Adenovirus-mediated decorin gene transfer prevents TGF-β-induced inhibition of lung morphogenesis

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1Center for Craniofacial Molecular Biology, Departments of Surgery and Pediatrics, and Cell and Developmental Biology Program, Childrens Hospital Los Angeles Research Institute, University of Southern California Schools of Dentistry and Medicine, Los Angeles, California 90033; and 2Molecular Virology and Immunology Program, Department of Pathology and Biology, Health Science Center, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Zhao, Jingsong, Patricia J. Sime, Pablo Bringas, J r., Jack Gauldie, and David Warburton. Adenovirus-mediated decorin gene transfer prevents TGF-β-induced inhibition of lung morphogenesis. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L412–L422, 1999.—Excessive transforming growth factor (TGF)-β signaling has been implicated in pulmonary hypoplasia associated with bronchopulmonary dysplasia, a chronic lung disease of human prematurity featuring pulmonary fibrosis. This implies that inhibitors of TGF-β could be useful therapeutic agents. Because exogenous TGF-β ligands are known to inhibit lung branching morphogenesis and cytodifferentiation in mouse embryonic lungs in vivo culture, we examined the capacity of a naturally occurring inhibitor of TGF-β activity, the proteoglycan decorin, to overcome the inhibitory effects of exogenous TGF-β. Intratracheal microinjection of a recombinant adenovirus containing decorin cDNA resulted in overexpression of the exogenous decorin gene in airway epithelium. Although exogenous TGF-β efficiently decreased epithelial lung branching morphogenesis in control cultures, TGF-β-induced inhibition of lung growth was abolished after epithelial transfer of the decorin gene. Additionally, exogenous TGF-β-induced antiproliferative effects as well as the downregulation of surfactant protein C were abrogated by decorin in cultured embryonic lungs. Moreover, lung branching inhibition by TGF-β could be restored by the addition of decorin antisense oligodeoxynucleotides in culture, indicating that decorin is both specifically and directly involved in suppressing TGF-β-mediated negative regulation of lung morphogenesis. Our findings suggest that decorin can antagonize bioactive TGF-β during lung growth and differentiation, establishing the rationale for decorin as a candidate therapeutic approach to ameliorate excessive levels of TGF-β signaling in the developing lung.

Lung morphogenesis, branching morphogenesis, alveolar saccular formation, cell proliferation, cell survival, migration, and extracellular matrix deposition (reviewed in Ref. 13). It is therefore likely that soluble factors instruct embryonic, fetal, and neonatal lung development by coordinated temporospatial autocrine/paracrine signaling.

Transforming growth factor (TGF)-β1, -2, and -3 are members of a large family of cytokines that includes activin, bone morphogenetic proteins, and Müllerian inhibiting substance. TGF-βs initiate their cellular action by binding primarily to three cell surface-receptor proteins termed TGF-β type I, type II, and type III receptors. On binding to TGF-βs, the TGF-β type III receptor (betaglycan) presents TGF-β directly to the TGF-β type II receptor, a serine/threonine kinase subunit (23, 24, 32). The TGF-β type II receptor kinase trans-phosphorylates the type I receptor after TGF-β type I-type II receptor heteromerization, which, in turn, displaces betaglycan from TGF-β ligand binding (25, 27). The TGF-β type I-type II receptor complex subsequently trans-phosphorylates intracellular Smad proteins and so eventually propagates a phosphorylation signal into the nucleus (6, 11, 21, 35).

The primitive lung epithelium undergoes cell proliferation, branching morphogenesis, and alveolar saccular formation as well as concomitant cell lineage differentiation to form an organ capable of conducting respiratory gases to a large, diffusible interface with the circulation. Lung development is now known to take place within a complex milieu of peptide growth factors, which may affect many parameters of cell behavior including cell proliferation, cell survival, migration, differentiation, and extracellular matrix deposition (reviewed in Ref. 13). It is therefore likely that soluble factors instruct embryonic, fetal, and neonatal lung development by coordinated temporospatial autocrine/paracrine signaling.

TGF-β signaling is an important negative regulator during embryonic lung development as demonstrated by gain- and loss-of-function studies in embryonic mouse lungs in organ culture as well as in transgenic and null mutant mice. TGF-β1 and TGF-β2 both inhibit pulmonary branching morphogenesis in culture (30, 39), whereas TGF-β3 null mutant mice have a specific neonatal lethal lung phenotype (18). On the other hand, abrogation of TGF-β type II receptor signaling, either with antisense oligodeoxynucleotides (ODNs) or with pulmonary epithelium-specific overexpression of a dominant-negative TGF-β type II receptor, significantly stimulates lung morphogenesis in culture and prevents TGF-β-induced downregulation of epithelial differentiation marker genes such as surfactant protein (SP) C (39, 40), indicating that physiological TGF-β signaling negatively modulates lung development. In contrast, overexpression of TGF-β1 in the alveolar epithelium with the human SP-C promoter generates a neonatal lethal, hypoplastic pulmonary phenotype with reduced saccular formation and abnormal epithelial differentiation, supporting the conclusion that exces-
sive TGF-β signaling can inhibit lung morphogenesis in vivo (42).

Aberrant expression of TGF-β, occurring as a result of lung disease and injury, could therefore perturb finely regulated TGF-β signaling and so result in abnormalities of lung growth, differentiation, and development. Pulmonary fibrosis is a prominent feature of bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, and increased concentrations of TGF-β1 have been found in the bronchoalveolar lavage fluid of human premature infants who develop a severe form of BPD (20). Excessive TGF-β signaling is known to induce a chronic fibrotic lung injury in rats as well as in the bleomycin model of chronic fibrosis (19, 31). Alveolar hypoplasia, a major sequela of neonatal hyperoxia, is also associated with high levels of TGF-β activity in premature lungs (3). Taken together, excessive TGF-β signaling appears to adversely disrupt the orderly temporal and molecular cascades that normally govern lung morphogenesis and cytodifferentiation. Therefore, therapeutic strategies to antagonize TGF-β signaling could ameliorate lung injury and augment lung repair in the developing lung.

Decorin belongs to the family of small leucine-rich proteoglycans that have been implicated as key regulators of both matrix assembly and cellular growth (15). Decorin consists of leucine-rich repeats of 20–24 amino acids and a single site for glycosaminoglycan side-chain attachment. Decorin is a 100-kDa proteoglycan found in the interstitial extracellular matrix, with a core protein of 45 kDa. Decorin specifically binds and neutralizes TGF-β ligands via its protein moiety, thus acting as a TGF-β inhibitor (37). Because decorin occurs naturally, it may be involved in the physiological regulation of the effects of TGF-β ligands. By sequestering bioactive TGF-β through formation of inactive TGF-β-decorin complexes, decorin could be useful as an antagonistic agent to downmodulate TGF-β signaling in the prevention and treatment of lung disease and injury.

In the present report, we used decorin to antagonize exogenous TGF-β-mediated inhibition of lung morphogenesis and cytodifferentiation in a well-characterized model of embryonic mouse lung morphogenesis ex vivo defined culture. A recombinant adenovirus expressing decorin was utilized, via an intratracheal microinjection to introduce the adenovirus, in a gene transfer approach to induce epithelium-specific decorin overexpression in embryonic mouse lungs in culture (40). We found that recombinant adenoviral expression of decorin in the pulmonary epithelium specifically overcame exogenous TGF-β-mediated negative regulation of lung epithelial branching. Decorin thus appears to be a candidate rational therapy to negatively regulate excessive TGF-β signaling during lung morphogenesis, injury, and repair.

MATERIALS AND METHODS

Recombinant adenovirus constructs. Construction of the recombinant adenovectors has been described in detail elsewhere (2, 31). Briefly, full-length human decorin cDNA was cloned into a shuttle vector containing the human cytomegalo-
Competitive PCR methodology for specific mRNA quantification of pulmonary genes has been described previously (38). Briefly, a set of primers (primers 1 and 2; Fig. 1A) were designed for murine decorin to amplify a cDNA fragment of 182 bp in size (29). Two composite primers were synthesized for decorin competitor construction (Fig. 1A); each composite primer had the target decorin primer sequence (solid boxes) incorporated into a stretch of sequence (open boxes) designed to hybridize to the opposite strand of a heterologous DNA fragment. The desired primer sequences (primers 1 and 2) were thus engineered into a competitor cDNA after PCR amplification, ensuring that both decorin cDNA and decorin competitor utilize the same set of primers in the decorin competitive PCR. The decorin competitor was 268 bp in length. Both decorin and its competitor PCR products were DNA sequenced to ensure their identities. Competitive PCR assay for biglycan was developed in a manner similar to that for decorin.

PCR amplification was carried out with a modification of a previously described assay for the TGF-β type II receptor (39). The PCR mixture containing a known amount of competitor was added to reverse-transcribed samples derived from 20 ng of total RNA or to dilutions of standard cDNA templates in a total volume of 50 µl. β-Actin competitive PCR as an internal control was routinely performed on the same samples. As a negative control for genomic or viral DNA, un-reverse-transcribed total RNA was also included in the competitive PCR assays.

Immunocytochemistry. Cultured lungs were fixed with paraformaldehyde and embedded into paraffin. Embryonic lung sections (5 µm thick) on HistoGrip-coated slides were subsequently prepared for immunohistochecmical study. Decorin antibodies were generous gifts from Dr. Larry Fisher (National Institute of Dental Research, National Institutes of Health, Bethesda, MD) and were used at the recommended concentrations (7). Biotinylation second antibody and streptavidin-peroxidase conjugate were used to detect bound antibody. Subsequent addition of aminoethylcarbazole chromogen generated a reddish precipitate surrounding the decorin antigen (Zymed, South San Francisco, CA). Normal rabbit serum, bovine serum albumin, and water were run in parallel with decorin antibodies to yield negative controls.

Densitometric and statistical analysis. Electrophoresis after PCR amplification was performed on 3% agarose gels (NuSieve 3:1, FMC Bioproducts, Rockland, ME) in a submarine gel unit (CBS Scientific, Del Mar, CA), where target and competitor PCR products were separated by size. DNA bands were visualized by staining with 5 µg/ml of ethidium bromide. Images were both photographed by Polaroid 667 films for a permanent record and captured by a computerized scanner for intensity measurement. The intensity was determined by densitometric analysis in pixels with ImageQuant band-analyzing software (Molecular Dynamics, Sunnyvale, CA). Means ± SD were calculated, and the significance of differences between means was evaluated by t-test (criterion for significance is P < 0.05).

RESULTS

Adenovirus-mediated epithelium-specific overexpression of human decorin in mouse lung explants in culture. To assess the effects of decorin on the TGF-β-mediated inhibition of embryonic lung development, we initiated the study by establishing an adenoviral transfer strategy to introduce decorin cDNA into embryonic lungs undergoing branching morphogenesis in culture. A replication-defective adenovirus carrying human decorin cDNA (AdDcn) under the control of a human cytomegalovirus promoter was prepared as described in MATERIALS AND METHODS. Because TGF-β ligands are known to inhibit embryonic lung epithelial branching morphogenesis in culture, intratracheal microinjection of recombinant adenovirus was administered on ED11.5 lung explants before culture.

To determine that the lung explants microinjected intratracheally with recombinant adenovirus express the transgene, cultured lungs microinjected with AdDcn or control virus were extracted for total RNA. The subsequent reverse-transcribed products from embryonic lungs were then used for PCR assays. Only lungs microinjected with AdDcn, not with control virus, expressed exogenous human decorin mRNA (Fig. 2A) when human-specific PCR primers to decorin cDNA were used. To measure the level of decorin transgene overexpression, a set of primers common to both human and mouse decorin were synthesized to anneal to identical sequences on their respective cDNAs. Competi-
tive PCR assays were developed to quantify mouse, human, or total (mouse plus human) decorin mRNA amounts present in the microinjected lungs. The construction of mouse decorin competitive PCR is shown as a paradigm in Fig. 1. Intratracheal microinjection of AdDcn yielded a pfu-dependent transgene overexpression of human decorin mRNA levels, whereas control virus microinjection at corresponding pfu titers resulted in basal levels of mouse decorin mRNA expression (Fig. 2A). However, overexpression of the human decorin transgene did not affect endogenous mouse decorin gene expression in comparison with medium and virus controls (Fig. 2A) as quantified by competitive PCR with a gene-specific primer set to amplify the murine decorin. Thus intratra-
cheal microinjection of AdDcn induced only a pfu titer-dependent overexpression of the exogenous human decorin gene but not of the endogenous mouse counterpart gene. Endogenous levels of other small leucine-rich proteoglycans, including biglycan and fibromodulin, were also not changed by AdDcn infection into embryonic lungs in culture (data not shown). Densitometric analysis of decorin competitive PCR electrophoretic pattern confirmed the virus dose-dependent induction of transgene expression in AdDcn-microinjected lungs (Fig. 2B). A 9- and a 23-fold overexpression of decorin transgene were present, respectively, when embryonic lungs were microinjected with AdDcn at 2 × 10^9 and 2 × 10^10 pfu/ml (P < 0.05), whereas infection with control virus at the above concentrations failed to elevate decorin gene expression. As a result, using the AdDcn-mediated gene transfer approach, we were able to achieve decorin gene overexpression during embryonic lung development in culture.

Decorin immunohistochemistry on cultured embryonic lungs microinjected with recombinant adenovirus was performed to confirm the overproduction of the transgene and localize the exogenous human decorin protein. A human-specific decorin antibody was used to localize cells with transgene expression in lungs infected intratracheally with AdDcn. Exogenous human decorin protein was detected in both proximal (Fig. 2f) and distal (Fig. 2g) airway epithelial cells with high intensities of immunostaining, whereas mesenchymal cells were not stained (Fig. 2c, arrowheads). Human decorin immunoreactivities in both major and terminal bronchial cells indicate that AdDcn microinjected into the tracheal lumen was able to fill the whole respiratory tract, achieving efficient transfection in epithelial cells lining both the proximal and distal airways. However, the above characteristic immunoreactive pattern was no longer observed when mouse-specific decorin antibody replaced human-specific decorin antibody (data not shown), further supporting the notion that exogenous human decorin transgene was overexpressed in lung epithelium when AdDcn was used for microinjection. Additionally, lungs microinjected with control virus yielded only background staining (Fig. 2c).

Thus we conclude that intratracheal microinjection of AdDcn, not of control virus, resulted in overexpression of the exogenous decorin transgene at both the mRNA and protein levels exclusively in the airway epithelium, not in the mesenchyme, in embryonic lungs in culture.

Intratracheal microinjection of AdDcn prevents exogenous TGF-β-mediated inhibition of embryonic lung branching morphogenesis in culture. Embryonic murine lungs (ED11.5) underwent extensive morphogenesis in culture to develop into a characteristic branching pattern (Fig. 3A, a and b). The use of the chemically defined, serumless culture system in the present study enabled us to separate the effects of decorin overexpression from those of hypoxia, reduced placental blood flow, or systemic metabolic effects mediated through the sympathoadrenal axis as well as allowing us to perform the functional blocking and pharmacological experiments in a precisely controlled manner. As Zhao et al. (39) have previously shown, both TGF-β1 (5 ng/ml; Fig. 3Ac) and TGF-β2 (0.1 ng/ml; Fig. 3Ae) inhibit lung branching morphogenesis in culture as quantified by the number of terminal branches, resulting in a hypoplastic phenotype. However, TGF-β-mediated dose-dependent lung branching inhibition was abolished when TGF-β neutralizing antibody was added in addition to the TGF-β ligand in lung culture (Fig. 3B, c and d). Analogous to the function of TGF-β neutralizing antibody, decorin is known to modulate soluble TGF-β by binding to it with high affinity, thereby inactivating TGF-β. Decorin was therefore overexpressed in the lung epithelium with intratracheal microinjection of AdDcn in cultured embryonic lungs. As shown in Fig. 3B, decorin transgene expression in embryonic lungs completely blocked the concentration-dependent inhibition of lung branching morphogenesis by either TGF-β1 or TGF-β2 (a and b, respectively). In lungs microinjected with AdDcn, 20 ng/ml of TGF-β1 failed to significantly inhibit lung branching (94.8% of medium control), whereas TGF-β1 of the same dose significantly decreased lung morphogenesis (64.7% of medium control; P < 0.05). Likewise, TGF-β2 (0.4 ng/ml) failed to alter lung branching in the presence of the decorin transgene (98.9% of medium control), whereas the same dose of TGF-β2 inhibited lung branching morphogenesis when the control virus was used for microinjection (67.3% of medium control; P < 0.05). Thus cultured lungs microinjected with AdDcn were resistant to exogenous TGF-β (Fig. 3A, d for TGF-β1 and f for TGF-β2) in comparison to TGF-β-treated lungs without decorin transgene overexpression in culture (Fig. 3A, c for TGF-β1 and e for TGF-β2). Our observation that administration of either TGF-β neutralizing antibody or decorin transgene overexpression prevented TGF-β-induced inhibition on embryonic lung epithelial branching morphogenesis suggests that decorin acts as an inhibitor of TGF-β ligands in a manner similar to that of TGF-β neutralizing antibodies.

To further examine the biological significance of decorin gene transfer into lung epithelium, various doses of decorin adenovirus were used for embryonic lung intratracheal microinjection (Fig. 4). Both TGF-β1 and TGF-β2 were able to significantly decrease lung branching in the presence of the control virus regardless of viral concentrations. However, a dose-dependent reversal of the TGF-β-mediated negative influence on lung morphogenesis was obtained in lungs microinjected with AdDcn and cultured in the presence of either TGF-β1 (5 ng/ml; Fig. 4, left) or TGF-β2 (0.1 ng/ml; Fig. 4, right). Cultured embryonic lung explants epitheially transfected with AdDcn at 2 × 10^10 pfu/ml showed complete restoration of lung branching morphogenesis from the TGF-β-mediated inhibition regardless of the presence of exogenously added TGF-β1 (95.5%) or TGF-β2 (103.4%). In comparison, both TGF-β1 and TGF-β2 efficiently decreased lung branching morphogenesis in cultured lungs transfected with control virus of the same dose (66.5% for TGF-β1 and 70.2% for
TGF-β2; P < 0.05). Therefore, the above observation that decorin recombinant adenovirus pfu dependently release TGF-β-induced inhibition on lung branching morphogenesis further supports the conclusion that decorin counteracts TGF-β-mediated negative signaling on epithelial branching morphogenesis during early embryonic lung development.

Restoration of TGF-β-mediated lung branching inhibition in AdDcn-microinjected embryonic lungs treated with human decorin antisense ODN. To ascertain the specificity of the effects reported in intratracheal microinjection of AdDcn prevents exogenous TGF-β-mediated inhibition of embryonic lung branching morphogenesis in culture, AdDcn-infected lung explants were treated with an antisense ODN complementary to the human decorin cDNA sequence (28). The results showed a dose-dependent suppression of the steady-state level of human decorin-specific transcripts in AdDcn-treated lungs cultured with human decorin antisense ODN as measured by human decorin competitive PCR (Fig. 5). Antisense ODN specific to human decorin (30 µM) decreased the exogenous decorin mRNA level to a remnant (4.4%) in comparison with medium control-treated AdDcn-infected lungs in culture, whereas either scrambled or sense ODN to human ODN at experimental doses showed no effect on exogenous decorin transgene expression in cultured embryonic lungs. In support of the above results, both endogenous decorin and biglycan mRNA amounts were quantified and confirmed not to be changed in response to human...
decorin ODN treatment in cultured lungs (Fig. 5A). Thus the human decorin antisense ODN markedly and specifically inhibited exogenous transgene expression in lung explants intratracheally microinjected with AdDcn, without negatively affecting endogenous gene expression in lungs in culture.

In the next step, human decorin antisense ODN was used to test the specificity of the effects of decorin transgene-mediated reversal on TGF-β-induced negative regulation of embryonic lung branching morphogenesis. Both TGF-β1 (5 ng/ml) and TGF-β2 (0.1 ng/ml) inhibit lung morphogenesis, as measured by the number of terminal sacs, in cultured lungs microinjected with the control virus regardless of the presence of either sense or antisense human decorin ODN in culture (Fig. 6). Exogenous decorin transgene overexpression in lung epithelium overcame the negative regulation of lung branching with either TGF-β1 or TGF-β2 added exogenously to the culture medium in the presence of either human decorin sense (Fig. 6) or scrambled (data not shown) ODN. In contrast, TGF-β-induced lung epithelial branching inhibition was restored in AdDcn microinjected lungs coadministered with antisense ODN to human decorin in culture (Fig. 5A). Therefore, the observation that TGF-β inhibited lung branching in AdDcn-microinjected lungs treated with human decorin antisense ODN indicates that decorin is specifically and directly involved in TGF-β-signaling-mediated growth control of embryonic lung epithelial branching.

Suppression of both cyclin A and SP-C gene expression by exogenous TGF-β is abolished in cultured embryonic lungs overexpressing decorin. TGF-β-induced growth arrest occurs late in the G1 phase and is accompanied by a reduction in the steady-state level of cyclin A mRNA in mammalian cells. Our data indicate that AdDcn induces refractoriness to TGF-β-induced lung branching inhibition and epithelial growth in lungs in culture. Therefore, cyclin A was used as a marker gene to evaluate the rate of cellular proliferation during embryonic lung development in the present study. As shown in the electrophoretic pattern of cyclin A competitive PCR (Fig. 7), we found that both TGF-β1 and TGF-β2 exert inhibitory effects on cyclin A mRNA expression in cultured lungs regardless of the control virus microinjection and the presence of sense control human decorin ODN. On the other hand, neither TGF-β1 nor TGF-β2 repressed cyclin A gene expression in lungs overexpressing decorin, indicating that decorin
The TGF-β signaling pathway has been implicated in the normal temporospatial pattern of lung morphogenesis and pulmonary-specific gene expression. Signaling molecules including TGF-β ligands, TGF-β receptors, and Smad-2, -3, and -4 have been the focus of studies to elucidate the functional significance of TGF-β signaling during normal lung development and pathogenesis (30, 39, 40, 42). However, little is known about the biological role of the TGF-β-interacting proteoglycans during lung growth and injury. Therefore, this is the first report to demonstrate that decorin can act as a TGF-β antagonist to attenuate exogenous TGF-β signaling during lung morphogenesis in culture, establishing the rationale that decorin, a naturally occurring TGF-β inhibitor, may be potentially useful in ameliorating TGF-β overproduction during lung growth, injury, and repair. Using a gene transfer approach in embryonic mouse lungs in culture, we discovered that decorin overexpression released the lungs from exogenous TGF-β-mediated branching inhibition to regain a normal phenotype of branching morphogenesis. Additionally, decorin transgene expression in the lung epithelium prevented both the TGF-β-induced antiproliferative effect and the downregulation of SP gene expression. The experimental evidence we present herein suggests that decorin might be a therapeutically useful component of a negative loop that can attenuate excessive TGF-β signaling during lung growth and development.

Decorin is capable of binding to TGF-β ligand with high affinity, resulting in the formation of inactive TGF-β-decorin complexes (12). Decorin therefore acts in an analogous manner to TGF-β neutralizing antibodies. In support of this notion, we showed that both decorin overexpression and the addition of TGF-β neutralizing antibodies are effective in blocking TGF-β-mediated inhibition of lung branching morphogenesis in culture. TGF-β is known to induce G1/S phase cell cycle arrest in lung epithelial cells as demonstrated by the reduction of cyclin A gene expression. Formation of either decorin-TGF-β or TGF-β-antigen-antibody complexes apparently inactivates TGF-β and therefore results in the release of TGF-β-induced pulmonary epithelial cell cycle arrest. However, unlike TGF-β antibodies, decorin is a naturally occurring inhibitor of TGF-β bioactivity. Decorin can also be overproduced at high levels in lung tissue with recombinant adenovirus-mediated gene transfer as presented in the present study. Excessive TGF-β signaling has been implicated in lung diseases such as BPD. A reduction in decorin mRNA levels in the whole lung and decreased decorin immunoreactivity have been detected in lungs developing hyperoxic injury (34).

Increased TGF-β production appears to be characteristic of several fibrotic diseases including hepatic cirrhosis, pulmonary fibrosis, and glomerular sclerosis. Administration of decorin protein inhibits the increased production of extracellular matrix and attenuates glomerular sclerosis in a rat model of glomerulonephritis (4). Gene transfer of decorin cDNA also increased the
decorin protein present in the kidney where it markedly ameliorated kidney fibrosis with a simultaneous reduction in the expression level of TGF-\(\beta\)1 in the renal glomeruli (17). In bleomycin-induced lung fibrosis, neutralization of TGF-\(\beta\) by systemic treatment with its antibodies reduced lung collagen accumulation (9). Likewise, decorin has been demonstrated to be as effective as TGF-\(\beta\) neutralizing antibodies in exerting an antifibrotic effect, including downregulation of matrix protein expression in a bleomycin hamster model of lung fibrosis (8). Therefore, decorin offers a novel rationale for therapeutic intervention that may be useful in treating fibrotic lung diseases associated with an overproduction of TGF-\(\beta\).

Decorin is a secreted protein that is deposited in the lung interstitium as localized by decorin immunohistochemistry in the present study. Decorin, like other leucine-rich proteoglycans, is a connective tissue organizer, orienting and ordering collagen fibrils by binding with collagen proteins, thereby establishing the exact topology of fibrillar collagenens in tissue. Besides TGF-\(\beta\), decorin can also bind to a variety of adhesive and nonadhesive proteins including fibronectin, thrombospondin, and various types of collagens (16). In null mutant animals, disruption of the decorin gene leads to skin fragility and abnormal collagen morphology, characterized by uncontrolled lateral fusion of fibrils (5). Being capable of simultaneously binding to both TGF-\(\beta\) ligands and collagen fibrils, decorin is able to tether TGF-\(\beta\) onto the interstitial tissue matrix, resulting in sequestration of TGF-\(\beta\) ligands. Therefore, we postulate that ectopically overexpressed decorin in lung epithelium after intratracheal microinjection of recombinant decorin adenovirus forms a steric barrier through formation of bioactive TGF-\(\beta\)-decorin heterodimers to prevent soluble TGF-\(\beta\) from accessing TGF-\(\beta\)-receptor complexes and thereby abolishes the negative effect of exogenous TGF-\(\beta\) signaling on lung growth and development.

Decorin antisense ODN experiments performed herein were designed to demonstrate that the observed refractoriness to exogenous TGF-\(\beta\)-mediated lung growth inhibition after decorin adenovirus injection was due directly and specifically to decorin overexpression in lung tissue. We also showed that antisense ODN to human decorin effectively suppresses exogenous decorin gene expression without affecting endogenous murine decorin gene expression in mouse lung explants transfected with AdDcn (14). Our results that the antisense ODN to human decorin restored exogenous TGF-\(\beta\)-mediated epithelial branching inhibition in AdDcn-infected lung cells or tissue indicate that decorin exerts specific anti-TGF-\(\beta\) effects during lung development. In support of the above conclusion, the expression levels of other related small leucine-rich proteoglycans including biglycan and fibromodulin were unaltered regardless of the presence of either decorin transgene or decorin antisense ODN. Thus it is decorin, not other related TGF-\(\beta\)-interaction proteoglycans, that is involved in antagonizing exogenous TGF-\(\beta\)-mediated negative regulation of lung growth and differentiation.

In addition to decorin, TGF-\(\beta\)-interacting proteins and proteoglycans such as betaglycan (TGF-\(\beta\)-type III receptor), biglycan, fibromodulin, and endoglin may also be implicated in the negative regulation of TGF-\(\beta\) signaling and therefore may affect key aspects of pulmonary morphogenesis and injury. Betaglycan is a TGF-\(\beta\)-ligand presenter that physically associates with TGF-\(\beta\)-type II receptor. Betaglycan increases the affinity between TGF-\(\beta\)-ligand and TGF-\(\beta\)-type II receptor (41). Endoglin is a dimeric TGF-\(\beta\)-1 and TGF-\(\beta\)-3 binding protein of endothelial cells that modulates cellular responses to TGF-\(\beta\)-signaling and can form heteromeric signaling complexes with TGF-\(\beta\)-type II and type I receptors (22). The endoglin gene is mutated in Osler-Weber-Rendu hereditary telangiectasia type 1, a condition that is characterized by large intrapulmonary arteriovenous malformations (26). Although we provide evidence of a functional role for decorin as a natural TGF-\(\beta\) inhibitor that can counteract increased TGF-\(\beta\)-signaling during lung morphogenesis, injury, and repair, the biological significance of other related TGF-\(\beta\)-associated proteoglycans remains to be elucidated.

Using an adenovector-mediated gene transfer strategy, we show the promise of decorin as a potential treatment or preventive measure for TGF-\(\beta\)-mediated lung diseases through decorin gene therapy. Intra-tracheal microinjection of recombinant adenovirus to introduce transgene expression may be of medical significance because of its simplicity, apparent safety, and lack of toxicity (1). The replication-deficient adenovirus has a wide spectrum of host cell range, including cells in both normal and pathological states. The adenovector offers a high efficiency of exogenous gene delivery and resultant transgene expression. The replication-defective virus is incapable of integrating into the host genome and thus does not negatively impact on intrinsic host cell function. Using the decorin recombinant adenovirus, we have shown the feasibility of achieving high expression of the decorin transgene in lung epithelial cells during embryonic lung development. The present report therefore supports the hypothesis that decorin, as a naturally occurring inhibitor of excessive TGF-\(\beta\) activity, may be particularly useful in designing novel strategies to treat lung diseases due to aberrant TGF-\(\beta\) production.

We conclude that decorin can play an important functional role in regulating TGF-\(\beta\) signaling during lung growth, differentiation, and development through sequestering bioactive TGF-\(\beta\) ligands. Decorin, as a naturally occurring biological molecule that antagonizes TGF-\(\beta\) bioactivity, may be potentially useful in designing new rational therapeutic strategies to ameliorate excessive TGF-\(\beta\) signaling in injured lungs. It is possible that decorin may, together with other proteoglycans, orchestrate TGF-\(\beta\)-mediated signaling on cellular growth, differentiation, and gene expression during lung development. We speculate that finely regulated TGF-\(\beta\) signaling through decorin may serve to modulate and therefore balance the positive functions of signaling by other peptide growth factor pathways,
including epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, and vascular endothelial growth factor, which exert positive and permissive influences on lung development.

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