Radical-Containing PM$_{0.2}$ Initiates Epithelial-to-Mesenchymal Transitions in Infant Airway Epithelial Cells

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Central Hypothesis

- Adult respiratory diseases result, in part, from environmental impact(s) that occur during a critical phase of pulmonary immuno-maturation.
  - Environment
  - Viral
  - Allergen
The Infant Lung

- At birth - saccular
  - Humans
    - 15% alveoli
  - Rodents
    - 0% alveoli
- Postnatal development
  - Humans
    - 3yr of age
  - Rodents
    - 4-7d, alveoli
    - 10d, respiratory bronchioles

*Pictures are artistic renditions of lung development and are designed to emphasize terminal acinus development and not the entire conducting airway system.

PM INDUCES ADVERSE PULMONARY EFFECTS IN INFANTS
WHAT IN PM IS RESPONSIBLE FOR ADVERSE PULMONARY EVENTS?
Atmospheric Fine Particles Contain Persistent Semiquinone-type Radicals

CS tar: 1e16 radicals/g

PM$_{2.5}$: 1e16 - 1e17 radicals/g
Combustion-Generated Fine Particles Contain Persistent Semiquinone-type Radicals

The existence of EPFRs represents a new paradigm for evaluating the toxicity of airborne PM2.5.
EPFRs in Baton Rouge PM2.5 PERSIST

\[ T_{1/2} = 21d \]
Problems with Studying Atmospheric PM Samples

- Size variation
- Sample variation
- Chemical complexity
  - Organic compounds
  - Metal ions
- Sufficient sample size
- Collection methodology (e.g. CAPs, extracts, etc.)
Laboratory Generated Combustion Samples

- Control
  - Size
  - Chemical composition
  - Sufficient quantities
  - In vivo inhalation studies

- More accurate assessment of potential risk posed by specific PM components
  - CHC/BHC
  - Radicals
Particle Systems

Environmentally Persistent Free Radical (EPFR)

EPFRs: $1 \times 10^{14}$ - $1 \times 10^{14}$ radicals/g

Dellinger et al., 2007
Hypothesis:

UFP generated from the combustion processes increases oxidative stress resulting in pulmonary damage and/or changes in lung architecture ultimately resulting in adult airways disease (i.e., asthma).
**In Vivo Acute Exposure Protocol**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Time (d)</th>
<th>Rat age (d)</th>
<th>Analysis</th>
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<td>Study Endpoints</td>
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<tr>
<td><strong>Lung Function</strong></td>
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<td>AHR</td>
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<tr>
<td>Resistance, elastance,</td>
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<tr>
<td><strong>Lung Histology</strong></td>
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<tr>
<td>Cellular inflammation</td>
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<tr>
<td>Mucus production</td>
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<tr>
<td><strong>Inflammation</strong></td>
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</table>
Inhalation Exposure to EPFRs Induces Physiologic Response in Neonates

EPFR Exposure Alters Neonatal Lung Architecture

Air

DCB230

Neonatal rats

EPFRs Increase Smooth Muscle Mass in the Peribronchial Region

Neonatal rats

EPFR Response Unique to Neonates

- Epithelial disorganization
- Increased smooth muscle mass and airway collagen deposition
- Correlated to increased airway hyper-responsiveness

Thevenot, et al. AJRCMB. In revision 2012.
In Vitro Exposure to EPFRs Suggests EMT

6hr Exposure to DCB230 (20 µm/cm²)

Thevenot, et al. AJRCMB. In revision 2012.
In Vitro Exposure to DCB230 Alters Epithelial Cell Morphology

Vehicle  DCB230  DCB230 + Uptake Blockers

Wortmann: macropinocytosis
Chlorpromazine: clathrin dependent endocytosis
Filipin III: calveole, cholesterol

Thevenot, et al. AJRCMB. In revision 2012.
# EMT-Related Gene Expression Increases in BEAS-2B Cells Exposed to DCB230

## Epithelial to Mesenchymal Transition Pathway Array

<table>
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<tr>
<th>Gene</th>
<th>Symbol</th>
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<td>E-cadherin</td>
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<td>Desmoplakin</td>
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<td>Occludin</td>
<td>OCLN</td>
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<td>Wingless Type 5B</td>
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<td>β-catenin</td>
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<td>Glycogen Synthase Kinase 3β</td>
<td>GSK-3β</td>
<td>-1.2</td>
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</table>

Thevenot, et al. AJRCMB. In revision 2012.
Experimental Methods

Mouse Neonate Nose Only Exposure Model

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<tr>
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</tbody>
</table>

DCB-230 200µg/m³ 20 min/d

EMT Gene and Protein Expression 24hr post-exposure

Analysis

Airway Remodeling

Air: Liquid Interface with Neonatal Airway Epithelial Cells

EMT
Summary of Results

- Infant exposures to EPFR-containing PM lead to long-term pulmonary consequences
  - Distinct pathologies
    - Inflammation
    - Remodeling (w/i 4d exposure) – EMT
      - In vivo
        » E-cad + aSMA
        » Bgal + aSMA
      - In vitro neonatal ALI
        » E-cad + aSMA
        » Expression of genes associated with EMT: ↑Snai1 + aSMA and ↓E-cad
  - Respiratory dysfunction
  - Uptake & Oxidative stress
    • ↑8-isoprostanes
    • ↓GSH:GSSG ratio

- Relevance:
  - Mechanistically link PM exposure to airway remodeling
  - Loss of epithelial integrity suggests a window of vulnerability to RTI

Impact

- **Global Environmental Health**
  - Role of inhalation exposures in the predisposition, development, and/or exacerbation of respiratory diseases
  - Mechanisms by which EPFR-containing PM
    - induces pulmonary inflammatory diseases
    - Increases susceptibility to LRTI
  - Potential therapeutic targets for preventing EPFR & PM induced pulmonary dysfunction

- **Environmental Policy**
  - Enhance monitoring practices to include EPFRs
  - Alter air quality standards for EPFR containing PM
Distribution of fluorescent microspheres in the lung

Fine spheres (red spheres, 1.6 μm, yellow arrow head)
Ultrafine spheres (green spheres, 0.2 μm, white arrow)
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