II. Immunology and Inflammation

4:45 PM
Too Much of a Good Thing? Considering Gene-Environment Interactions in Health and Disease

Lee Niswander, Ph.D. – Chair of Molecular, Cellular, and Developmental Biology, University of Colorado Boulder
Too much of a good thing?
Considering gene-environment interactions in health and disease.
Developmental Biology to understand the causes of birth defects
Developmental Origins of Health and Disease

Neural Tube Defects (spinal cord/brain)
NTDs = Failure of Neural Tube Closure  
~1:1000 births worldwide

**Spina Bifida:** failure of lower neural tube to close  
- Increased mortality  
- Life-long morbidities  
  - Neurologic/Neurosurgical  
  - Urologic  
  - Orthopedic  
  - Psychological

**Anencephaly:** failure of cranial neural tube to close  
- Lethal
Early Brain and Spinal Cord Development: Neural Tube Closure

- **mouse**: E8.25 – 10.25
- **human**: Week 3-4

**Diagram:**
- Panels A-D depict the neural tube closure process from different stages.
- Key annotations include:
  - **Neural Plate**, **Non-neural Ectoderm**, **Notochord**, **Mesoderm**, **Neural crest**.
Early Brain and Spinal Cord Development: Neural Tube Closure

Coordinate:
- Patterning
- Growth
- Differentiation
- Cell death
- Cell movements
- Cell architecture
- Tissue interactions
- Physical forces

Diagram showing stages of neural tube closure with labels for neural plate, non-neural ectoderm, notochord, mesoderm, and neural crest.
Early brain and spinal cord formation

Week 3-4: Before woman knows she is pregnant

Timing of closure is critical

Best treatment for NTD is to close the defect...surgery (postnatal or fetal)

Prevention
Strategies for Prevention: Gene-Environment Interactions

Environmental risk factors for NTDs

- Teratogens:
  - valproic acid, carbamazepine, trimethoprim
- Maternal obesity
- Maternal diabetes/hyperglycemia
- Maternal hyperthermia
- Maternal nutrient deficiencies:
  - Folic acid, zinc, iron
Benefits of Folic Acid Fortification

- Folic acid studies began in 1960s, landmark random clinical trials in 1990s

- Mandatory U.S. grain supply fortification started January 1998

- ~35% decrease in NTDs in the U.S.

- How does folic acid prevent NTDs?

- Which mutations/gene pathways benefit from folic acid?
Folic acid is needed for the production of purines, thymidylate, and SAM

Is there a strong correlation between folate pathway mutants and NTDs? NO
How does folic acid act during neural tube closure?

Folic acid (synthetic) is reduced by dihydrofolate reductase (DHFR) and then converted to a biologically active form, 5-methyl-tetrahydrofolate (5-meTHF), by serinehydroxymethyl-transferase (SHMT) and 5,10-methylenetetrahydrofolate reductase (MTHFR). 5-meTHF is then used in the biosynthesis of purines and thymidylate, the synthesis of methionine from homocysteine, and the biosynthesis of S-adenosylmethionine (SAM), the universal methyl donor for cellular methylation reactions, including DNA and protein methylation.

Drugs that act as folate antagonists have continued to be implicated as risk factors for NTDs. Women who took folate antagonists in their first trimester were more than sixfold more likely to have an NTD-affected pregnancy. These included DHFR-inhibitors, such as methotrexate, which inhibit the conversion of folate to its active form, and the antiepileptic drug valproic acid (an HDAC inhibitor).

Folate is a water-soluble vitamin that is found naturally in many foods, especially green, leafy vegetables. Dietary folates, in the form of tetrahydrofolates (THF), play an important role in one-carbon metabolism (Figure 3). FA is a synthetic form of folate that is more stable than the naturally occurring form. Both FA and folate must be reduced by dihydrofolate reductase (DHFR) and then converted to a biologically active form, 5-meTHF, by SHMT and MTHFR. 5-meTHF is then used in the biosynthesis of purines and thymidylate, the synthesis of methionine from homocysteine, and the biosynthesis of SAM, the universal methyl donor for cellular methylation reactions, including DNA and protein methylation.
How does folic acid act during neural tube closure?

Folate replete
Long-term fortification & supplementation

Folate deficient

Cell proliferation and survival

Methylation changes? Epigenetic regulation?

FIGURE 3
Schematic of folate in one-carbon metabolism. Synthetic and dietary folates are reduced to tetrahydrofolates (THFs), which are converted to the biologically active 5-methyltetrahydrofolate (5-methyl-THF) by SHMT and MTHFR. 5-methyl-THF acts as a methyl donor for purine and thymidylate synthesis and generates the major cellular methylation donor S-adenosylmethionine (SAM).

Abbreviations: AHCY, S-adenosylhomocysteine hydrolase; DHFR, dihydrofolate reductase; MAT, methionine adenosyltransferase; MTHFD, methylenetetrahydrofolate dehydrogenase; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; MTs, methyltransferases; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine-hydroxymethyltransferase.
Chromatin modifying enzymes and neural tube defects

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
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<tbody>
<tr>
<td>Baf155</td>
<td>Chromatin remodeling</td>
</tr>
<tr>
<td>Baf47</td>
<td>Chromatin remodeling</td>
</tr>
<tr>
<td>Brg1</td>
<td>ATPase of chromatin remodeling</td>
</tr>
<tr>
<td>Nap 1/2</td>
<td>Histone Chaperone- nucleosome assembly</td>
</tr>
<tr>
<td>CBP</td>
<td>HAT/Transcriptional activation</td>
</tr>
<tr>
<td>P300</td>
<td>HAT/Transcriptional activation</td>
</tr>
<tr>
<td>GCN 5</td>
<td>Histone Acetyltransferase</td>
</tr>
<tr>
<td>HDAC4</td>
<td>Histone Deacetylase</td>
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<tr>
<td>Sirt1</td>
<td>Deacetylase</td>
</tr>
<tr>
<td>Brd2</td>
<td>Histone Modification</td>
</tr>
</tbody>
</table>

Copp and Greene, J Pathology 2009
Harris and Juriloff, Birth Defects Res 2010

Valproic acid: histone deacetylase inhibitor

Crabtree et al 2010
How does folic acid act during neural tube closure?
Genetics of neural tube closure using mouse models

- What are the genes involved?

Mouse studies
Genetic Screens

wildtype

Exencephaly
Cranial NTD

Spina bifida
Caudal NTD

- How do these genes work?

- What goes wrong to cause neural tube defects?
Niswander lab contributions to understanding neural tube closure

**Patterning/cilia**
- Ift88 (null & hypomorph)
- Ift52
- 3poly
- C2cd3 (null & hypomorph)
- Inturned (null & hypomorph)
- Fuzzy
- Mks1
- Ccdc40
- PigN
- Pgap1
- Snx3
- Tmem132a

**Migration**
- Phactr4

**Tissue Interactions**
- Hectd1
- Baf155

**Cell adhesion**
- Grhl2
- Frem2
- AP2α
- Ryr1
- p38IP (null & hypomorph)

**Cell architecture**
- Shroom3
- Grhl3

**Proliferation**
- mLin41
- Phactr4
- Wdr62
- Gcn5

**Differentiation**
- Fpn1
- Pax3
- Zic2

**Environmental Factors**
- Folic acid
- Iron
- Zinc

**Environmental Factors**
- Folic acid
- Iron
- Zinc
Impacting child health

Mouse models of NTDs

Human NTD genomic information

Animal models: causative role & genetic interplay

Environmental Impact

Fetal surgery
Biomaterials, Stem Cells, NTD modeling
Mouse NTD models to uncover the genetics of responsiveness to folic acid

To better reflect current US folic acid intake:

- Moderate and enriched folic acid diets that correlate with pre- and post-fortification diets
  - Long-term diet over multiple generations

What pathways or cellular functions are responsive?

Additional therapies for folic acid non-responsive NTDs?
Mouse NTD models to uncover the genetics of responsiveness to folic acid

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- Folic acid
- Iron
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**Differentiation**
- Fpn1
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- Zic2
Low folic acid levels can increase the risk for NTD.

Is NTD prevention always due to rescue?

No, early embryonic lethality.
Low folic acid levels can increase the risk for NTD.

**Balance?** Might there be a dose that exceeds a beneficial level in the context of genetic mutation?

Unexpected increased NTD risk on enriched folate diet.

Can length of exposure affect the outcome? Yes,.... epigenetic?
Folic Acid Responsive in Mouse Models

11 cases
Non-responsive

Human Molecular Genetics, 2011
Folic Acid Response in Mouse Models

No response to FA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Response</th>
</tr>
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<tbody>
<tr>
<td>Frem2</td>
<td>Non-responsive</td>
</tr>
<tr>
<td>Grhl2</td>
<td>Detrimental response</td>
</tr>
<tr>
<td>Shroom3</td>
<td>Beneficial response</td>
</tr>
<tr>
<td>Pax3</td>
<td>11 cases</td>
</tr>
<tr>
<td>Zic2</td>
<td>Long-term High FA Diet</td>
</tr>
<tr>
<td>3Poly</td>
<td>Long-term Control FA Diet</td>
</tr>
<tr>
<td>Grhl2het</td>
<td>Shroom3 short-term FA beneficial</td>
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</tbody>
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Human Molecular Genetics, 2011
Folic Acid Response in Mouse Models

No response to FA

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<tbody>
<tr>
<td>Frem2</td>
<td>11 cases</td>
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</tr>
<tr>
<td>Grhl2</td>
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</tr>
<tr>
<td>Shroom3</td>
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</tr>
<tr>
<td>Pax3</td>
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<tr>
<td>Zic2</td>
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Human Molecular Genetics, 2011
Folic Acid Response in Mouse Models

- No response to FA
- Beneficial response
- Detrimental response

Frem2         Grhl2        Shroom3        Pax3             Zic2           3Poly           Grhl2het

- Non-responsive: 11 cases
- Beneficial response: 8 cases
- Detrimental response: 3 cases

Link to cilia?
Ciliopathies
Folic Acid Response in Mouse Models

Moderate folic acid levels are beneficial for cilia mutants.
Moderate folic acid levels are beneficial for cilia mutants

Human patient cell lines (primary cilia)
Moderate folic acid levels are beneficial for multi-ciliated cells

**Ependymal flow** (multi-ciliated cells in brain ventricles that move CSF)

- **Moderate folic acid diet**
- **Enriched folic acid diet**

![Moderate folic acid diet](image1)

![Enriched folic acid diet](image2)

![Directionality histograms for moderate folic acid diet](image3)

![Directionality histograms for enriched folic acid diet](image4)
Moderate folic acid levels are beneficial for multi-ciliated cells

Ependymal flow (multi-ciliated cells in brain ventricles that move CSF)

Moderate folic acid diet

Enriched folic acid diet

Moderate folic acid diet

Enriched folic acid diet

Intu het

L3P het

Bead Flowrate µm/s

Moderate

Enriched

Heterozygous (female)

Heterozygous (female)
Moderate folic acid levels are beneficial for multi-ciliated cells

Ependymal flow (multi-ciliated cells in brain ventricles that move CSF)
Moderate folic acid levels are beneficial for multi-ciliated cells

Ependymal flow (cilia in brain ventricles that move CSF)
Increased variability in gene expression as a contributor to NTD risk?

The genetics of an individual may determine the appropriate balance in folic acid supplementation.
Inconsistent regulation of gene expression as a contributor to NTD risk

Baf155 mutant
ATP-dependent chromatin remodeling complex

Laura Harmacek
William Pavan (NIH)
Michael Salbaum
(Pennington Biomed Res, LA)

Developmental Neurobiology 2013
Balance? Too MUCH, as well as too little may be problematic.

Mutations can shift this balance.

Is NTD prevention always due to rescue? No, early embryonic lethality.

Might some gene mutations and cellular processes benefit from moderate levels of folic acid? Yes, cilia and others.

The genetics of an individual may determine the appropriate balance in folic acid supplementation.
Approaches to Understand the Causes of NTDs

**Genetics**
- Forward Genetic Screens
- KOMP

**Environment**
- Folic Acid
- Zinc, Iron

**Modeling Human NTD Mutations**
- Gleeson (UCSD)
- Wang (Fudan Univ)
- Zhang (Beijing)

**Dynamic Imaging**

**Patient iPSCs**
- Maternal-Fetal Center
- R. Marwan, MD
Lori Bulwith  
Heather Clancy  
David Engelhardt  
Eric Jaffe  
Jianfu Chen (Univ of Southern California)  
Amanda Graf (Nationwide Children’s Hospital)  
Laura Harmacek (National Jewish Health Center)  
Tae-Hee Kim (Hospital for Sick Kids)  
Aimin Liu (Penn State Univ)  
Amber Marean (Univ of CO, Colorado Springs)  
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Jonathan Wilde (MIT)  
Irene Zohn (Children’s National Medical Center)  
Ying Zhang (Harvard Univ)  

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