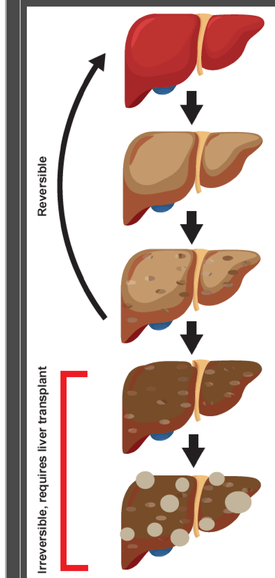
Cyclophilin D knockout promotes cell death pathways in preventing HCC development in a streptozotocin-induced mouse model of diabetes-linked NASH

W. T. Stauffer[1], J. Kuo[2], M. Bobardt[1], D. R. Ure[3], R. T. Foster[3], and P. Gallay[1]

[1]Department of Immunology & Microbiology, Scripps Research, La Jolla, California 92037, USA.
[2]Arena Pharmaceuticals, San Diego, California 92121, USA.
[3]Hepion Pharmaceuticals, Edison, New Jersey 08837, USA.

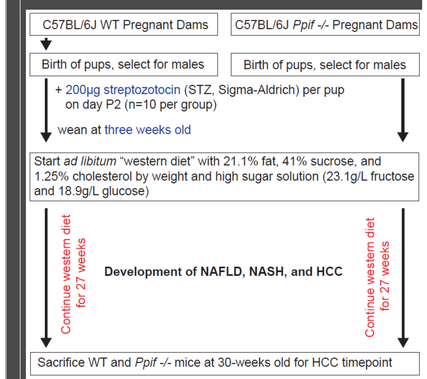
PREMISE



- ~25% of the adult population has some form of fatty liver.
- Associated with obesity, diabetes, and metabolic syndrome.
- Non-alcoholic fatty liver disease (NAFLD) features simple steatosis or lipid accumulation.
- NAFLD may progress to non-alcoholic steato-hepatitis (NASH) with inflammation and hepatocyte damage.
- NAFLD and NASH can be reversed.
- Further scarring can induce cirrhosis and the development of hepatocellular carcinoma (HCC), which cannot be reversed.

- Previous studies[1,2] have shown cyclophilin (Cyp) inhibitor drugs can prevent HCC development. It is unknown which Cyp family member(s) is the cause.
- Cyclophilins are a family of peptidylprolyl isomerases with diverse functions. CypD is a component of the mitochondrial permeability transition pore (mPTP).

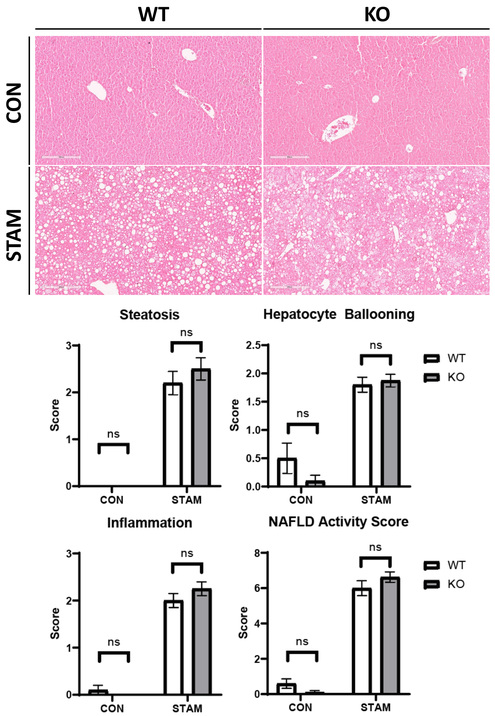
METHODS



- The STAM model[3] mimics diabetes in mice by destroying pancreatic beta cells.
- Combined with western diet, STAM reliably produces NAFLD/NASH and HCC.
- Comparing wild-type (WT) mice to *Ppif* (CypD) knockout (KO) mice reveals the necessity for CypD in HCC development.

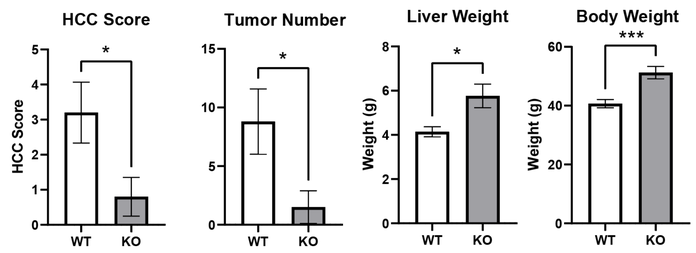
RESULTS

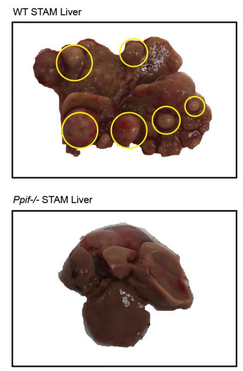
WT and CypD KO Mice Both Develop NASH in the STAM Model



- Under STAM, both WT and CypD KO mice developed significant steatosis, hepatocyte ballooning, and inflammation, as determined by histological analysis of hematoxylin & eosin (H&E)-stained liver tissue sections.
- Combining scores for all three categories yields the NAFLD activity score (NAS). Both WT and KO STAM mice had average NAS of greater than 6, which is consistent with NASH. ns=not significant (p>0.05) by unpaired student's t-test. Representative images of H&E-stained liver sections are above on the left.
- Our scoring system for NAS is as follows: for ballooning: 0 (no changes), 1 (few ballooned cells), and 2 (many prominent balloon cells); for inflammation: 0 (no changes), 1 (minimal infiltration with no major inflammatory foci), 2 (mild with <2 inflammatory clusters), 3 (moderate with 2–4 foci of leukocytes), and 4 (severe infiltration with >4 inflammatory clusters); for steatosis: 0 (<5% steatosis), 1 (5–33%), 2 (33–66%), and 3 (>66% steatosis).

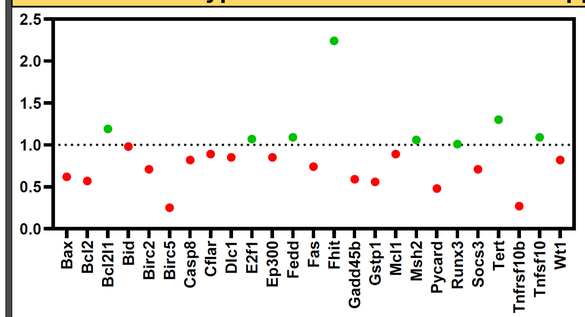
CypD Knockout Prevents HCC Development in the STAM Model





- CypD KO mice developed significantly less tumor burden after STAM compared to WT. *p≤0.05, ***p≤0.001 by unpaired student's t-test. Representative images of livers are on the right. HCC tumors are circled.
- Our scoring system for tumor burden is as follows: Scores included 0 (no detectable nodules), 1 (no more than 4 small ≤0.5cm nodules with no larger nodules), 2 (more than 4 small nodules with no larger nodules), 3 (no more than 2 medium-sized (<1cm) nodules with no larger nodules), 4 (3 or more medium-sized nodules with no larger nodules), 5 (no more than 1 large (≥1cm) nodule), 6 (no more than 2 large nodules), and 7 (3 or more large nodules).

CypD KO in the STAM Model Suppresses the Oncogene *Birc5*



- To explore possible consequences of CypD KO and impaired mPTP activity in HCC development, we conducted a preliminary PCR array on post-STAM WT and CypD KO livers of relevant apoptosis and cell death gene transcripts.
- While a majority of apoptotic gene-transcripts were down regulated in CypD KO STAM livers relative to WT (red dots), some anti-apoptotic genes-transcripts were also decreased, most especially *Birc5* (red dots).
- *Birc5* is anti-apoptotic and is elevated in a variety of cancers, including HCC, though it is not known whether it is regulated by CypD.
- Further investigation is required to confirm these findings and determine if there is a mechanistic link between *Birc5* induction and HCC prevention in this model.

CONCLUSIONS

- CypD KO prevents in the development of HCC in this model of diabetes-induced NAFLD/NASH.
- This suggests CypD inhibition is a viable target for the treatment of NAFLD/NASH and is in agreement with previous studies which showed global cyclophilin-inhibitor drugs had similar effects.
- Future studies include determining the mechanism(s) of action of CypD KO in preventing HCC development. Furthermore, it must be determined whether the role of CypD in the mPTP or the suppression of Birc5 play roles critical to this process.

REFERENCES

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DISCLOSURES

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- Stauffer, Kuo, Bobardt, and Gallay declare no competing financial interests. Ure and Foster are employees of Hepion Pharmaceuticals Inc.

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