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High Tidal Volume Ventilation Activates Smad2 and Upregulates Expression of Connective Tissue Growth Factor in Newborn Rat Lung

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ABSTRACT: High tidal volume (V_T) ventilation plays a key role in ventilator induced lung injury and bronchopulmonary dysplasia. However, little is known about the effect of high V_T on expression of growth factors that are critical to lung development. In a previous study, we demonstrated that connective tissue growth factor (CTGF) inhibits branching morphogenesis. In this study, we investigated the effect of high V_T on CTGF expression in newborn rat lungs. Newborn rats were ventilated with normal V_T (10 mL/kg) or high V_T (25 mL/kg) for 6 h. Nonventilated animals served as controls. We found that high V_T upregulated CTGF expression. To identify the potential signaling pathways mediating high V_T induction of CTGF, newborn rats were ventilated with high V_T for 1 or 3 h. Temporal expression of TGF-βs, p-Smad2, Smad7, and CTGF was analyzed. High V_T ventilation did not change gene expression of TGF-βs and Smad7 but induced rapid and sustained expression of p-Smad2 that precedes increased CTGF expression. CTGF and p-Smad2 were localized in bronchiolar epithelial cells, alveolar walls and septa. These data suggest that high V_T ventilation activates the Smad2 pathway, which may be responsible for downstream induction of CTGF expression in newborn rat lungs. **(*Pediatr Res* 63: 245–250, 2008)**

Mechanical ventilation is essential for managing prematurely born infants with respiratory failure. However, ventilation with high tidal volume (V_T) can lead to ventilator induced lung injury (VILI) and bronchopulmonary dysplasia (BPD) (1–3). The lung pathology of BPD is characterized by fewer and larger alveoli, dysmorphic and decreased capillary network, and variable interstitial fibrosis, suggesting abnormal lung development and injury repair processes (4,5). Most of the studies in VILI and BPD have been focused on high V_T induced lung proinflammatory response (6–9). Little is known about the effect of high V_T on expression of growth factors that are key to lung development and injury repair in neonatal lungs.

Connective tissue growth factor (CTGF) belongs to the CCN family of early gene products with a high degree of amino acid sequence homology and 38 conserved cysteine residues (10,11). CTGF promotes fibroblast proliferation, extracellular matrix (ECM) production, myofibroblast differentiation, and cell adhesion and migration (12–16). CTGF is a potent profibrotic cytokine and its mRNA and protein levels have been correlated with the degree of lung fibrosis in bleomycin-treated mice and in human fibrotic lung disorders (17–19). Previous studies have demonstrated that mechanical stress drastically induces CTGF expression in cultured fibroblasts (20,21). CTGF is closely linked to TGF-β. Studies have demonstrated that TGF-β is a major inducer of CTGF expression in a variety of tissues and organs including the lung (11,22–24). Activation of the Smad pathway plays a key role in TGF-β induction of CTGF expression (25,26). TGF-β stimulation of fibroblast proliferation, collagen synthesis, and myofibroblast differentiation is mediated *via* a CTGF-dependent pathway (13–15). Recent data suggest that CTGF plays a role in lung development and neonatal lung injury. Using an embryonic lung explant model, we have demonstrated that endogenous expression of CTGF and TGF-β induced CTGF expression is mediated *via* the Smad2 pathway (27). Furthermore, addition of recombinant CTGF into the culture medium inhibited branching morphogenesis (27). A recent study in a neonatal rat model showed that hyperoxia exposure increased CTGF mRNA and protein expression, which precedes the fibrotic phase of lung injury (28). These data suggest that CTGF is a negative regulator for lung morphogenesis and may play a role in hyperoxia-induced lung injury and fibrosis in the neonatal lung. However, the role of CTGF in high V_T induced lung injury in neonates is unknown.

We hypothesized that high V_T ventilation induces CTGF expression in newborn rat lungs. To test this hypothesis, newborn rats were ventilated with normal or high V_T. CTGF mRNA and protein expression were evaluated in the lungs of these animals and compared with control nonventilated animals. Temporal expression of TGF-βs, phosphorylated Smad2 (p-Smad2), a key intracellular transducer of TGF-β responses,

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Abbreviations: BPD, bronchopulmonary dysplasia; **CTGF,** connective tissue growth factor; **ECM,** extracellular matrix; **E_TCO_2,** end-tidal CO_2; **GAPDH,** glyceraldehyde-3-phosphate dehydrogenase; **HE,** hematoxylin and eosin; **PEEP,** positive end-expiratory pressure; **PIP,** peak inspiratory pressure; **p-Smad2,** phosphorylated Smad2; **VILI,** ventilator induced lung injury; V_T, tidal volume

and Smad7, a inhibitory Smad was also investigated to identify the potential signal transduction pathway mediating high V_T induced CTGF expression in newborn rat lungs.

MATERIALS AND METHODS

Animal preparation and ventilator protocol. The animal protocol was approved by the Animal Care and Use Committee of the University of Miami School of Medicine. Newborn Sprague–Dawley rats (7–14 d old) were anesthetized by intraperitoneal injection of ketamine (40 mg/kg) and xylazine (4 mg/kg) and tracheotomized. To compare the effects of normal V_T and high V_T on expression of CTGF, animals were randomly assigned to one of the following three groups:

Group 1: control (no-ventilation)
Group 2: ventilation with normal V_T (10 mL/kg) for 6 h
Group 3: ventilation with high V_T (25 mL/kg) for 6 h

The normal V_T and high V_T were based on a recent study of VILI in newborn rats (9). To determine the potential signal transduction pathways mediating CTGF expression, two additional groups of animals were studied:

Group 4: ventilation with high V_T (25 mL/kg) for 1 h
Group 5: ventilation with high V_T (25 mL/kg) for 3 h

The animals were ventilated using a time-cycled ventilator (Sechrist Infant Ventilator model iv-100B, Anaheim, CA) with positive end-expiratory pressure (PEEP) of 3 cm H_2O, inspiratory time of 0.2 s, and 40% oxygen (O_2). Airflow was measured by a heated pneumotachograph (Model 8430B, flow range 0–3 L/min, linearity 0.2%, Hans Rudolph, Inc. Kansas City, MI) placed between the tracheostomy tube and the ventilator circuit. The differential pressure output from the pneumotachograph was measured with a transducer (MP45, Validyne Engineering Co., Northridge, CA) and amplified by a pressure amplifier (Gould Instrument, Cleveland, OH). The flow signal was electronically integrated to obtain V_T as described before (29). The peak inspiratory pressure (PIP) was adjusted to maintain a V_T in the desired range. The end-tidal CO_2 (E_TCO_2) was measured intermittently (every 30 min) by a Micro-Capnometer (Columbus Instrument, Columbus, OH) *via* the side port of the tracheostomy tube and was maintained at the same level for normal and high V_T groups by adjusting the ventilator rate and dead space. Heart rate, airflow, V_T, PIP, and PEEP were monitored continuously throughout the experiments. Body temperature was continuously monitored with a rectal thermal probe (Yellow Springs Instrument Co., Yellow Springs, OH), and maintained within the normal range for age (35.8–36.2°C) by using a servo-controlled radiant warmer. Control animals breathed air and received anesthesia and tracheotomy as described before euthanasia. At the end of the experiment, animals were euthanized with an overdose of pentobarbital and lungs were removed for subsequent analysis.

Lung histology. The left lungs from control and 6 h ventilated animals were infused with 4% paraformaldehyde *via* a tracheal catheter under 20 cm H_2O pressure and fixed overnight at 4°C. Fixed lung tissues were paraffin-embedded and 5 μm sections were stained by the standard hematoxylin and eosin (HE) method. Lung histology was examined by a pathologist unaware of the experimental conditions.

RNA isolation and quantitative real-time (RT)-PCR. Total RNA was isolated from frozen right lung tissue using Trizol reagent (Invitrogen, Carsbad, CA) and then treated with DNase to remove possible DNA contamination. Two micrograms of total RNA was reverse-transcribed in a 20 μL reaction by using a first-strand cDNA synthesis kit according to the manufacturer's protocol (Invitrogen, Carsbad, CA). The quantitative real-time RT-PCR was performed on a Light-cycler (Roche, Indianapolis, IN). Each reaction included diluted first-strand cDNA, rat IL-6, TNF-α, CTGF, TGF-β1, TGF-β2, TGF-β3, or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers, and RT^2 Real-Time PCR SYBR Green master mix according to the manufacturer's instructions (Superarray, Frederick, MD). Real-time RT-PCR conditions were 95°C for 15 min, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. RNase-free water was used as a negative control. For each target gene, a standard curve was established by performing a series dilution of first-strand cDNA. The mRNA expression levels of these factors were determined from the standard curve and normalized to GAPDH.

Northern blot analysis. Twenty microgram of total RNA from each lung sample was electrophoresed on 1.2% agarose gel containing formaldehyde and transferred to a nylon membrane. A 1 kb human CTGF cDNA fragment that has been cloned into the pRc/CMV plasmid was used to generate radiolabeled CTGF probe. A 497 bp mouse GAPDH cDNA was prepared by RT-PCR using primers 5′-ACCACAGTCCATGCCATCAC-3′ (position 593–613) and 5′-TCCACCACCCTGTTGATGTA-3′ (position 1090–1110). The CTGF and GAPDH cDNAs were radiolabeled with [^{32}P]dCTP using the DNA labeling kit and purified using Quick Spin Columns (Amersham Biosciences, Piscataway, NJ). The hybridization was first carried out with [^{32}P]-labeled CTGF probe, then stripped and rehybridized with [^{32}P]-labeled GAPDH probe as described (30). The intensities of mRNA bands on the autoradiography were quantified by Quantity One Imaging Analysis Program (Bio-Rad, Richmond, CA). The CTGF mRNA levels were determined after normalization to GAPDH.

Western blot analysis. Total protein was extracted from frozen right lung tissues with a lysis buffer from Active Motif according to the manufacturer's protocol (Carsbad, CA). The protein concentrations were measured by BCA protein assay using commercial kits from Pierce Biotechnology Inc (Rockford, IL). Seventy-five micrograms of total protein was fractionated by SDS-PAGE on a 10% Tris-glycine precast gel (Bio-Rad) and then transferred to a nitrocellulose membrane (Amersham). The membrane was incubated with a primary antibody for CTGF (1:1000 dilution), total Smad2/3 (1:500 dilution), Smad7 (1:500) (Santa Cruz Biotechnology Inc, Santa Cruz, CA), p-Smad2 (1:500 dilution) (Chemicon, Temecula, CA) or 1:10000 diluted mouse anti-β-actin antibody (Sigma Chemical Co., St. Louis, MO) overnight at 4°C, then incubated for 1 h at room temperature with the respective HRP-conjugated secondary antibodies. Antibody bond proteins were detected using ECL chemiluminescence method (Amersham). The intensities of protein bands were quantified by Quantity One Imaging Analysis Program. The CTGF and Smad7 levels were determined after normalization to β-actin and p-Smad2 levels were normalized to total Smad2/3.

Immunohistochemistry. Localization of CTGF, p-Smad2, or total Smad2/3 protein expression was determined by immunostaining with a rabbit CTGF antibody (Torrey Pines Biolabs, Huston, TX), a rabbit p-Smad2 antibody (Chemicon) or a goat total Smad2/3 antibody (Santa Cruz). The dilution for all three antibodies was 1:1000. These antibodies are specific and have been used in many published studies (31,32). Nonimmune IgG was used as the negative control. The antibody bond positive cells were detected with a biotinylated secondary antibody, streptavidin–biotin-peroxidase complexes and DAB substrate (Vector, Burlingame, CA).

Statistical analysis. Data are presented as mean ± SD. Comparisons were performed using one-way ANOVA followed by the Student-Newman-Keuls test. A p value of less than 0.05 was considered significant.

RESULTS

Physiologic data. As shown in Table 1, the control rats and 6-h ventilated animals had similar age and weight. As expected, the PIP for the high V_T group was significantly higher than the PIP for the normal V_T group. The E_TCO_2 was in the same range for both normal and high V_T ventilated groups.

Lung histology. The most striking finding was severe overdistension of the distal airspaces and thinning of the alveolar septum seen in the high V_T ventilated lungs (Fig. 1). The normal V_T lungs appeared similar to the control lungs.

Expression of IL-6 and TNF-α mRNA. Ventilation with normal or high V_T for 6 h did not change mRNA expression of TNF-α compared with the control group (data not shown). However, high V_T ventilation resulted in near threefold increase in IL-6 mRNA expression (3.12 ± 1.72) compared with the control group (1.11 ± 0.42) and normal V_T group (1.45 ± 0.5) ($p = 0.011$).

Expression of CTGF mRNA. When compared with the control group, ventilation with normal V_T for 6 h did not affect

Table 1. *Physiological data*

	Control	Normal V_T	High V_T
Age (d)	10 ± 1.8	11.2 ± 2.1	10 ± 1.4
Weight (g)	23.1 ± 5.6	26.1 ± 8.1	21.5 ± 5.6
PIP (cm H_2O)		12.0 ± 0.99	17.5 ± 3.45*
E_TCO_2 (mm Hg)		40.3 ± 0.81	39.5 ± 2.31

Data are mean ± SD from seven animals per group.
* $p < 0.002$ high V_T *vs.* normal V_T.

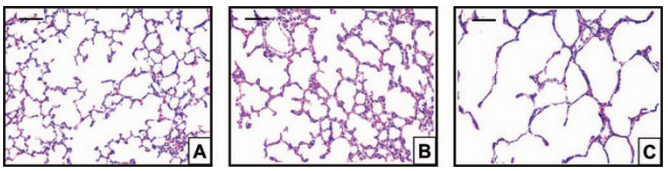

Figure 1. Histology of control, normal, and high V_T ventilated lungs. Lung tissue sections from control (*A*), normal V_T (*B*), and high V_T (*C*) ventilation for 6 h were stained by standard H&E method. The histology of normal V_T lungs was similar to that of controls. Severe alveolar over-distension and thinning of the septa were noticed in the high V_T ventilated lungs. Images were taken under 200× magnifications. Bars = 40 μm.

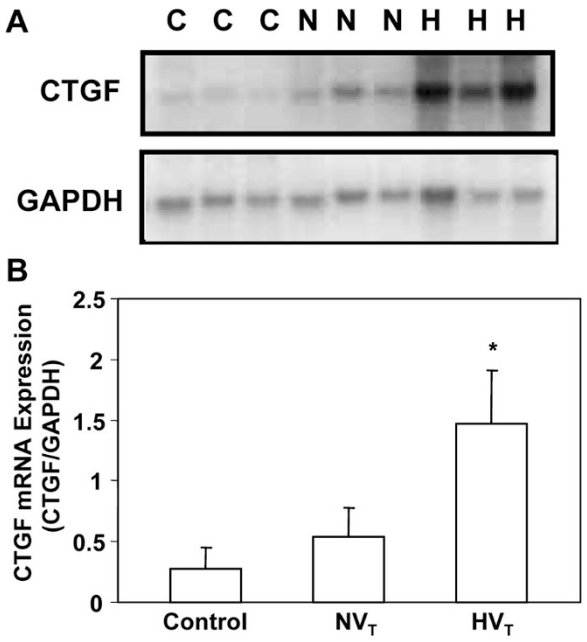

Figure 2. Induction of CTGF mRNA expression by mechanical ventilation is V_T-dependent. *A*, Representative autoradiographs from Northern blot analysis. C: control; N: normal V_T for 6 h; H: high V_T for 6 h. *B*, Densitometric quantification of Northern blot analysis. Data are mean ± SD from seven animals in each group. Increased CTGF mRNA was detected in high V_T ventilated lungs. *$p < 0.001$ compared with control and normal V_T ventilation.

CTGF mRNA expression. However, ventilation with high V_T for 6 h significantly upregulated CTGF mRNA expression compared with both control and normal V_T groups (Fig. 2).

Expression of CTGF protein. Consistent with CTGF mRNA expression, normal V_T ventilation for 6 h did not change CTGF protein expression compared with the control group. However, high V_T ventilation for 6 h significantly increased CTGF protein expression compared with both control and normal V_T groups (Fig. 3).

Localization of CTGF protein expression. As shown in Fig. 4, CTGF protein was sparsely detected with moderate intensity in alveolar walls and septa, and weak intensity in bronchiolar epithelial cells in control and normal V_T ventilated lungs (Fig. 4*A*,*B*). However, CTGF protein was abundantly detected with strong intensity in alveolar walls and septa, and bronchiolar epithelial cells in high V_T ventilated lungs (Fig. 4*C*).

Temporal effect of high V_T on mRNA expression of TGF-β1, TGF-β2, TGF-β3, and CTGF. There was no significant difference in mRNA expression of the three TGF-βs among control and the groups ventilated with high V_T (Table 2). High

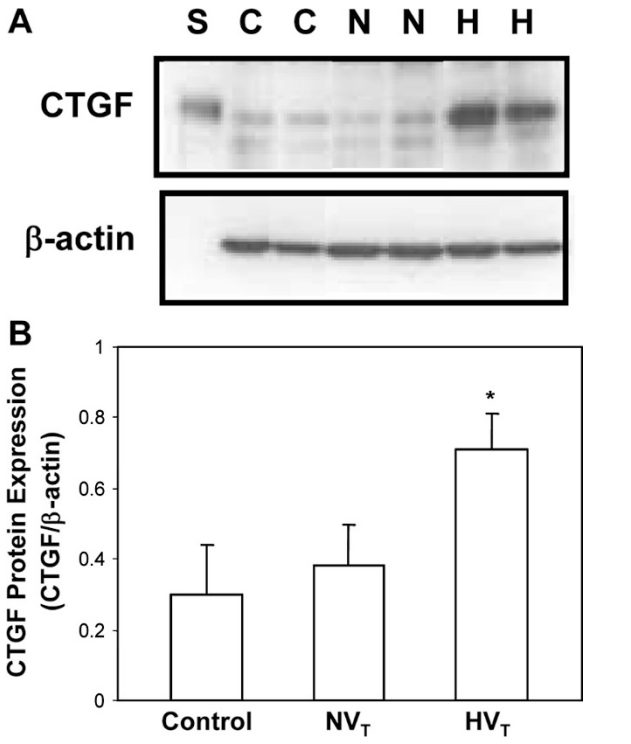

Figure 3. High V_T ventilation upregulates CTGF protein expression. CTGF protein was detected by Western blot with a CTGF specific antibody in control, normal V_T and high V_T ventilated lungs (6 h) and normalized by β-actin. *A*, Representative Western blot images. S: CTGF standard. *B*, Densitometric quantification of Western blot analysis. Results are mean ± SD from control ($n = 6$), normal V_T ($n = 5$) and high V_T ($n = 4$) groups. High V_T increased CTGF protein expression compared with both control and normal V_T group. *$p < 0.001$.

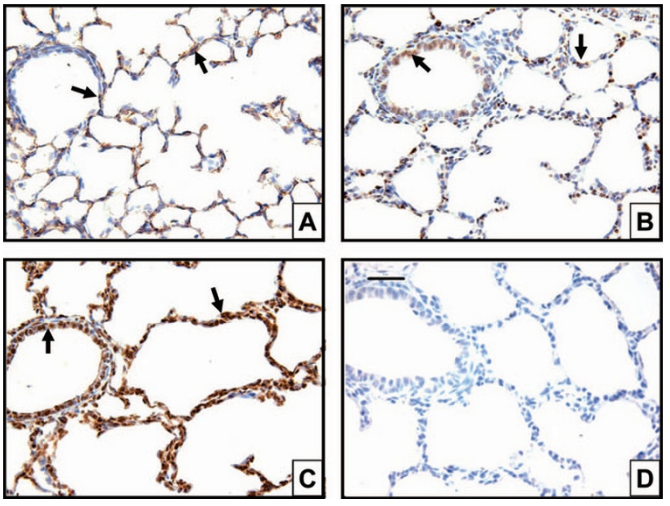

Figure 4. Localization of CTGF protein by immunohistochemistry. Lung tissue sections were immunostained with a CTGF specific antibody. CTGF positive cells (brown color) are indicated by arrows. *A*, control; *B*, normal V_T ventilation (6 h); *C*, high V_T ventilation (6 h); *D*, negative control stained with nonimmune IgG. CTGF was detected with stronger intensity and more abundance in high V_T ventilated lungs when compared with control and normal V_T ventilated lungs. Images were taken under 400× magnifications. Bar = 20 μm.

V_T ventilation for 1 or 3 h did not change CTGF mRNA expression, but increased CTGF mRNA expression at 6 h (Table 2) which was also demonstrated by Northern blot analysis described above (Fig. 2). Active TGF-β1 was mea-

Table 2. *Gene expression of TGF-β1, TGF-β2, TGF-β3 and CTGF*

		Ventilation duration (h)		
Gene	Control	1	3	6
TGF-β1	0.016 ± 0.011	0.017 ± 0.006	0.015 ± 0.001	0.013 ± 0.007
TGF-β2	0.024 ± 0.009	0.013 ± 0.005	0.014 ± 0.011	0.019 ± 0.004
TGF-β3	0.022 ± 0.011	0.017 ± 0.003	0.018 ± 0.007	0.019 ± 0.010
CTGF	0.047 ± 0.015	0.080 ± 0.003	0.078 ± 0.001	0.220 ± 0.012*†

mRNA levels were determined by real-time RT-PCR and normalized by GAPDH. Data are mean ± SD from six animals per group.

* $p < 0.001$ compared with control group.

† $p < 0.002$ compared with 1 and 3 h ventilated groups.

sured by ELISA (without activation) in protein extracts from control and high V_T ventilated lungs and there was no difference among the groups (data not shown).

Expression of Smad7 and p-Samd2 in response to high V_T ventilation. Western blot analysis revealed that high V_T ventilation did not change Smad7 expression at any time point (control: 0.45 ± 0.18; 1 h: 0.48 ± 0.07; 3 h: 0.51 ± 0.07; 6 h: 0.35 ± 0.13, $p = 0.37$). In contrast, high V_T ventilation induced rapid and sustained p-Smad2 expression. As demonstrated in Fig. 5, high V_T significantly induced p-Smad2 expression as early as 1 h and this lasted for up to 6 h. These results suggest that activation of Smad2 precedes CTGF induction by high V_T. On immunohistochemistry, p-Smad2 was detected with weak intensity in alveolar walls and septa and bronchiolar epithelial cells in control lungs (Fig. 6*A*). However, p-Smad2 was detected with strong intensity in alveolar walls and septa, and bronchiolar epithelial cells in 6 h high V_T ventilated lungs (Fig. 6*C*). In contrast, total Smad-2 was detected with similar intensity in both control (Fig. 6*B*) and high V_T (Fig. 6*D*) ventilated lungs. The localization of p-Smad2 was similar to that of CTGF.

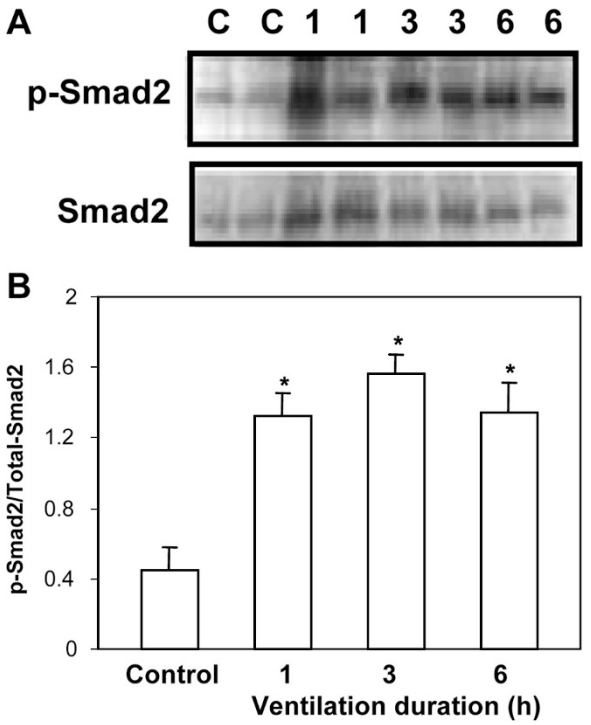

Figure 5. High V_T upregulates p-Smad2 expression. Expression of p-Smad2 was determined by Western blot analysis using a p-Smad2 specific antibody and normalized by total Smad2 in high V_T ventilated lungs. *A*, Representative Western blot images. Ventilation durations (h) are indicated on the top of the images. *B*, Densitometric quantification of Western blot analysis. Results are mean ± SD from four animals in each group. High V_T ventilation increased p-Smad2 expression from 1 to 6 h compared with control groups. *$p < 0.001$.

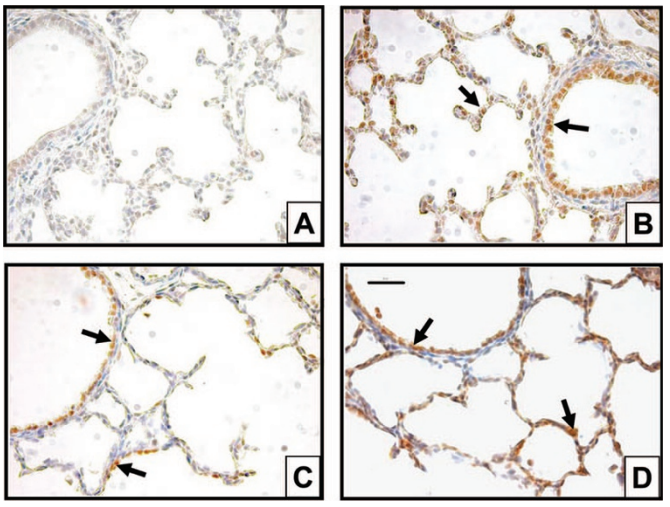

Figure 6. Localization of p-Smad2. Lung tissue sections were stained with a p-Smad2 or a total Smad2 specific antibody. Positive cells for p-Smad2 and total Smad2 (brown color) are indicated by arrows. *A*, control stained with a p-Samd2 antibody; *B*, control stained with a total Smad2 antibody; *C*, high V_T ventilated lung stained with a p-Smad2 antibody; *D*, high V_T ventilated lung stained with a total Smad2 antibody. P-Smad2 was strongly detected in bronchiolar epithelial cells and alveolar walls and septa in high V_T ventilated lungs. Images were taken under 400× magnifications. Bar = 20 μm.

DISCUSSION

The data of this study demonstrate that high V_T ventilation results in alveolar over-distension and upregulation of CTGF expression in newborn rat lungs. High V_T ventilation also induces rapid and sustained p-Smad2 expression that precedes CTGF induction. To the best of our knowledge, this is the first report to demonstrate that high V_T ventilation is associated with activation of the Smad2 pathway and induction of CTGF expression in an *in vivo* neonatal animal model.

Despite the fact that aggressive ventilation is a major contributor in the pathogenesis of BPD, little is known about the molecular basis of volume induced lung injury in the immature lung. Although many studies in volume induced lung injury and BPD have been conducted in large animal models such as baboons and lambs, these animals are difficult to maintain and expensive (33,34). The lung developmental stage of premature infants at risk for BPD is at the prealveolar stage, *i.e.*, later canalicular and saccular stages (35,36). The lungs of newborn rats are at the saccular stage that rapidly progresses to the alveolar stage in the first 2 weeks after birth (37,38). Although the newborn rat lung is not surfactant deficient, its structure closely resembles the lung structure of premature infants at risk for BPD. We ventilated newborn rats (7–14 d) to investigate the effect of high V_T on lung expression of CTGF mRNA and protein. Consistent with our hypothesis, high V_T ventilation dramatically upregulated expression of

CTGF mRNA and protein in newborn rat lung. *In vitro* studies have demonstrated that the CTGF gene is one of the most striking genes induced by mechanical stress (20,21). CTGF is a multifunctional cytokine that promotes fibroblast proliferation, ECM production, and myofibroblast differentiation (13–15). Overexpression of CTGF is associated with pulmonary fibrosis in adult human and animal models (17–19). Our data suggest that high V_T induced CTGF expression may cause profibrotic responses in the neonatal lung.

Previous studies have indicated that TGF-β is a key inducer of CTGF expression in a variety of tissues and organs (11,22–24). In the present study, we took a step further to identify the potential upstream signal transduction pathway that mediates high V_T induced CTGF expression by focusing on the TGF-β and Smad2 axis. TGF-β is a family of closely related peptides including TGF-β1, TGF-β2, and TGF-β3. TGF-βs initiate their action by binding to the TGF-β receptors on the cell membrane and phosphorylate Smad2/3. The p-Smad2/3 forms a complex with Smad4, translocates to the nucleus and binds to the target gene promoters to activate gene transcription (39). Smad7 is an inducible intracellular inhibitor that decreases Smad2/3 phosphorylation by blocking their access to TGF-β receptors (39). Studies have indicated that TGF-β activated Smad2/3 signal transduction pathway plays a key role in TGF-β induction of CTGF expression (25,26). We have previously shown that TGF-β induces CTGF expression *via* the Smad2-dependent pathway during early embryonic lung development (27). In the present study, we examined the temporal relationship of Smad7, TGF-βs, Smad2, and CTGF in response to high V_T ventilation in newborn rat lungs. We demonstrated that high V_T induction of CTGF expression is independent of decreased inhibitory Smad7. Although mRNA expression of TGF-βs and active TGF-β1 were not altered by high V_T at any time point, rapid and sustained expression of p-Smad2 was induced by high V_T and that preceded CTGF mRNA and protein expression. We also showed that p-Smad2 and CTGF are expressed in similar locations. These data demonstrate a temporal and spatial association of p-Smad2 and CTGF expression suggesting that high V_T ventilation may activate the Smad2 signal transduction pathway which in turn results in upregulation of CTGF expression. Further studies are needed to determine whether high V_T activation of the Smad2 pathway is TGF-β-dependent or TGF-β-independent and if Smad2 activation mediates CTGF induction.

The present study was not designed to evaluate the effect of O_2 concentration on CTGF expression. All animals were ventilated with 40% O_2 which is necessary to keep their hemodynamic function stable during the 6 h experiments. The fact that the animals ventilated with normal V_T did not exhibit any change in lung histology or CTGF expression suggests that the increased CTGF was caused by high V_T and not by O_2.

We did not measure arterial blood gases due to the difficulty to obtain sufficient blood for such analysis. However, we closely monitored the E_TCO_2, which was kept in the same range between the normal V_T and high V_T groups by adjusting the dead space and ventilator rates. This eliminated the possible effect of different $PaCO_2$ and pH on the results.

TNF-α is known for its ability to inhibit the TGF-β dependent Smad2/3 pathway and CTGF expression (40). A recent study has demonstrated that antenatal endotoxin exposure increases expression of TGF-β and p-Smad2 but decreases CTGF expression in lungs of preterm lambs (41). The decreased CTGF expression was explained by the concurrent elevation of TNF-α. In the present study, high V_T ventilation did not increase TNF-α expression. This may explain why high V_T ventilation induced significant expression of p-Smad2 and CTGF in newborn rat lungs.

Our model did not intend to identify the downstream CTGF effects in response to high V_T because of the relatively short duration of ventilation. Recent data from our laboratory indicate that CTGF is a negative regulator of lung branching morphogenesis (27). CTGF is associated with TGF-β induced fibroblast to myofibroblast differentiation that may interfere with secondary septal formation and alveolar development (15). In addition, CTGF is also a profibrotic cytokine that may result in interstitial fibrosis (13,14). We speculate that prolonged overexpression of CTGF in the alveolar septa may inhibit alveolar development, produce interstitial fibrosis and result in BPD-like architecture in the immature lung.

In conclusion, the present study demonstrates that high V_T ventilation activates the Smad2 pathway and induces CTGF expression in newborn rat lungs. These results suggest an important role of this pathway in high V_T induced lung injury in neonates.

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