The Role of MicroRNAs in NEC

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September 26, 2016
Objectives

• Review the pathogenesis of NEC.
• Describe the mechanism by which breast milk is protective against NEC.
• Discuss the role of microRNAs in NEC.
Introduction

- Necrotizing enterocolitis is the leading cause of death from GI disease in the premature infant
- Etiology remains incompletely understood

Premature infant with NEC

Intraoperative
How does NEC happen?

Background

- TLR4 activation within intestinal epithelium leads to mucosal injury
  - Through accelerated enterocyte apoptosis
  - Impaired mucosal proliferation

- Since TLR4 is important in NEC development, strategies to limit TLR4 signaling may offer a preventative approach
Background

- NEC is up to 6x more common in infants fed formula vs. breast milk
- The specific protective agent and mechanism mediating protection is unclear
Background

- EGF is involved in intestinal development
  - present in breast milk and amniotic fluid
- EGF enhances proliferation of epithelial cells and heals damaged mucosa
- EGF is involved in regulation of cell replication, cell movement and cell survival

Hypothesis

• We hypothesized that breast milk inhibits TLR4 signaling within the neonatal intestinal epithelium via EGFR activation, and attenuates the severity of experimental NEC.
Induction of mouse experimental NEC

**Neonatal mouse pups**

- Breast fed controls
- Gavage NEC formula every 3 hours for 12hrs
- Hypoxia (5% O2, 95% N2) twice daily
  - NEC in 4 days
  - Free air
Decreased intestinal EGFR expression is associated with NEC development in mice.

Good et al., PNAS, 2012
Decreased intestinal EGFR expression is associated with NEC development in humans

Good et al, PNAS, 2012
Breast Milk Extraction
Breast milk inhibits TLR4 signaling in vivo

Control  LPS  LPS+BM  LPS+BM+Cetux

Total Flux (photons/sec) x 10^4

Normalized to control

Saline  LPS  BM  BM+Cetux

TLR4 qRT-PCR

IL-6 qRT-PCR

Good et al., Mucosal Immunology, 2015
Breast milk attenuates NEC via EGFR activation

Wild-type

Control

NEC

NEC+BM

NEC+BM+Cetux

NEC+IBM

NEC+IBM+EGF

Good et al, Mucosal Immunology, 2015
Breast milk attenuates NEC severity via EGFR

EGFR$^{\Delta IEC}$

Control  | NEC  | NEC+BM
---|---|---

i  | ii  | iii

* iNOS qRT-PCR

Good et al, *Mucosal Immunology*, 2015
Breast milk inhibits NEC-mediated apoptosis and enhances crypt proliferation via EGF

Good et al, Mucosal Immunology, 2015
Breast milk inhibits NEC-mediated apoptosis and enhances crypt proliferation via EGFR

Good et al, *Mucosal Immunology*, 2015
Breast milk inhibits TLR4 signaling in intestinal epithelial cells via EGFR activation

Good et al, Mucosal Immunology, 2015
Interim Summary

• We have shown that breast milk is protective against NEC by inhibiting TLR4 with the activation of EGFR.
• EGFR signaling protected against TLR4-mediated enterocyte apoptosis and enhanced enterocyte proliferation in NEC.
• Thus, it is particularly important to study the gene regulation of EGFR in NEC as a therapeutic or preventative target in NEC.
**MicroRNA Background**

- Noncoding RNA about 22 nucleotides long

- Functions in RNA silencing and therefore post-transcriptional regulation of gene expression

- Initially expressed as pri-miRNAs in clusters that undergo post-transcriptional processing to produce mature miRNAs
miRNA Background

• MiRNAs are differentially expressed in the intestine.
• Implicated in IBD, intestinal inflammation, gut barrier function.
Observation:

miRNAs are known to be differentially expressed in intestinal inflammatory states

Hypothesis:

miRNAs are differentially expressed in premature infants with NEC
Methods

• Univ. of Pittsburgh approved IRB protocol

• Inclusion/Exclusion Criteria
  • Premature infants admitted to NICU
  • NEC patients with Bell’s Stage II or greater
  • Excluded patients with congenital anomalies

• Samples
  • Intestinal resections: At NEC diagnosis or at stoma closure (Resolved NEC)
  • Serum: At time of NEC diagnosis or age-matched controls
NEC is Associated with Differential Expression of miRNAs
miRNA-17~92 Background

- MiR17~92 cluster made up of miRNA-17, 18a, 19a, 19b, 20a, and 92a
- These miRNAs are known to be differentially expressed in intestinal inflammatory states.
- Components of the miR-17~92 cluster have increased intestinal and serum expression in inflammatory states of IBD.
Embryonic small intestine of miR-17~92ko mice display blunted villiform structures.
MicroRNAs

• Previously our lab has shown that EGFR expression is decreased in NEC

• Protective effects seen in NEC with EGFR activation

• miRNA-17 is predicted to target EGFR

Good et al, PNAS, 2012
Good et al, Mucosal Immunology, 2015

MicroRNAs
Expression of Intestinal miRNA-17
Increased in NEC

Pompa et al, in preparation
Serum miRNA-17 is Increased in NEC

*P<0.05

Pompa *et al.*, in preparation
Serum MiR-17 is increased in medical and surgical NEC

*P<0.05

Pompa et al, in preparation
Observation:
miRNA-17 is increased in the intestine and blood of infants with NEC

Hypothesis:
Infants with increased miRNA-17 have increased intestinal barrier dysfunction and decreased cellular proliferation
miRNA-17 expression is increased in surgical NEC

Pompa et al, in preparation
Increased miR-17, decreased proliferation, tight junctions and EGFR in NEC

Pompa et al, in preparation
MiR-17 targets EGFR

Pompa et al, in preparation
Conclusions

- The miRNA-17~92 cluster is upregulated in the intestines of infants with NEC.
- miRNA-17 is upregulated in the intestine and serum of infants with medical and surgical NEC.
- MiR-17 targets EGFR.
- miRNA-17 may play an important role in the maintenance of the gut barrier in NEC pathogenesis.
Future Directions

• Characterize the intestinal cell populations that express miRNAs with flow cytometry.
• Evaluate susceptibility of intestinal specific miRNA-17~92ko mice NEC model.
• Determine the effects of Mir-17~92 cluster on intestinal development in the mouse.
Future Directions

- Determine the effect of overexpressing Mir-17 in neonatal mouse enteroids with and without NEC.
Acknowledgements

Good Lab
Anthony Pompa, MD (Pediatric Resident)
Congrong Ma (Technician)
Alexa Bolock (Technician)
Zerina Hodzic (Medical Student)
Olivia Parks (Undergraduate Student)
Kara Touscany (Undergraduate Student)
Onome Oghifobibi, MD (Pediatric Resident)
Sonali Agrawal (Undergraduate Student)
Tyler McCullough (Undergraduate Student)

David Hackam Lab, Hopkins
Chhinder Sodhi, PhD
Hongpeng Jia, MD
Charlotte Egan, PhD
Yuki Yamaguchi, PhD
Peng Lu, PhD
Amin Afrazi, MD, PhD
Maria Branca
Tom Prindle
Samantha Weyandt

Funding Sources
National Institutes of Health K08DK101608
CHP Department of Pediatrics
UPMC Competitive Medical Research Fund

Jay Kolls Lab, Pitt
Pawan Kumar, PhD
Kara Kracinovsky

Gary Silverman Lab, Wash U
Cliff Luke, PhD

Jackie Ho Lab, Pitt
Yu Leng Phua

John Ozolek, MD, Pitt
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