta interferon were not affected by the *relA* antisense oligomer treatment. In corroboration with the conditioned-medium experiments, these results suggested that *relA* antisense oligomers specifically interfere with cytokine expression.

Exogenous TGF-β1 abrogates the in vitro effects of *relA* antisense oligomers. We next performed reconstitution experiments by adding purified TGF-β to the K-BALB cells treated with *relA* antisense oligomers (Fig. 4). The loosely adherent K-BALB cells, following treatment with *relA* antisense oligomers for 24 h, began to spread and attach within 18 to 24 h of treatment with TGF-β1 (10 ng/ml), and the effects were maintained for 72 h, whereas untreated K-BALB cells continued to grow in suspension in the presence of *relA* antisense oligomers for up to 96 h following a single treatment with the oligomers (Fig. 4A). Similar results were obtained with TGF-β2 (not shown). The murine B-16 melanoma cells also responded identically in the reconstitution experiments (not shown). In parallel experiments, addition of purified GM-CSF (5 ng/ml), TGF-α (5 ng/ml), or epidermal growth factor (10 ng/ml) did not alter the ability of *relA* antisense oligomers to cause a block of cellular adhesion.

Subsequently we investigated the growth of *relA* antisense oligomer-treated K-BALB cells in soft agar in the presence of these cytokines (Fig. 4B). The inhibition of K-BALB cells' ability to form soft-agar colonies in the presence of *relA* antisense oligomers was reversed by the addition of TGF-β1 (Fig. 4B) but not by GM-CSF, TGF-α, or epidermal growth factor (data not shown). These results suggested that RelA NF-κB activity might regulate TGF-β expression and that *relA* antisense oligomers inhibit cellular adhesion and growth via TGF-β, either directly or indirectly.

Lack of upregulation of NF-κB activity by TGF-β. We reasoned that the reversal of effects of *relA* antisense oligomers by TGF-β1 might be due to an upregulation of NF-κB activity by TGF-β, thereby relieving the antisense oligomer effects. To clarify this, we performed electrophoretic gel mobility shift assays with the nuclear extracts of the K-BALB cells treated with *relA* antisense oligomers in the presence or absence of TGF-β1 (Fig. 5A). In comparison with cells treated with HCl-bovine serum albumin (HCl-BSA), TGF-β1-treated cells showed no upregulation of NF-κB activity. The inhibition of NF-κB activity was observed in the *relA* antisense oligomer-treated cells, and this inhibition was not overcome by addition

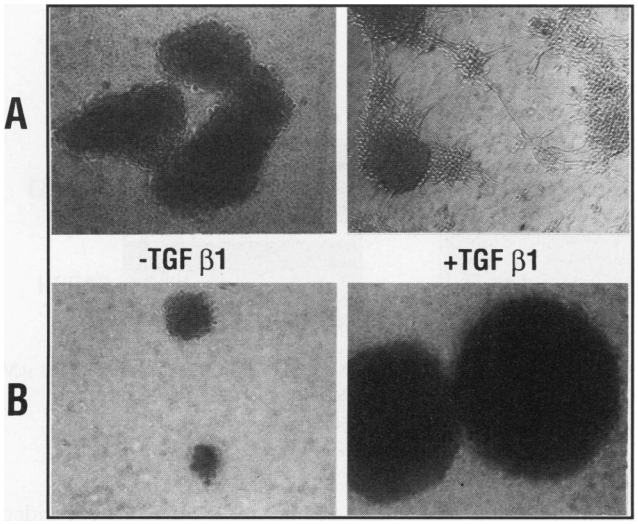

FIG. 4. Exogenous TGF-β1 reverses in vitro effects of *relA* antisense oligomers. (A) Adhesion. K-BALB cells were plated in the presence of 20 μM *relA* antisense oligomers for 48 h, treated with 10 ng of TGF-β1 per ml for 24 h or left untreated, and photographed. Magnification, ×34. (B) Soft agar growth. K-BALB cells were treated with 20 μM *relA* antisense oligomers for 72 h, plated onto soft agar in the presence of oligomers with or without 10 ng of TGF-β1 per ml, and photographed at day 10. Magnification, ×34.

of TGF-β. The K-BALB cells had a very high basal level of nuclear NF-κB activity. This activity was inhibited by *relA* antisense oligomers within 4 h of the treatment of the cells (>60%) and was seen up to 48 h following a single treatment with the *relA* antisense oligomers (Fig. 5B). The cytoplasmic NF-κB activity in detergent-disrupted extracts was also inhibited by antisense *relA* oligomer treatment (data not shown). The authenticity of the NF-κB activity in the K-BALB nuclear extracts was demonstrated (Fig. 5C) by antibody-based supershift assays. The nuclear NF-κB activity detected in vehicle (PBS)-treated cells was further retarded in electrophoretic mobility by the addition of either the RelA or the NFKB1 antibodies. The reduction in nuclear NF-κB activity in antisense *relA* oligomer-treated cells is further reflected by the reduction in supershifted activity by the RelA and NFKB1 antibodies.

TGF-β1 antisense oligomers mimic the in vitro effects of *relA* antisense oligomers. To address whether TGF-β1 is a relevant downstream target for *relA* antisense oligomer-mediated inhibition of fibroblast-derived tumor cells, we utilized antisense phosphorothioates to TGF-β1 (Table 1). Treatment of K-BALB cells with TGF-β1 antisense but not sense or scrambled phosphorothioates resulted in a pronounced block of cellular adhesion (Fig. 6A) to the substratum. Addition of exogenous TGF-β1 (10 ng/ml) to the TGF-β1 antisense oligomer-treated cells reversed the block of cellular adhesion (not shown). Exposure of K-BALB cells to antisense TGF-β1 oligomers or to a neutralizing antibody to TGF-β also inhibited soft-agar colony formation (not shown). Treatment of K-BALB cells with TGF-β1 antisense oligomers inhibited TGF-β1 mRNA in comparison with treatment with the control oligomers (Fig. 6B).

DISCUSSION

The studies described in this report were undertaken to establish the molecular basis of *relA* antisense oligomer-

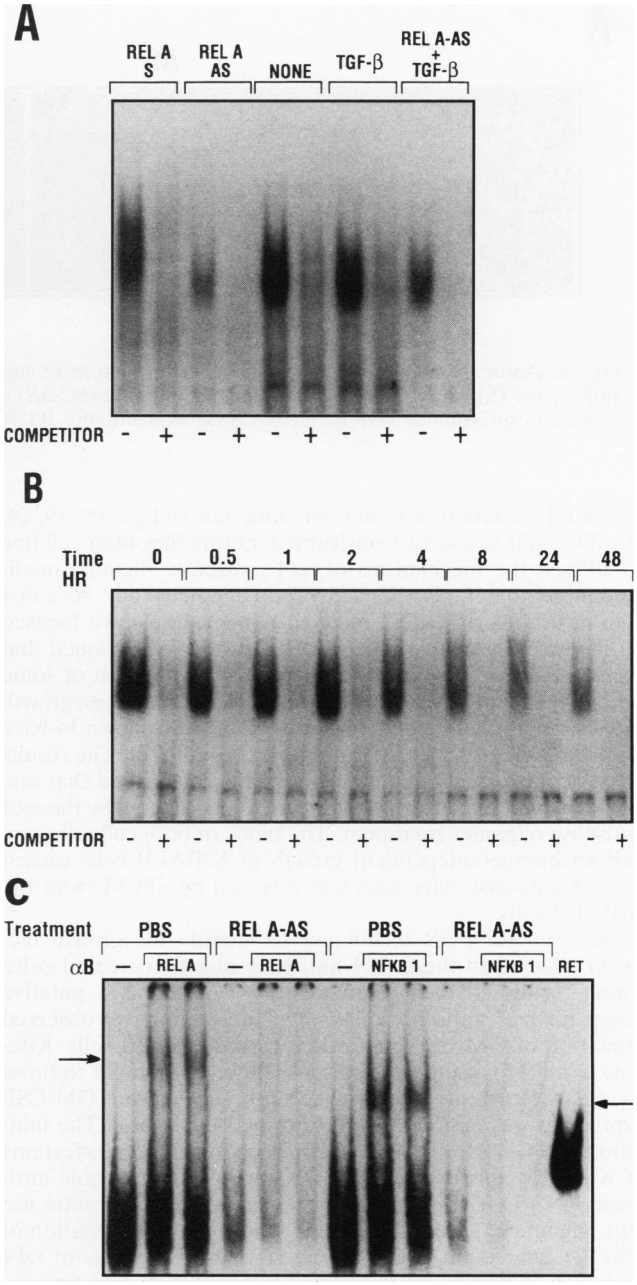

FIG. 5. Exogenous TGF-β1 does not cause upregulation of NF-κB activity in K-BALB cells. (A) Lack of upregulation of NF-κB activity by TGF-β1. K-BALB cells were treated with 20 μM *relA* sense (S) or antisense (AS) oligomers, nothing (HCl-BSA vehicle instead of TGF-β1), TGF-β1 (10 ng/ml), or *relA* antisense oligomers (20 μM) and TGF-β1 (10 ng/ml) for 72 h. Nuclear extracts were analyzed for NF-κB activity. (B) Rapid inhibition of NF-κB activity in K-BALB cells by *relA* antisense oligomers. K-BALB cells were treated with 20 μM *relA* antisense oligomers, and at the indicated time, nuclear extracts were prepared and were analyzed for NF-κB activity by electrophoretic mobility shift assay in the presence or absence of a 25-fold molar excess of double-stranded, unlabelled competitor. (C) Verification of the composition of the K-BALB NF-κB activity. K-BALB cells were treated for 48 h with either vehicle (PBS) or *relA* antisense oligomers (20 μM), and the nuclear extracts from these cells were used in a supershift assay. RelA and NFKB1 antibodies (1 and 2 μl) were added to the binding reaction mixtures, and the complexes were resolved on a 4% nondenaturing gel. The positions of supershifted RelA and NFKB1 components are indicated by arrows. RET, reticulocyte lysate.

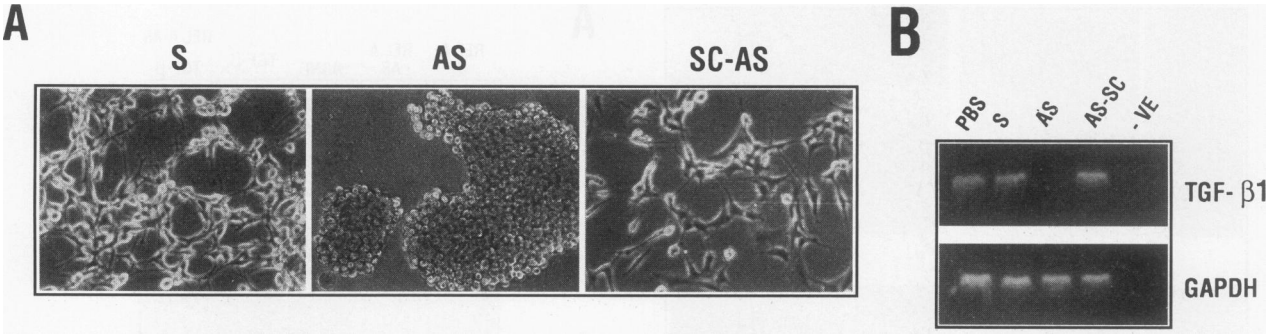

FIG. 6. Antisense TGF-β1 oligomers mimic in vitro effects of antisense *relA* oligomers. K-BALB cells were plated in the presence of 20 μM TGF-β1 sense (S), antisense (AS), or scrambled antisense (SC-AS) oligomers and were analyzed for adhesion as in Fig. 1 (magnification, ×37) (A) and for expression of TGF-β1 mRNA by semiquantitative RT-PCR as in Fig. 3 (B). -VE, template-negative control.

mediated inhibition of tumor cell adhesion and growth (9, 24, 30). We utilized a k-*ras*-transformed murine fibroblast cell line to address the mechanisms of *relA* antisense oligomer-mediated inhibition of NF-κB activity for a detailed study. As a first step in deciphering these molecular mechanisms, we focused on in vitro aspects of K-BALB cell growth. We reasoned that RelA NF-κB activity may be crucial to the function of some soluble mediator of cell growth, since diverse cytokines, growth factors, and growth factor receptors have been shown to have NF-κB binding sites at their promoter regions (7). The results of our conditioned-medium experiments established that one or more autocrine growth factors are likely affected by the *relA* antisense oligomer treatment. The block of both cell adhesion and anchorage-independent growth of K-BALB cells caused by *relA* antisense oligomers was reversed by SFCM from the K-BALB cells.

We used RT-PCR techniques to identify the growth factor(s) affected in these *relA* antisense oligomer-treated cells. These results enabled us to identify TGF-β1 as a putative target for *relA* antisense oligomers. In addition, we observed inhibition of GM-CSF expression in these treated cells. Kitajima et al. (16), using *relA* antisense oligomers similar to those used in the current study, also recently showed that GM-CSF expression was inhibited in transformed fibroblasts. The inhibition of TGF-β1 mRNA was also seen in stable transfectants of K-BALB cells expressing a dexamethasone-inducible antisense RNA to *relA*, thus corroborating the effects of antisense *relA* oligomers. To establish a link between downregulation of TGF-β1 expression and the growth-inhibitory effects of *relA* antisense oligomers, we performed reconstitution experiments. Interestingly, the addition of TGF-β1 but not of GM-CSF, epidermal growth factor, or TGF-α reversed the antigrowth and antiadhesion effects of *relA* antisense oligomers.

We next investigated whether the TGF-β1-mediated reversal of antigrowth effects by *relA* antisense oligomers could be due to upregulation of RelA NF-κB activity by TGF-β. Our results established that TGF-β1 does not cause an induction of NF-κB activity. Hence, the reversal of the effects of *relA* antisense oligomers on K-BALB cells by TGF-β1 could not be due to a relief of antisense oligomer effects through activation of NF-κB. Direct inhibition of TGF-β1 expression by TGF-β1 antisense oligomers also resulted in an identical block of K-BALB cell adhesion. These results established a link between RelA and TGF-β1 in fibroblast-derived tumor cells. K-BALB cells had a very high basal level of NF-κB activity. Antisense *relA* oligomer treatment of K-BALB cells resulted in a rapid inhibition of nuclear NF-κB activity. The time point at which inhibition of NF-κB became apparent (4 h) coincided with the block of adhesion in response to treatment with antisense *relA* oligomers. Antibody-based supershift experiments confirmed the authenticity of the NF-κB complex.

Transient transfections of a TGF-β1–chloramphenicol acetyltransferase reporter construct, PHTG-2 (15), into the K-BALB cells followed by treatment with *relA* antisense oligomers did not show an inhibition of TGF-β1 promoter-driven chloramphenicol acetyltransferase activity (data not shown), suggesting that inhibition of TGF-β1 mRNA by antisense *relA* oligomers does not occur at the TGF-β1 promoter level. The lack of inhibition by antisense *relA* phosphorothioates was not due to the high levels of exogenous expression of a transiently transfected gene, since a similar lack of correlation was seen in stable transfectants of the PHTG-2 reporter construct in the same K-BALB cells (not shown). This is not surprising, since no NF-κB binding sites are present in the TGF-β1 promoter sequence (15). Our results raise the possibility that antisense *relA* oligomer-mediated inhibition of TGF-β1 mRNA expression may involve posttranscriptional mechanisms such as mRNA stability or processing. Alternatively, polyadenylation of TGF-β1 mRNA could be inhibited by antisense *relA* oligomers, which could result in destabilization and degradation of mRNA.

TGF-β is a 25-kDa homodimeric molecule which belongs to a family of structurally related, multifunctional polypeptides (20, 21, 23, 31, 32). TGF-β has complex biological effects that alter cellular growth and differentiation. The subtypes of TGF-β (TGF-β1, TGF-β2, and TGF-β3) are growth stimulatory to fibroblasts in semisolid-agar cultures but growth inhibitory to most epithelial cells, endothelial cells, lymphoid cells, and myeloid cells, both in vitro and in vivo (23). The growth-stimulatory effects of TGF-βs on fibroblasts may be indirect and probably involve induction of platelet-derived growth factor (19). In fibroblasts, oncogenes such as *ras* cause upregulation of the TGF-β promoter function (5). TGF-β1 and TGF-β2 act as potent stimulators of extracellular-matrix production in fibroblasts by increasing the synthesis of extracellular-matrix components (10–12) and by reducing extracellular-matrix degradation by stimulating production of protease inhibitors (23). Interference at the extracellular-matrix level has been postulated in diverse actions of TGF-βs on cell morphology, adhesion, and phenotype (11).

In addition to K-BALB fibroblasts, several transformed epithelial cells are growth inhibited by treatment with antisense *relA* oligomers (9). However, in epithelial cells, the antisense oligomer-mediated inhibition of RelA NF-κB activity was not associated with inhibition of TGF-β1 expression,

and exogenous TGF-β1 could not reverse the growth- and adhesion-blocking effects of antisense *relA* oligomers (not shown). These results are not surprising since TGF-β1 is growth inhibitory to epithelial cells, although it is growth stimulatory to fibroblasts in vitro (23). These results further support the concept that regulation of gene expression by RelA NF-κB activity is dependent on cell type and can be complex.

Inhibition of expression of the same target gene was recently demonstrated by two different means of inhibiting NF-κB function. We have recently shown (30) that antisense *relA* oligomers inhibit the surface expression of a neutrophil-specific integrin, CD11b, in HL-60 cells. Alternatively, Eck et al. (4), utilizing a double-stranded phosphorothioate NF-κB consensus sequence which served as an in vivo competitor to inhibit the function of the NF-κB complex, recently demonstrated a similar block of CD11b expression in HL-60 cells. Since the CD11b promoter has no NF-κB site (25), these results suggest that regulation of genes by NF-κB is complex and can involve indirect mechanisms.

Several recent reports have demonstrated the physical association of RelA NF-κB with various transcription factors (13, 14, 27, 33–35). We have recently shown that RelA NF-κB function is essential for induction of the Sp-1 transcription factor by phosphorothioates in diverse cell lines (26). A double-stranded phosphorothioate NF-κB consensus sequence described by Eck et al. (4) did not block adhesion of K-BALB cells in our adhesion assays, in spite of a complete inhibition of NF-κB activity (data not shown). In addition, the double-stranded κB thioate competitor did not inhibit the soft-agar colony formation of K-BALB cells (data not shown). A similar lack of inhibition of adhesion and growth in soft agar was seen in diverse transformed cell lines, including B-16 (melanoma), DU-145 (prostate carcinoma), and SW-480 (colon carcinoma), upon treatment with the κB thioate competitor, despite a complete inhibition of NF-κB activity (not shown). These results are not surprising, since RelA homodimers may potentially bind to RelA-specific sequences present in the promoter regions of specific genes in addition to binding to NF-κB sites. Eventual identification of such a RelA-specific sequence present in the natural promoters of genes that regulate adhesion would help to clarify this issue. In the context of our recent observations that *relA* antisense oligomers but not NFKB1 antisense oligomers cause a block of cellular adhesion, in vitro growth of diverse transformed cells, and in vivo inhibition of tumorigenicity (9, 22, 24, 30), our current results suggest that RelA, acting either as a homodimer or as a heterodimer with other nuclear factors, can regulate distinct genes in tumor cell growth.

For the K-BALB fibrosarcoma cells, we propose a model that would explain the inhibitory effects of antisense *relA* oligomers on cell adhesion and growth in agar: TGF-β1 facilitates both cell adhesion and agar growth of fibroblasts; antisense *relA* oligomers, by inhibiting TGF-β1 expression, prevent both of these in vitro effects. Whether the in vivo inhibition of tumorigenicity by *relA* antisense oligomers we recently demonstrated in these cells also involves TGF-β remains to be clarified.

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