Determination of Sex-Specific Differences in EGFR and ATF2 in the Mouse Liver

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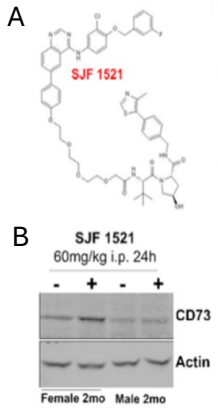
UNC RESEARCH

Introduction

- Currently, there is an abundance of research which observe the sex-specific differences in the clinical presentation, onset, progression, and prognosis for liver diseases. For example, chronic liver disease is one of the top leading causes of death in men, but not in women
- CD73 (also known as ecto-5'-nucleotidase) is a transmembrane protein which converts AMP into adenosine, which can provide a protective or deleterious role in the liver, depending on the injury type
- Silencing of EGFR (epidermal growth factor receptor) via the proteolytic inhibitor SJF-121 resulted in decreased expression of CD73 in males, but not in females
- EGFR phosphorylates ERK, which in turn phosphorylates ATF2. ATF2 modifies gene expression to turn on gene that have a restorative effect on liver injury
- Investigating downstream pathways affected by CD73 may provide insight that advances future clinical therapies for liver diseases in a sex-dependent manner
- **Hypothesis:** Inhibition of CD73 expression will result in decreased expression of both EGFR and ATF2 in male mice, but not in female mice

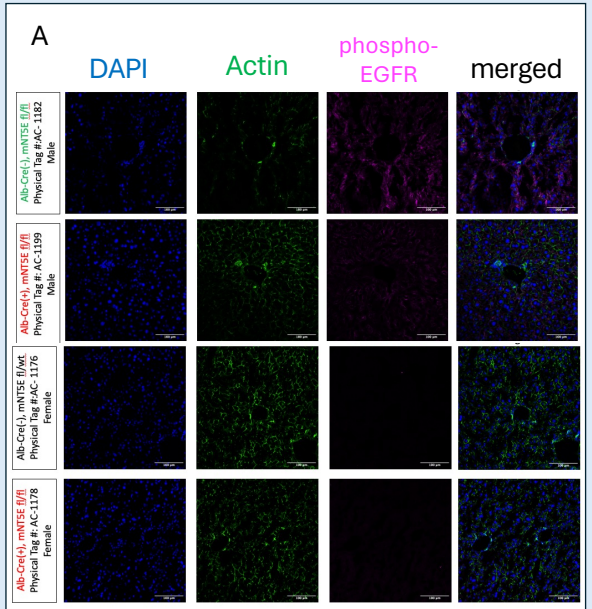
Preliminary Work/Methods



- Investigated how degradation of EGFR affected CD73 using a proteolysis-targeting compound called SJF-121
- We generated a liver-specific CD73 knock-out model using the Cre-loxP system to delete the CD73-encoding gene (Nt5e) from hepatocytes
- Mouse livers were extracted, and sections were stained with our proteins of interest (phospho-EGFR, phospho-ATF2). Pan-actin included as a control for protein concentration.

Figure 1. A) Molecular structure of SJF 1521, with two active domains for binding and a linker region **B)** Immunoblot showing difference in CD73 levels between age-matched SJF 1521- treated mice and controls

Results



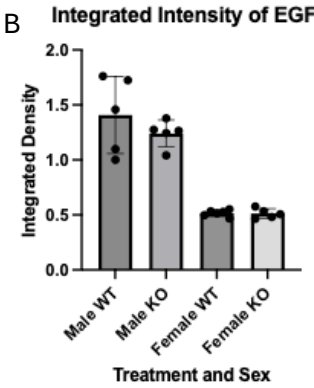
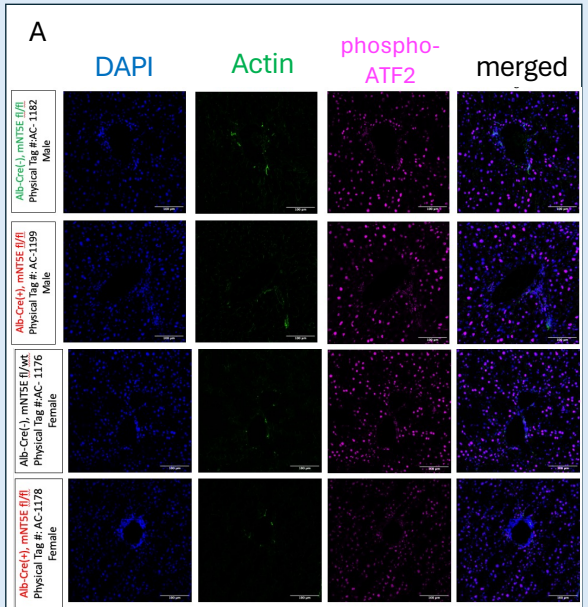


Figure 2. A) Confocal imaging showing mouse liver sections stained for DAPI (blue), pan-actin (green), and phospho-EGFR (red). **B)** Integrated intensity of phospho-EGFR for male and female mice, with pan-actin used to normalize between groups (n=5 for each treatment group)

Results



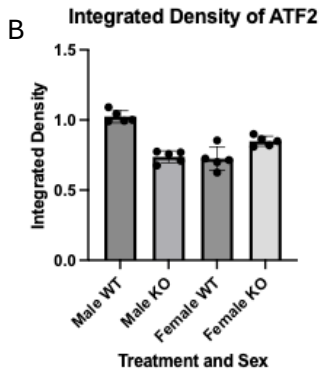


Figure 3. A) Confocal imaging showing mouse liver sections stained for DAPI (blue), pan-actin (green), and ATF2 (red). **B)** Integrated intensity of ATF2 for male and female mice, with pan-actin used to normalize between groups (n=5 for each treatment group)

Conclusions

- Our findings suggest that there is a significant difference in phosphorylated EGFR between male and female mice, both in CD73 KO and control
- However, there is no statistically significant difference in EGFR expression within sex groups
- For ATF2, CD73 KO resulted in a statistically significant difference in ATF2 expression for males, but not for females
- Possible pathway could be involved including ATF2 which is more dependent on CD73 in males but not in females

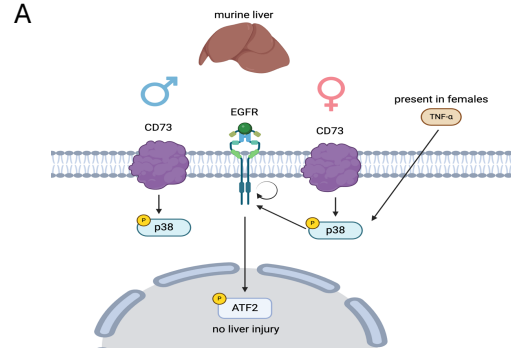


Figure 3. A) Proposed pathway potentially explaining the differences in EGFR between male and female mice. Female mice have high levels of TNF-α, which phosphorylates a kinase involved in recycling EGFR

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