The Environment-Genome Interplay and the Emergence of Neuro-epigenetics

Lubo Zhang

The Lawrence D. Longo, MD Center for Perinatal Biology
Loma Linda University School of Medicine
OPPORTUNITIES AND CHALLENGES

- Environmental and Life Style Impact on Development
- Influence of Early Life Events on Health and Disease
- Understanding Complex Molecular Interactions
Developmental Programming of Health and Disease

- Environmental Exposure
- Programmable Phenotype
- Genomic Variation
- Epigenomic Variation
Malnutrition
Hypoxia
Smoking & Drugs
Ischemic Heart Disease
Hypertension
Atherosclerosis
Diabetes
Stroke and Neurological Disorders
Cancer
Malnutrition
Hypoxia
Smoking & Drugs
Hypoxic-ischemic encephalopathy (HIE) is a major cause of acute neonatal brain injury and mortality, as well as chronic neurological disabilities.

Mechanisms underlying neonatal HIE remain largely elusive.

Fetal stress may be of importance in programming of HI-sensitive phenotype in the developing brain.
Animal Model

Hypoxia (10.5% O₂)

E15 - E21  |  Birth  |  P10 pups

Brain gene expression

HI brain injury Rice-Vannucci model

Sprague Dawley Rats
Male                   Female

Infarct size
(% whole brain)

* *

Control
Hypoxia

Neurobiol Dis 2014;65:172-179
What are the molecular mechanisms underlying fetal stress-induced increase in hypoxic-ischemic vulnerability of neonatal brains?
Epigenetic Modification of Gene Repression

Active chromatin

Nucleosome

Ac

Ac

TF

Histone deacetylase

DNA methyltransferase

Inactive chromatin

TF

Me

Me

P

TF

Transcription inhibited

DNA

Exon

Exon

Exon

Exon

mRNA

Protein

Translation inhibited

mRNA + microRNA

NEJM 2008;359:61-73
- Global hypomethylation
- Gene-specific hypermethylation (GR)
- Micro RNAs (micro RNA 210)
%5-methylcytosine (fold of control E21)

- **Normoxia**
- **Hypoxia**

**E21**

**P30**

- *
- #

Exp Neurol 2016;275:1-10
<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Hippocampus</th>
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<tr>
<td><strong>Saline</strong></td>
<td><img src="5-mC_Saline_Cortex.png" alt="Image" /></td>
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<td><strong>5-Aza</strong></td>
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*Exp Neurol 2016;275:1-10*
Aza saline
T2 map
HRS

Saline
5-Aza

Saline 5-Aza
*
*
*

0 5 10 15
Saline 5-Aza

% Brain lesion volume

Total Core Penumbra

* * *

Exp Neurol 2016;275:1-10
**Rotarod test**

- **Saline**
- **5-Aza**

**Open Field test**

- **Saline**
- **5-Aza**

**Zero Maze test**

- **Saline**
- **5-Aza**
Gene-specific hypermethylation (GR)

- Glucocorticoids are essential for the brain development and play a central role in the response to stress.

- Glucocorticoid receptors (GRs) are abundantly expressed in the developing brain with dynamic and complicated ontogeny in a tissue-specific manner.

- GR gene transcription is permanently determined or programmed by perinatal events.
E21 fetal brain

P10 neonatal hippocampus

Neurobiol Dis 2014;65:172-179
Proximal Promoters

J Mol Cell Cardiol 2016;91:160-171
GR promoter

E21 fetal brain

P10 neonatal hippocampus

Hypoxia-induced changes (fold of control)

Exon 1s: 4 5 6 7 9 10 11

Female

Male

Control

Hypoxia

mRNA (fold of control)

Exon 1s: 7 11

Female

Male

* Neurobiol Dis 2014;65:172-179
5'-SP1-Egr-1-exon 17

5'-SP1-exon 111

5-methylcytosine (% control)

Control

Hypoxia

Neurobiol Dis 2014;65:172-179
**Neurobiol Dis 2016;89:202-212**

The diagram illustrates the infarct size (%) in different treatment groups. The y-axis represents the infarct size in percentage, ranging from 0% to 30%. The x-axis lists different treatment conditions.

- **Control**
- **DEX (10 ng)**
- **DEX+RU486 (0.5 µg)**
- **DEX+RU486 (1 µg)**

The diagram shows a significant decrease in infarct size with the DEX+RU486 (0.5 µg) treatment compared to the control and other treatment groups. * denotes statistical significance.
Micro RNAs (micro RNA 210)

- MiR210 is the Master Hypoxamir of a specific group of miRs termed “Hypoxamirs” that are regulated by hypoxia.

- Mature miR210 of 22 nt is highly homologous and is identical among human, ovine and bovine, and rodents.

- MiRs silence gene expression by binding to 3’UTR of transcripts via their seed sequences at 5’ ends (nucleotides 2-8), resulting in transcript degradation or translational inhibition of the target genes.
Relative miR-210 level

- Sham
- HI

* Significant difference

Neurobiol Dis 2016;89:202-212
Neg. Ctrl  |  miR-210 mimic
---|---
GR  |  β actin

Neg. Ctrl  |  miR-210 mimic
---|---
ISCU  |  β actin

GR protein (fold of control)

<table>
<thead>
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<tr>
<td>0.0</td>
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</tr>
<tr>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.8</td>
<td>1.0</td>
</tr>
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<td>1.2</td>
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ISCU protein (fold of control)

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* Neurobiol Dis 2016;89:202-212
Neg. Ctrl
miR-210 mimic

Infarct size (%)

B

Control

miR-210

Neurobiol Dis 2016;89:202-212
*Neurobiol Dis 2016;89:202-212*
NeuN/TUNEL

Neg. Ctrl
LNA

Cortex

Hippocampus

TUNEL positive cells (%)

TUNEL
NeuN/TUNEL

Neg. Ctrl
LNA

Neurobiol Dis 2016;89:202-212
Hypoxia

miR-210

ISCU

GR

Mitochondrial dysfunction

Brain injury

Oxidative stress

Neuro-inflammation
Therapeutic potential of intranasal administration of miR210 antagonomir after neonatal hypoxic-ischemic insult on short-term brain injury and long-term neurobehavioral function recovery.
miR210-LNA (100 pmol)  
Negative control (100 pmol)  
Negative control (200 pmol)  
miR210-LNA (200 pmol)

Infarct size (%)  

- Negative control (100 pmol)  
- miR210-LNA (100 pmol)  
- Negative control (200 pmol)  
- miR210-LNA (200 pmol)

* * 

Neurobiol Dis 2016;89:202-212
Neurobiol Dis 2016;89:202-212
Water maze test: Spatial Learning

Zero Maze Test

Rotarod Test

Neurobiol Dis 2016;89:202-212
$r^2 = 0.4535$

$p < 0.05$

Cortex width index

Rotarod trial 1 (s)

Neg. Ctrl

LNA

Neurobiol Dis 2016;89:202-212
Summary

- Adverse intrauterine environment and fetal stress result in epigenetic programming of hypoxic-ischemic (HI)-sensitive phenotype in the developing brain.

- Fetal hypoxia induces both global hypomethylation and gene-specific hypermethylation (GR) in the developing brain, which play a key role in antenatal stress-mediated increase in the risk of hypoxic-ischemic encephalopathy (HIE).

- MicroRNA 210 is the Master Hypoxamir and translationally suppresses ISCU and GR protein expression, leading to mitochondrial dysfunction and increases in oxidative stress and neuroinflammatory response in the brain.

- Intranasal administration of microRNA 210 antagonim and hydrocortisone presents a therapeutic potential in neonatal hypoxic-ischemic brain injury and improves long-term neurobehavioral function recovery.
Questions?