NASH Pathogenesis
Role of Cell Death and Sterile Inflammation
STOPNASH SYMPOSIUM

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Disclosures
Co-inventor on pending and issued patents filed by the Cleveland Clinic and UCSD that refer to the use of biomarkers in fatty liver disorders.

And

Scientific Advisory Board: Gilead, Takeda, Mitsubishi-Tanabe, Raptor, Conatus

And

My presentation does not include discussion of off-label or investigational use

Outline

- Pathogenic Pathways
  - Hepatocyte cell death in NASH progression
  - Cell death - sterile inflammation loop
  - NLRP3 Inflammasome as driver of inflammation and fibrosis
  - Summary and Conclusions
Non-Alcoholic Fatty Liver Disease (NAFLD)

- Spectrum of disease
  - NAFL: fatty liver (steatosis)
  - NASH: steatosis + inflammation + liver injury
  - Fibrotic NASH: steatosis + inflammation + liver injury + fibrosis


NAFLD Pathogenesis: complex interaction and crosstalk between environmental factors, host genetics and gut microflora

Hepatocyte Cell Death is a Prominent Feature of Human NASH

Normal Liver  NASH

Feldstein AE…..Gores GJ. Gastroenterology 2003;125:437-443
Hepatocyte Cell Death
Expanding View

Wree A.....Feldstein AE. Nature Reviews Gastroenterology and Hepatology, 2013

Cell Death and Sterile Inflammation
Loop in NASH

NLRP3 Inflammasome
Driver of Inflammatory Response in NASH
Experimental and Human NASH are Associated with NLRP3 Inflammasome activation in the liver

Csak et al. HEPATOLOGY 2011;54:133-144

Hepatocytes Express NLR Components and Palmitic Acid and LPS Exposure Results in Caspase 1 Activation and IL-1β Production

NLRP3-inflammasome Activates Hepatic Stellate Cells

Watanabe et al. Am J Physiol Gastrointest Liver Physiol 2009
HSC Activation and Collagen Deposition During Diet-Induced Steatohepatitis are Markedly Decreased in Caspase-1-KO Mice


High Fat-induced Early Fibrogenesis is Prevented in Caspase-1 Knockout Mice.


http://www.plosone.org/article/info:doi/10.1371/journal.pone.0056100

What is the specific contribution of NLRP3 activation to liver disease?

What are the main NLRP3 modulators in NASH?

What is the role of NLRP3 activation in various cell types in the liver?

What is the importance of the downstream inflammatory and cell death pathways in NLRP3 mediated liver pathology?
Development of NLPR3 Knockin Mice

<table>
<thead>
<tr>
<th>Model Name</th>
<th>Cre recombinease</th>
<th>NLPR3 expression</th>
<th>Constitutive/Inducible</th>
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<tbody>
<tr>
<td>NLRP3-CreT</td>
<td>LCAT - Lecithin:Cholesterol acyltransferase</td>
<td>Hepatic stellate cells</td>
<td>Constitutive</td>
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<tr>
<td>NLRP3-CreL</td>
<td>LysM - Lysozyme</td>
<td>Myeloid cells</td>
<td>Constitutive</td>
</tr>
<tr>
<td>NLRP3-CreA</td>
<td>Alb - Albumin</td>
<td>Hepatocytes</td>
<td>Constitutive</td>
</tr>
<tr>
<td>NLRP3-CreT</td>
<td>ERT - Estrogen Receptor</td>
<td>Tamoxifen-induced</td>
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NLRP3 Activation Leads to Severe Liver Inflammation


NLRP3 Activation Leads to HSC Activation and Collagen Deposition

NLRP3 Activation Leads to Hepatocyte Pyroptosis


Pyroptosis and Amplification of Inflammasome Response


Serum Caspase-1 levels are Increased in NASH mouse model and are modulated by NLRP3 Inflammasome

Drug: MCC950
Nat Medicine. Feb 2015
Serum Caspase-1 Activity in human NAFLD

Steatosis Grade

- Serum Caspase-1 Activity
  - Presence: p=0.3
  - Absent: p=0.037

Inflammation

- Serum Caspase-1 Activity
  - Presence: p=0.013
  - Absent: p=0.001

Ballooning

- Serum Caspase-1 Activity
  - Presence: p<0.001
  - Absent: p=0.001

Development of inducible Nlrp3 knock-in mice

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NLRP3 Inflammasome Activation is Required for Fibrosis Development in NAFLD

1. Dietary fatty liver model in Nlrp3<sup>NAIP<sup>Cre</sup>/mice:

2. Dietary NASH model in Nlrp3<sup>-/-</sup> mice:

Long term chronic Nlrp3 activation

Increased collagen deposition and activation of hepatic stellate cells in chronic Nlrp3 activation

Nlrp3 inflammasome and HSC activation
NAFLD pathogenesis involves a complex interaction between environmental factors, host genetics and gut microflora and depends on both intrahepatic and extrahepatic events.

Hepatocyte cell death and sterile inflammation may result in a feed-forward self-perpetuating loop that triggers liver damage and fibrosis.

NLRP3 Inflammasome is a key component of this loop.

Studies using constitutively activated NLRP3 demonstrated that NLRP3 is not only required for hepatic inflammation and fibrosis, but NLRP3 activation is sufficient for hepatic inflammation and fibrosis.

Future studies to better identify the key driver of NLRP3 activation, the cell specificity and the importance of various downstream pathways in the development of liver pathology and in particular liver fibrosis during NASH development may result in novel therapies.

Collaborators
- Stanley Hazen (CC)
- Thomas McIntyre (CC)
- Maurizio Parola (Univ. of Torino)
- Laura Nagy (CC)
- Leon Adams (Perth, Australia)
- Jonathan Smith (CC)
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