Mixed Lineage Kinase 3 Mediates Release of C-X-C Motif Ligand 10-Bearing Extracellular Vesicles from lipotoxic hepatocytes

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Disclosure

• Nothing to disclose

Fatty Liver Disease Significance in Children

• A public health problem
• Striking increase in prevalence
  • The most common liver disease
  • 10% (2.5 millions) of US teens have NAFLD
  • 3% have NASH
• 59% of adolescent undergoing bariatric surgery have NAFLD
• Rapid progression of NASH to cirrhosis in pediatric patients
• A common cause of liver transplantation in adults

1Welsh et al., J. Pediatr. 2013
2Schwimmer et al., Pediatrics 2006
3Kanthakos et al, Gastroenterology 2015
MLK3<sup>−/−</sup> Mice are Protected Against Diet-Induced Steatohepatitis

**Hypothesis**
MLK3 mediates obesity-induced liver injury & inflammation in NASH by promoting hepatocyte release of chemotactic EVs.

**Questions**
1. Do lipotoxic hepatocytes release extracellular vesicles (EVs) by an MLK3-dependent pathway?
2. Do MLK3-generated hepatocyte EVs contain chemokines and induce macrophage chemotaxis?
3. Is hepatoprotection against NASH in MLK3<sup>−/−</sup> mice associated with a reduction of hepatocyte EV release?

**Approach**
- Lipotoxicity
  - Lysophosphatidylcholine (LPC, 20 μM)<sup>1</sup>
- Extracellular vesicles
  - Isolation by ultracentrifugation
  - Nanoparticle tracking analysis (NTA) by Nanosight NS300<sup>2</sup>
- MLK3 inhibition
  - Genetic: MLK3<sup>−/−</sup> primary mouse hepatocytes (PMH)
  - Pharmacological: MLK3 inhibitors (URMC, CLFB from Califia Bio Inc., San Diego, CA)<sup>1</sup>

<sup>1</sup>Kakisaka et al. Am J Physiol Gastrointest Liver Physiol 2011
<sup>2</sup>Wolin et al. Am J Physiol Gastrointest Liver Physiol 2011
Lipotoxic Hepatocytes Release EVs by an MLK3-dependent Pathway

![Graph A](#)

**Question**

Is there chemotactic cargo in lipotoxic EVs?

**Approach**

- Mass Spectrometry (MS)
- Western blot
- Immunogold-electron microscopy

CXCL10 Is Highly Enriched in Lipotoxic EVs in an MLK3-dependent Manner

![Graph B](#)
**Question**
Do EVs induce macrophage chemotaxis by a CXCL10-dependent mechanism?

**Approach**
- Migration assay
  - Modified Boyden chamber
  - 4-hour migration assay
  - 10 µm pore membrane
  - Chemoattractant (EVs, CXCL10)
  - Migrated macrophages

**Lipotoxic EVs Induce Macrophage Chemotaxis in a CXCL10-dependent Manner**

![Bar graph showing migration of macrophages (%)]

- Control
- EV
- CXCL10
- CXCL10 Ab

**Question**
Is hepatoprotection against NASH in Mlk3−/− mice associated with reduction in hepatocytes EVs release?

**Approach**
- Mice: C57BL/6J wild type and Mlk3−/− mice
- Diet: FFC1 & Chow diet for 6 months
- Plasma ALT level measurement by a veterinary chemistry analyzer
- CXCL10
  - In mouse plasma EVs by ELISA

*Charlton et al. Am J Physiol Gastrointest Liver Physiol 2011*
**Mlk3** in a Dietary NASH Mouse Model Protects against Liver Injury by Reducing Hepatocyte EV Release

### Conclusion

- Hepatocyte
- Lipotoxic Insult
- CXCL10 Rich Lipotoxic EVs
- MLK3 Inhibitors (URMC & CLFB Calixa Bio Inc., San Diego, CA)

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