Mechanistic Mediation of Flame Retardants in Preterm Birth

Ramkumar Menon, MS, PhD
Professor, Department of Obstetrics and Gynecology and Cell Biology
Director, Division of Basic and Translational Research
The University of Texas Medical Branch,
Galveston, TX, USA
Executive Director, Preterm Birth International Collaborative (PREBIC, Inc.)

World Prematurity Day November 17th

Webinar
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Happy & Healthy Baby!

Definition of preterm birth:
Babies born alive before 37 completed weeks of pregnancy

- 15 million/year
- 1 million neonatal deaths
- Life-long disabilities
- Adult-onset diseases
Preterm birth: A complex syndrome

- Genetic
- Epigenetic
- Infection
- Autoimmune
- Uterine distensions
- Autoimmune
- Endocrine dysfunctions
- Allergies
- Epigenetic
- Oxidative stress
- Inflammation
- Placental senescence and apoptosis
- Decidual activation
- Membrane Weakening o & rupture
- Myometrial activation
- Cervical ripening
- Preterm labor

- Body Mass Index
- Socio-economic
- Epigenetic
- Physiological Stress
- Psychological
- Nutrition
- Ethnicity/Race
- Epigenetic
- Decidual Hemorrhage
- Hydramnios

- Environment*
Polybrominated Diphenyl Ethers (PBDE)

- Environmental pollution is a well-established risk factor for preterm birth.

- Polybrominated Diphenyl Ethers (PBDE) - one of the most prevalent organic pollutants

- PBDEs have been applied to numerous consumer products over the past 40 years.
  - PBDEs function as flame retardants
  - Commercial PBDE is a mixture of different PBDE congeners.
  - Structurally similar, contains a central biphenyl structure surrounded by up to 10 bromine atoms
  - Environmental pollutants are endocrine disruptors, many with pro-estrogenic functions

PBDE-47 (2,2',4,4'-tetrabromodiphenyl ether)  

PBDE-99 (2,2',4,4',5-pentabromodiphenyl ether)

Flame Retardant and Adverse Perinatal Outcome (FRAPO) – PIs - Getahun, Darios, Peltier, Morgan R & Menon R (co-I)
Increased PBDE Concentration is Associated with Preterm birth

Cumulative empirical distribution of maternal plasma PBDE in women who deliver at term or preterm

Effect of PBDE levels on the odds of delivering preterm.
Data adjusted for maternal race, marital status, and age. Adjusted odds ratios ± 95% CI. Bars that cross 1 are not statistically significant.
Inflammation is Associated with Term and Preterm Labor

**Physiologic Activation**
- Fetal signals of organ maturity
- Feto-maternal endocrine factors

**Pathologic Activation**
- Risk exposures

Increased inflammation and oxidative stress

Cervical Ripening

Myometrial activation/contractions (Labor)

Delivery
Mechanistic Mediation of Flame Retardants in Preterm Birth

Basic Anatomy of the Maternal-Fetal Interface

- Amniotic Fluid
- Placenta
- Cervix

Fetal Membranes
- Amnion
- Chorion

Maternal Side
- Decidua
- Myometrium

Amniotic Fluid
Production of Reactive Oxygen Species by PBDE

Environmental toxin (PBDE) or Cigarette smoke extract (CSE)

Reactive Oxygen Species 
Damage due to OS 
Development of pathology
Oxidative Damage

Amnion

Chorion nuclei

Chorion E R

Chorion mitochondria

Oxidative Damage Associated Signaling Activation

DNA Damage Repair

ROS

DNA Damage

p38 MAPK

p53

p19

p16

p21

CDK 4/6

CDK 2

Cyclin E

Cyclin D

G1

S

Cancer

Senescence/Apoptosis

The Cell Cycle

Cell with chromosomes in the nucleus

G1

S

G2

DNA synthesis

CDK

cyclin

CDK 4/6

CDK 2

Cyclin E

Cyclin D
PBDE-99 Induces p38MAPK Activation

PBDE-47 48 hrs
PBDE-47 72 hrs
Control 48 hrs
Control 72 hrs

PBDE-99 48 hrs
PBDE-99 72 hrs
Control 48 hrs
Control 72 hrs

P-p38MAPK
Total p38MAPK
Actin

PBDE Induces Cellular Senescence

Control

PBDE - 47

PBDE - 99

% of SP

PBDE treated amnion cells with SP

Senescence associated secretory phenotype (SASP)
- Increased inflammatory cytokines
- IL-6, IL-8, TNF-a, GM-CSF
- MMP9

➢ p53 activation was not seen in response to OS
Extracellular Vesicles – As Carriers of Specific Cargo

- Natural nanoparticles
- 30-200 nm size
- Released from all cells
- Considered as waste dispensers
- Represent physiologic state of cell
- Contain proteins, nucleic acids, lipids and other materials
- Can be involved in paracrine signaling

Can Fetal Exosomes cause Inflammation in Maternal Cells?

Exosomal Characteristics from PBDE Treated Fetal Cells

Size

Quantity

Control
PBDE-47
PBDE-99

Exosome cargo from OS induced fetal cells
• HMGB1
• CD9 Exosome marker
How Fetus Communicates with Mother to cause Preterm Birth?

Testing exosomal communication using organ on a chip (OOC) for fetal – maternal interface
Impact of Cadmium toxicity during pregnancy and pathologic mechanism at the feto-maternal interface

Created an in vitro ascending model of infection. Physiologically validated in vivo using animal models

Recreated cervical remodeling process in vitro
Fetal-Maternal Interface-On-Chip

Fetal Membrane Organ on a Chip (FMi-OOC)
Fetal Maternal Interface-On-Chip

- Bright field
- Trichome
- CK-18/Vimentin
- Viability

AEC
AMC
CT
Decidua
Engineering of Exosomes to load HMGB1 and its Characterization

1. EXOSOME ISOLATION From AEC
2. NANOPARTICLE TRACKING ANALYSIS
3. ELECTROPORATION rhHMGB1
4. CLEANING OF ENGINEERED EXOSOMES

Particle size
Morphology by TEM
Exosome markers

HMGB1 + - - -
Electroporation + + - -

Exoview data

Exosomes containing 10ng HMGB1 was used for experiments.

Electroporation did not impact uptake of exosomes by recipient cells.
Two types of exosomes derived from amnion epithelial cells (AECs) were tested:

1. Endogenous exosomes – Derived from RFP Cells → red exosomes
2. Exogenous exosomes – Derived from AEC, electroporated to contain HMGB1 (eHMGB1) → labelled with Green dye

Theoretical concept

Summary

Experimental approach and outcome

- **Preterm Birth**
- **Chamber #1** (AECs)
- **Chamber #2** (AMCs)
- **Chamber #3** (CTCs)
- **Chamber #4** (DEC)

START MONITORING UNTIL BIRTH

IA EXOSOMES

E17 E17.5 E18 E18.5 E19 E20

INTRA AMNIOTIC INJECTION

START MONITORING UNTIL BIRTH

TERM BIRTH

IA EXOSOMES

E17 E17.5 E18 E18.5 E19 E20

Placenta

Fat membrane

Endometrium

Amniotic fluid

Exosomes Uptaken by fetal membrane & uterine cells

Enhanced inflammation

Preterm Birth
Exosomal delivery of NF-κB inhibitor delays LPS-induced preterm birth and modulates fetal immune cell profile in mouse models.

Samantha Sheller-Miller, Enkhtugaa Radnaa, Jee-Kwang You, Yipyoung Choi, Younggon Kim, Yu Na Kim, Eunsoo Kim, Lauren Richardson, Chulhee Cho, Ramkumar Menon.

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Conclusions

- Congener specific PBDE function can induce preterm birth
  - Health issues for neonates and potentially their mothers
- Environmental toxicants can generate ROS induced cellular damage
- Activate fetal membrane cell senescence leading to preterm birth
- Senescence associated inflammation is propagated via exosomes that can cause untimely activation (preterm) of maternal uterine tissues
  - Quiescent muscular myometrium gets activate to a labor phenotype

Future Directions

- Mechanistic impacts of environmental pollutants are hardly studied during pregnancy
- Impacts vulnerable population – Reproductive age women and their children
- Poorly funded area of research

- Exosomes and organ on chip technology offers valuable tools to study pollutant effects during pregnancy
- Biomarker potential (fetal exosomes in maternal blood)
- Delivery of drugs using exosomes as vehicles to cross placental barrier
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It’s about saving babies!