Latent adenoviral infection induces production of growth factors relevant to airway remodeling in COPD

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Ogawa, Emiko, W. Mark Elliott, Fiona Hughes, Thomas J. Eichholtz, James C. Hogg, and Shizu Hayashi. Latent adenoviral infection induces production of growth factors relevant to airway remodeling in COPD. Am J Physiol Lung Cell Mol Physiol 286: L189–L197, 2004.—Previous studies showed an association between latent adenoviral infection with expression of the adenoviral E1A gene and chronic obstructive pulmonary disease (COPD). The present study focuses on how the adenoviral E1A gene could alter expression of growth factors by human bronchial epithelial (HBE) cells. The data show that connective tissue growth factor (CTGF) and transforming growth factor (TGF)-β1 mRNA and protein expression were upregulated in E1A-positive HBE cells. Upregulation of CTGF in this in vitro model was independent of TGF-β secreted into the growth medium. Comparison of E1A-positive with E1A-negative HBE cells showed that both expressed cytokeratin but only E1A-positive cells expressed the mesenchymal markers vimentin and α-smooth muscle actin. We conclude that latent infection of epithelial cells by adovirus E1A could contribute to airway remodeling in COPD by the viral E1A gene, inducing TGF-β1 and CTGF expression and shifting cells to a more mesenchymal phenotype.

chronic obstructive pulmonary disease (COPD) is a worldwide health problem that is a major cause of mortality and morbidity (49). The loss of lung elastic recoil caused by emphysema and small-airway obstruction are responsible for the gradual decline in forced expiratory volume in 1 s (FEV1) that characterizes this condition. Although cigarette smoking is the major cause of these lesions, only a susceptible minority of smokers develop COPD. There is growing evidence that lung inflammation is amplified in severe COPD and persists even after cessation of smoking (51, 53). Our working hypothesis is that latent adenoviral infection is one of the factors that might amplify lung inflammation, and we have presented human data (51) demonstrating the presence of the viral E1A gene and its expression in the lungs from smokers (14, 43) as well as animal (58) and cell culture studies (31) to support this view. We postulate that a small population of lung epithelial cells carrying the adenoviral E1A gene can amplify cigarette smoke-induced lung inflammation to produce the airway and parenchymal lesions responsible for COPD. The present study concerns the inflammatory changes that lead to collagen and related ECM deposition, elastin degradation, and induction of abnormal elastin in COPD (4, 17, 39).

Two growth factors that are prime candidates as regulators of collagen and matrix deposition are connective tissue growth factor (CTGF) and transforming growth factor (TGF)-β. CTGF (3), a member of the ctfg/cyr61/nov (CCN) family, is a 38-kDa cysteine-rich peptide that promotes the proliferation of fibroblasts (12) and increases their production of ECM (12, 24, 60). Several studies showed that CTGF mRNA is overexpressed in the fibrotic lesions that occur in many organs including the lung (10, 26, 28, 34). However, CTGF is also expressed during normal tissue repair. TGF-β, on the other hand, regulates cellular functions in several ways besides those involved in ECM production (42). TGF-β is also a potent inducer of CTGF (27), and together CTGF and TGF-β are important in appropriate tissue repair (6, 27).

In this study we examined the effect of adenoviral E1A on the regulation of the CTGF and TGF-β genes in human bronchial epithelial (HBE) cells and the effect of steroids on the expression of these growth factors. Phenotypic changes induced by E1A in these cells that may be related to the remodeling process were also investigated.

MATERIALS AND METHODS

Reagents. LPS from Escherichia coli 0111:B4 was obtained from Sigma (St. Louis, MO), recombinant human (rh) tumor necrosis factor (TNF)-α from Calbiochem (La Jolla, CA), and rhTGF-β from BD Biosciences PharMingen (San Diego, CA). The antibodies used were obtained as follows: for neutralization of TGF-β from R & D Systems (Minneapolis, MN); for immunocytochemistry and Western blotting of vimentin (monoclonal) from Santa Cruz Biotechnology (Santa Cruz, CA) and of acidic and basic cytokeratin (monoclonal clones AE1 and AE3) and α-smooth muscle actin (α-SMA) (monoclonal IA4) from DAKO (Mississauga, ON, Canada); and for Western blotting of CTGF (polyclonal) from Torrey Pines Biolabs (Houston, TX), of β-actin from Sigma, and of adenoviral E1A (monoclonal) from Calbiochem. Fluticasone was provided by GlaxoSmithKline (Stevenage, UK).

Cell culture. The HBE cells used in this study were previously described in detail (22). They include primary cells, HC34, HC35, and HC57, from each of three different patients. These cells do not express E1A mRNA or protein. Cell lines from these same patients that were transfected with the pSV2neo plasmid carrying the adenoviral E1A and EIB genes under the control of their viral promoters and that expressed the E1A gene are HA34, HA35-1, and HA57, respectively.

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One cell line from the patient from whom HC35 and HA35-1 were developed was transfected with this plasmid DNA but did not express E1A mRNA or protein. It is referred to as HA35-2 and was used as an additional E1A-negative control for HA35-1. All these cells were analyzed for CTGF expression by Northern blot analysis. The E1A-positive HA35-1 and E1A-negative HA35-2 were mainly used in this study, and in all respective experiments dealing with these two cell lines similar passages were used.

The HBE cells were cultured in bronchial/tracheal epithelial cell basal medium with growth factors (BEGM; Clonetics, San Diego, CA), and the medium was changed every 48–72 h. Subconfluent cells were used at the start of each experiment. Mink lung epithelial cells [Mv1Lu; American Type Culture Collection (ATCC), Manassas, VA] were cultured in MEM with 2 mM l-glutamine, 1.5 g/l sodium bicarbonate, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate, and 10% FBS. Human fetal lung fibroblasts (ATCC) and primary human lung fibroblasts from lungs of patients resected for a primary bronchogenic carcinoma were cultured in DMEM with 10% FBS. Patients provided written consent for the use of their cells, and the studies were approved by the institutional review board of the University of British Columbia. Pulmonary artery smooth muscle cells (PASMC2169; Clonetics) were cultured in smooth muscle growth medium with growth factors and 5% FBS (SmGM-2; Clonetics).

**Northern blot analysis.** Transfected and untransfected HBE cells were grown in 6-cm dishes. After 24 h of incubation in fresh BEGM when they were subconfluent, cells were exposed to 10 μg/ml LPS or 100 U/ml TNF-α. For steroid pretreatment, 10 nM dexamethasone was added 30 min before addition of the inflammatory stimulus. Before investigating the effect of TGF-β1 on CTGF mRNA expression in the HBE cells, we confirmed the activities of the rhTGF-β1 and pan-specific TGF-β1 neutralizing antibody used in these investigations by 3H-labeled thymidine incorporation by mink lung epithelial cells (25). HBE cells were then incubated in BEGM with 10 μg/ml pan-specific TGF-β1 antibody and/or 5 ng/ml rhTGF-β. Six hours after each of the above treatments total RNA was extracted with TRIzol reagent (GIBCO BRL) and 15–20 μg of the RNA was electrophoresed through a 1.3% agarose gel containing formaldehyde, transferred to Hybond-N membranes, and fixed under ultraviolet illumination. Hybridization was performed with DNA probes labeled with [α-32P]dCTP. A specific probe for human CTGF was made by PCR. Briefly, the I.M.A.G.E. Consortium clone cDNA 1013266 encoding the CTGF precursor mRNA including the 3′ untranslated region (38) was used as a PCR template, and the sequence of the PCR product was confirmed by sequence analysis. The probe for human TGF-β1 was a generous gift from Dr. Rik Derynck (Genentech, South San Francisco, CA) (9). The specific probe for human α-SMA was a cDNA obtained by RT-PCR of RNA extracted from E1A-positive HBE cells (HA35-1). The cDNA was subcloned into pBluescript, and its sequence was confirmed by sequence analysis. A 1.2-kb PstI fragment of rat GAPDH cDNA that cross-hybridized with human GAPDH was used as a control for RNA loading of the gel. Autoradiographic densities of the mRNA bands were measured with the public domain ImageJ program from the US National Institutes of Health (NIH), which is available at http://rsfl.info.nih.gov/NIH-image. The density of the CTGF or TGF-β1 mRNA band was corrected by comparison with the GAPDH band in the same lane.

**TGF-β1 immunoassays.** After 24-h incubation of E1A-positive or -negative HBE cells in fresh medium, the medium was changed and then sampled 24 or 48 h later. The concentration of TGF-β1 was determined with the TGF-β1 E_{max} Immunoassay System (Promega, Madison, WI) according to the manufacturer’s protocol. The kit is designed to detect biologically active TGF-β1, the total concentration of TGF-β1 in the culture medium after acidification with 1 N HCl, which converted latent TGF-β1 to the active form, and this was compared with the amount of active TGF-β1 in the nonacidified medium. To correct for differences in the number of E1A-negative and -positive cells, cell counts were made with the methylene blue assay (13).

**Immunocytochemistry.** Cells grown on glass cover slips in BEGM were fixed with methanol for 5 min and air dried. After 1-h incubation with universal blocking reagent (Sigma), cells were incubated overnight at 4°C with primary antibodies, monoclonal anti-human cytokeratin, monoclonal anti-vimentin, or monoclonal α-SMA, followed by incubation with goat anti-mouse IgG for 30 min and detected with the alkaline phosphatase anti-alkaline phosphatase method (31).

**Western blot analysis.** Cells were lysed with buffer containing PBS with 1% Nonidet P-40 and a protease inhibitor cocktail (Sigma). The protein concentration was measured by the Bio-Rad protein assay (Bio-Rad, Hercules, CA). Samples of 30 μg of protein from HBE cells were electrophoresed through 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad). Membranes were blocked with 5% skim milk in Tris-buffered saline (TBS) and 0.1% Tween 20 and then incubated overnight at 4°C with the primary antibodies, anti-CTGF, anti-β-actin, anti-cytokeratin, anti-α-SMA, anti-vimentin, or anti-E1A, in 5% skim milk in TBS and 0.1% Tween 20. Five micrograms of protein from pulmonary artery smooth muscle cells were used as a positive control for α-SMA expression. The membranes were then incubated with horseradish peroxidase-conjugated anti-mouse antibody (DAKO), and a chemiluminescence kit (Promega) was used for detection.

**Transient transfection and luciferase assay.** The pGL3 Basic luciferase vector (Promega) driven by a 1.362-bp full-length TGF-β1 promoter was a kind gift from Dr. S.-J. Kim (NIH, Bethesda, MD). Relative to the transcription initiation site at +1, the promoter spans from −1362 to +1 of the TGF-β1 gene (33). For transient transfection, trypsinized E1A-negative HA35-2 cells were resuspended in bronchial/tracheal epithelial cell basal medium with 10 mM dextran and 0.1 mM DTT at a concentration of 5.0 × 106 cells/ml. The cells (1.9 × 102 cells/cuvette) were transiently cotransfected with 10 μg of the reporter construct, a control vector pCMV-β-gal (4 μg) for β-galactosidase (β-gal) assay, a gift from Dr. H. Luo (University of British Columbia, Vancouver, BC, Canada), and either the E1A expression vector (pE1Aneo) that contains E1A gene or control vector without E1A (pSVneo) (31) by electroporation with a Gene Pulser II (Bio-Rad) at 400 V and 950 μF in 0.4-cm cuvettes. The cells were then plated on 12-well culture dishes, allowed to attach for 24 h, and incubated for another 48 h in fresh BEGM before lysing with reporter lysis buffer (Promega). Luciferase activity in the cell lysates was measured with a luciferase assay system (Promega), and results from transfections with the empty pGL3 basic vector were used for background correction. To correct for differences in transfection efficiency, β-gal activity (48) was used to normalize the corresponding luciferase activity.

**Statistical analysis.** ANOVA with the post hoc Fisher’s test was used for comparisons among groups. Values of \( P < 0.05 \) were considered statistically significant.

**RESULTS**

**CTGF mRNA induction in E1A-positive HBE cells.** To determine whether adenovirus E1A affects CTGF mRNA expression by HBE cells, Northern blot analysis was performed on RNA from E1A-transfected HBE cells that express this viral protein and compared with controls not expressing E1A 6 h after incubation with or without inflammatory stimulus (Fig. 1). CTGF mRNA levels were very low or undetectable in either HA35-2, the E1A-transfected cell that does not express E1A (Fig. 1A), or the primary HBE cells HC34 and HC57 (Fig. 1C) and HC35 (data not shown), which do not express E1A. In contrast, basal CTGF mRNA levels were significantly higher.
in the corresponding E1A-expressing HBE cells from the same patient sources (Fig. 1) but this was lower than that found in human fetal lung fibroblasts or primary human lung fibroblasts (data not shown). Neither LPS nor TNF-α affected the expression of CTGF mRNA in any of the HBE cells (Fig. 1, B and C). However, after 6.5 h of incubation with fluticasone, CTGF mRNA levels were further upregulated in E1A-expressing HA35-1 and HA57 cells (Fig. 1). This elevated expression by fluticasone was not affected by LPS or TNF-α stimulation (Fig. 1, A and B) and returned to basal levels after 24-h incubation (data not shown). Fluticasone did not affect CTGF mRNA in cells not expressing E1A (Fig. 1).

**TGF-β1 mRNA expression in E1A-positive HBE cells.** The expression of TGF-β1 mRNA in the three E1A-expressing HBE cells used in this study, but not in E1A-negative cells, and the fact that this expression is not affected by LPS or TNF-α stimulation have already been demonstrated (22). However, because TGF-β1 was identified as a strong inducer of CTGF gene expression (27), it was important to confirm in this study that the effect of E1A on TGF-β expression had not been altered by passing these cells during the intervening period between these two studies. As expected, TGF-β1 mRNA was very low or almost undetectable in Northern blots from the E1A-negative cell line HA35-2 whereas it was constitutively expressed in the HA35-1 E1A-positive cells and this expression was unaltered by LPS or TNF-α stimulation (data not shown). In addition, TGF-β1 mRNA levels were not affected by fluticasone alone or in combination with LPS or TNF-α in either cell line (Fig. 2). Because E1A-transfected HBE cells that do not express E1A are a more appropriate control for transfected E1A-positive HBE cells than untransfected primary HBE cells, the remainder of this study concentrated mainly on
TGF-β1 concentration in growth medium. To measure TGF-β1 in the medium we used an ELISA that detects the active form of this growth factor. Because TGF-β1 is commonly secreted as a latent, biologically inactive form by cultured cells (35), we used this ELISA before and after acidification of the growth medium to convert the latent form to the active form. TGF-β1 was not detected in the medium alone (data not shown). As shown in Fig. 3, the medium sampled after 24 and 48 h of incubation of the E1A-negative HA35-2 and E1A-positive HA35-1 HBE cells in BEGM contained TGF-β1 and, consistent with mRNA expression, the E1A-positive HBE cells secreted more TGF-β1 than the E1A-negative cells. This TGF-β1 was mostly in the latent form, with little active form secreted. We compared TGF-β1 production between E1A-negative and E1A-positive HBE cells after normalizing for cell number and confirmed that this correction E1A-positive HBE cells produced significantly more of the latent form of TGF-β1 than negative cells (data not shown).

TGF-β did not affect CTGF mRNA expression in E1A-transfected HBE cells. We examined whether TGF-β1 secreted by HBE cells into the medium affects CTGF mRNA expression in these cell lines. The rhTGF-β used in this study was confirmed to inhibit [3H]thymidine incorporation by mink epithelial cells (data not shown), and the neutralizing antibody was shown to block this TGF-β1 effect dose-dependently (data not shown). Figure 4 shows that addition of rhTGF-β1 to the growth medium did not affect CTGF and TGF-β1 mRNA expression in the E1A-negative HA35-2 or the E1A-positive HA35-1 HBE cells. Furthermore, the antibody capable of neutralizing both TGF-β1 and TGF-β2 did not affect the expression of CTGF and TGF-β1 mRNAs.

CTGF protein expression in E1A-positive HBE cells. To confirm the expression of CTGF protein by E1A-positive HBE cells, Western blot analyses were performed with whole cell lysates from the E1A-negative HA35-2 or E1A-positive HA35-1 cells. As shown in Fig. 5, more CTGF was expressed in the E1A-positive HBE cells than in the negative cells and this protein expression paralleled the mRNA expression (Fig. 6).
At the same time β-actin protein levels in these two cell lines were similar (Fig. 5).

**Possibility of phenotypic change in HBE cells by adenovirus E1A.** Because TGF-β, in particular TGF-β1, and CTGF are mainly produced by mesenchymal cells, our next step was to determine whether any further changes toward a mesenchymal phenotype occur in E1A-positive HBE cells or whether their epithelial characteristics are retained. Figure 6 shows that by immunocytochemical analyses both E1A-positive cells, HA35-1 and HA57, and the corresponding E1A-negative primary HBE cells, HC35 and HC57, respectively, as well as the transfected but E1A-negative counterpart of HA35-1, namely, HA35-2, all expressed the epithelial cytokeratin marker (Fig. 6, A-1, B-1, C-1, D-1, and E-1), whereas both E1A-positive HA35-1 and HA57 cells increased their expression of two markers of mesenchymal cells, vimentin (Fig. 6, C-2 and E-2) and α-SMA (Fig. 6, C-3 and E-3), compared with the corresponding E1A-negative cells. Western blot analysis (Fig. 7) confirmed that whereas both E1A-negative and -positive HBE cells express cytokeratin, although higher-molecular-mass forms (50–60 kDa) are found in E1A-positive cells, the E1A-positive cells increased their expression of vimentin and α-SMA compared with the corresponding E1A-negative controls. At the same time, β-actin levels were similar in all these cells, demonstrating that protein loading was not different, except for the E1A-negative HC57 (Fig. 7). Western blot analysis also confirmed the expression of the viral E1A protein by the positive HBE cells, whereas the E1A-negative controls did not express this viral protein (Fig. 7).

![Fig. 6. Immunocytochemical staining of cytokeratin, vimentin, and α-SMA in E1A-negative and -positive HBE cells. The primary HBE cells (HC35 and HC57), the transfected but E1A-negative cells (HA35-2), and the transfected E1A-positive cells (HA35-1 and HA57) were grown on glass coverslips in BEGM, fixed in methanol, and incubated with primary antibodies, either monoclonal anti-human cytokeratin (A-1, B-1, C-1, D-1, and E-1), monoclonal anti-vimentin (A-2, B-2, C-2, D-2, and E-2), anti-α-SMA (A-3, B-3, C-3, D-3, and E-3), or, as a negative control, mouse IgG (A-4, B-4, C-4, D-4, and E-4). Detection was by the alkaline phosphatase anti-alkaline phosphatase technique. A, B, and D: E1A-negative HC35, HA35-2, and HC57 HBE cells, respectively. C and E: E1A-positive HA35-1 and HA57 HBE cells, respectively. Bar, 50 μm.

![Fig. 7. Expression of cytokeratin, vimentin, α-SMA, adenovirus E1A, and β-actin in E1A-negative and -positive HBE cells. The primary HBE cells (HC35 and HC57), the transfected but E1A-negative cells (HA35-2) and the transfected E1A-positive cells (HA35-1 and HA57) were grown until subconfluent in BEGM and analyzed by Western blotting. Representative autoradiograms from 1 of 3 independent experiments are shown. The membranes were probed with anti-cytokeratin, anti-vimentin, anti-α-SMA, anti-E1A antibody, or anti-β-actin, which was used as an internal control.](image-url)
As shown in Fig. 8, expression of α-SMA mRNA paralleled its protein expression (Fig. 7), that is, it was significantly upregulated in E1A-positive HA35-1 cells compared with E1A-negative HA35-2 cells (Fig. 8). However, its expression in these E1A-positive HBE cells was lower than that in pulmonary artery smooth muscle cells (data not presented). Treatment with rhTGF-β1 or neutralizing antibody to TGF-β did not affect α-SMA mRNA expression in either E1A-negative or E1A-positive cells (Fig. 7).

E1A predominates over E1B in regulating transcription of TGF-β. To distinguish between the regulation of the TGF-β1 gene by adenovirus E1A or E1B in HBE cells transfected with the pSV2neo plasmid carrying the adenoviral E1A and E1B genes, HA35-2 cells that were transfected with this plasmid but do not express E1A were transiently cotransfected with the luciferase reporter driven by the TGF-β1 promoter and either pE1Aneo or the control vector. In HA35-2 cells cotransfected with pE1A, the transcriptional activity of TGF-β1 promoter was increased 1.8 times compared with the same cells transfected with the control vector (Fig. 9).

**DISCUSSION**

This study demonstrates that the presence of adenoviral E1A in HBE cells resulted in the constitutive expression of CTGF and TGF-β1 mRNA by these cells. This E1A-regulated CTGF expression was independent of the pathway through which TGF-β1 induces CTGF expression. The steroid fluticasone upregulated E1A-induced CTGF mRNA expression. Other phenotypic changes including the expression of two mesenchymal markers, α-SMA and vimentin, were also induced by E1A in these cells. These results suggest that latent adenoviral infection of HBE cells with expression of the viral E1A gene could stimulate connective tissue matrix deposition in the airway wall and in this manner play a role in the pathogenesis of the airway remodeling process observed in COPD.

Adenovirus E1A was first identified as an oncoprotein (20). Since then it has been shown to regulate both viral and host genes by a multitude of mechanisms. E1A does not act as a transcription factor because it does not bind DNA. However, E1A can affect the transcription of genes by binding to the retinoblastoma gene product (RB) (59), interaction with DNA binding domains of transcription factors that allow its recruitment to promoters of genes (40), and interaction with the cellular coactivators p300 and CREB-binding protein (CBP) (2, 41). As discussed below, it is through such interactions that E1A could affect the expression of CTGF and TGF-β. However, because these HBE cells were transfected with a combination of E1A and E1B, the contribution of E1B to the induction of these growth factors was taken into consideration. Because we found that transient transfection of an E1A expression plasmid induced a 1.8-fold increase in TGF-β1 promoter activity in E1A-negative HBE cells and this increase was similar to the increase we found in TGF-β1 mRNA in E1A-expressing HBE cells, we favor a greater contribution to the regulation of the TGF-β1 gene expression in these cells by E1A than by E1B. That this is also true for CTGF is supported by results from lung epithelial A549 cells transfected with a plasmid carrying only adenovirus E1A (31), where CTGF mRNA expression was higher compared with cells transfected with a control plasmid (T. J. Eichholtz, unpublished observation).

The predominant sources of TGF-β1 in the lung are the mesenchymal cells; lung epithelial cells produce little of this isoform of TGF-β (52). When adenovirus E1A is present in HBE cells, however, TGF-β1 mRNA expression is upreg-
lated. Taking into consideration that E1A is a nuclear protein known to interact with the host transcriptional apparatus, especially at the level of the transcription factors (2, 40, 41, 59), this viral protein most likely regulates TGF-β1 expression through documented interactions with those factors that bind to the 5′-flanking region of the TGF-β1 gene (36, 37, 54). The report that the promoter activity of TGF-β1 was repressed by the 12S E1A protein (7) is contradictory to our result of increased TGF-β1 expression in E1A-expressing HBE cells. A possible difference between the two studies that might account for this is that our HBE cells express both 12S and 13S E1A (22) and that although 12S E1A can repress transactivation by c-jun, the 13S product cooperates with this subunit of activator protein (AP)-1 to stimulate transcription (8).

The low level of CTGF mRNA expression in E1A-negative HBE cells is consistent with reports of the absence of CTGF expression in lung epithelial cells in primary culture (34) and of low levels of expression by HBE cells (11). On the other hand, CTGF expression has been documented in epithelial cells of some organs, particularly during periods of growth, differentiation, secretory activity, or wound healing (50, 55, 56). The increased CTGF mRNA in HBE cells as a result of adenovirus E1A transfection reported here may be related to similar events in the lung. Although the transcriptional regulation of the CTGF gene has not been fully elucidated, the presence of an AP-1 (37) and a NF-1-like binding site in its promoter region reminiscent of the TGF-β1 gene suggests that regulation by E1A may be similar between the two genes.

That steroids, in combination with E1A, augment the expression only of CTGF and not of TGF-β in HBE cells indicates that there are differences in the regulation of the two growth factors by the viral transactivator. Steroids act by binding to and activating their receptors, which in turn translocate to the nucleus and bind to steroid-responsive elements of genes or interact with other proteins involved in controlling gene transcription. The interaction of the steroid receptor with two such proteins, adenovirus E1A (47) and the transcription factor AP-1 (30), which in turn can interact with each other as noted above, could result in increased expression of the CTGF gene. The absence of a similar control of the TGF-β1 gene suggests that different mechanisms are used by E1A to modulate the expression of these two growth factors.

Another possible source of CTGF gene activation is the TGF-β constitutively expressed by the E1A-positive HBE cells. The main regulatory sequences responding to TGF-β in the 5′-flanking region of the CTGF gene have been identified as the SMAD binding site in fibroblasts that express a scleroderma phenotype (23) and as the TGF-β-responsive element in skin fibroblasts (21). E1A binding to the coactivators CBP and p300 could block TGF-β signaling downstream of SMAD (46). If this were the case, E1A would be expected to down-regulate CTGF. However, this is not the case in HBE cells. Moreover, because the TGF-β secreted by the E1A-positive HBE cells was in the latent form, TGF-β activation of these pathways is not expected. Further evidence favoring a regulation of CTGF independent of TGF-β in the E1A-positive HBE cells is the following. Both neutralization of TGF-β1/β2 in the medium and addition of rhTGF-β did not affect the constitutive expression of CTGF mRNA. Also, TNF-α, which inhibits TGF-β/SMAD signaling (57) and suppresses TGF-β-induced CTGF in fibroblasts (1), did not suppress E1A-induced CTGF.

Together, our results favor a process by which E1A induces the expression of CTGF independent of TGF-β, and this process may simultaneously block control by TGF-β.

Possible mechanisms by which latent adenovirus infection of the airway epithelial cells in lungs of patients with COPD (14) could promote airway remodeling, particularly by connective tissue deposition (49), are suggested by the results of this study. In the lung CTGF has been shown to target fibroblasts to produce collagen (26) and related ECM protein (18) and to stimulate smooth muscle cells to proliferate (16). TGF-β1 and CTGF can cause epithelial cells from different tissues to transdifferentiate into myofibroblasts (15, 19, 29). Therefore, our findings of the induction of TGF-β1 and CTGF expression in HBE cells by adenovirus E1A suggests that if these growth factors were induced in airway epithelial cells in lungs of patients with COPD by a latent adenovirus infection they could initiate similar tissue remodeling. The acquisition of a more mesenchymal phenotype, that is, the expression of the mesenchymal markers α-SMA and vimentin, by E1A-positive HBE cells might be, although not tested directly in our study, a consequence of E1A-induced CTGF expression. Also, although the TGF-β1 induced in the HBE cells in culture by adenovirus E1A was in the latent form, this latent TGF-β1 would mostly likely be activated in the lung (32). These include activation by plasmin (44), thrombomodulin (5), and integrin α,β6 (45).

The complex pathogenesis of COPD involves several aspects of the inflammatory process. We previously reported (51) that many of the inflammatory cells present in the advanced stage of emphysema are increased in relation to the number of epithelial cells expressing E1A. The results reported here of E1A-induced expression of the growth factors CTGF and TGF-β by bronchial epithelial cells suggest that E1A could also stimulate connective tissue deposition in the airway inflammatory response in COPD. Although our in vitro studies of HBE cells suggest a role for adenovirus E1A in the airway remodeling process in COPD, further studies are needed to clarify the in vivo role of these growth factors in the pathogenesis of this disease. Furthermore, the possibility that E1B might contribute to the induction of growth factors in our cell lines needs to be clarified.

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