TRANSCRIPTIONAL REGULATION IN VILI

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VENTILATOR INDUCED LUNG INJURY

Modified from Gattinoni L, Protti A CMAJ 2008; Image by: Lianne Friesen and Nicholas Woolridge
Effect of static inflation on alveolar epithelial permeability

45 cm H$_2$O peak airway pressure

- 5 min
- 20 min
Full thickness tears of the alveolar wall

Maintenance of ventilation and oxygenation compatible with life required use of peak inspiratory pressures as high as 53 cm H$_2$O while supported by pressure-control ventilation at a frequency of 32 breaths/min, positive end-expiratory pressure of 17 cm H$_2$O, and Fio$_2$ of 1.0 mm Hg.
Tensegrity I. Cell structure and hierarchical systems biology

Donald E. Ingber
Departments of Surgery and Pathology, Children’s Hospital and Harvard Medical School, Enders 1007, 300 Longwood Avenue, Boston, MA 02115, USA

Figure A: Illustration of cell structure with tension and compression forces.

Figure B: Diagram showing tension and compression forces in the extracellular matrix (ECM) with microtubules (MTs) and intermediate filaments (IFs).

Panel A: Fluorescence microscopy image of cell structure.

Panel B: Close-up of cell structure with additional tension forces indicated.
MECHANOSENSORS ON THE CYTOPLASMIC MEMBRANE


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**Diagram:**
- Growth factor receptor
- Stretch-activated ion channel
- Kinase
- Tight junction
- Adherens junction (cadherins)
- Gap junction (connexins)
- Actin filament
- α-actinin
- Tensin
- Vinculin
- Paxillin
- Talin
- Integrins
- Extracellular matrix
MECHANOTRANSDUCTION

dos Santos and Slutsky, AJP 2000
Injurious Ventilatory Strategies Increase Cytokines and c-fos m-RNA Expression in an Isolated Rat Lung Model

Lorraine Tremblay,* Franco Valenza,‡ Sergio P. Ribeiro,‡ Jingfang Li,‡ and Arthur S. Slutsky‡
*Division of General Surgery and Division of Thoracic Surgery, The Toronto Hospital, Toronto, M5G 1L7; and ‡Division of Respiratory Medicine, Mount Sinai Hospital, Samuel Lunenfeld Research Institute, University of Toronto, Toronto, M5G 1X5 Canada

JCI, 1997
Northern Blot Analysis

Promoter Analysis

Localization and regulation of c-fos and c-jun protooncogene induction by systolic wall stress in normal and hypertrophied rat hearts

(cardiac hypertrophy/calcium/aortic stenosis)

HERIBERT SCHUNKERT*1, LOTTHAR JAHN*, SEIGO IZUMO*, CARL S. APSTEIN1, AND BEVERLY H. LORELL*1

Preliminary characterisation of mecanoresponsive regions of the c-fos promoter in bone cells

Matthew A. Peake, Alicia J. El Haj*
Early Changes in Lung Gene Expression due to High Tidal Volume

Egr1

Cytokines
Growth factors
Injury
Receptor

MAPKs

Cytosol
Phosphorylation
Nucleus

Egr-1

EBS
NFkB
Sp1
CRE
Negative Feedback
Serum Response Elements
Inhibitory domain

Ngiam AJPLCMP 2007
Early Growth Response-1 Worsens Ventilator-induced Lung Injury by Up-Regulating Prostanoid Synthesis

Nicola Ngiam1, Vanya Peltкова1, Doreen Engelberts1, Gail Otulakowski1, Martin Post1, and Brian P. Kavanagh1,2,3

1Physiology and Experimental Medicine, 2Department of Critical Care Medicine, and 3Department of Anesthesia, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada

A

B

C

D

AJRCCM, 2010
Genetic and Pharmacologic Evidence Links Oxidative Stress to Ventilator-induced Lung Injury in Mice

NRF2 PROTECTS CELLS FOR OXIDATIVE STRESS BY INDUCING TRANSCRIPTION OF ANTI-OXIDANT GENES
Functional polymorphisms in the transcription factor \textit{NRF2} in humans increase the risk of acute lung injury


**TABLE 4.** Multivariable analysis of the association of NRF2 -617 genotype and ALI risk

<table>
<thead>
<tr>
<th>Clinical Variable</th>
<th>Adjusted Odds Ratio for NRF2 -617 CA (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted (base model)</td>
<td>6.44 (1.34, 30.8)</td>
<td>0.021</td>
</tr>
<tr>
<td>Adjusted for:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>6.37 (1.26, 32.1)</td>
<td>0.025</td>
</tr>
<tr>
<td>Gender</td>
<td>6.51 (1.31, 32.2)</td>
<td>0.020</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>5.37 (1.03, 28.1)</td>
<td>0.046</td>
</tr>
<tr>
<td>Mechanism of trauma</td>
<td>5.26 (1.08, 25.7)</td>
<td>0.040</td>
</tr>
<tr>
<td>Total crystalloid fluid, ml</td>
<td>9.16 (1.04, 80.8)</td>
<td>0.046</td>
</tr>
<tr>
<td>Packed red blood cells, units</td>
<td>6.46 (1.25, 33.3)</td>
<td>0.026</td>
</tr>
<tr>
<td>Fresh frozen plasma, ml</td>
<td>5.70 (1.16, 27.9)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

CI, confidence interval; APACHE II, Acute Physiology and Chronic Health Evaluation II (22). The reported odds ratio is for the association of NRF2-617 CA genotype and ALI, adjusted for each clinical variable, matched on race and ISS strata using conditional logistic regression methods. Clinical variables are baseline variables collected during the first 24 h after trauma.
The zinc finger protein Gfi1 acts upstream of TNF to attenuate endotoxin-mediated inflammatory responses in the lung

Jianmin Jin¹, Hui Zeng¹, Kurt-Werner Schmid², Martin Toetsch², Stefan Uhlig³ and Tarik Möröy¹

Ex-vivo Overventilation model

Zinc Finger Transcriptional Repressor


Phelan, Curr Opin Hema 2010
Modulation of Lipopolysaccharide-Induced Gene Transcription and Promotion of Lung Injury by Mechanical Ventilation

William A. Altemeier,²* Gustavo Matute-Bello,⁎ Sina A. Gharib,⁎ Robb W. Glenny,⁎ Thomas R. Martin,⁎ and W. Conrad Liles⁎

The Journal of Immunology, 2005, 175: 3369–3376
PRIMA (PRomoter Integration in Microarray Analysis)

and based on the position weight matrices of approximately 500 known eukaryotic TFs (TRANSFAC database).
A. Up-Regulation with TNFα plus Mechanical Stretch

B. Morphology

C. Src pY416

D. F-actin

E. NFκB (p65)

Beas2b Cells
(immortalized distal bronchial airway epithelial cells)

Microarray Analysis
(Affymetrix U133plus2)

dos Santos, Physiol Gen 2004
PROMOTER ANALYSIS OF CYCLIC STRETCH-DEPENDENT GENES

(a) Enrichment for Genes containing ATF3 promoter binding sequences

(b) Cyclic Stretch Profile

(c) ATF3 Promoter Regulatory Sequences

Akram et al. AJRCC 2011
ATF3 is a Cyclic-Stretch Sensitive Transcription Factor

(a) ATF3 gene expression

(b) ATF3 protein expression

Akram et al. AJRCCM 2011
ATF3 IS REGULATED BY MECHANICAL VENTILATION

Akram et al. AJRCCM 2011
EXPRESSION OF MEMBERS OF THE ATF3 TRANSCRIPTION COMPLEX IN RESPONSE TO BIOPHYSICAL AND/OR BIOMOLECULAR STIMULATION IN BEAS2B

Adapted from Gilchrist et al Nat Med 2006
ATF3 −/− LEADS TO INCREASED PULMONARY EDEMA

Akram et al. AJRCCM 2011
ATF3 −/− LEADS TO INCREASED NEUTROPHIL INFILTRATION
ATF3 REGULATES NF-KB AND AP1

ALI/ARDS/VILI

Biomolecular Injury (TNFα, IL1β, IL8, LPS)

Lung Injury

Biophysical Injury (repetitive cyclic stretch)

Inflammation

Oxidative Stress

Apoptosis

Resolution of Tissue Injury

ATF3 REGULATES NF-KB AND AP1

ALI/ARDS/VILI

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Resolution of Tissue Injury
GENERATION OF ATF3 CHIMERAS BY ADOPTIVE BONE MARROW TRANSFER

Donor
Male ATF3 deficient (ATF3 -/-)

Recipient
Female C57/b6 (ATF3 +/+)

Chimera: WT$^{ATF3}$
Parenchymal cells will be ATF3 +/+  
Myeloid derived cells will be ATF3 -/-

Donor
Male C57/b6 (ATF3 +/+)

Recipient
Female ATF3 deficient (ATF3 -/-)

Chimera: ATF3$^{WT}$
Parenchymal cells will be ATF3 -/-  
Myeloid derived cells will be ATF3 +/+
TWO-HIT VILI PROTOCOL

- **Chimeric mice**: 20-25g
- **First Hit**: LPS inhalation $10\mu g/kg$
- **Second Hit**: Mechanical Ventilation for 3 hrs: $V_t = 6 \text{ ml/kg}$. PEEP $= 2 \text{ cmH}_2\text{O}$; FiO 0.4
ALL ATF3-CHIMERA ARE SUSCEPTIBLE TO VILI

(a) Whole Lung Histology Two - hit
(LPS inhalation plus HV ventilation)

(b) Lung Injury Score
(LPS inhalation plus HV ventilation)

Chimeras lacking ATF3 in either resident or circulating cells show increased lung injury

Shan et al. manuscript submitted
WT MICE RECONSTITUTED WITH ATF3 KO BONE MARROW SHOW INCREASED NEUTROPHIL INFILTRATION AFTER VILI

(a) Bronchoalveolar lavage Cytospin

(b) Total Neutrophil Count

Chimeras lacking ATF3 in myeloid cells show increased neutrophil infiltration

Shan et al. manuscript submitted
Chimeras lacking ATF3 in parenchymal cells show increased levels of inflammatory mediators.
ATF3 KO MICE RECONSTITUTED WITH WT BONE MARROW SHOW INCREASED PULMONARY EDEMA

Chimeras lacking ATF3 in resident cells show increased alveolar edema
ABSENCE OF ATF3 RESULTS IN MARKED LOSS OF TIGHT AND ADHERENS JUNCTION PROTEIN EXPRESSION

Western Blot

<table>
<thead>
<tr>
<th></th>
<th>Occludin</th>
<th>E-Cadherin</th>
<th>Beta-Actin</th>
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<tbody>
<tr>
<td>WT/WT</td>
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<td></td>
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<tr>
<td>ATF3/ATF3</td>
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<tr>
<td>ATF3/WT</td>
<td></td>
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</tr>
</tbody>
</table>

Chimeras lacking ATF3 in resident cells show decreased junctional protein expression during VILI
ATF3 LOSS AND GAIN OF FUNCTION
ATF3 is involved in preserving epithelial cell membrane function
ATF3 is involved in regulating expression of adhesion proteins.
SUMMARY

- ATF3 transcriptional activation is induced during VILI
- ATF3 confers protection from VILI
- ATF3 is expressed in both myeloid and non-myeloid cells
- ATF3 chimeras – BOTH are symptomatic.
- But defect may be due to cellular specific susceptibility

- Highlights the unique role of ATF3 in different cells

- Decoupling of inflammation and membrane permeability?

Provides novel insight into pathogenesis of VILI
ACKNOWLEDGEMENTS

- Mentors
  - Arthur S. Slutsky
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