

Final Report - Oxalosis and Hyperoxaluria Foundation

Title of study: Identification and Characterization of Pharmacological Chaperones for AGT.

Biochemical studies of misfolded alanine:glyoxylate aminotransferase (AGT) proteins associated with primary hyperoxaluria type I have suggested that these proteins may benefit from factors that increase protein stability. An increase in stability can be induced by chemicals that interact with a protein and that enhance its ability to fold in a “native” or normal conformation. Such chemicals, termed “pharmacological chaperones”, can restore protein function and are easily delivered in drug-based treatments. While such an approach to treat AGT has promise, simple straightforward assays are required to screen large numbers of compounds to identify stabilizing compounds. The goals of this study were to generate and test two novel genetic assays to identify pharmacological chaperones for AGT that could be scaled up for high-throughput screens.

The first assay involved a yeast complementation approach, in which the yeast homolog of AGT, *AGX1*, is replaced by the human AGT protein. In yeast, *AGX1* is not essential for viability. However, *AGX1* is involved in biosynthesis of glycine, and yeast lacking *AGX1* in a *shm1*, *shm2*, *gly1* background are unable to grow on media that contains ethanol unless glycine is added. We replaced yeast *AGX1* with its human counterpart, AGT, in yeast lacking *SHM1*, *SHM2*, and *GLY1*, and found that the human protein was able to substitute for the yeast one, and that we were able to generate yeast that are dependent on human AGT for growth. We then tested to see if mutant versions of AGT that are associated with primary hyperoxaluria would confer reduced growth on these yeast. We found that growth of yeast expressing PH1-associated AGT mutations showed reduced or no growth in a manner that correlated with the severity of the mutation(s), indicating that the yeast complementation assay should provide a good platform on which to screen for stabilizing factors.

We generated a second genetic assay in yeast for directly assessing folding/misfolding of AGT variants. In this assay, the AGT protein was inserted in the middle of a reporter protein, DHFR. Studies suggest that the stabilities of the two proteins can be directly linked in this chimeric molecule, such that a decrease in stability of AGT would be observed as a decrease in stability of the DHFR reporter. The outcome would be a reduction in DHFR activity, which can be linked to growth of yeast. Testing of this approach showed that while wild-type AGT expressed in the DHFR construct showed good growth in yeast, PH1-associated mutants of AGT showed reduced growth. These results indicated that, as with the above assay, this misfolding assay should be useful in screening for stabilizing factors.

In concert with the above approaches, we also examined stability of AGT and PH1-associated mutants *in vitro*. We initiated a mass-spectrometry based study of AGT that allows measurement of stability differences of different AGT variants, and stability changes in the presence of ligands PLP and AOA. This mass-spectrometry based approach allowed us to directly compare the stabilities of AGT wild-type and mutant proteins, and the gains in stability upon ligand binding.

Finally, one of the goals of our work was to carry out test screens of compounds with the above yeast assays. We carried out screens of several thousand compounds with each assay, but were unable to identify a compound that could rescue the poor growth of

the yeast. We suspect, however, that a much higher number of compounds needs to be screened to find such a compound that directly stabilizes AGT.

Much of the work described above has been submitted for publication, and several publications are planned in the future. We also plan to continue our studies of AGT in yeast, as our work funded through the OHF has also provided us with preliminary data that was used to obtain a 4 year R01 grant through the NIH.