Early biomarkers and potential mediators of ventilation-induced lung injury in very preterm lambs

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Abstract

Background: Bronchopulmonary dysplasia (BPD) is closely associated with ventilator-induced lung injury (VILI) in very preterm infants. The greatest risk of VILI may be in the immediate period after birth, when the lungs are surfactant deficient, still partially filled with liquid and not uniformly aerated. However, there have been very few studies that have examined this immediate post-birth period and identified the initial injury-related pathways that are activated. We aimed to determine if the early response genes; connective tissue growth factor (CTGF), cysteine rich-61 (CYR61) and early growth response 1 (EGR1), were rapidly induced by VILI in preterm lambs and whether ventilation with different tidal volumes caused different inflammatory cytokine and early response gene expression.

Methods: To identify early markers of VILI, preterm lambs (132 d gestational age; GA, term ~147 d) were resuscitated with an injurious ventilation strategy (VT 20 mL/kg for 15 min) then gently ventilated (5 mL/kg) for 15, 30, 60 or 120 min (n = 4 in each). To determine if early response genes and inflammatory cytokines were differentially regulated by different ventilation strategies, separate groups of preterm lambs (125 d GA; n = 5 in each) were ventilated from birth with a VT of 5 (VG5) or 10 mL/kg (VG10) for 135 minutes. Lung gene expression levels were compared to levels prior to ventilation in age-matched control fetuses.

Results: CTGF, CYR61 and EGR1 lung mRNA levels were increased ~25, 50 and 120-fold respectively (p < 0.05), within 30 minutes of injurious ventilation. VG5 and VG10 caused significant increases in CTGF, CYR61, EGR1, IL-1, -6 and IL-8 mRNA levels compared to control levels. CTGF, CYR61, IL-6 and IL-8 expression levels were higher in VG10 than VG5 lambs; although only the IL-6 and CYR61 mRNA levels reached significance.

Conclusion: CTGF, CYR61 and EGR1 may be novel early markers of lung injury and mechanical ventilation from birth using relatively low tidal volumes may be less injurious than using higher tidal volumes.

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Introduction

The lungs of very preterm infants have an immature distal airway structure, with a thick air/blood barrier and a small surface area for gas-exchange. They are surfactant deficient because undifferentiated epithelial cells predominate with few type II alveolar cells. As a result, very preterm infants often require respiratory support in the minutes following birth. Although essential for survival, mechanical ventilation of very preterm infants is closely associated with a high risk of developing bronchopulmonary dysplasia (BPD). BPD is characterised by a simplification of airways, a cessation of alveolarisation, hypercellularity, variable fibrosis and capillary dysplasia [1].

Ventilator induced lung injury (VILI) in preterm infants is associated with many different forms of mechanical ventilation [2-7]. The inflammation that results from VILI is thought to play an important role in the pathogenesis of BPD. VILI promotes the recruitment of inflammatory cells such as neutrophils and macrophages and induces many pro-inflammatory cytokines, transcription factors and growth factors leading to abnormal lung development [8,9]. These factors include interleukin (IL)-1β, IL-6, IL-8, IL-10, tumour necrosis factor (TNF)-α, transforming growth factor (TGF)-β1, nuclear factor (NF)-κB and interferon-γ [8,10-13]. Although these factors are elevated in response to VILI, a detectable increase can take many hours or days [14], making it difficult to define the initial injury-related pathways involved [9,15]. Identifying the initial injury pathways is critical as the greatest risk of injury may be during the period immediately after birth when the lungs are partially liquid-filled, are surfactant deficient and are not uniformly aerated [16-18]. However, it is unclear whether the above factors are reliable markers of lung injury in studies that are of short duration e.g. investigations of the neonatal resuscitation period.

One of the histological hallmarks of BPD is hypercellularity of the lung [1] and we have recently demonstrated that VILI rapidly stimulates lung cell proliferation in the immature lung [19]. The early response genes connective tissue growth factor (CTGF), cysteine-rich 61 (CYR61) and early growth response factor 1 (EGR1) are known to promote cell proliferation [20,21] and we have recently shown that they are rapidly activated in response to a fetal lung growth stimulus [22]. Previous studies have also demonstrated that these genes are activated in response to lung injury in adults [23-27], but their role in VILI in the preterm neonate is unknown. Thus, our first aim was to investigate whether these early response genes are activated within 15 min-2 h of an injurious insult to the lungs of preterm lambs, before pathological changes to the lung have occurred. To determine their usefulness as early markers of lung injury, we compared their change in expression with changes in the expression of the inflammation genes IL-1, IL-6, IL-8 and TGF-β1, TNF-α protein levels and NF-κB activity, which have previously been associated with VILI in neonates [8,11,13]. Our second aim was to determine if the mRNA levels of these genes could differentiate between ventilation strategies likely to induce only a mild degree of VILI. To address that aim we determined the mRNA levels of CTGF, CYR61, EGR1, IL-1β, IL-6 and IL-8 in preterm lambs resuscitated from birth using tidal volumes of 5 or 10 mL/kg. Based on the known roles of CTGF, CYR61 and EGR1, it is possible that their aberrant expression contributes to abnormal lung development in very preterm infants destined to develop BPD.

Methods

Animal experiments

Delivery and ventilation of lambs

All experimental procedures on animals were approved by the Monash University Animal Ethics Committee. Pregnant Merino × Border Leicester ewes at 125 or 132 days of gestational age (GA; term is ~147 d) were anaesthetised and the fetal head and neck were exposed for catheterisation and intubation. The fetus was then delivered and ventilated as described below for 135 min. Arterial blood samples were collected every 5 min for the first 15 min and then every 10 min until the end of the experiment. The peak inspiratory pressure (PIP), positive end expiratory pressure (PEEP), mean airway pressure (Paw), tidal volume (VT), inspiratory and expiratory times, ventilation rate, arterial blood pressure and heart rate were recorded using a data acquisition system (PowerLab, ADInstruments Pty. Ltd., Castle Hill, NSW, Aust.). The alveolar-arterial oxygen difference (AaDO2) was calculated using the equation: (Pbarometric - PH2O) × FiO2 - (PaCO2/0.8) - PaO2. Control fetuses at the same gestational ages were used to indicate the levels of gene expression prior to ventilation.

Time-course for the activation of early response genes caused by injurious ventilation (IV)

Preterm lambs delivered at 132 d gestation (n = 16) were resuscitated and mechanically ventilated from birth using a Dräger “Babylog 8000+” (Dräger Medical, Lubeck, Germany). For the first 15 min after birth, lambs were ventilated with an injurious ventilation (IV) protocol, consisting of a tidal volume (VT) of 20 mL/kg in the absence of a PEEP. After 15 min, lambs were ventilated using a VT of 5 mL/kg and 8 cmH2O PEEP for a further 15 (LI 15), 30 (LI 30), 60 (LI 60) or 120 (LI 120) mins (n = 4 for each group).

Affect of tidal volume on the activation of early response genes

Preterm lambs delivered at 125 d GA were resuscitated and mechanically ventilated using the Dräger “Babylog 8000+” set to deliver a guaranteed VT of either 5 (VG5) or 10 (VG10) mL/kg with 8 cmH2O of PEEP for 135 min...
from birth (15 minute resuscitation stabilisation period followed by 2 h of ventilation; n = 5 in each group). The ventilation settings and experimental protocol for these studies have been described previously [28].

Post-mortem examination and tissue collection
At the end of each experiment lambs were humanely killed with an overdose of sodium pentobarbitone (i.v.). The lungs were removed, weighed and the left bronchus was ligated. The left lung was cut into small sections and snap frozen in liquid nitrogen for analysis of CTGF, CYR61, EGR1, IL-1, IL-6, IL-8 and TGF-β mRNA levels, active NF-κB levels and TNF-α protein concentrations. The right lung was fixed via the airways, using 4% paraformaldehyde at 20 cmH2O for light microscopy.

Tissue analysis
Active NF-κB protein levels
NF-κB protein activity was measured in lung tissue using an electromobility gel-shift assay. Lung nuclear proteins were extracted [29] from lung tissue and the protein concentration was determined using a BioRad DC Protein Assay kit (Sigma Aldrich, Australia). Nuclear protein (8 μg) was incubated on ice for 20 min with 2 μl binding buffer (100 mM HEPES, 50 mM MgCl2, 50% glycerol, 10 mM EDTA, 500 mM potassium glutamate), 1 μl DTT, 1 μl poly dIdC and 1 μl of a double stranded 32P-kb DNA probe containing the cognate κB motif (5’-AGTTGAG-GGGACTTTCC-3’; total volume 20 μl). Samples were then electrophoresed for 2 h at 110 V at room temperature in a 5% non-denaturing polyacrylamide (19:1 Acrylamide:Bis-acrylamide) gel with 0.5× TBE buffer. The gel was then dried onto Whatmann 3 mm chromatography paper in a gel drier (Speed Gel SG210D, Savant Instruments, USA) and exposed to a storage phosphor screen for 24 – 48 h at room temperature. The relative levels of active NF-κB bound to the κB motif were quantified by measuring the total integrated density of each band using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). To compare values from different electromobility gel-shift assays, values from each treatment group were expressed as a percentage of the mean value obtained from the same electromobility gel-shift assay. The concentration of TNF-α protein in lung tissue was measured using a modified antibody-sandwich method of the enzyme-linked immunosorbent assay [30]. Tissue samples were homogenised in 1× PBS and centrifuged at 2,500 rpm for 20 min. Supernatant, plasma or standards (50 μl) were incubated overnight in a 96-well microtitre plate precoated with 50 μl of TNF-α mouse ascites monoclonal antibody (diluted 1:250 in 3 mM NaN3, 20 mM Na2CO3, 30 mM NaHCO3) and blocked with 1% skim milk powder in PBS. Plates were washed five times in PBS with 20% Tween 20 (Wash buffer), then incubated for 2 h with 50 μl of rabbit anti-TNF-α polyclonal antisera (1:5 dilution in 0.001 M PBS/5%BSA). The plates were then washed with buffer and incubated for 1 h with 50 μl of sheep anti-rabbit horseradish peroxidase (diluted 1:1000 in 0.01 M PBS/5% BSA). The plates were then washed, 100 μl tetramethyl benzidine/dimethyl sulphoxide was added and the plates were incubated for 10 – 15 min in the dark before the colour reaction was stopped using 0.5 M sulphuric acid. An automatic plate reader (Original Labsystems Multiskan RC, USA) measured the absorbance (at 450 nm) and the levels of TNFα in each sample were determined by interpolation of the standard curve.

TGFB-1 gene expression
TGFB-1 mRNA levels in lung tissue were quantified by Northern Blot analysis as previously described [31]. The total integrated density of the TGFB-1 mRNA transcript was divided by the total integrated density of the 18S rRNA band for that sample to account for minor differences in total RNA loading between lanes. As a result, the band densities are presented as a ratio of the 18S rRNA band density and, therefore, have no units.

Quantitative real-time polymerase chain reaction
EGR1, CTGF, CYR61, IL-1, IL-6 and IL-8 mRNA levels in lung tissue were measured using quantitative real-time polymerase chain reaction (qRT-PCR). The primers used for amplification of these genes, the gene accession numbers and the regions amplified are shown in Table 1. Total RNA was extracted, DNase-treated and 1 μg was reverse transcribed into cDNA (M-MLV Reverse Transcripate, RNase H Minus, Point Mutant Kit; Promega, Madison,

Table 1: Primers used for quantitative real-time PCR

| Gene | GenBank Accession # | Nucleotides amplified | Upstream primer 5'-3' | Downstream primer 5'-3' |
|------|---------------------|-----------------------|-----------------------|------------------------|
| EGR1 | DQ239634            | 444–532               | AGGGTCACTGTGGAAGGTC   | GCAGCTGAAGTCAAAGGAA    |
| CTGF | DQ239672            | 407–469               | TATAGCTCCAAGCAAGGTC   | AGGAAACTTGACCTGAGGCA   |
| CYR61| DQ239638            | 286–354               | ATGCTGAGGACACTTGGTGA  | GTGTAAGCCTGAGGAGATAC   |
| IL-1 | NM_00109465         | 353–473               | CGTGGCAGGGTCTGTGAG    | CTGGTGAAGAGGAGTTGGAGAG |
| IL-6 | NM_00109392         | 598–705               | CGGAAGGTTATCATCACC    | CCCAGGAAACTACCAACATCA  |
| IL-8 | NM_00109401         | 438–520               | CCTCACTGAAGGATGCAATGA | TGACAACCTACACAGACCC    |
| 18S  | X01117              | 1495–1673             | GTCTGTGATGCCCCCTAGATGC| AAGCTTTAGGCCCGCATTACC  |
W1). qRT-PCR was performed using a Mastercycler® ep gradient S realplex real-time PCR system (Eppendorf, Germany) using 20 μl reactions, containing 1 μl cDNA template (1.5 μg/μl for IL-6, 1 μg/μl for IL-1, IL-8 and CTGF, 500 ng/μl for EGR1 and 200 ng/μl for CYR61 and 18S), 1 μl of each forward and reverse primer (10 μM for IL-1, IL-6, IL-8, CYR61 and 18S and 4 μM for CTGF and EGR1), 10 μl SYBR green (Platinum® SYBR® Green qPCR SuperMix-UDG; Invitrogen Life Technologies, Carlsbad, CA) and 7 μl of nuclease-free water. The thermal profile used to amplify the PCR products included an initial 2 min incubation at 95°C, followed by 35–40 cycles of; denaturation at 95°C for 3 sec, annealing at 59°C (IL-1, IL-6 and EGR1) or 60°C (IL-6, CTGF and CYR61) for 20 sec and elongation at 72°C for 20 sec. The fluorescence was recorded after each 72°C step. Dissociation curves were performed to ensure that a single PCR product had been amplified for each primer pair. Each sample was measured in triplicate and a control sample, containing no template, was included in each run. A threshold value (C_T value) for each sample was determined. Minor differences in the amount of cDNA template added to each reaction were adjusted by subtracting the C_T value for the gene of interest (ΔC_T). To enable comparisons between assays, a calibrator sample (in quadruplicate) was run in each assay. The average C_T value for the calibrator sample was subtracted from the ΔC_T of each sample (ΔΔC_T). The mRNA levels of genes of interest were normalized using the equation 2−ΔΔC_T and the results were expressed relative to the mean mRNA levels of the gene of interest in non-ventilated control fetuses.

**Light microscopy and immunohistochemistry for EGR1 and CYR61**

Each lobe of each right lung was cut into 5 mm slices. Every second slice was subdivided into 3 sections and 6 sections were chosen at random from each lobe, cut into ~1 cm × 1 cm sections and embedded in paraffin. Paraffin blocks were randomly selected and 5 μm sections were incubated at 60°C for 2 h, deparaffinised in xylene, rehydrated using graded alcohol washes and washed in PBS and either stained with Haemotoxylin and Eosin (H&E) or treated further for immunohistochemistry. Sections used for immunohistochemistry were then boiled in sodium citrate (0.01 M, pH 6.0) for 20 mins (in a microwave, on high) to enhance antigen retrieval. Sections were then washed in PBS (CYR61 2 × 5 min; EGR1 3 × 5 min) and incubated (CYR61 5 min; EGR1 30 min) in hydrogen peroxide (3%) to block endogenous peroxidase activity. They were then rinsed in water (CYR61 only), washed in PBS and incubated in blocking/permeabilisation buffer (10% normal goat serum and 0.1% TritonX-100 in 0.05 M TrisHCl for CYR61 sections or 25% normal goat serum and 5% BSA in 0.05 M TrisHCl for EGR1 sections) in a humidity chamber (CYR61 30 min; EGR1 45 min, at room temp). The sections were then incubated with the primary antibodies (CYR61 Cat# sc-13100; EGR1 Cat# sc-189, Santa Cruz Biotechnology, California, USA) diluted in DAKO antibody diluent (CYR61, diluted 1:150; EGR1 diluted 1:200) for either 90 min at room temperature (CYR61) or overnight at 4°C (EGR1). Sections were then washed in PBS (0.1% Tween-20) for 5 mins (×3) and incubated with a biotinylated secondary antibody (goat anti-rabbit diluted 1:700; Vector laboratories, Burlingame, CA) in PBS/0.1% Tween 20 (CYR61) or Dako antibody diluent (EGR1) for 1 hour at room temperature. The sections were again washed in PBS (0.1% Tween 20) for 5 mins (×3) before the secondary antibody was detected using the Vectastain ABC detection kit (Vector laboratories). The sections were washed, dehydrated and permanently mounted. Sections that lacked the primary antibodies or the secondary antibody were also included.

Sections were viewed under a light microscope and images were captured at a magnification of 1000× using a digital camera. Analysis was performed on images using ImagePro Plus (Media Cybernetics, MD) on 5 fields of view per section using 3 randomly chosen sections (from different regions of the lungs). For each field of view, the area of tissue positively stained for EGR1 or CYR61 was measured and expressed as a percentage of the total area of tissue. The percentage of stained tissue for each lamb was then averaged for each experimental group. Analysis was performed on the alveolar region of the lung, taking care to avoid areas containing major airways or blood vessels.

**Data analysis**

Data are expressed as the mean ± SEM with the level of statistical significance set at p < 0.05. PaCO₂, pHa, SaO₂, FiO₂ and PIP were analysed using a 2-way repeated measures ANOVA. The immunohistochemistry data was analysed by a nested ANOVA. The relative amounts of active NF-κB (all three bands summed) and the mRNA levels of TGF-β, CTGF, CYR61, EGR1, IL-6, IL-8 and IL-1 were compared between groups using one-way ANOVA. Significant differences indicated by ANOVA were subjected to a least significant difference post-hoc test to identify differences between individual time points and treatment groups.

**Results**

**Activation of early response genes following IV**

All blood gas and ventilation parameters were similar in the four groups of lambs exposed to 15 mins of IV immediately after birth (LI 15, LI 30, LI 60, LI 120). Thus, only data from the lambs ventilated for 2 hrs after the 15 min IV protocol (LI 120) are presented in Fig 1.

**Blood gas parameters**

Throughout the 135 min experimental period, the SaO₂ remained at or higher than 95% (Fig. 1). The FiO₂ was initially reduced from 0.60 ± 0.18 to 0.27 ± 0.03 at the end of the experiment.
Blood gas parameters following 15 minutes of injurious ventilation. The alveolar-arterial difference in oxygenation (AaDO₂) (A), oxygen saturation (SaO₂) (B), fraction of inspired oxygen (FiO₂) (C), arterial pH (pHa) (D) and partial pressure of CO₂ in arterial blood (PaCO₂) (E) in preterm lambs at 132 days of gestation resuscitated at birth using an injurious ventilation strategy then ventilated gently for 120 minutes. Values are mean ± SEM. The black bar indicates 15 min of ventilation with 20 mL/kg VT and 0 cmH₂O of positive end-expiratory pressure. The asterisks (*) represent values significantly different (p < 0.05) to the initial (5 min) time point.
of the 15 min IV period (V, 20 mL/kg, 0 cmH2O PEEP),
but it was necessary to gradually increase the FiO2 to a maximum of 0.47 ± 0.13 at 70 mins after completion of
the IV period. The AaDO2 was significantly reduced from
739.6 ± 213.1 mmHg to 285.9 ± 38.0 mmHg by the end
of the 15 min IV period and then remained at this level for
the duration of the experiment. During the 15 min IV
period, the PaCO2 and pHa remained unchanged at 15 ±
1 mmHg and 7.66 ± 0.02, respectively. However, during
the remainder of the experimental period, the PaCO2
gradually increased, reaching a maximum of 64 ± 6
mmHg, and the pHa gradually decreased, reaching a minimum of 7.18 ± 0.04 (Fig. 1).

**Ventilation parameters**
During 15 min of IV, the PIP required to administer a Vt
of 20 mL/kg (in the absence of PEEP) decreased (p < 0.02)
from 54 ± 2 cmH2O at 3 min after birth to 47 ± 3 cmH2O
by the end of the 15 min IV period. Within 10 min of
change in ventilation strategy, the PIP required to deliver
a Vt of 5 mL/kg with 8 cmH2O PEEP was reduced (p <
0.001) to 32 ± 1 cmH2O. The required PIP did not change
further during the remainder of the 120 min ventilation
period. However, because of the increasing PaCO2 and
decreasing pH, it was necessary to gradually increase the ventilation rate from 36.3 ± 6.6 breaths/min at the end of
the 15 min IV period to 87.1 ± 18.5 breaths/min at the
completion of the experiment. As a result, the mean air-
way pressure at the end of the 15 min IV period was simi-
lar to that at completion of the experiment (15.2 ± 0.5 vs
15.6 ± 0.6 cmH2O).

**Indicators of lung injury**
The level of active NF-κB within lung tissue did not signif-
ically change for up to 2 h following 15 min of IV; the
levels were similar at 15 (78.2 ± 7.9%), 30 (93.2 ±
27.0%), 60 (109.9 ± 22%) and 120 (70.4 ± 23.3%) min
after IV compared with values prior to ventilation mea-
sured in age-matched control fetuses (100.0 ± 5.8%). Sim-
ilarly, TGF-β1 mRNA levels in lung tissue were similar at
15 (96.4 ± 2.0%), 30 (99.7 ± 4.2%), 60 (98.3 ± 14.1%)
and 120 (99.1 ± 13.6%) minutes after IV, compared with
the levels before ventilation in age-matched control
fetuses (100.0 ± 3.8%). TNF-α protein levels could not be
detected in plasma or tissue homogenates in ventilated
lambs or in unventilated age-matched control fetuses.

IV induced a large and sustained increase in IL-1, IL-6 and
IL-8 mRNA levels; 28.3 ± 16.6, 25.6 ± 13.9 and 74.1 ± 20.4 fold increase respectively (p < 0.05), compared with pre-
ventilation control values, within 15 mins of completing
IV (Fig 2). Although IL-1 mRNA levels had returned to
control levels at 120 mins after completion of the IV
period, IL-6 and IL-8 mRNA levels remained significantly
elevated (p < 0.05) at 11.0 ± 3.2 and 42.8 ± 11.3 fold,
respectively, above pre-ventilation control values at this
time (Fig 2).

IV also induced a time-dependent increase in mRNA lev-
els for CTGF, EGR1 and CYR61. The expression levels of
all three genes were significantly higher (p < 0.05) at every
time point after IV, than the pre-ventilation mRNA levels
in age-matched control fetuses. CTGF mRNA levels
increased 15.5 ± 3.8 fold at 15 mins and increased further
to 24.4 ± 2.1 fold the control values at 30 mins after the
IV period. CTGF mRNA levels in lung tissue then declined
to 10.9 ± 2.7 fold at 60 mins and to 7.8 ± 1.5 fold of the
control values at 120 mins after the IV period (Fig 3A).
Compared with the values prior to ventilation in age-
matched control fetuses, EGR1 and CYR61 mRNA levels
increased by 123.7 ± 7.0 and 51.3 ± 11.4 fold, respec-
tively, at 15 mins after the IV period. EGR1 and CYR61
mRNA levels in lung tissue then declined to 43.9 ± 8.8 and
29.1 ± 4.3 fold above control values at 30 mins, to 13.8 ±
4.1 and 13.7 ± 3.5 fold at 60 mins, and to 11.1 ± 2.7 and
5.6 ± 1.5 fold, respectively, at 120 mins after the IV period
(Fig. 3A).

The increase in CYR61 and EGR1 gene expression was
reflected by a gradual, but marked, increase in the percent-
age of lung tissue stained positive for these proteins (Fig
3B); representative histological sections immunostained
for CYR61 and EGR1 are shown in Figure 4. The percent-
age of lung tissue labelled positive for the CYR61 and
EGR1 proteins increased from 3.0 ± 1.4 and 11.2 ± 1.2%
before ventilation in control fetuses to 16.8 ± 2.9 and 31.1
± 1.6%, respectively (p < 0.05), at 2 hours after IV (Fig.
3B). Sections of lung tissue that lacked the primary anti-
odies or the secondary antibody showed no evidence of
staining. CTGF protein levels could not be determined as
none of the commercial antibodies tested recognised
evine CTGF.

**Affect of tidal volume on the activation of early response
genes**

**Blood gas and ventilation parameters and indices of lung injury**
The blood gas and ventilation parameters for these studies
have been presented in detail previously [28]. The co-effi-
cient of variation of the delivered VT was 6.5 ± 0.3%. The
PIP and Paw delivered to VG10 lambs was significantly
higher (p < 0.05) than the PIP and Paw delivered to VG5
lambs throughout the 15 minute resuscitation and 2 h
ventilation period (Fig 5). PaCO2 values were significantly
lower (p < 0.05) in the VG10 group than the VG5 group
throughout the 15 minute resuscitation period and 2 h
ventilation period. pHa values were significantly higher (p
< 0.05) in lambs ventilated at 10 mL/kg compared with
lambs ventilated at 5 mL/kg during the resuscitation
period but were not different from the 5 mL/kg lambs
during the 2 hour ventilation period. The SaO2 and AaDO2
60 minutes after the IV period. mRNA levels were significantly higher than the levels in age-matched control fetuses (T = 0 values). The increase in IL-1 mRNA levels following injurious ventilation was also greater in the VG10 lambs (92.2 ± 52.4 fold) than in the VG5 lambs (28.9 ± 4.8 fold, p < 0.05), both of which were significantly higher than the levels before ventilation in control fetuses (1.0 ± 0.3; p < 0.001). The increase in IL-8 mRNA levels was also greater in the VG10 lambs (92.2 ± 52.4 fold) than in the VG5 lambs (28.9 ± 4.8 fold) and both groups were significantly higher than control levels (1.0 ± 0.4; p < 0.001), however, due to the large degree of variation between lambs the differences between the two ventilated groups were not statistically significant.

The lung mRNA levels of EGR1, CYR61 and CTGF were also significantly increased (p < 0.01) in both ventilated groups of lambs, compared to the levels before ventilation in age-matched control fetuses (Fig 7). The fold increase in EGR1 mRNA levels relative to control levels (1.0 ± 0.2; p < 0.001) was similar in VG5 (14.8 ± 2.6 fold) and VG10 (14.6 ± 2.5 fold) lambs. The fold increase in CYR61 mRNA levels was greater in the VG10 (21.2 ± 4.9 fold; p < 0.01) lambs than in the VG5 treated lambs (8.8 ± 1.4 fold) and both were significantly greater than the levels prior to ventilation in control fetuses (1.0 ± 0.1; p < 0.01). The increase in mRNA levels for CTGF was also greater in the VG10 (11.8 ± 4.1 fold) lambs than in the VG5 treated lambs (6.5 ± 1.1 fold) but the difference between the ventilated groups failed to reach statistical significance. Both groups of ventilated fetuses had significantly higher CTGF mRNA levels than the control fetuses (1.0 ± 0.4; p < 0.001).

Discussion

Ventilator-induced lung injury (VILI) is closely associated with BPD in very preterm infants [1] and is thought to trigger an inflammatory response which results in abnormal lung development. However, the specific mechanisms by which mechanical ventilation causes lung injury in very preterm infants are largely unknown, as are the pathways resulting in the abnormal lung development that characterise BPD. We have recently demonstrated that VILI in the immature lung induces a rapid increase in distal lung cell proliferation [19] which is consistent with the fibroblast proliferation seen in infants with BPD [1]. We have also identified a number of early response genes (CTGF, EGR1 and CYR61) that regulate cell proliferation and are thought to play a role in normal lung develop-

![Interleukin mRNA level](image-url)
CTGF, CYR61 and EGR1 mRNA levels following injurious ventilation. (A) CTGF, CYR61 and EGR1 lung mRNA levels and (B) the percentage of tissue staining positive for CYR61 and EGR1 protein in preterm lambs at 132 days of gestation resuscitated at birth using an injurious ventilation (IV) strategy for 15 minutes, then ventilated gently for 15–120 minutes. All values are mean ± SEM and expressed as a fold change relative to values in unventilated age-matched control fetuses (T = 0 values). The mRNA levels of CTGF, CYR61 and EGR1 were significantly higher (p < 0.05) than the levels prior to ventilation (T = 0), at all time points after IV. The asterisks (*) indicate protein levels of CYR61 and EGR1 that were significantly higher (p < 0.05) than the levels before ventilation measured in age-matched control fetuses.
CTGF, EGR1

tion following VILI in preterm lambs. We found that injury and disease [24-27], we investigated their activa-
part of the very early response to lung injury in very pre-
VILI, suggesting that these proteins and genes do not form
mal lung development that follows VILI, is explained at
ferentially expressed during high and low tidal volume

Figure 4
EGR1 and CYR61 protein levels in lung tissue follow-
injurious ventilation. Lung tissue sections stained for
EGR1 and CYR61 proteins using immunohistochemical tech-
niques. The lung tissue sections shown are representative of
the sections from unventilated age-matched control fetuses
and preterm lambs at 2 hours after a 15 minute period of
injurious ventilation (IV). The brown stain represents lung
tissue containing the EGR1 or CYR61 protein. Slides incu-
bated without the primary or secondary antibodies did not
show any evidence of brown staining (data not shown).

CTGF
CTGF and CYR61 are members of the CCN protein family
which in mammals consists of 6 proteins (CYR61, CTGF,
nephroblastoma-overexpressed1; NOV1 and the Wnt-
induced secreted proteins; WISP-1, WISP-2 and WISP-3;
[34]). The CCN family are secreted matricellular proteins
that form interactions between the extracellular matrix
and cell adhesion molecules, leading to diverse cellular
responses including cell proliferation, extracellular matrix
production, angiogenesis, adhesion, migration, apoptosis
and growth arrest [34].

CTGF inducers lung fibroblast proliferation, myofibroblast
differentiation [35] and the expression of collagen and
other extracellular molecules [34]. CTGF has increased
expression (0.3 fold) in fetal sheep lungs undergoing
accelerated lung growth [22] and CTGF knockout mice die
at birth of respiratory failure due to defects in the rib cage
and pulmonary hypoplasia [36]. Although these data
indicate that CTGF is important for normal lung growth,
abnormally elevated levels of CTGF expression are also
implicated in the pathogenesis of adult human lung dis-
ases such as idiopathic pulmonary fibrosis [24] and
chronic obstructive pulmonary disease [26]. In the adult
mouse, bleomycin-induced pulmonary fibrosis [23] and
hyperoxia-induced lung injury [25], also exhibit elevated
CTGF mRNA levels. As fibroblast proliferation, myofi-
broblast differentiation, hypercellularity and pulmonary
fibrosis are commonly associated with VILI in very pre-
term infants [1] and fetal sheep [19], it is possible that
abnormally high CTGF expression following VILI (~25 fold in the current study) may contribute to the pathogen-

EGR1

A primary aim of this study was to determine the degree
and rapidity of increase in expression of CTGF, CYR61
and EGR1 following injurious ventilation, in comparison
to that of inflammatory factors that have previously been
associated with VILI in neonates [8,11,32]. In the present
study TNFα protein was not detectable, while NF-κB activ-
ity and TGF-1 mRNA levels did not change within 2 hr of
VILI, suggesting that these proteins and genes do not form
part of the very early response to lung injury in very pre-
term lambs. In contrast, the increases in IL-1, IL-6 and IL-
8 after injurious ventilation support the findings of other
studies that have also found these inflammatory cytokines
are increased at 2–3 h after injurious ventilation from
birth [32,33]. Our study extends those findings to demon-
strate that IL-1, IL-6, IL-8, CTGF, CYR61 and EGR1 all
responded very rapidly (within 15 minutes of an injurious
resuscitation period) and to levels substantially higher
(25–125 fold) than those in unventilated controls. These
data suggest that the cascade of events leading to lung
inflammation and lung remodelling can be rapidly initi-
atated during the immediate resuscitation period after birth.
The abnormally high expression levels of these genes was
not only limited to resuscitation with high tidal volumes
without PEEP, but also occurred in response to ventilation
regimens similar to those commonly used for preterm
infants.

CYR61 and CTGF are increased at 2–3 h after injurious ventilation, in comparison
CTGF, CYR61, IL-6

and also acts as an early response gene. CYR61 acts syner-
gistically with other growth factors to potentiate their
mitogenic effects on endothelial, epithelial and fibroblast
cells [20,37] as well as to promote collagen and cartilage

CTGF

CTGF induces lung fibroblast proliferation, myofibroblast
differentiation [35] and the expression of collagen and
other extracellular molecules [34]. CTGF has increased
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Figure 5
Blood gas and ventilator parameters during VG5 and VG10 ventilation strategies. Arterial pH (pHa) (A), partial pressure of CO₂ in arterial blood (PaCO₂) (B), alveolar-arterial oxygen difference (AaDO₂) (C), peak inspiratory pressure (PIP) (D) and mean airway pressure (Paw) (E) in preterm lambs mechanically ventilated from birth at 125 days of gestation. Lambs were mechanically ventilated with either 5 (VG5) or 10 (VG10) mL/kg. Values are mean ± SEM and the asterisks represent values significantly different (p < 0.05) between VG5 and VG10.
Figure 6
Histological evidence of lung injury in lambs ventilated with VG5 and VG10 ventilation strategies. Representative haematoxylin and eosin stained lung tissue sections in preterm lambs mechanically ventilated from birth at 125 d of gestation with a tidal volume of 10 mL/kg (A) or 5 mL/kg (B) and unventilated control fetuses (C). Hyaline membranes are shown with arrows and detached epithelial cells are shown with arrowheads.
Figure 7

Interleukin-1, -6 and -8, EGR1, CYR61 and CTGF mRNA levels in control fetuses and following VG5 and VG10 ventilation strategies. IL-1, IL-6 and IL-8 (A) and EGR1, CYR61 and CTGF (B) mRNA levels in unventilated age-matched control fetuses and in preterm lambs mechanically ventilated from birth at 125 days of gestation with either 5 (VG5) or 10 (VG10) mL/kg. The values are mean ± SEM and expressed as a fold-change relative to the mean levels in unventilated control fetuses. The asterisks (*) represents values significantly greater (p < 0.001) than values before ventilation measured in age-matched control fetuses. The hash (#) represents values significantly greater than those in the VG5 lambs (p < 0.05).
production [34]. Depending on the cellular milieu, the primary role of CYR61 is thought to be the regulation of angiogenesis by promoting the proliferation of endothelial cells and the production of angiogenic molecules such as vascular endothelial growth factor [38,39]. Interestingly, CYR61 also up-regulates the expression of inflammatory genes, including IL-1, as well as modulators of the extracellular matrix such as proteases and their inhibitors [40]. Similar to CTGF, CYR61 expression is also increased (~0.3 fold) in fetal sheep lungs undergoing accelerated growth [22] and abnormally high levels of CYR61 have been implicated in the pathogenesis of chronic obstructive pulmonary disease [26] in humans as well as lung injury in adult rodents induced by hyperoxia [25] or volutrauma [27]. Based on its known roles, the large and rapid increase in CYR61 expression (~50 fold in the current study) may contribute to the abnormal lung pathology caused by VILI via several mechanisms. It may contribute to the hypercellularity and fibrosis by directly stimulating the proliferation of fibroblasts and epithelial cells and may upset the normal balance of angiogenic factors, contributing to dysmorphic capillary growth. It may also contribute to the sustained inflammation and abnormal tissue repair that can occur in response to VILI and is an antecedent of BPD in very preterm infants. Our results indicate that increased CYR61 expression may play a key role in initiating the cascade of events caused by VILI, as CYR61 protein levels in lung tissue were increased 6-fold within two hours of VILI.

EGR1 is a transcription factor that is rapidly expressed by diverse stimuli that induce growth, differentiation and apoptosis [41]. EGR1 up-regulates the expression of cell cycle regulatory proteins, growth factors, cytokines such as IL-1β, TNFα and TGFβ and other transcription factors including itself and matrix proteins [21,42-45]. EGR1 is up-regulated in the fetal sheep during accelerated lung growth [22] and in hemi-pneumonectomy induced compensatory lung growth in adult mice suggesting that it may play a role in regulating normal lung growth [46]. However, EGR1 expression is also increased by volutrauma in the adult rat lung [47] and it plays a pivotal role in the response to pulmonary ischaemia-reperfusion injury in the adult mouse [48]. In humans it has been implicated in the pathogenesis of chronic obstructive pulmonary disease [26,49] and vascular pathologies where it can cause vascular lesions, suppress the growth of damaged endothelial cells and modulate vascular tone [reviewed in [43]]. These roles for EGR1, suggest the high levels of its expression induced by VILI (~125 fold in the current study), may contribute to abnormal lung development by its ability to induce cell proliferation, impair vascular development, produce matrix proteins and induce cytokines that promote inflammation.

Regardless of whether CTGF, CYR61 and EGR1 are critical mediators of abnormal lung development caused by VILI, they are likely to be early markers of lung injury. All three genes were very rapidly elevated in response to the injurious ventilation strategy. More importantly, when taken together, the expression levels of IL-6, IL-8, CTGF and CYR61 appeared to differentiate between ventilation strategies causing different degrees of lung injury. Expression levels of all four genes were lowest in lambs mechanically ventilated with a tidal volume of 5 mL/kg and were higher in lambs mechanically ventilated with 10 mL/kg that exhibited gross and histological evidence of lung injury. In contrast, EGR1 and IL-1 appeared not to be sufficiently sensitive to detect any differences between the ventilation strategies. Although the 135 minute ventilation period did not allow time for changes in lung structure to manifest histologically, other evidence indicated that VG10 lambs incurred more lung injury than VG5 lambs. This evidence included the presence of hyaline membranes, detached epithelial cells, red blood cells in the distal lung parenchyma, the presence of blood stained tracheal aspirates, the production of pneumothoraces and subpleural air leaks, and the high PIP required to achieve the tidal volume of 10 mL/kg [28].

Conclusion
The current international guidelines for neonatal resuscitation (ILCOR) provide little guidance on the most appropriate resuscitation techniques that minimise lung injury in the immediate newborn period when the lungs are partially liquid-filled and not uniformly aerated. Our data indicate that VILI during the immediate newborn period can rapidly (within 15 mins) initiate changes in gene expression which are abnormal and likely to potentiate inflammation and to promote abnormal lung development. Furthermore, our studies indicate that resuscitation and mechanical ventilation at birth with relatively high tidal volumes is potentially more injurious than with relatively low tidal volumes. We also conclude that CTGF, CYR61, EGR1, IL-1β, IL-6 and IL-8 are likely to be useful biomarkers of VILI in the newborn, particularly in studies of short duration.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MW identified EGR1, CTGF and CYR61 as likely candidate genes, oversaw the molecular and histological component of the analyses and prepared the manuscript. MP performed the animal experiments and the TGF-β1, TNF-α and NF-kB analyses. VZ performed the real-time PCR and immunohistochemical analyses. KC supervised the animal experiments and TC provided intellectual input into
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References

1. Coislon JJ: Pathology of new bronchopulmonary dysplasia. Scand J Thorac Cardiovasc Surg 2003, 8:73-81.
2. Gerstmann DR, Minton SD, Stoddard RA, Meredith KS, Monaco F, Bertrand JM, et al.: The Provo multicenter early high-frequency oscillatory ventilation trial: improved pulmonary and clinical outcome in respiratory distress syndrome. Pediatrics 1996, 98:1044-1052.
3. Stewart TE, Meade MO, Cook DJ, Granton JT, Hodder RV, Lapinskas SE, et al.: Evaluation of a ventilation strategy to prevent baro-trauma in patients at high risk for acute respiratory distress syndrome. N Engl J Med 1998, 338:355-361.
4. Kucher M, Modanlou HD, Brudno DS, Clark FI, Cohen RS, Ryan RM, et al.: Multicenter controlled clinical trial of high-frequency jet ventilation in preterm infants with uncomplicated respiratory distress syndrome. Pediatrics 1997, 100:593-599.
5. Tsuno K, Prato P, Kolobow T: Acute lung injury from mechanical ventilation at moderate airway pressures. J Appl Physiol 1990, 69:956-961.
6. Parker JC, Hernandez LA, Peavy KJ: Mechanisms of ventilator-induced lung injury. Crit Care Med 1993, 21:131-143.
7. Dreyfuss D, Saumon G: Ventilator-induced lung injury: lessons from experimental studies. Am J Respir Crit Care Med 1998, 157:294-323.
8. Bose CL, Dammann CE, Laughon MM: Bronchopulmonary dysplasia and inflammatory biomarkers in the premature neonate. Arch Dis Child Fetal Neonatal Ed 2008, 93:F455-F461.
9. Kotecha S, Chan B, Azam N, Silverman M, Shaw RJ: Increase in interleukin-8 and soluble intercellular adhesion molecule-1 in bronchoalveolar lavage fluid from premature infants who develop chronic lung disease. Arch Dis Child Fetal Neonatal Ed 1995, 72:260-264.
10. Pugin J, Dunn I, Jollivet P, Tassaux D, Magenat JL, Nicod LP, et al.: Activation of human macrophages by mechanical ventilation in vitro. Am J Physiol 1998, 275:L1040-L1050.
11. Kotecha S. Cytokines in chronic lung disease of prematurity. Eur J Pediatr 1996, 25:14-17.
12. Held HD, Boeztcher S, Hamann L, Uhlig S: Ventilation-induced chemokine and cytokine release is associated with activation of nuclear factor-kappaB and is blocked by steroids. Am J Respir Crit Care Med 2001, 163:711-716.
13. Coislon JJ, Winter VT, Siler-Khodr T, Yoder BA: Neonatal chronic lung disease in extremely immature baboons. Am J Respir Crit Care Med 1999, 160:1333-1346.
14. Gronneb P, Gotze-Speer B, Oppermann M, Effert H, Speer CP: Association of pulmonary inflammation and increased microvascular permeability during the development of bronchopulmonary dysplasia: a sequential analysis of inflammatory mediators in respiratory fluids of high-risk preterm neonates. Pediatrics 1994, 93:712-718.
15. Ambalavanan N, Novak ZE: Peptide Growth Factors in Tracheal Aspirates of Mechanically Ventilated Preterm Neonates. Pediatr Res 2003, 53:240-244.
16. Bjorklund LJ, Ingimarsson J, Curstedt T, Larsson A, Robertson B, Werner O: Lung recruitment at birth does not improve lung function in immature lambs receiving surfactant. Acta Anaesthesiol Scand 2001, 45:966-993.
17. Bjorklund LJ, Ingimarsson J, Curstedt T, John J, Robertson B, Werner O, et al.: Manual ventilation with a few large breaths at birth compromises the therapeutic effect of subsequent surfactant replacement in immature lambs. Pediatr Res 1997, 42:348-355.
18. Kada K, Jobe AH, Ikegami M: Tidal volume effects on surfactant treatment responses with the initiation of ventilation in preterm lambs. J Appl Physiol 1997, 83:1054-1061.
19. Allison BJ, Crossley KJ, Flecknroe SJ, Davis PG, Morley CJ, Harding R, et al.: Ventilation of the very immature lung in utero induces injury and BPD-like changes in lung structure in fetal sheep. Pediatr Res 2008, 378:392.
20. Brigstock DR: The connective tissue growth factor/cytostrein-rich 61/nephroblastoma overexpressed (CCN) family. Endocr Rev 1999, 20:189-206.
21. Goecker G, Crozat A, Pardee AB: The immediate-early gene Egr-1 regulates the activity of the thymidine kinase promoter at the G0-to-G1 transition of the cell cycle. Mol Cell Biol 1994, 14:5242-5248.
22. Sozo F, Wallace MJ, Zahra VA, Filby CE, Hooper SB: Gene expression profiling during duration fetal lung injury identifies genes likely to regulate development of the distal airways. Phys Genomics 2006, 24:105-113.
23. Lasky JA, Ortiz LA, Tonthat B, Hoyle GW, Corti M, Athas G, et al.: Connective tissue growth factor mRNA expression is upregulated in bleomycin-induced lung fibrosis. Am J Physiol 1998, 275:L365-L371.
24. Pan LH, Yamauchi K, Uzuki M, Nakanishi T, Takigawa M, Inoue H, et al.: Type II alveolar epithelial cells and interstitial fibroblasts express connective tissue growth factor in IPF. Eur Respir J 2001, 17:1220-1227.
25. Perkowski S, Sun J, Singhal S, Santiago J, Leikaf GD, Aibdela SM: Gene expression profiling of the early pulmonary response to hyperoxia in mice. Am J Respir Cell Mol Biol 2003, 28:682-696.
26. Ning W, Li CJ, Kamikins N, Fehgahi-Bostwick CA, Alber SM, Di YP, et al.: Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease. Proc Natl Acad Sci USA 2004, 101:i14895-i14900.
27. Ma SF, Grigoryev DN, Taylor AD, Nonas S, Sammami S, Ye SQ, et al.: Bioinformatic identification of novel early stress response genes in rodent models of lung injury. Am J Physiol Lung Cell Mol Physiol 2005, 289:L468-L477.
28. Proby ME, Hooper SB, Dargaville PA, McCallon N, Harding R, Morley CJ: Effects of tidal volume and positive end-expiratory pressure during resuscitation of very premature lambs. Acta Paediatr 2005, 94:1764-1770.
29. Deryckere F, Davis PG: A one-hour minipreparation technique for extraction of DNA-binding proteins from animal tissues. Biotechniques 1994, 16:405.
30. Egan PJ, Rother JL, Andrewes AE, Seow HF, Wood PR, Nash AD: Characterization of monoclonal antibodies to ovine tumor necrosis factor-alpha and development of a sensitive immunosay. Vet Immunol Immunopath 1994, 41:259-274.
31. Lines A, Nardo L, Phillips LD, Possmayer F, Hooper SB: Alterations in lung expansion affect surfactant protein A, B and C mRNA levels in fetal sheep. Am J Physiol 1999, 276:L239-L245.
32. Hillman NH, Moss TM, Kallapur SG, Bachurski C, Pillow Jj, Polglase GR, et al.: Brief, large tidal volume ventilation initiates lung injury and a systemic response in fetal sheep. Am J Respir Crit Care Med 2007, 176:537-541.
33. Naik AS, Kallapur SG, Bachurski CJ, Jobe AH, Michna J, Kramer BW, et al.: Effects of ventilation with different positive end-expiratory pressures on cytokine expression in the preterm lamb lung. Am J Respir Crit Care Med 2001, 164:494-498.
34. Brigstock DR: The CCN family: a new stimulus package. J Endo 2003, 178:169-175.
35. Grotendorst GR, Duncan MR: Individual domains of connective tissue growth factor regulate fibroblast proliferation and myofibroblast differentiation. FASEB J 2005, 19:729-737.
36. Baguma-Nibasheka M, Kablar B: Pulmonary hypoplasia in the connective tissue growth factor (Ctgf) null mouse. Dev Dyn 2008, 237:485-493.
37. Sakamoto S, Yokoyama M, Aoki M, Suzuki K, Kakehi Y, Saito Y: Induction and function of cytochrome Cytostre in prostatic stromal and epithelial cells: Cytostre1 is required for prostatic cell proliferation. Prostate 2004, 61:305-317.
38. Zhou D, Herrick DJ, Rosenbloom J, Chaqour B: Cytostre1 Mediates the Expression of VEGF, [alpha]1Integrin and (alpha)-Actin Genes through Cytoskeleton-Based Mechanotransduction

http://respiratory-research.com/content/10/11/19
Mechanisms in Bladder Smooth Muscle Cells. *J Appl Physiol* 2005, 98:2344-2354.

39. Kubota S, Takigawa M. CCN family proteins and angiogenesis: from embryo to adulthood. *Angiogenesis* 2007, 10:1-11.

40. Chen CC, Mo FE, Lau LF. The angiogenic factor Cyr61 activates a genetic program for wound healing in human skin fibroblasts. *J Biol Chem* 2001, 276:47329-47337.

41. Lee SL, Sadovsky Y, Swirnoff AH, Polish JA, Gods P, Gavrila G, et al.: Luteinizing hormone deficiency and female infertility in mice lacking the transcription factor NGFI-A (Egr-1). *Science* 1996, 273:1219-1221.

42. Fu M, Zhu X, Zhang J, Liang J, Lin Y, Zhao L, et al.: Egr-1 target genes in human endothelial cells identified by microarray analysis. *Gene* 2003, 315:33-41.

43. Silverman ES, Collins T. Pathways of Egr-1-mediated gene transcription in vascular biology. *Am J Pathol* 1999, 154:665-670.

44. Reynolds PR, Cosio MG, Hoidal JR. Cigarette smoke-induced Egr-1 upregulates proinflammatory cytokines in pulmonary epithelial cells. *Am J Respir Cell Mol Biol* 2006, 35:314-319.

45. Khachigian LM, Collins T. Inducible expression of Egr-1-dependent genes. A paradigm of transcriptional activation in vascular endothelium. *Circ Res* 1997, 81:457-461.

46. Landesberg LJ, Ramalingam R, Lee K, Rosengart TK, Crystal RG: Upregulation of transcription factors in lung in the early phase of postpneumonectomy lung growth. *Am J Physiol Lung Cell Mol Physiol* 2001, 281:L1138-L1149.

47. Copland IB, Kavanagh BP, Engelberts D, McKerlie C, Belik J, Post M: Early changes in lung gene expression due to high tidal volume. *Am J Respir Crit Care Med* 2003, 168:1051-1059.

48. Yan SF, Fujita T, Lu J, Okada K, Shan ZY, Mackman N, et al.: Egr-1, a master switch coordinating upregulation of divergent gene families underlying ischemic stress. *Nat Med* 2000, 6:1355-1361.

49. Zhang W, Yan SD, Zhu A, Zou YS, Williams M, Godman GC, et al.: Expression of Egr-1 in late stage emphysema. *Am J Pathol* 2000, 157:1311-1320.
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