Discovery of Novel Small Molecule Inhibitors of a Stress Granule Protein G3BP1 in Neurodegeneration

Rukayat Aromokeye, Savio Cardoza, Jiqiang Fu, Martha Ackerman-Berrier, Lei Wang, Hongmin Li, Gregory Thatcher*, and Haining Zhu*
Department of Pharmacology and Toxicology, University of Arizona, Tucson, Arizona, USA.

Stress granules are membraneless organelles consisting of RNA and proteins. Dysregulated stress granules have been linked to cancer, viral diseases, and neurodegenerative diseases. Formation of stress granules is mediated by G3BP1 (Ras GTPase-activating protein-binding protein 1), hence, G3BP1 interacts with many cellular proteins to regulate their participation in stress granules. Accumulation of these proteins has been associated with a broad range of neurodegenerative pathologies such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and Alzheimer's disease (AD). Here, we describe the development of a high-throughput time resolved fluorescence resonance energy transfer (TR-FRET) screening assay to identify inhibitors of the interaction between G3BP1 and its interacting partner USP10 (ubiquitin specific protein 10). We designed and expressed 6his-G3BP1 and biotin-USP10 that were used in conjunction with commercially sourced fluorescent labeled anti-his antibody and streptavidin to design a robust, low-volume, 384-well format TR-FRET assay suitable for high-throughput screening (HTS). We screened a library of 41,554 compounds and identified 342 primary positives ("hits"). Subsequent testing of hit compounds was performed in the primary screening assay, as well as in a counter screen employing an unrelated protein pair and identical fluorescent detection reagents. We found that 66 molecules showed specific, dose-dependent inhibition of the G3BP1:USP10 interaction, with IC50s of less than 1.5uM to 60uM. We further confirmed the binding of 32 of these compounds to G3BP1 using surface plasmon resonance (SPR) with dissociating constants (K_D) of 32nM to 46uM. Five compounds were further selected based on their structural activity relationship, chemotype, IC50 in TR-FRET and binding constant for further testing in phenotypic assay of stress granule formation. We identified 2 compounds with significant phenotypic characteristics in stress granule formation and lead optimization studies led to the identification of a potent ligand of G3BP1. We have described the successful development of an HTS assay targeting inhibitors of the G3BP1:USP10 interaction; the identification of specific, dose-dependent inhibitors in TR-FRET assay, SPR and stress granule formation phenotypic assay suggests that this may be a useful approach for G3BP1-related drug discovery efforts.

Funded by: NIH/NINDS R01NS115507

Presenter name and contact information:
Rukayat Aromokeye
PhD. Candidate in Pharmacology and Toxicology
R. Ken Coit College of Pharmacy
The University of Arizona
Phone number: (615) 602-2428
Email: raromoke@arizona.edu