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

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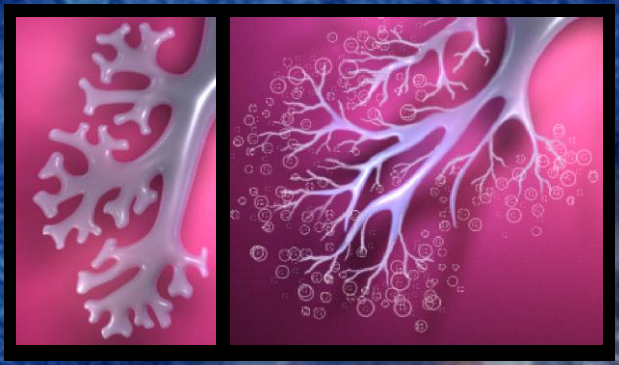
Central Hypothesis

- 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



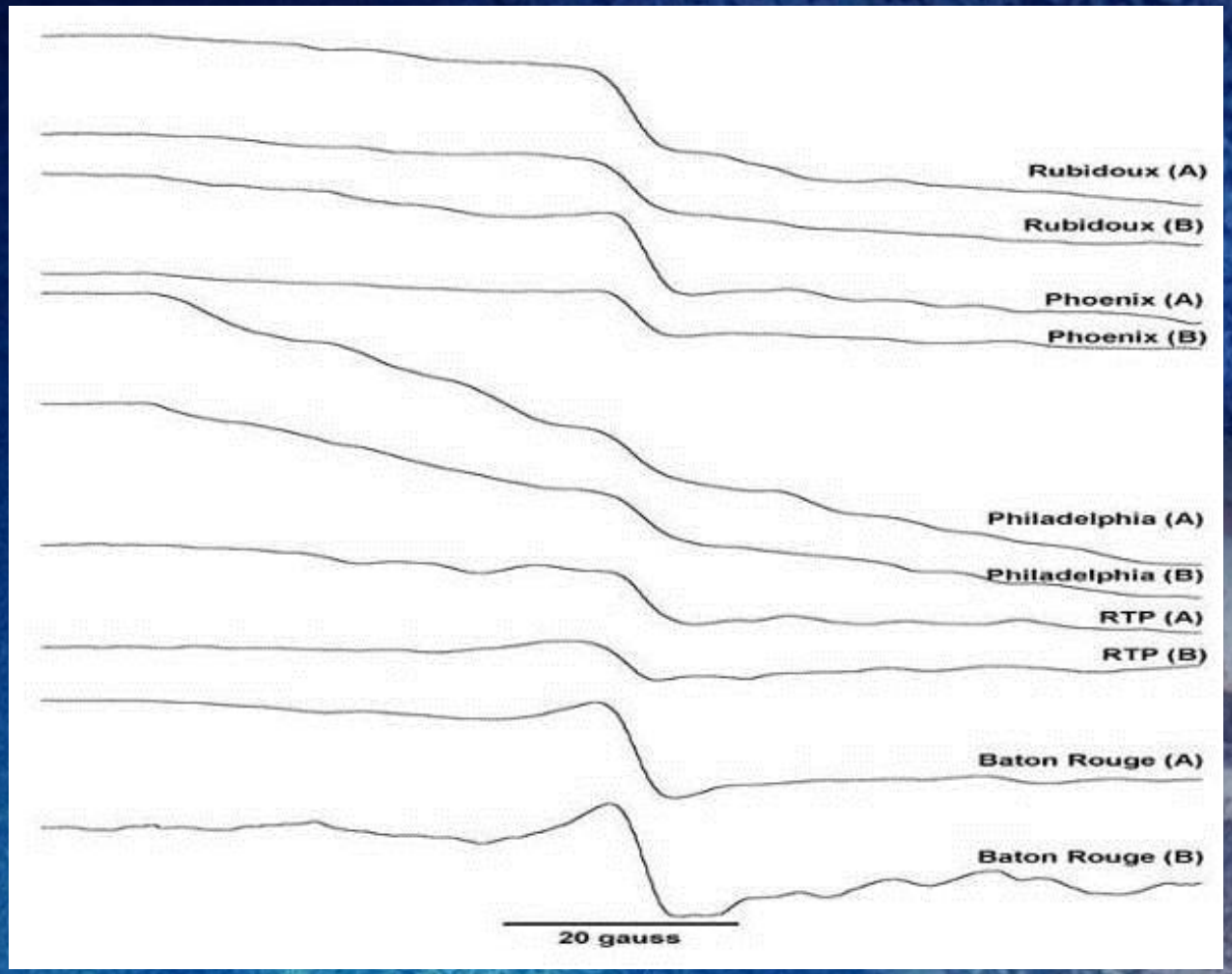
*Pictures are artistic renditions of lung development and are designed to emphasize terminal acinus development and not the entire conducting airway system

Behrman: Nelson Textbook of Pediatrics, 16th ed., 2000. Langston C, et al. *Am Rev Respir Dis.* 1984;129:607-613.

PM INDUCES ADVERSE PULMONARY EFFECTS IN INFANTS

WHAT IN PM IS RESPONSIBLE FOR ADVERSE PULMONARY EVENTS?

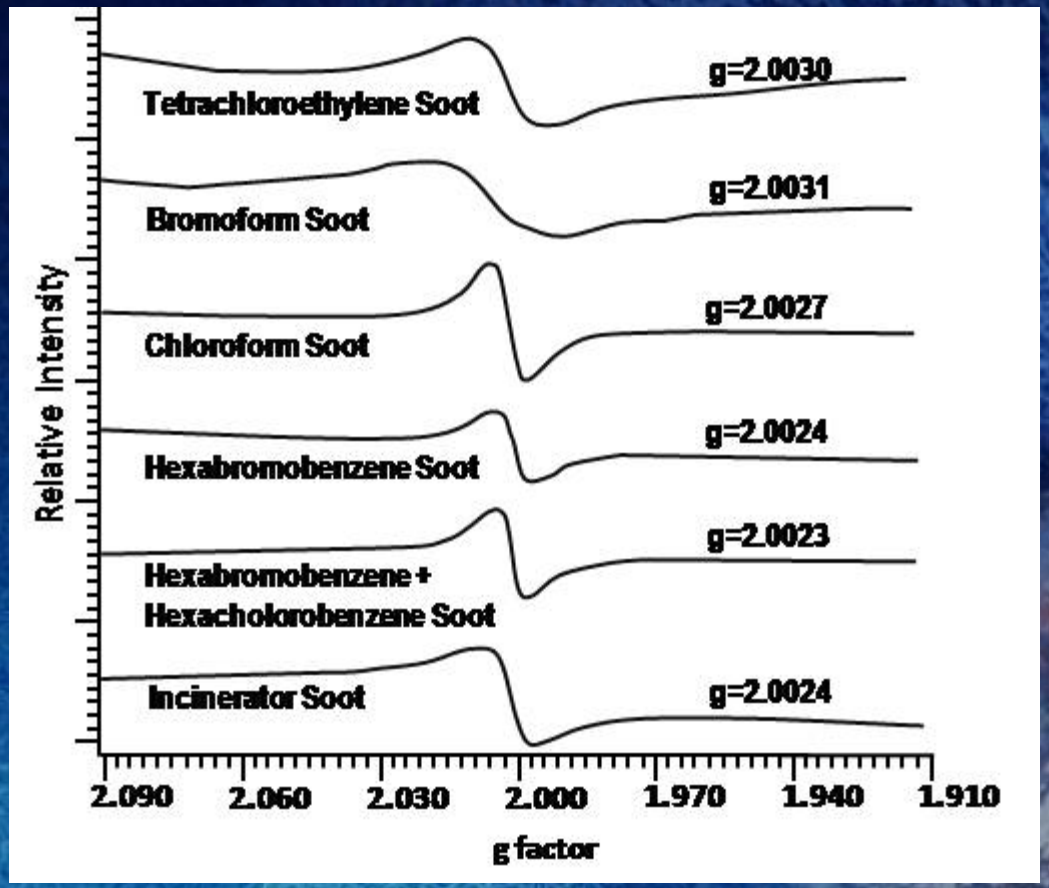
Atmospheric Fine Particles Contain Persistent Semiquinone-type Radicals



CS tar: 1e16 radicals/g

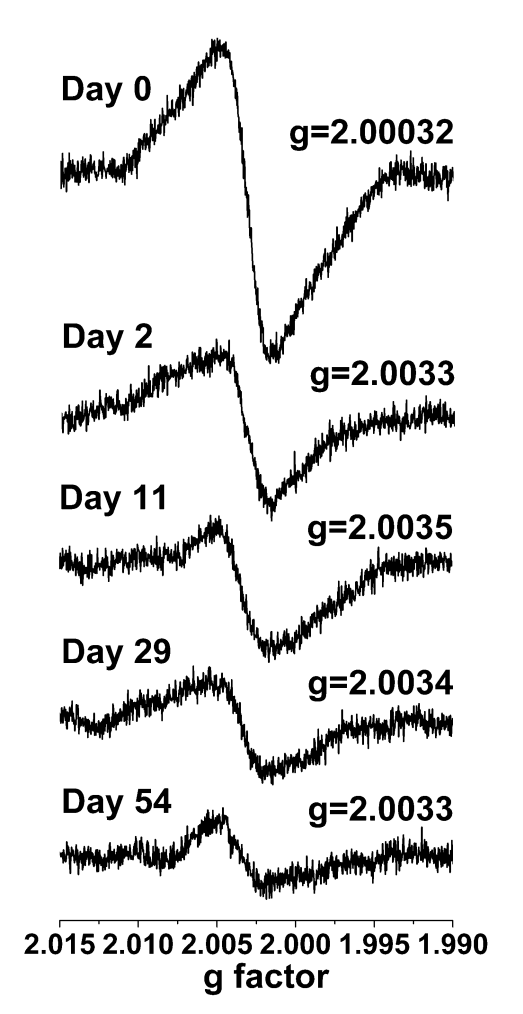
$PM_{2.5}$:1e16 - 1e17 radicals/g

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



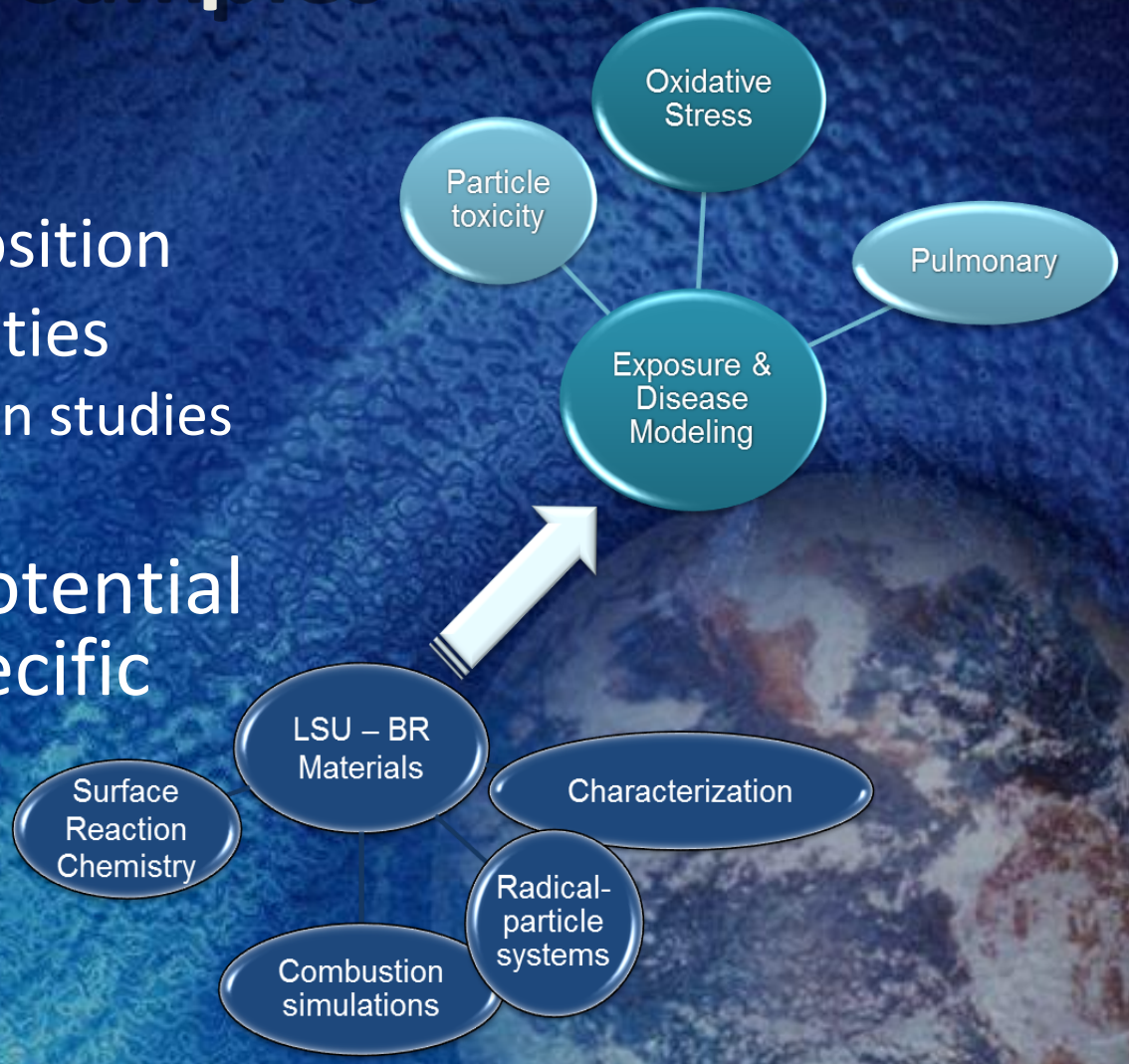
$T_{1/2}$ = 21d

Problems with Studying Atmospheric PM Samples

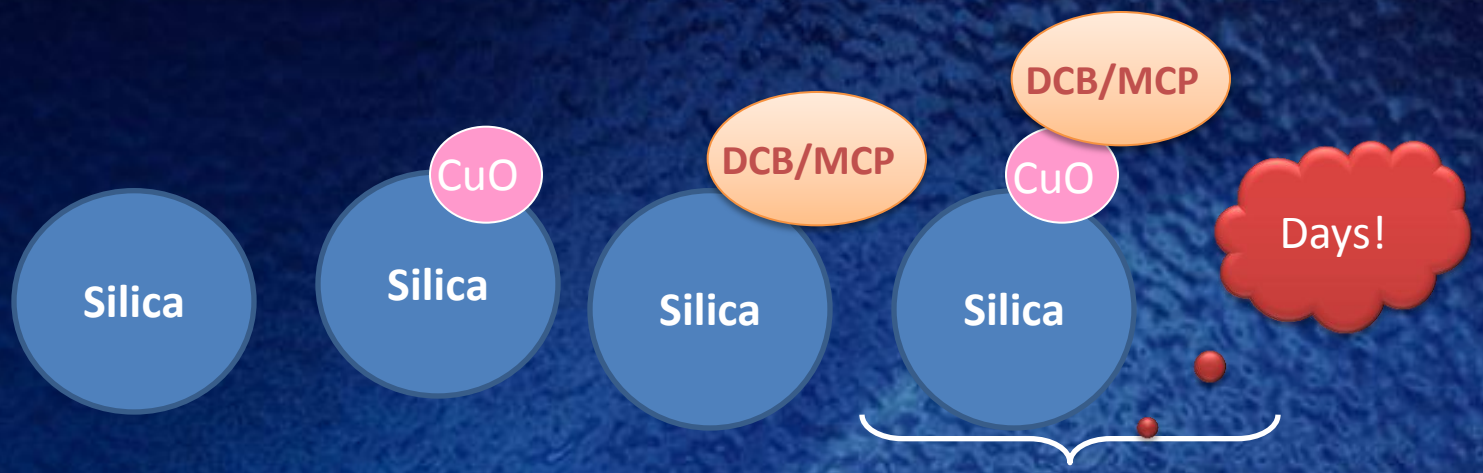
- Size variation
- Sample variation
- Chemical complexity
  - Organic compounds
  - Metal ions
- Sufficient sample size
- Collection methodology (e.g. CAPs, extracts, etc.)

Laboratory Generated Combustion Samples

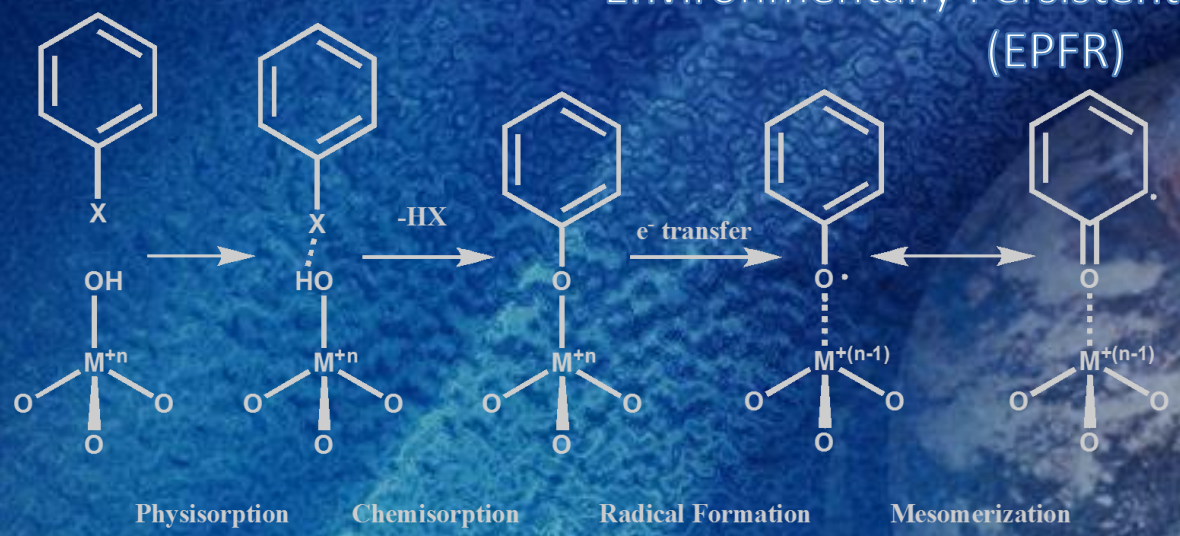
- Control
  - Size
  - Chemical composition
  - Sufficient quantities
    - In vivo inhalation studies
- More accurate assessment of potential risk posed by specific PM components
  - CHC/BHC
  - Radicals



Particle Systems



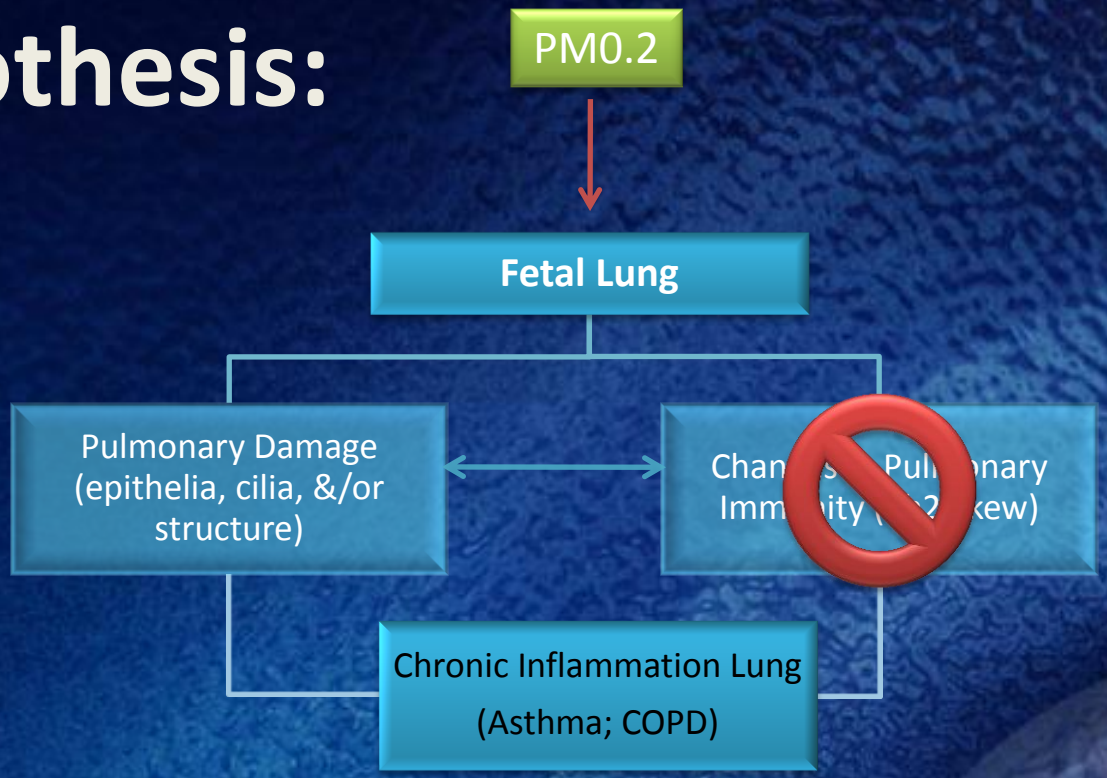
Environmentally Persistent Free Radical (EPFR)



Dellinger et al., 2007

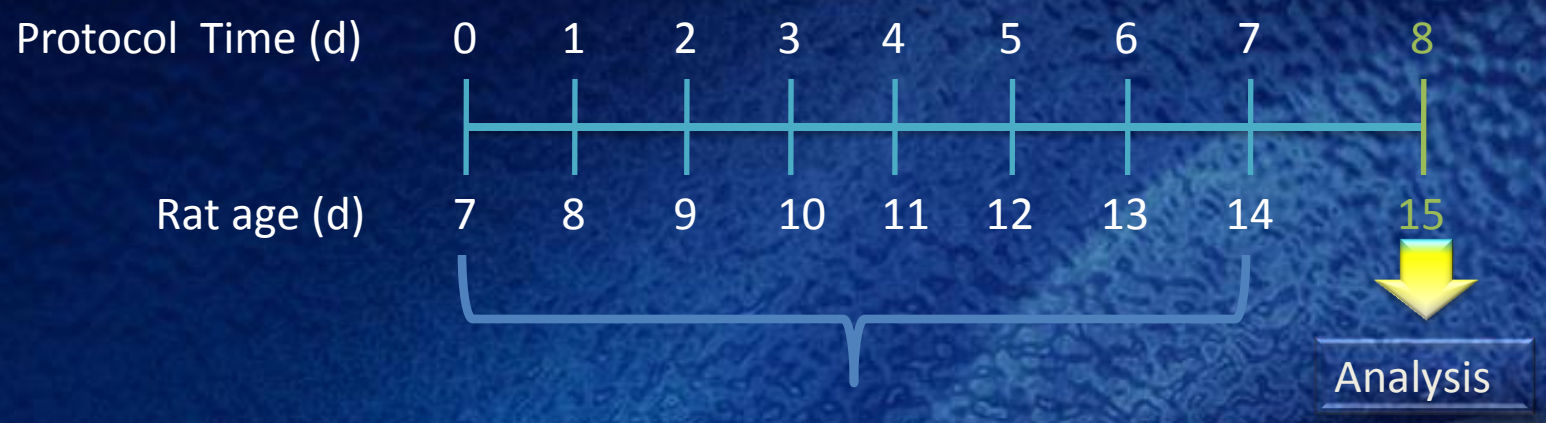
EPFRs:1e14 - 1e14 radicals/g

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



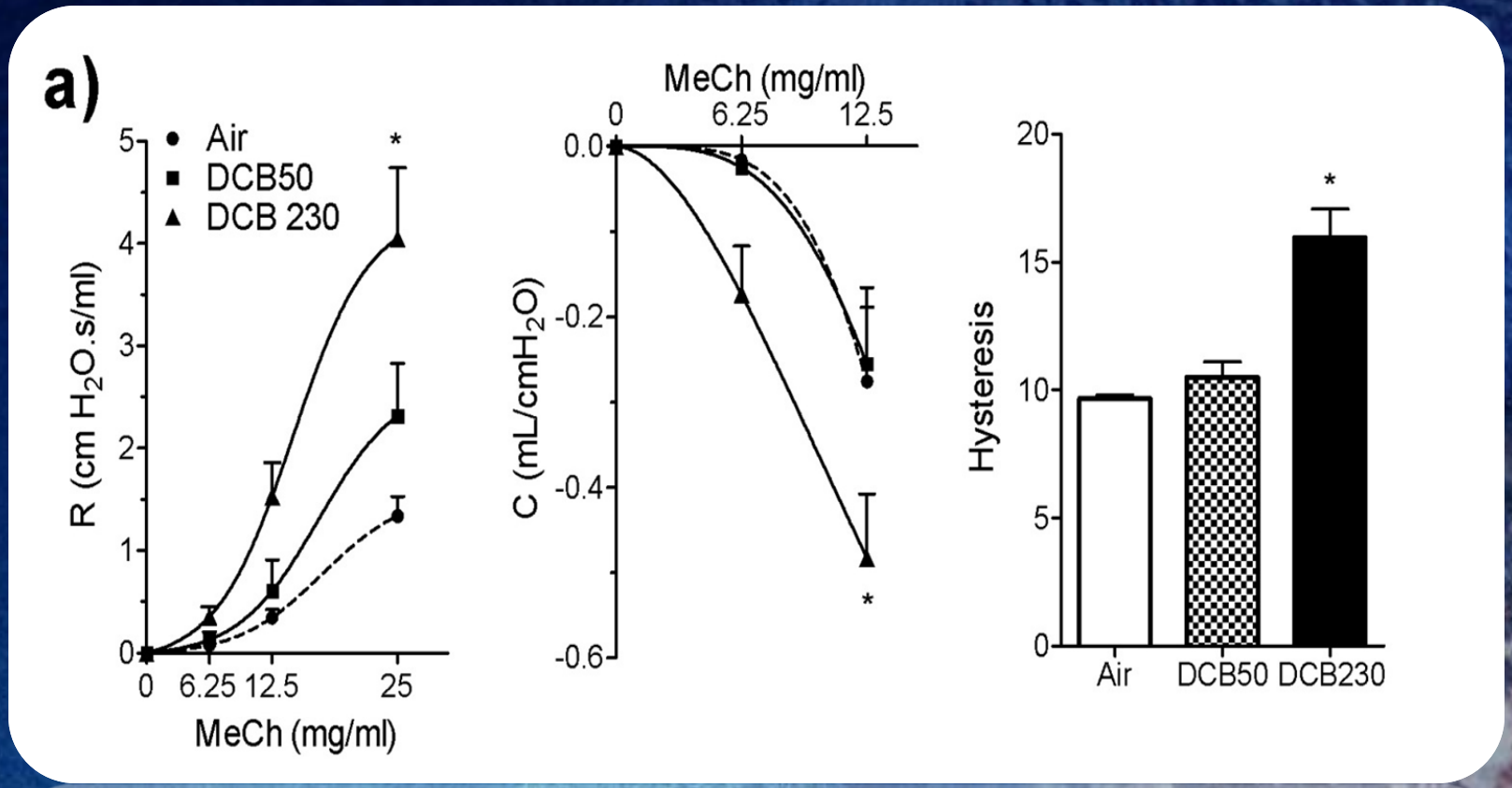
Study Endpoints

Lung Function
- AHR
- Resistance, elastance,

Lung Histology
- Cellular inflammation
- Mucus production

Inflammation
- BAL: cell type & numb

Inhalation Exposure to EPFRs Induces Physiologic Response in Neonates



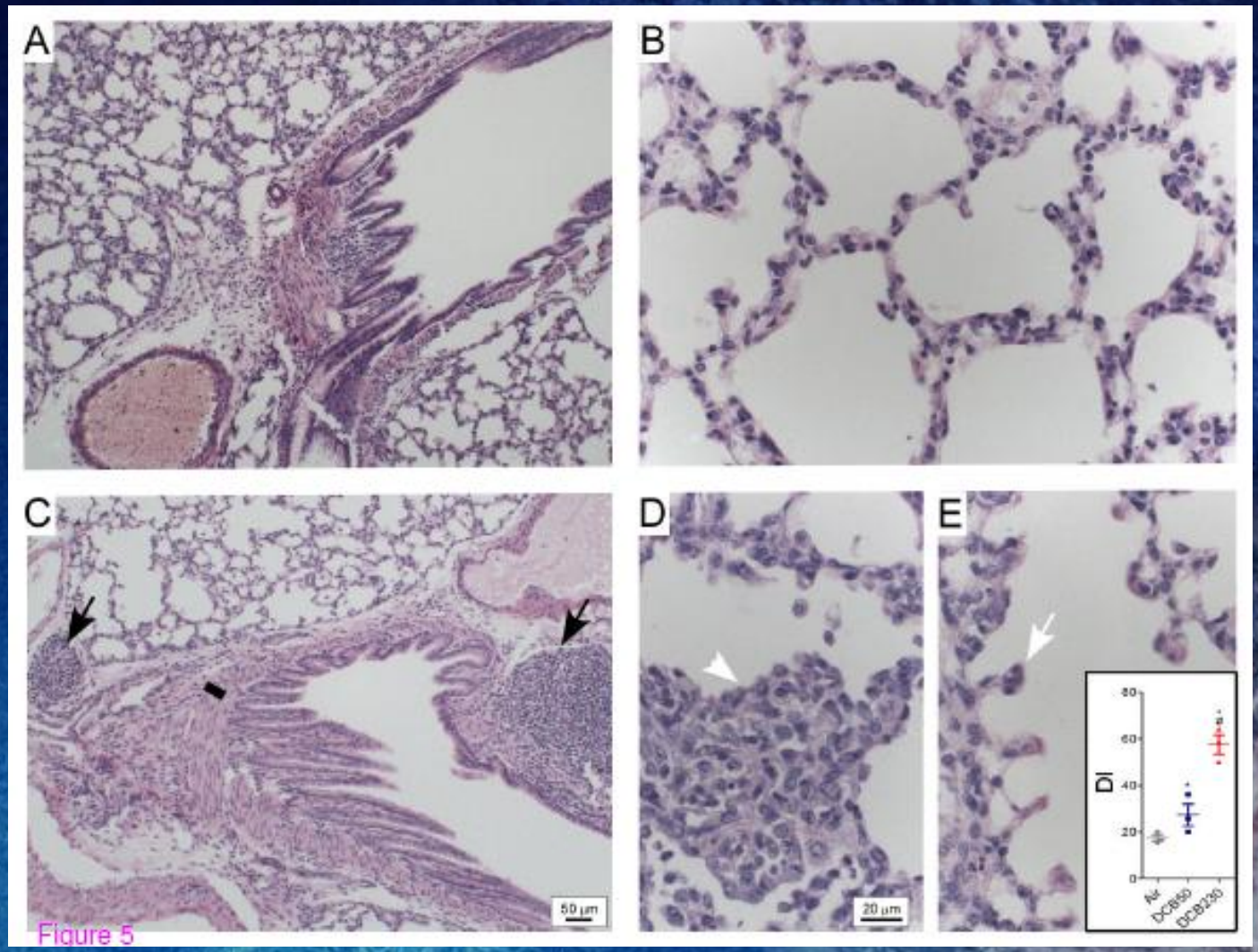
Neonatal rats

Balakrishna S, Saravia J, et al. PFT. 2011;8:11.

EPFR Exposure Alters Neonatal Lung Architecture

Air

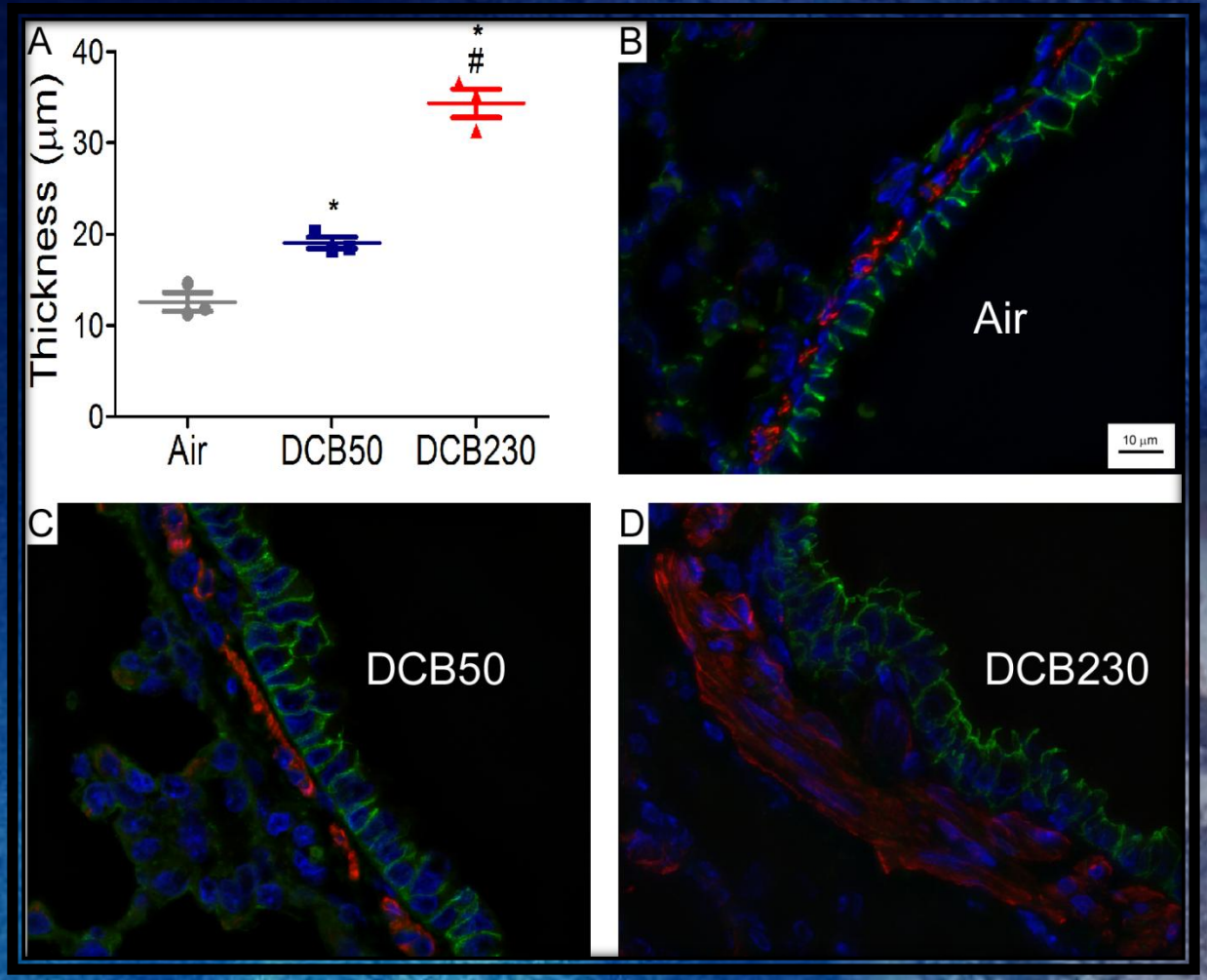
DCB230



Neonatal rats

Balakrishna S, Saravia J, et al. PFT. 2011;8:11.

EPFRs Increase Smooth Muscle Mass in the Peribronchial Region

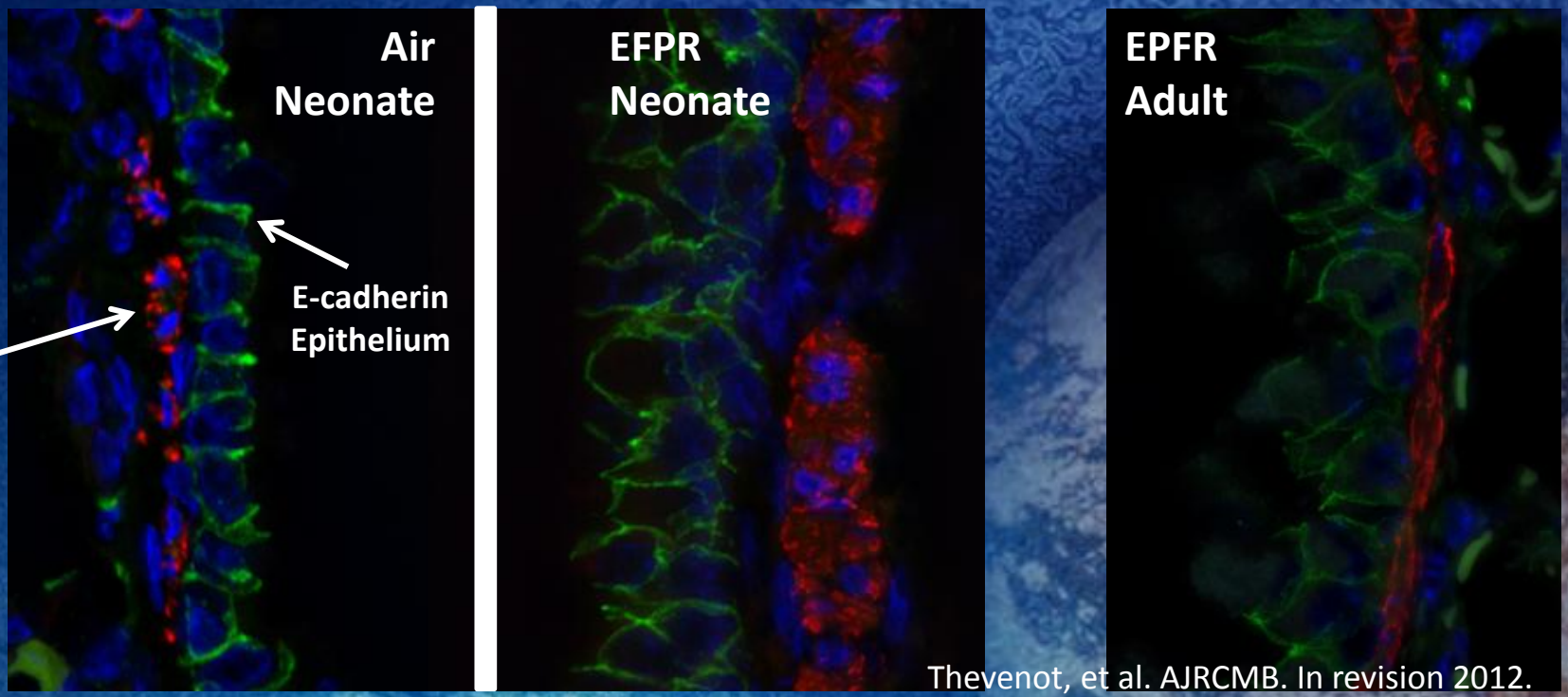


Neonatal rats

Balakrishna S, Saravia J, et al. PFT. 2011;8:11.

EPFR Response Unique to Neonates

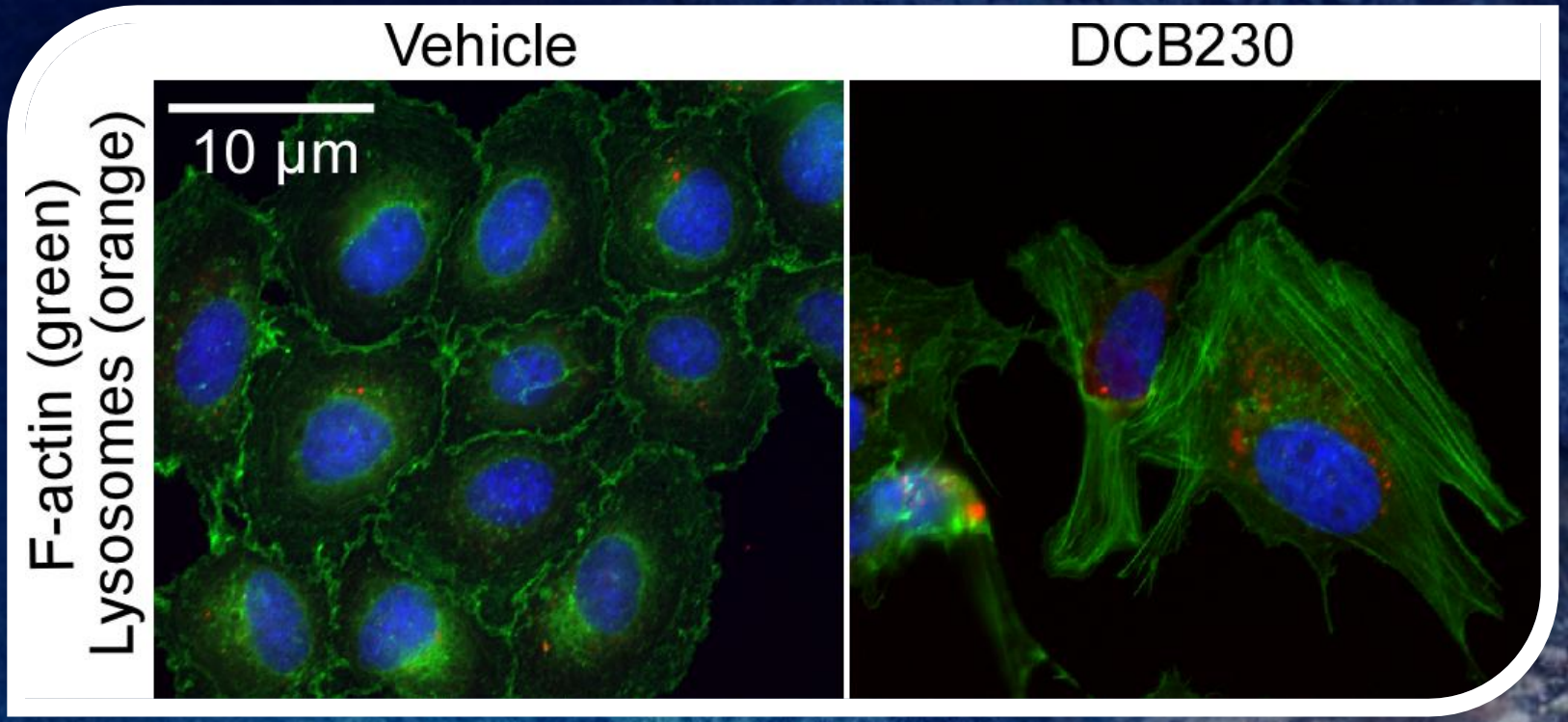
- Epithelial disorganization
- Increased smooth muscle mass and airway collagen deposition
- Correlated to increased airway hyper-responsiveness



Mice

Thevenot, et al. AJRCMB. In revision 2012.

In Vitro Exposure to EPFRs Suggests EMT

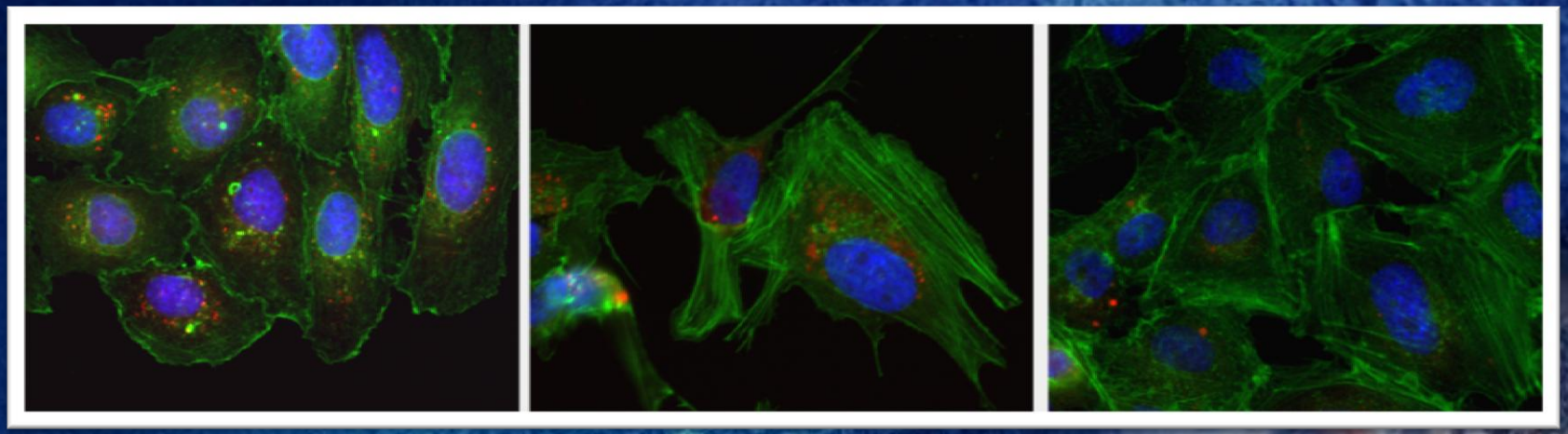


6hr Exposure to DCB230 (20 µm/cm^2)

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.

F-actin, lysosomes, nuclei

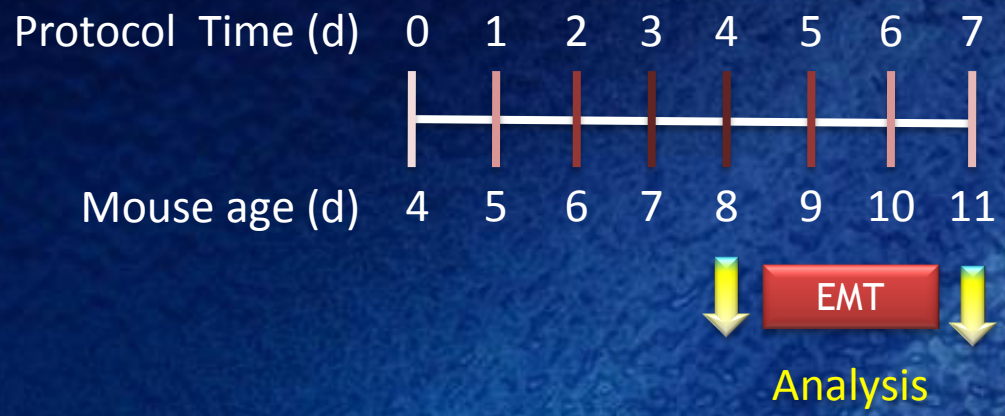
EMT-RELATED GENE EXPRESSION INCREASES IN BEAS-2B CELLS EXPOSED TO DCB230

Epithelial to Mesenchymal Transition Pathway Array				
	Gene	Symbol		Fold Change
Cell:Cell Junctions	E-cadherin	*CDH1*	↓	-1.5
	Desmoplakin	*DSP*	↓	-1.8
	Junctional Adhesion Molecule	*F11R*	—	-1.4
	Vimentin	*VIM*	—	1.3
	Fibronectin 1	*FN1*	—	1.3
	Occludin	*OCLN*	—	-1.2
E-cadherin Repression and Mesenchymal Phenotype	Snail	*SNAI1*	↑	1.8
	Slug	*SNAI2*	↑	2.1
	Smuc	*SNAI3*	↑	3.9
	Zinc Finger E-box Binding Homeobox 1	*ZEB1*	↑	1.5
	Zinc Finger E-box Binding Homeobox 2	*ZEB2*	↑	1.6
Remodeling and ECM	Versican	*VCAN*	↑	3
	Matrix metalloproteinase 3	*MMP3*	↑	40
	Matrix metalloproteinase 9	*MMP9*	↑	3
	Plasminogen Activator Inhibitor	*SERPINE1*	↑	12.8
WNT Signaling Pathway	Frizzled 7	*FZD7*	—	1.3
	Wingless Type 11	*WNT11*	↑	5.9
	Wingless Type 5A	*WNT5A*	↑	3.9
	Wingless Type 5B	*WNT5B*	↑	5.7
	β-catenin	*CTNNB1*	—	-1.4
	Glycogen Synthase Kinase 3β	*GSK-3β*	—	-1.2

Thevenot, et al. AJRCMB. In revision 2012.

Experimental Methods

Mouse Neonate Nose Only Exposure Model



DCB-230
200µg/m³
20 min/d

Airway Remodeling

EMT Gene and Protein Expression 24hr post-exposure

Air:Liquid Interface with Neonatal Airway Epithelial Cells

Summary of Results

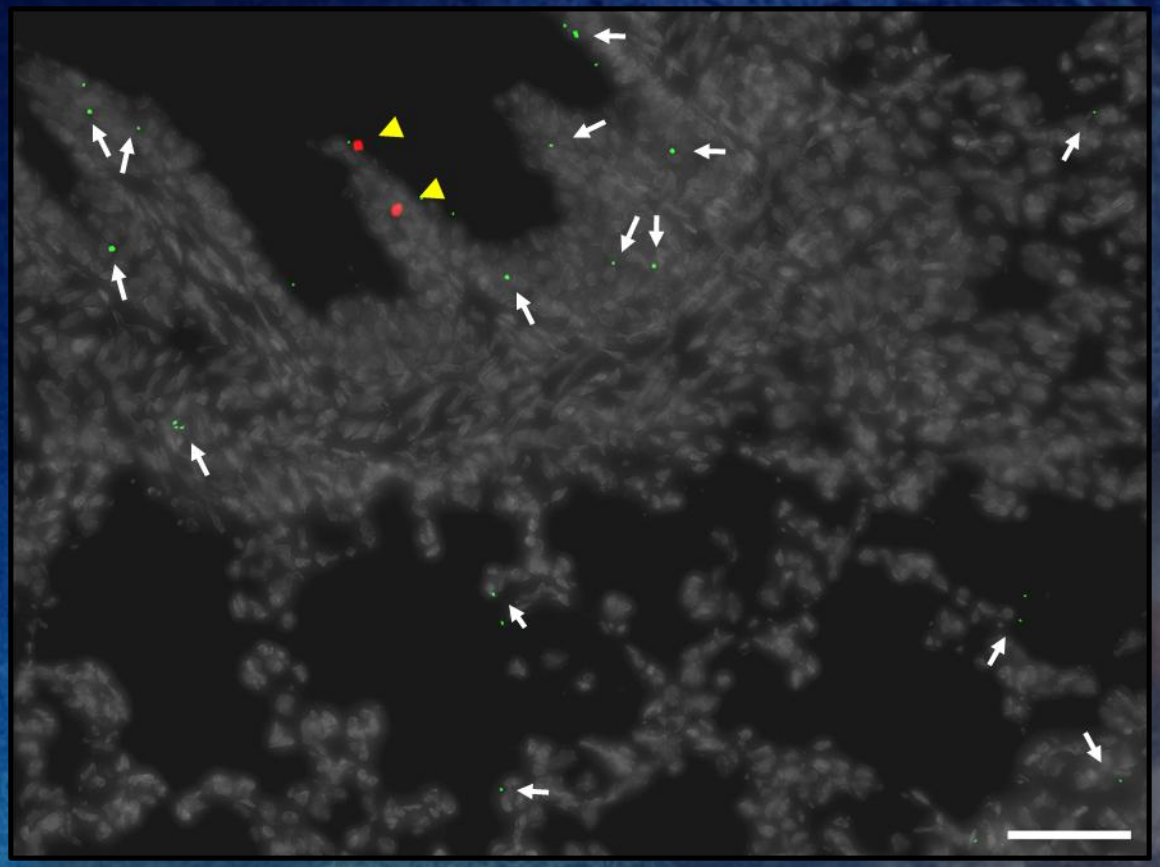
- Infant exposures to EPFR-containing PM lead to long-term pulmonary consequences
  - Distinct pathologies
    - Inflammation
    - Remodeling (w/i 4d exposure) – EMT
      - In vivo
        - » E-cad + aSMA
        - » Bgal + aSMA
      - In vitro neonatal ALI
        - » E-cad + aSMA
        - » Expression 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

Balakrishna S, et al. PFT. 2011;8:11.
Wang P, et al. AJRCMB. 2011. 45: 977-983

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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    - **Joseph Giaimo**
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