NK cells from homozygous NLG4-/- (KO) mice inhibit liver fibrosis through PI3K/AKT/mTOR pathway and decreased interleukin-4

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17/06/2016 Eilat
Abstract MP-196: Recombinant β-neuroxin as a therapeutic target to inhibit fibrosis through alleviation in NK cells cytotoxicity following inhibition of neuroligin-4 (nlg4) receptor
Presenter:
Johnny Amer (Israel)

Abstract MP-197: Tgf-beta2 inhibition in endothelial cells ameliorates liver fibrogenesis and inflammation
Presenter:
Anne Dropmann (Germany)

Abstract MP-199: Association of controlled attenuation parameter and glycosylated hemoglobin in patients with fatty liver
Presenter:
Anita Arslanow (Germany)

Abstract MP-201: Early changes in non invasive assessment of liver fibrosis in hepatitis c virus-infected patients treated with daas: preliminary reports
Presenter:
Claudia Iegri (Italy)

Abstract MP-213: Survival of apoptosis-primed activated hepatic fibroblasts is bcl-xl dependent
Presenter:
Anja Moncsek (Switzerland)

Abstract MP-202: The change of liver and spleen stiffness measured by shear-wave elastography after transjugular intrahepatic portosystemic shunt predicts outcome.
Presenter:
Jonel Trebicka (Germany)
Disclosures

None
Proposal: NLG4 is mediating HSC/NK synapse

- Autism
- Schizophrenia
Confocal NK/HSC interplay:
NLG4:βNeuroxin are involving immune Synapse
Gene expressions of impaired NK cells in cirrhosis

Neuroligin 4: is a postsynaptic membrane protein that mediates synapse formation between neurons
AIM

To assess the role of PI3K/AKT/mTOR pathway & NLG4 in NK cell killing
DESIGN

WT

NLG4-/-

CCl₄ i.p injections
2X/wk for 6 wks

Livers

Serum

✓ Lymphocytes immune alterations
✓ aSMA activations and expressions
✓ PI3K/AKT/mTOR expressions

ALT levels
Methods

✓ Serum ALT levels.

✓ RT-PCR is used to quantify and to analyze the gene expression of liver αSMA and PI3K/AKT/mTOR.

✓ Fluorescence-activated cell sorting (FACS) is used to evaluate the changes on the NK cells. Count, activations (CD107a), CD8, CD4, F4/08 (monocytes) and primary HSCs activations, apoptosis (Annexin V) and proliferations (CFSE).
RESULTS
In vivo CCl₄ model: WT vs. NLG4⁻/⁻

**Serum ALT (IU/ml)**

- WT Naïve
- WT FIB (1W)
- WT FIB (5W)
- NLG4⁻/⁻ Naïve
- NLG4⁻/⁻ FIB (1W)
- NLG4⁻/⁻ FIB (5W)

**P<0.05**

**αSMA/βActin ratio**

- WT Naïve
- WT CCl₄ (6w)
- NLG4⁻/⁻ Naïve
- NLG4⁻/⁻ CCl₄ (6w)

**P<0.05**
FACS analysis of liver lymphocytes

NK+ CD3-

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<tr>
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<th>Naïve WT</th>
<th>WT 6wks</th>
<th>Naïve -/-</th>
<th>NLG-/- 6wks</th>
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<tr>
<td>P-value</td>
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Percentage of CD8, CD4, and F480 cells in Naïve WT, WT 6wks, Naïve -/-, and NLG-/- 6wks.
Isolated pHSCs from livers of NLG4-/- mice showed:
Liver NK cells PI3K/AKT/mTOR pathway

**PI3K**

**mTOR**

**Akt 1**

**% IL-4**

Liver NK cells PI3K/AKT/mTOR pathway

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**NK1.1 expressions (FACS)**
**HSC apoptosis:**

- aSMA+/Ann V+ (%)

**NK activation:**

- NK+/aKIR+ (%)

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<tr>
<th>NK cell</th>
<th>WT</th>
<th>-/-</th>
<th>WT</th>
<th>-/-</th>
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<tr>
<td>CCl4</td>
<td>-</td>
<td>-</td>
<td>+</td>
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Conclusions

- Homozygous NLG4−/− mice exert anti-fibrotic profile through increase in NK cells and decreased CD8 cells.

- Alterations in NK cells phenotypes were observed through increased activity associated with increased pHSCs killings and consequently decreased in αSMA quantitations.

- NK activations and potentials to kill are suggested to be associated with elevated expressions of PI3K/AKT/mTOR accompanied in decreased in the pro-fibrogenic marker; IL4.

- NLG4 modulations could be a potential target for fibrosis treatments.
Thank you

Acknowledgments
Holy Family Hospital, Nazareth

Nils Brose; Abteilung Moleklare Neurobiolgie, Germany

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