MAYO CLINIC

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

10/10/2015

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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[1,2]
  - 3% have NASH[2]
- 59% of adolescent undergoing bariatric surgery have NAFLD[3]
- Rapid progression of NASH to cirrhosis in pediatric patients
- A common cause of liver transplantation in adults

[1]*Welsh et al., J. Pediatr. 2013*
[2]*Schwimmer et al., Pediatrics 2006*
[3]Xanthakos et al, Gastroenterology 2015

Mlk3$^{-/-}$ Mice are Protected Against Diet-induced Steatohepatitis

Saturated Free Fatty Acid

↓

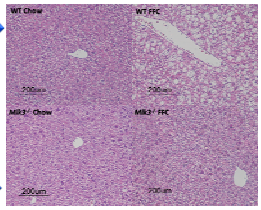
Mixed Lineage Kinase (MLK) 3

↓

c-Jun N-terminal Kinase (JNK)

↓

Liver Injury & Inflammation



WT: Wild Type, **Mlk:** Mixed Lineage Kinase
FFC: High Fat, Fructose, and Cholesterol

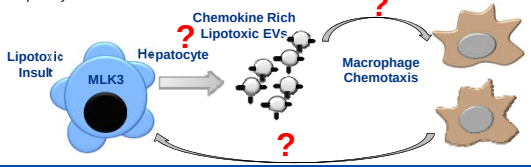
Han et al. Science 2013
Jaeschke et al. Mol Cell 2007
Ibrahim et al. Liver Int 2013

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*$^{-/-}$ mice associated with a reduction of hepatocyte EV release?



Question

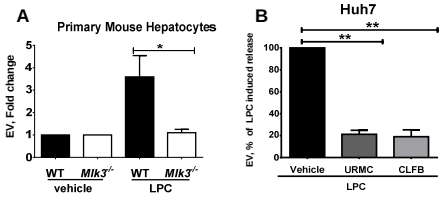
Do lipotoxic hepatocytes release EVs by an MLK3-dependent pathway?

Approach

- Lipotoxicity
  - Lysophosphatidylcholine (LPC, 20 μM)[1]
- Extracellular vesicles
  - Isolation by ultracentrifugation
  - Nanoparticle tracking analysis (NTA) by Nanosight NS300
- MLK3 inhibition
  - Genetic: *Mlk3*$^{-/-}$ primary mouse hepatocytes (PMH)
  - Pharmacological: MLK3 inhibitors (URMC, CLFB from Califia Bio Inc., San Diego, CA)

[1]Kakisaka et al. Am J Physiol Gastrointest Liver Physiol 2011

Lipotoxic Hepatocytes Release EVs by an MLK3-dependent Pathway



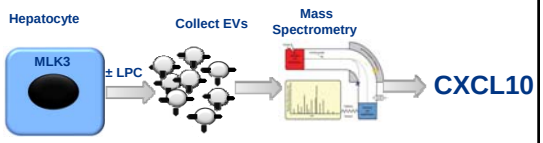
* p<0.05, **p<0.01

Question

Is there chemotactic cargo in lipotoxic EVs?

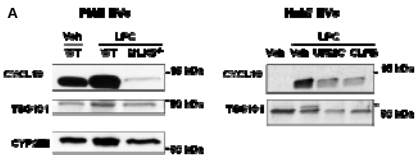
Approach

- Mass Spectrometry (MS)

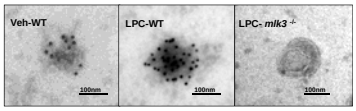


- Western blot
- Immunogold-electron microscopy

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



B CXCL10-Immunogold-electron microscopy

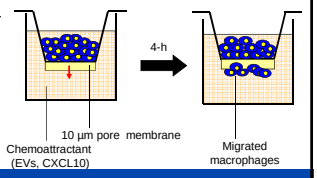


Question

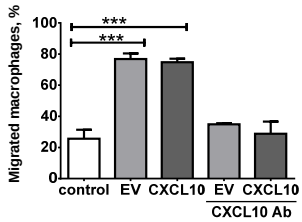
Do EVs induce macrophage chemotaxis by a CXCL10-dependent mechanism?

Approach

- Migration assay
  - Modified Boyden chamber



Lipotoxic EVs Induce Macrophage Chemotaxis in a CXCL10-dependent Manner



***p<0.001

Question

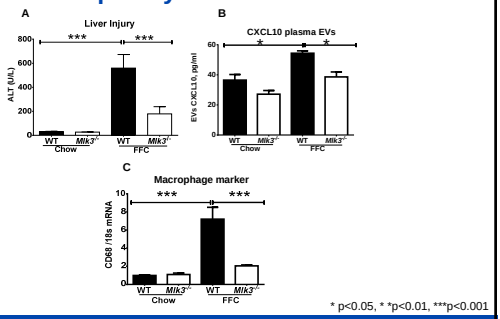
Is hepatoprotection against NASH in *Mlk3*$^{-/-}$ mice associated with reduction in hepatocytes EVs release?

Approach

- Mice: C57BL/6J wild type and *Mlk3*$^{-/-}$ mice
- Diet: FFC[1] & Chow diet for 6 months
- Plasma ALT level measurement by a veterinary chemistry analyzer
- CXCL10
  - In mouse plasma EVs by ELISA

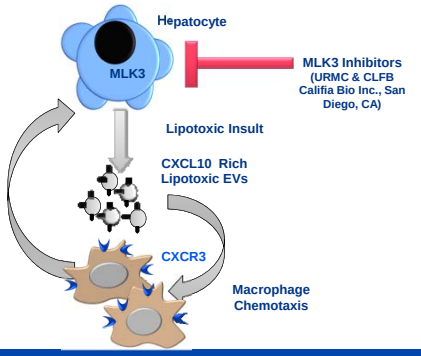
[1]Charlton et al. Am J Physiol Gastrointest Liver Physiol 2011

Mlk3$^{-/-}$ in a Dietary NASH Mouse Model Protects against Liver Injury by Reducing Hepatocyte EV Release



* p<0.05, * *p<0.01, ***p<0.001

Conclusion



Acknowledgement

- Dr. Gores and laboratory members
- Center for Clinical and Translational Science (CCaTS), KL2 program
- Mayo Center for Cell Signaling in Gastroenterology, Pilot and Feasibility program
- Pediatric Gastroenterology Division