

Modulation of human bronchial epithelial cell IIICS fibronectin mRNA *in vitro*

E. Rosi*, J.D. Beckmann**, P. Pladsen**, S.I. Rennard**, D.J. Romberger***

Modulation of human bronchial epithelial cell IIICS fibronectin mRNA in vitro. E. Rosi, J.D. Beckmann, P. Pladsen, S.I. Rennard, D.J. Romberger. ©ERS Journals 1996.

ABSTRACT: Fibronectin (Fn) is an extracellular matrix glycoprotein which is involved in wound repair, including repair of injured airway epithelium. Bronchial epithelial cells (BECs) are known to produce Fn which has enhanced chemotactic activity compared to serum Fn. Alternative splicing of the Fn gene is an important mechanism by which cells regulate the production of Fn. Human BECs produce Fn which contains the EIIIA region, but the expression of IIICS region variants has not previously been reported. Our purpose was to better define the molecular characteristics of human BEC Fn by determining the expression of alternative splice variants of the IIICS region of Fn of human BECs *in vitro*.

Human bronchial epithelial cells obtained from bronchoscopy were cultured. To examine the presence of IIICS messenger ribonucleic acid (mRNA) variants, we synthesized oligonucleotide primers complementary to the published human fibronectin complementary deoxyribonucleic acid (cDNA) sequence of the IIICS domain for use in polymerase chain reactions (PCR) with total ribonucleic acid (RNA) extracted from cultured human BECs. To examine the modulation of IIICS mRNA expression, the 428 base pair (bp) DNA fragment generated in the PCR was oligo-labelled with ³²[P]-deoxycytidine triphosphate (dCTP) for use as a probe for Northern blot analysis. Human BECs were cultured in the presence and absence of transforming growth factor-β (TGF-β) and agents which influence cyclic adenosine monophosphate (cAMP) including isoproterenol and dibutyl cAMP (db-cAMP). Total RNA from cultures was extracted, electrophoresis performed, and Northern blots obtained. Blots were hybridized with IIICS probe, total Fn cDNA, and tubulin cDNA.

It was found that human BECs in culture expressed the five known human IIICS variants. TGF-β enhanced the expression of IIICS mRNA in a concentration- and time-dependent fashion. Isoproterenol and db-cAMP both reduced the expression of IIICS mRNA and attenuated the TGF-β induction. Changes in IIICS mRNA paralleled changes in total Fn mRNA, suggesting that these agents do not selectively modulate only the IIICS domain of Fn.

We conclude that human airway epithelial cell Fn *in vitro* does contain mRNA for five IIICS variants, and that IIICS mRNA can be modulated by TGF-β and agents which influence cAMP. It is unknown whether alterations in IIICS variants contribute to the functional differences previously observed between airway epithelial cell Fn and plasma-derived Fn.

Eur Respir J., 1996, 9, 549–555.

Fibronectin (Fn) is an extracellular matrix glycoprotein known to play an important role in many cellular processes involved in wound repair [1]. In the lung, it has been demonstrated that Fn is involved in normal development, especially in animal models [2]. Furthermore, overexpression of Fn is associated with fibrosis in patients with interstitial lung disease [3]. The importance of Fn in airways has been suggested by observations, such as an increase of Fn in tracheal lavage fluid of children with respiratory distress who progress to bronchopulmonary dysplasia [4].

Bronchial epithelial cells are among potential sources of Fn in the airways [5, 6]. Fn derived from bronchial

epithelial cells *in vitro* is capable of influencing the behaviour both of fibroblasts and airway epithelial cells. Specifically, it has been shown that bronchial epithelial cells produce chemotactic activity for fibroblasts and epithelial cells, which is largely Fn [5, 7]. This epithelial cell-derived Fn is more potent as a chemotactic agent than plasma-derived Fn. This suggests that epithelial cell-derived Fn has unique properties which may influence the repair of injured airways.

Variations in Fn generally occur due to differences in amino acid sequences or posttranslational modifications [8]. It is now well-established that there is one gene for Fn which can be spliced in three specific regions, accounting

*Clinica Medica III, Policlinico di Careggi, Firenze, Italy. **Dept of Internal Medicine, University of Nebraska Medical Center, Omaha, NE, USA. *Research Service of the Dept of Veterans Affairs Medical Center, Omaha, NE, USA.

Correspondence: D.J. Romberger
Dept of Internal Medicine, Pulmonary and Critical Care Medicine Section
600 South 42nd Street
Omaha, NE
USA 68198 5300

Keywords: Airway epithelium
fibronectin gene regulation
messenger ribonucleic acid splicing

Received: April 20 1995
Accepted after revision November 14 1995

This work was sponsored in part by Veteran's Administration Merit Review funding (DJR).

for the known variations in amino acid sequence. Differential splicing in three different domains (EIIIA, EIIB and IIICS) can generate in man at least 20 different Fn polypeptides [8]. The EIIIA and EIIB domains are spliced to be either included or excluded in total, whilst splicing of the human IIICS (or variable) domain can generate five different variants. Alternative splicing of Fn is known to be cell and tissue specific [9]. WANG *et al.* [10] have demonstrated that guinea-pig tracheal epithelial cells in culture produce one of the alternative splice variants, EIIIA, which is modulated by transforming growth factor- β (TGF- β). The expression of IIICS Fn variants in airway epithelial cells has not been determined.

Differential splicing is thought to result in functional properties of Fn [11]. The IIICS splice variants may be particularly relevant to cellular processes of inflammation. At least two of the variants of the IIICS region, CS-1 and CS-5, have been shown to promote cell attachment and spreading [9]. The CS-1 region is able to interact with the integrin $\alpha_4\beta_1$ (also known as very late activation antigen-4 (VLA-4)) [12], which is predominantly expressed on lymphocytes, monocytes and eosinophils. Recently, the CS-1 region has been implicated in the inflammatory process both of rheumatoid arthritis and bacterial-induced arthritis [13, 14]. ELICES *et al.* [13] demonstrated that CS-1 is preferentially expressed on the synovial endothelium of rheumatoid arthritis patients, and that adherence of T-cell lines to the endothelium in rheumatoid arthritis was primarily mediated by CS-1 on the endothelial cells. WAHL *et al.* [14] demonstrated that systemic administration of Fn peptides, including a CS-1 peptide, can attenuate the development of chronic inflammation in an animal model of bacterial-induced arthritis.

In airway diseases characterized by inflammation, such as asthma and chronic bronchitis, the interaction of inflammatory cells and the mediators they release may influence the repair of the damaged epithelium. Regulation of expression of bronchial epithelial cell Fn splice variants, and IIICS variants in particular, may be involved in directing epithelial cell-inflammatory interactions. The purpose of this study work was: 1) to determine the pattern of IIICS variants present in human bronchial epithelial cells *in vitro*; and 2) to examine the effect of substances known to regulate Fn gene expression on the modulation of IIICS Fn expression in cultured human airway epithelial cells. The effect of TGF- β , was investigated, as it is a potent stimulator of epithelial cell Fn production and has been shown to alter IIICS splicing pattern in some other cell lines [15].

The effect of a beta-adrenergic receptor agonist, isoproterenol (iso), on IIICS messenger ribonucleic acid (mRNA) was also examined, as beta-adrenergic receptor agonists are commonly used in airway disease and little is known about their influence on epithelial cell Fn. Beta-adrenergic receptor agonists increase cyclic adenosine monophosphate (cAMP) and other cAMP agents, such as dibutyryl cAMP (db-cAMP) have been shown to regulate total Fn production in other cell types [16–19]. However, the effect of cAMP on cellular Fn production

is highly dependent on cell type, with both stimulation and suppression of Fn observed. The influence of cAMP on human bronchial epithelial total Fn mRNA or IIICS Fn mRNA *in vitro* has not previously been described. Thus, the effect of two agents which increase cAMP, iso and db-cAMP, on airway epithelial Fn and IIICS Fn were examined.

Materials and methods

Media and supplements

LHC-D, a growth factor deficient medium, contains LHC basal medium (Biofluids, Rockville, MD, USA), 0.5 μM phosphoethanolamine/ethanolamine (Sigma, St Louis, MO, USA), 0.11 mM calcium (Fisher, Springfield, NJ, USA), 50 $\text{U}\cdot\text{mL}^{-1}$ penicillin and streptomycin (Gibco, Grand Island, NY, USA), 2 $\mu\text{g}\cdot\text{mL}^{-1}$ fungizone (Gibco), and trace elements. LHC-9 medium contains LHC-D medium with 5 $\mu\text{g}\cdot\text{mL}^{-1}$ bovine insulin (Sigma), 5 $\text{ng}\cdot\text{mL}^{-1}$ epidermal growth factor (Sigma), 10 $\mu\text{g}\cdot\text{mL}^{-1}$ bovine transferrin (Sigma), 10 nM 3,3',5' triiodothyronine (Biofluids), bovine pituitary extract (50 μg protein $\cdot\text{mL}^{-1}$; Pel Freeze, Rogers, AR, USA), 0.2 μM hydrocortisone (Biofluids), 0.5 $\mu\text{g}\cdot\text{mL}^{-1}$ epinephrine (Sigma), and 0.1 $\mu\text{g}\cdot\text{mL}^{-1}$ retinoic acid (Sigma). LHC-9/RPMI 1640 (RPMI 1640, Gibco) at 1:1 mix was used to support the growth of these cells, as described previously for bovine bronchial epithelial cells [20].

Other reagents utilized in cell culture experiments included TGF- β from R&D Systems (Minneapolis, MN, USA), isoproterenol (iso), and db-cAMP from Sigma.

Cell culture

Human bronchial epithelial cells (HBECs) were obtained from bronchoscopy brushings (approximately 6–8 brushes in 3–4 locations) of patients undergoing bronchoscopy for clinical reasons, after obtaining informed consent and with the approval of the Institutional Review Board of the University of Nebraska Medical Center and the Human Studies Subcommittee of the Research and Development Committee of the Omaha Veteran's Administration Medical Center. Brushings from three patients with diagnoses of chronic bronchitis, cardiovascular disease, and bronchoalveolar carcinoma were utilized. Cells were processed using the technique of KELSEN *et al.* [21]. Cells were maintained in culture in serum-free medium, LHC9/RPMI 1640 (1:1), at 37°C in 5% $\text{CO}_2/95\%$ air. Cells were passaged no more than eight times prior to use in experiments. Using a cytokeratin stain, cells were found to be 98–99% epithelial. Staining was performed with mouse anti-human cytokeratin (Dako; clone MNF116; isotype immunoglobulin G1 (IgG1); broad-spectrum molecular weights (MWs) of 45, 46, 56.5 kDa). For the experiments, HBECs were plated in 35 mm tissue culture plastic dishes coated with Vitrogen, 0.03 $\text{mg}\cdot\text{mL}^{-1}$ (Celtrix, Santa Clara, CA, USA). When monolayer cultures reached

approximately 70% confluence, cells were exposed to varying concentrations of TGF- β , isoproterenol or db-cAMP in media deficient in growth factors, LHCD/RPMI 1640 (1:1), for varying lengths of time prior to harvest of cell layers for ribonucleic acid (RNA). The various conditions were examined in triplicate.

RNA extraction

Total cellular RNA was isolated from monolayer cultures by the method of CHOMCZYNSKI and SACCHI [22], utilizing guanidine thiocyanate extraction. Concentration and purity of samples were determined by spectrophotometric analysis. Integrity of RNA was evaluated by electrophoresis in 1% agarose/formaldehyde gel [23], and visualized with ethidium bromide staining.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total cellular RNA (5 μ g) was denatured at 95°C for 5 min and incubated at 42°C for 60 min in 20 μ L of a mixture consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl₂, 1 U- μ L⁻¹ RNasin (RNA-Guard; Pharmacia, Pisataway, NJ, USA), 100 pM Random hexamer (Pharmacia), 1 mM each of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP) (Perkin Elmer, Norwalk, CT, USA), and 200 U Superscript Reverse Transcriptase (Gibco BRL). For each reaction mixture 10 μ L of the reverse transcription product was added to the PCR buffer (1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.01% gelatin) (Perkin Elmer), 1 mM of each primer, 1 mM each of dATP, dCTP, dGTP, dTTP (Perkin Elmer), and 2.5 U AmpliTaq polymerase (Perkin Elmer).

Oligonucleotide primers were synthesized that are complementary to the published human fibronectin complementary deoxyribonucleic acid (cDNA) sequence that lies between 23 base pairs (bp) outside of the 3' end and 16 bp outside the 5' end of the IIICS domain [24, 25]. The sequences were 5'GAATAATCAGAAGAGCGAGC3' and 5'CCATGAGATGGTTGTCTGAG3'.

The reaction mixture was overlaid with paraffin oil and the DNA sequence was amplified in a thermal cycler (MJ Research, Watertown, MA, USA). Fifteen, 20, or 30 cycles were performed with denaturation at 95°C for 2 min, annealing at 42°C for 1 min, and extension at 72°C for 1 min. The amplification products were separated electrophoretically on a 1% agarose gel and visualized with ethidium bromide staining. The 428 bp DNA fragment was excised from the agarose gel and oligolabelled with ³²P-dCTP (3,000 Ci·mmol⁻¹; Amersham, Arlington Heights, IL, USA) for use as a probe for Northern blot analysis.

DNA sequencing

Specificity of the PCR reaction was confirmed by sequencing of the PCR product using a 380B synthesizer.

Northern blot analysis

For Northern blot analysis, 10 μ g of total RNA was electrophoresed on 1% formaldehyde-agarose gel and transferred to nylon membranes (Scheicher and Schuell, Keene, NH, USA). After prehybridization at 42°C in a solution containing 5 \times Denhart's solution, 5 \times SSPE buffer, 1% sodium dodecyl sulphate (SDS), blots were hybridized overnight with ³²P-oligolabelled probe for IIICS Fn at 42°C in a solution containing 2 \times Denhart's solution, 5 \times SSPE, 0.5% SDS, 50% formamide, and 5% dextran sulphate. Membranes were then washed at 23°C in 2 \times SSPE and 0.1% SDS for 30 min, and 0.1 \times SSPE and 0.1% SDS for 1 h, and exposed to film for 4–18 h with an intensifying screen.

Autoradiographs were analysed with a densitometer (Hoeffer Scientific Instruments, San Francisco, CA, USA). Oligolabelling and hybridization with mouse β 5 tubulin cDNA (from Don Cleveland, Baltimore, MD, USA) were also performed as above to demonstrate equivalent RNA loading. A human Fn cDNA probe (gift from F. Baralle, Oxford, UK) was used to demonstrate total Fn mRNA. Results are expressed as ratio of IIICS/control (tubulin) or total Fn/control densitometry signals.

Results

To study the expression of IIICS Fn mRNA by HBECs, RT-PCR was performed using synthesized oligonucleotide primers complementary to flanking regions of the human IIICS Fn cDNA sequence. Electrophoresis of the PCR product demonstrated five bands of approximately the correct size for the five known human Fn IIICS splice variants (fig. 1). Due to their similarity in size, the 353 bp and the 335 bp products appeared as either a doublet or a single band. The heaviest band (428 bp) was excised from the gel and sequenced using a 380B synthesizer. The sequencing showed 98% identity with human IIICS cDNA according to published data [24]. The 2% error is not significant, being due to either sequencing misreading or PCR-generated misincorporation.

HBECs were also exposed to 200 pM TGF- β for 24 h prior to RNA extraction for use in RT-PCR. No difference, in terms of relative intensity of staining by ethidium bromide or number of bands, was observed between cells with and without TGF- β exposure, even when PCR was performed for a lower number of cycles (15–20). This suggests that TGF- β exposure does not change the relative expression of the five IIICS variants identified in cultured airway epithelial cells.

TGF- β exposure caused an increase in HBEC steady-state IIICS mRNA expression by Northern blot analysis (fig. 2). Both a concentration- and time-dependent increase in IIICS mRNA expression was observed. There was no effect after 6 h and a minimal increase after 12 h of exposure with 50 and 200 pM TGF- β . After 24 h, IIICS expression was increased 3.2 fold (by densitometry) with exposure to 50 pM and 5 fold with exposure to 200 pM TGF- β . The increase persisted at 48 h. The maximal effect was observed with exposure to 200 pM

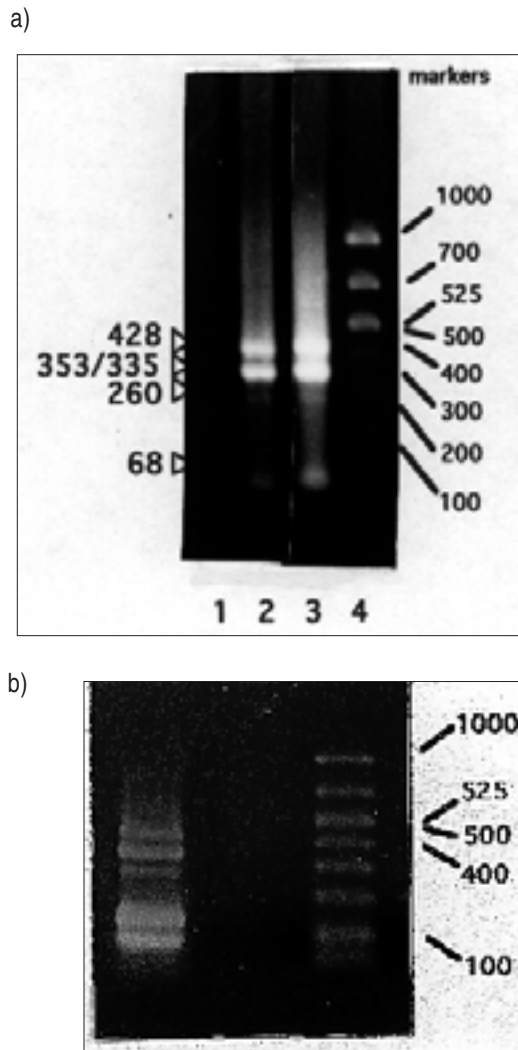


Fig. 1. – a) Ethidium bromide stained agarose gel of PCR generated HBEC IIICS mRNA. Oligonucleotide primers were synthesized that are complementary to human fibronectin cDNA sequence as noted in the text. Using total RNA derived from cultured HBECs (with and without pretreatment with TGF- β for 24 h), RT-PCR was performed with the primers (30 cycles) and five bands appropriate for the five known human IIICS splice variants were observed (noted on the left). Due to their similarity in size, the 353 bp and the 335 bp products appeared either as a doublet or a single band. Lane 1: negative control (no RT product in the PCR mix); and lane 2: HBECs untreated; lane 3: HBECs treated with 200 pM TGF- β for 24 h; lane 4: size markers. b) A single lane of the ethidium bromide stained agarose gel showing the five bands with size markers on the right. PCR: polymerase chain reaction; HBEC: human bronchial epithelial cells; mRNA: messenger ribonucleic acid; cDNA: complementary deoxyribonucleic acid; RNA: ribonucleic acid; TGF- β : transforming growth factor- β ; RT: reverse transcriptase; bp: base pair.

TGF- β at 24 h. The autoradiograph data shown in figure 2 is from one experiment with conditions examined in triplicate and is representative of results of duplicate experiments. Thus, TGF- β appears to upregulate the expression of IIICS Fn mRNA in cultured human airway epithelial cells. TGF- β is known to modulate the total Fn mRNA expression of bronchial epithelial cells [10, 26]. In HBECs, TGF- β induced comparable increases in the expression of IIICS mRNA and total Fn mRNA (fig. 3). Therefore, TGF- β does not preferentially increase

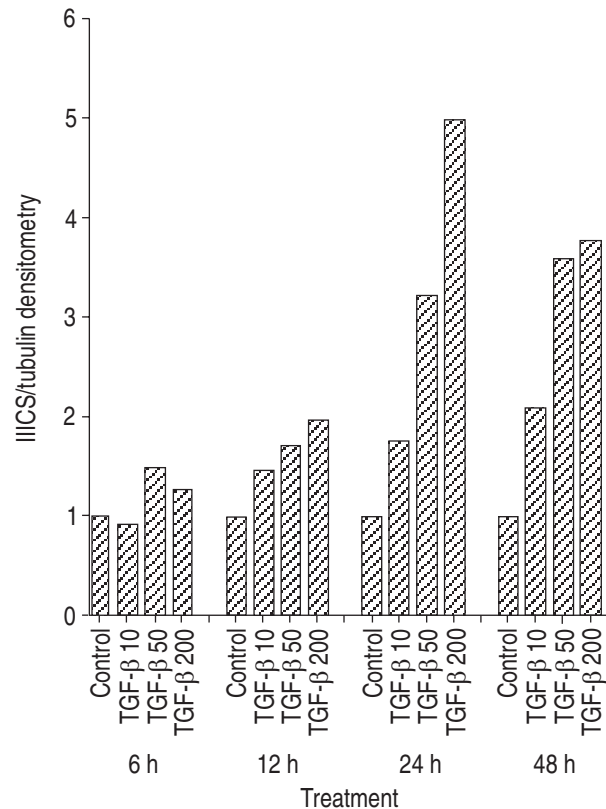


Fig. 2. – The effect of TGF- β on HBEC IIICS mRNA expression. Cells were treated with TGF- β 10, 50, 200 pM for 6, 12, 24 and 48 h. Northern blots were prepared with 10 μ g of total cytoplasmic RNA. Blots were hybridized with 32 P-IIICS probe and 32 P-tubulin probe. Densitometry values were obtained from an autoradiograph (representative of duplicate experiments) and expressed as IIICS mRNA/tubulin mRNA ratio. For abbreviations see legend to figure 1.

IIICS mRNA, but increases IIICS expression in proportion to induction of total Fn mRNA expression.

Agents that stimulate intracellular cAMP production are known to modulate the expression of total Fn mRNA in certain cell types, particularly fibroblasts [19]. Therefore, it was decided to evaluate whether the beta-adrenergic agonist, isoproterenol, and db-cAMP modulate the expression of HBEC IIICS and total Fn mRNA. The expression of IIICS Fn mRNA was reduced after exposure to isoproterenol for 24 h (fig. 3). In repeat experiments ($n=5$), IIICS mRNA expression of isoproterenol exposed cells was 54–88% (using densitometry) of that of control, non-isoproterenol treated HBECs. The maximally effective concentration was 10^{-6} M (10^{-4} to 10^{-8} M examined). The reduction of IIICS mRNA expression persisted at 48 h of isoproterenol exposure. Furthermore, concomitant exposure of HBECs both to isoproterenol and TGF- β resulted in an attenuation of the TGF- β induction of IIICS mRNA (fig. 3). The attenuation did not occur at 24 h of TGF- β and isoproterenol exposure, but was present after 48 h of exposure. The attenuation was only 30% in figure 3, but ranged 30–52% in repeat experiments. Parallel changes were seen with total Fn mRNA in all conditions. Thus, the beta-adrenergic agonist isoproterenol, which acts in part through cAMP, inhibits IIICS mRNA expression and attenuates the TGF- β induction of IIICS mRNA.

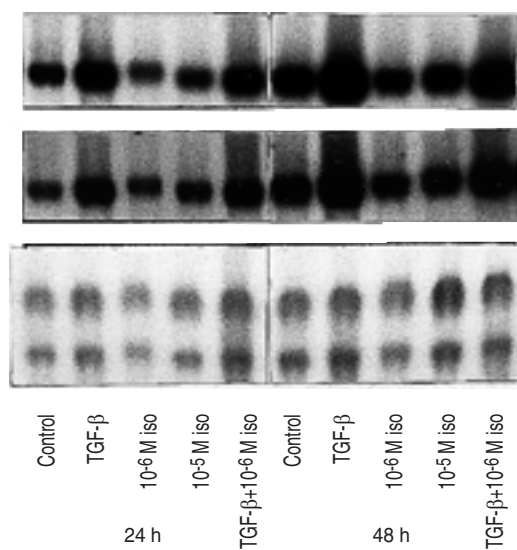


Fig. 3. – Northern blot of total cytoplasmic RNA from cultured HBECs. Cells were exposed to isoproterenol (iso) 10^{-6} and 10^{-5} M, TGF- β 200 pM, TGF- β and isoproterenol concomitantly for 24 and 48 h. The blots were hybridized with 32 P-IIICS probe, 32 P-fibronectin probe and 32 P-tubulin probe. The autoradiograph shown is representative of five replicate experiments. Fn: fibronectin. tub: tubulin. For further abbreviations see legend to figure 1.

Exposure to the cAMP derivative, db-cAMP, resulted in a reduction of IIICS expression, similar to isoproterenol (fig. 4). The most significant reduction of constitutive IIICS mRNA expression was observed at a concentration of 10^{-3} M db-cAMP. At 24 h of exposure, db-cAMP-treated cells demonstrated IIICS mRNA expression which was 35% that of control, untreated cells. This decrease persisted at 48 h of exposure, but was 59% of control at that time-point. HBECs treated simultaneously with TGF- β and db-cAMP also demonstrated a slight attenuation of the TGF- β induction of IIICS mRNA (fig. 4) (densitometry values are from a single autoradiograph, representative of results obtained in duplicate experiments). Both after 24 and 48 h of exposure, there was an approximate 25% reduction of expression in db-cAMP and TGF- β exposed cells compared to TGF- β exposed cells. Again, parallel changes were observed in total Fn mRNA expression. Thus, the cAMP derivative, db-cAMP, reduces IIICS mRNA expression and the TGF- β induction of IIICS mRNA.

RT-PCR was also performed with cultured HBECs that were exposed to isoproterenol (10^{-6} M) and db-cAMP (10^{-3} M) for 24 h prior to harvest of RNA. In a similar fashion to the results seen with TGF- β exposed HBECs, there was no obvious change in the pattern of IIICS splicing demonstrated on the ethidium bromide stained gel after electrophoresis of PCR products (data not shown). This suggests that neither isoproterenol nor db-cAMP alter the relative expression of the individual IIICS variants, but rather that they influence the expression of mRNA for all the IIICS variants.

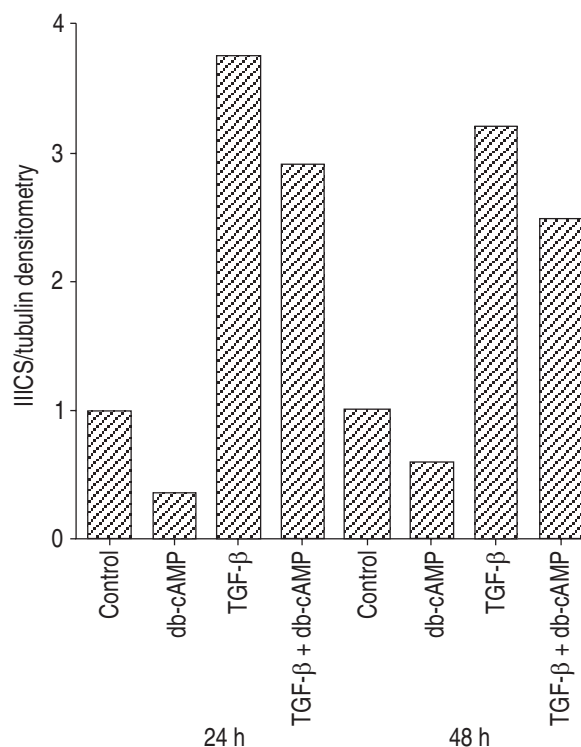


Fig. 4. – The effect of db-cAMP on HBECs IIICS mRNA expression. Cells were treated with db-cAMP 10^{-3} M, TGF- β 200 pM, TGF- β 200 pM + db-cAMP 10^{-3} M for 24 and 48 h. Northern blots were prepared with 10 μ g of total cytoplasmic RNA. Blots were hybridized with 32 P-IIICS probe and 32 P-tubulin probe. Densitometry values are expressed as IIICS mRNA/tubulin mRNA ratio. The data shown are from a single autoradiograph, representative of duplicate experiments. db-cAMP: dibutyl cyclic adenosine monophosphate. For further abbreviations see legend to figure 1.

Discussion

In this study, we demonstrated that human bronchial epithelial cells in culture expressed IIICS Fn mRNA. This expression was enhanced by TGF- β exposure in a concentration- and time-dependent fashion, and was partially attenuated by exposure to isoproterenol and db-cAMP. Isoproterenol and db-cAMP also reduced the constitutive expression of IIICS Fn mRNA. The bronchial epithelial cells expressed all five known variants of the human IIICS region. The relative expression of these five variants did not appear to be altered by TGF- β exposure or exposure to isoproterenol or db-cAMP. Furthermore, modulation of the IIICS mRNA expression by TGF- β , isoproterenol, and db-cAMP paralleled the modulation of the total Fn mRNA expression in these cells.

Fibronectin mRNA can be alternatively spliced in three different regions, specifically EIIIA (or ED-A), EIIIB (or ED-B) and IIICS (or variable, V). Whilst EIIIA and EIIIB are either included or excluded, IIICS domain has three regions and, in human fibronectin, at least five different splicing variants can arise from their combination. Fn produced by various cell types is characterized by variable expression of alternative splice regions. For example, plasma Fn, which is largely derived from hepatocytes, does not contain the EIIIA region. The proportion of IIICS containing Fn is dependent on cell type and

is also developmentally regulated [9, 27]. The presence of IIICS mRNA has previously been described in a variety of human cell lines [9, 15]. In this work, we observed that human bronchial epithelial cells *in vitro* also express Fn mRNA containing the IIICS region.

There has been much investigation of the functional significance of the Fn splice variants, including those derived from the IIICS region. Peptides derived from the IIICS segment can promote cell attachment and spreading [11]. Cell adhesion activity has been demonstrated for two different peptides, CS-1 and CS-5, derived from the IIICS domain. CS-1, containing the active PEILDV sequence, and CS-5, containing the active REDV sequence, exhibited adhesion activity for melanoma cells [28]. Furthermore, CS-1 is known to be a binding site for VLA-4 ($\alpha_4\beta_1$) integrin [9, 29]. It has been demonstrated that CS-1 is expressed in the synovial endothelium of patients with rheumatoid arthritis, but not in persons without [13]. In addition, the adhesion of T-cells expressing $\alpha_4\beta_1$ integrin in rheumatoid arthritis synovium was blocked by CS-1 peptide, suggesting a role for CS-1 in the pathogenesis of rheumatoid arthritis inflammation. Thus, changes in IIICS Fn expression may be an important mechanism regulating inflammatory processes.

Bronchial inflammation is a predominant feature of such airway diseases as asthma or chronic bronchitis. Bronchial epithelial cells are known to produce Fn which is particularly effective at promoting chemotaxis both of fibroblasts and bronchial epithelial cells *in vitro* [5, 7]. It is not known whether alternative splicing accounts for the enhanced chemotactic activity of airway epithelial cell Fn or whether post-translational modifications of Fn may be responsible. From our current work, we know that human bronchial epithelial cell Fn mRNA contains the IIICS region. We have not yet characterized the functional significance of this expression. We can speculate that the IIICS region may be involved in the enhanced ability of airway epithelial cell Fn to influence chemotaxis of fibroblasts and bronchial epithelial cells. Additionally, since the IIICS region of Fn is known to interact with the $\alpha_4\beta_1$ integrin, it is possible that IIICS Fn of bronchial epithelial cells may help direct inflammatory cells, particularly macrophages and lymphocytes, in the airway epithelium. However, the specific roles of IIICS Fn in the airway epithelium are yet to be delineated.

TGF- β is known to modulate Fn production in guinea-pig and bovine airway epithelial cells *in vitro* [10, 26], and to be produced by bronchial epithelial cells [30]. A modification in the pattern of IIICS Fn splicing induced by TGF- β has been reported in certain malignant cell lines [15], whilst in mesangial cells no alteration has been observed [31]. In our study, TGF- β seemed not to change the pattern of IIICS mRNA variants as shown by electrophoresis of RT-PCR products. Also, the increasing expression of IIICS Fn mRNA did not differ from the increasing expression of total Fn mRNA. This suggests that in human airway epithelial cells *in vitro* TGF- β upregulates expression of Fn mRNA, including the IIICS region, but does not selectively alter IIICS splicing.

The effect of cAMP on total Fn mRNA expression has been extensively examined [16–19, 32] and varies with cell type. Induction of Fn expression in HT1080 fibroblasts has been observed with exposure to db-cAMP, 8-bromo-cAMP, forskolin and 3-isobutyl-1-methylxanthine (IBMX) [19]. In contrast, db-cAMP specifically decreased Fn synthesis and mRNA expression in granulosa cells [17], and articular chondrocytes [18, 32]. Our data show that in human bronchial epithelial cells, exposure to isoproterenol and db-cAMP reduces IIICS Fn mRNA expression. It is unclear what the role of cAMP attenuation of IIICS Fn mRNA might be in airway epithelial cells, which *in vivo* may be frequently exposed to beta-adrenergic agonists that can influence cAMP. Depending on the specific role(s) of IIICS Fn in airway inflammation, such modulation of the IIICS Fn might function in either a positive or negative fashion to influence ongoing repair in the airways.

In conclusion, we have described the expression of the IIICS messenger ribonucleic acid region in human airway epithelial cell fibronectin from cells *in vitro*. IIICS messenger ribonucleic acid is modulated in proportion to total fibronectin messenger ribonucleic acid expression by transforming growth factor- β and cyclic adenosine monophosphate stimulating agents, isoproterenol and dibutyryl cyclic adenosine monophosphate. Further understanding of the role of epithelial cell fibronectin in airway inflammation and repair will require better characterization of the fibronectin splice variants expressed and their functional significance.

Acknowledgements: The authors express their appreciation for photographic assistance to A. Heires and secretarial assistance to S. Latenser.

References

1. Clark RAF. Potential roles of fibronectin in cutaneous wound repair. *Arch. Dermatol* 1988; 124: 201–206.
2. Roman JE, Crouch E, Pickles K, McDonald JA. Role of fibronectin and its receptor in mammalian lung development. *Am Rev Respir Dis* 1989; 139: 197A.
3. Rennard SI, Crystal RG. Fibronectin in human bronchopulmonary lavage fluid: elevation of patients with interstitial lung disease. *J Clin Invest* 1982; 69: 113–122.
4. Gerdes JS, Yoder MC, Douglas SD, Paul M, Harris MC, Polin RA. Tracheal lavage and plasma fibronectin: relationship to respiratory distress syndrome and development of bronchopulmonary dysplasia. *J Pediatr* 1986; 108: 601–606.
5. Shoji S, Rickard KA, Ertl RF, Robbins RA, Linder J, Rennard SI. Bronchial epithelial cells produce lung fibroblast chemotactic factor: fibronectin. *Am J Respir Cell Mol Biol* 1989; 1: 13–20.
6. Stoner GD, Katoch Y, Foidart J-M, Trump BF, Steinert PM, Harris CC. Cultured human bronchial epithelial cells: blood group antigens, keratin, collagens and fibronectin. *In Vitro* 1981; 17: 577–587.
7. Shoji S, Ertl RF, Linder J, Romberger DJ, Rennard SI. Bronchial epithelial cells produce chemotactic activity for bronchial epithelial cells. *Am Rev Respir Dis* 1990; 141: 218–225.
8. Hynes RO. Fibronectins. *In: Rich A, ed. Springer Series*

- in Molecular Biology. New York; Springer-Verlag, 1990.
9. Hershberger RP, Culp LA. Cell type specific expression of alternatively spliced human fibronectin IIICS mRNAs. *Mol Cell Biol* 1990; 10(2): 662–671.
 10. Wang A, Cohen DS, Palmer E, Sheppard D. Polarized regulation of fibronectin secretion and alternative splicing by transforming growth factor- β . *J Biol Chem* 1991; 266(24): 15598–15601.
 11. Guan J, Trevithick JE, Hynes RO. Retroviral expression of alternatively spliced forms of rat fibronectin. *J Cell Biol* 1990; 110: 833–847.
 12. Guan J-L, Hynes RO. Lymphoid cells recognize an alternatively spliced segment of fibronectin *via* $\alpha_4\beta_1$. *Cell* 1990; 60: 53–61.
 13. Elices MJ, Tsai V, Strahl D, *et al.* Expression and functional significance of alternatively spliced CS-1 fibronectin in rheumatoid arthritis microvasculature. *Am Soc Clin Invest* 1994; 93: 405–416.
 14. Wahl SM, Allen JB, Hines KL, *et al.* Synthetic fibronectin peptides suppress arthritis in rats by interrupting leukocyte adhesion and recruitment. *J Clin Invest* 1994; 94: 655–662.
 15. McKay NG, Hunter DJ, Haites NE, Power DA. Regulation of alternative splicing of the fibronectin IIICS domain by cytokines. *Biochem Biophys Res Commun* 1994; 199(2): 1005–1011.
 16. Coats WD Jr, Brecher P. Cyclic AMP suppresses fibronectin expression in the rabbit aorta *in vitro*. *Arterioscler Thromb* 1993; 13: 1668–1679.
 17. Bernath VA, Muro AF, Vitullo AD, Bley MA, Baranao JL, Kornblihtt AR. Cyclic AMP inhibits fibronectin gene expression in a newly developed granulosa cell line by a mechanism that suppresses cAMP responsive element-dependent transcriptional activation. *J Biol Chem* 1990; 265(30): 18219–18226.
 18. Burton-Wurster N, Leipold HR, Lust G. Dibutyl cyclic AMP decreases expression of ED-A fibronectin by canine chondrocytes. *Biochem Biophys Res Commun* 1988; 154(3): 1088–1093.
 19. Dean DC, Newby RF, Bourgeois S. Regulation of fibronectin biosynthesis by dexamthasone, transforming growth factor- β , and cAMP in human cell lines. *J Cell Biol* 1988; 106: 2159–2170.
 20. Beckmann JD, Takizawa H, Romberger D, *et al.* Serum-free culture of fractionated bovine bronchial epithelial cells. *In Vitro Cell Dev Biol* 1992; 28A: 39–46.
 21. Kelsen SG, Mardini IA, Zhou S, Benoivic JL, Higgins N. A technique to harvest viable tracheobronchial epithelial cells from living human donors. *Am J Respir Cell Mol Biol* 1992; 7: 66–72.
 22. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162: 156–159.
 23. Maniatis T, Fritsch EF, Sambrook J. *In: Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, 1982.
 24. Owens RJ, Kornblihtt AR, Baralle FE. Fibronectin, the generation of multiple polypeptides from a single gene. *In: Oxford Series on Eukaryotic genes.* 1986; 3: pp. 141–160.
 25. Kornblihtt A, Vibe-Pedersen K, Baralle FE. Human fibronectin: cell specific alternative mRNA splicing generates polypeptide chains differing in the number of internal repeats. *Nucleic Acids Res* 1984; 12: 5853–5868.
 26. Romberger D, Beckmann J, Claassen L, Ertl R, Rennard S. Modulation of fibronectin production of bovine bronchial epithelial cells by transforming growth factor- β . *Am J Respir Cell Mol Biol* 1992; 7: 149–155.
 27. Norton PA, Hynes RO. Alternative splicing of chicken fibronectin in embryos and in normal and transformed cells. *Mol Cell Biol* 1987; 7(12): 4297–4307.
 28. Humphries M, Akiyama S, Komoriya A, Olden K, Yamada K. Identification of an alternatively spliced site in human plasma fibronectin that mediates cell type specific adhesion. *J Cell Biol* 1986; 103: 2637–2647.
 29. Kocher O, Kennedy SP, Madri JA. Alternative splicing of endothelial cell fibronectin mRNA in the IIICS region. *Am J Pathol* 1990; 137(6): 1509–1524.
 30. Sacco O, Romberger D, Rizzino A, Beckmann J, Rennard S, Spurzem J. Spontaneous production of TGF- β_2 by primary cultures of bronchial epithelial cells: effects on cell behavior *in vitro*. *J Clin Invest* 1992; 90: 1379–1385.
 31. McKay NG, Khong TF, Haites NE, Power DA. The effect of transforming growth factor- β_1 on mesangial cell fibronectin synthesis: increased incorporation into the extracellular matrix and reduced p/ but no effect on alternative splicing. *Exp Mol Pathol* 1993; 59: 211–224.
 32. Leipold HR, Burton-Wurster N, Steinmeyer J, Vernier-Singer MS, Lust G. Fibronectin and keratin sulfate synthesis by canine articular chondrocytes in culture is modulated by dibutyl cyclic adenosine monophosphate. *J Orthop Res* 1992; 10: 33–48.