Effects of *in silico*-identified ligands of the mitochondrial protein C1qbp on cell death in fibroblasts

College of Veterinary Medicine
University of Missouri

Jordan A. Altug, Christopher P. Baines

Department of Biomedical Sciences, College of Veterinary Medicine, University of Missouri, Columbia, MO

Veterinary Research Scholars Program
University of Missouri

BACKGROUND

- The top two causes of death in the United States are heart disease & cancer
- Mitochondrial dysfunction is an underlying cause of many cardiac diseases & cancers and, thus, the proteins involved are therapeutic molecular targets
- Complement 1q Binding Protein (C1qbp) is a homotrimeric mitochondrial matrix protein that is highly expressed in cardiac and cancer cells
- We have shown that upregulation of C1qbp inhibits cell death whereas depletion of C1qbp promotes cell death
- Thus, pharmaceutical “activation” or “inhibition” of C1qbp have potential as therapeutical interventions

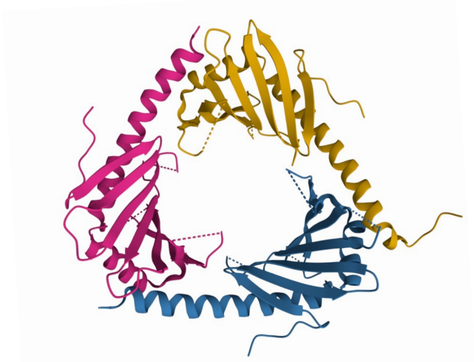

Figure 1: Structure of C1qbp. The crystal structure of C1qbp (Protein Data Bank: 3RPX) showing its homotrimeric composition.

- An *in silico* screen of 8 million chemical compounds against the crystal structure of C1qbp was conducted
- Identified compounds predicted to bind either the monomeric or trimeric forms of C1qbp

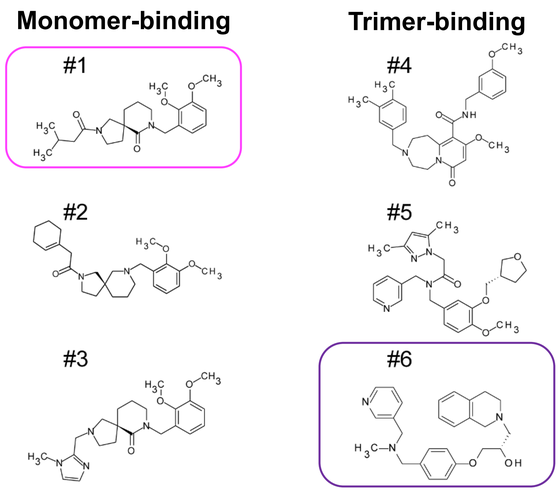

Figure 2: Identification of C1qbp-binding ligands. Top 6 C1qbp binding ligands identified by *in silico* screening of a chemical library against the crystal structure of C1qbp. Ligands #1-3 are predicted to bind the monomer, ligands #4-6 to the trimer.

OBJECTIVE

- To evaluate the effects of these putative C1qbp-binding ligands on cell death in cultured cells

METHODOLOGY

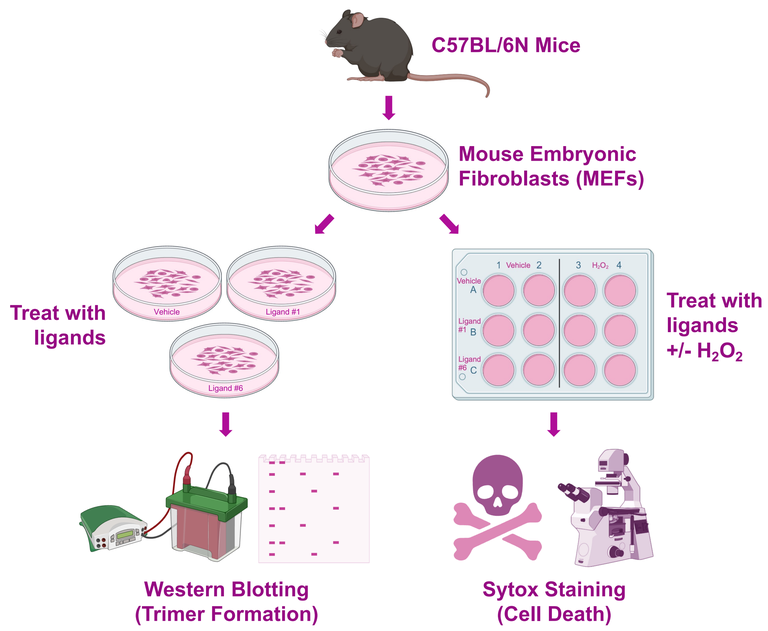

Figure 3: Schematic of methods. In the 1st set of experiments, MEFs were treated with vehicle or C1qbp ligands #1 and #6 and trimer formation assessed by Western blotting. In the second set, MEFs were treated with vehicle or the C1qbp ligands followed by vehicle or H_2O_2. Cell death was then assessed by Sytox staining. Illustration created with BioRender.com.

RESULTS: C1qbp binding ligands do not affect trimer formation

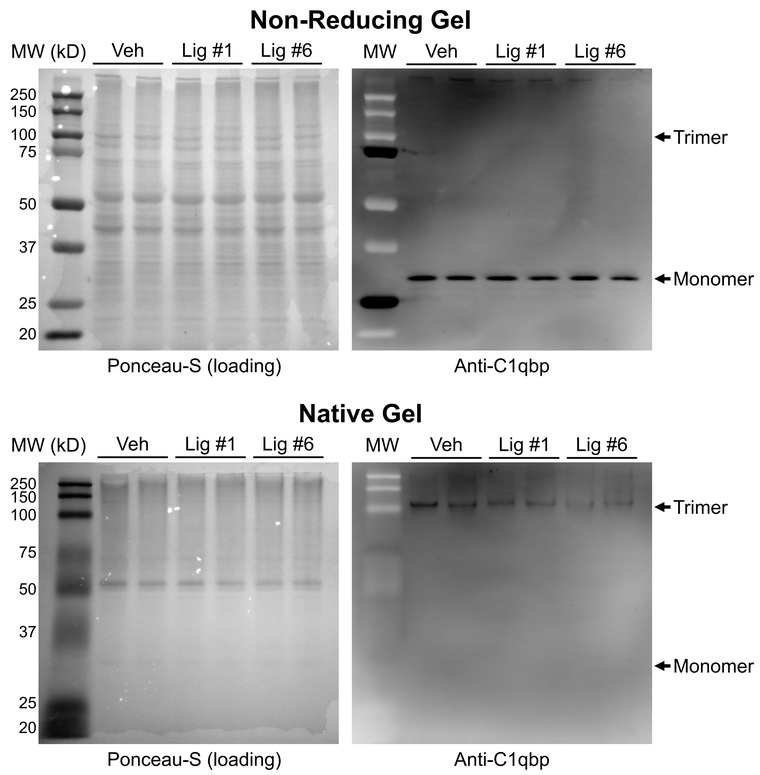

Figure 4: Effects of C1qbp ligands on monomer vs trimer formation. MEFs were treated with either vehicle (DMSO) or 100μM of ligands #1 or #6 for 4hrs, and then harvested and lysed. The cell lysates were then run on either non-reducing or native gels and Western blotted for C1qbp.

RESULTS: C1qbp binding ligands increase H_2O_2-induced cell death

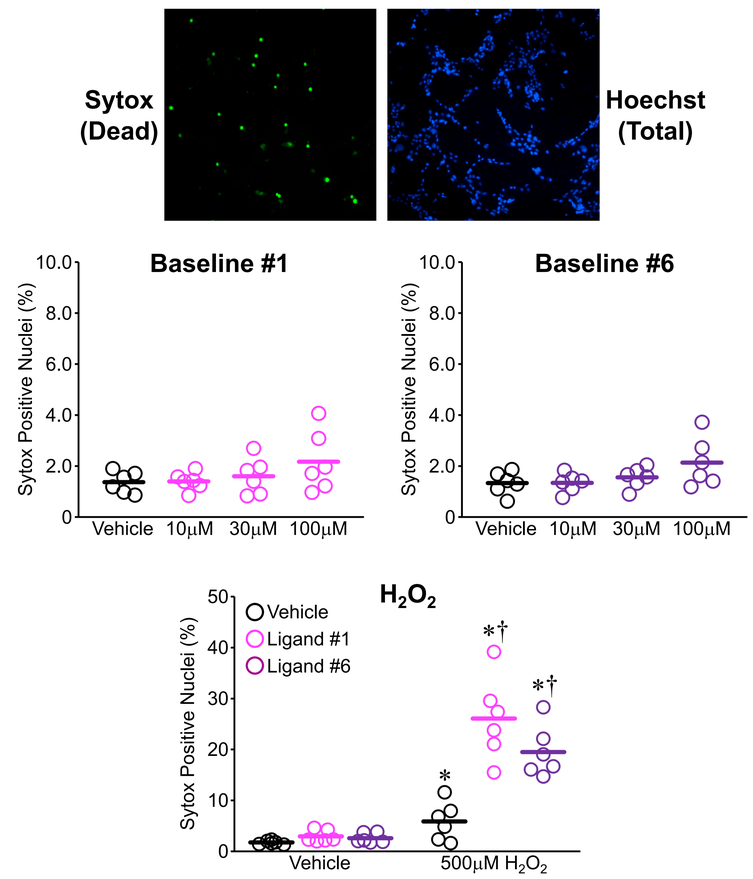

Figure 5: Effects of C1qbp ligands on cell death. MEFs were either treated with vehicle or 10-100μM of ligands #1 or #6 for 4hrs or pre-treated for 30min with vehicle or 100μM of the ligands followed by vehicle or 500μM H_2O_2 for 4hrs. Cell death was then measured by Sytox staining. *p<0.05 vehicle vs H_2O_2, †p<0.05 vehicle vs ligand (Two-Way ANOVA with Scheffe’s post-hoc test).

SUMMARY & CONCLUSIONS

- Native gels showed that C1qbp does indeed exist as a trimer and that this was not affected by the C1qbp-binding ligands
- The ligands did not significantly affect baseline cell death. However, they markedly increased sensitivity to H_2O_2-induced cell death
- This suggests that the ligands may act as “inhibitors” by disrupting the pro-survival function of C1qbp

FUTURE DIRECTIONS

- Repeat experiments in C1qbp-depleted MEFs to test if the ligands are truly working through C1qbp binding
- Test the effects of the other top “hits” from the screen
- Test the effects of the ligands in cardiac and cancer cells

(Research grant: CPB RIF fund; Stipend support: MU CVM)