Background: Tauopathies are a group of sporadic and familial neurodegenerative diseases, including Alzheimer's disease (AD) [1-5]. These diseases share the common feature of neurofibrillary tangles (NFT) mainly composed of hyperphosphorylated microtubule-associated protein Tau (MAPT), which is central to the progressive neuronal dysfunction and damage in tauopathy patients[6-10]. The exact molecular mechanisms of NFT formation remains unclear. MAPT exon 10 encodes the second microtubule-binding repeat. Its alternative splicing (AS) produces three or four microtubule-binding repeats, termed 3R-Tau and 4R-Tau[11-13](Figure 1A). Approximately equal amount of 3R- and 4R-Tau are found in healthy brain, whereas the distorted 3R/4R Tau ratio is associated with tauopathy[14]. Several trans-acting factors have been reported to regulate Tau Exon 10 AS, mainly based on their consensus binding motifs[14-19]. However, a comprehensive list of modulators that contribute to Tau exon 10 AS regulation is still missing, which is crucial knowledge to unveil the mechanism that connects Tau exon 10 AS with neurodegeneration. **Methods:** We prepared a Tau mini-gene cassette that contains complete sequences of Tau exons 9, 10, and 11, along with partial sequences of Tau introns 9 and 10 (Figure 1C). In the SH-SY5Y neuroblastoma cell line that stably expresses the Tau mini-gene cassette, we performed RNA-antisense purification (RAP) proteomic experiments to identify RNA-binding proteins (RBPs) that interact with the primary mRNA (pre-mRNA) of the Tau mini-gene (Figure 2A). These RBPs represent candidate splicing factors that contribute to Tau exon 10 AS regulation. **Results:** We checked Tau Exon 10 AS profile in neuroblastoma cell lines (Figure 1B,1D and 1E) and identified a list of RBPs that interact with the pre-mRNA of the Tau mini-gene by performing RAP proteomics in SH-SY5Y cells (Figure 2). We are currently validating these novel RNA-protein interactions in the SH-SY5Y cells as well as cortical neurons derived from human induced pluripotent stem cells (iPSC). **Conclusions:** We discovered a comprehensive list of RBPs that interact with the Tau pre-mRNA. Further validation assays are needed in postmortem human brain samples and human iPSC-derived cortical neurons to confirm the roles of these newly-identified RBPs in Tau exon 10 AS regulation in human neurons. References 1. Spillantini, M.G. and M. Goedert, *Tau pathology and neurodegeneration.* Lancet Neurol, 2013. **12**(6): p. 609-22. 2. Wang, Y. and E. Mandelkow, *Tau in physiology and pathology.* Nat Rev Neurosci, 2016. **17**(1): p. 5-21. 3. Caillet-Boudin, M.L., et al., Brain pathology in myotonic dystrophy: when tauopathy meets spliceopathy and RNAopathy. Front Mol Neurosci, 2014. **6**: p. 57. 4. Crary, J.F., et al., Primary age-related tauopathy (PART): a common pathology associated with human aging. Acta Neuropathol, 2014.**128**(6): p. 755-66. 5. Sergeant, N., A. Delacourte, and L. Buee, *Tau protein as a differential biomarker of tauopathies.* Biochim Biophys Acta, 2005. **1739**(2-3): p. 179-97. 6. Pei, J.J., et al., *Distribution, levels, and activity of glycogen synthase kinase-3 in the Alzheimer disease brain*. J Neuropathol Exp Neurol, 1997. **56**(1): p. 70-8. 7. Noble, W., et al., *Cdk5 is a key factor in tau aggregation and tangle formation in vivo*. Neuron, 2003. **38**(4): p. 555-65. 8. Hanger, D.P., et al., *Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis*. J Biol Chem, 2007. **282**(32): p. 23645-54. 9. Coutadeur, S., et al., *A novel DYRK1A (dual specificity tyrosine phosphorylation-regulated kinase 1A) inhibitor for the treatment of Alzheimer's disease: effect on Tau and amyloid pathologies in vitro*. J Neurochem, 2015.**133**(3): p. 440-51. 10. Cruz, J.C., et al., *Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles*. Neuron, 2003. **40**(3): p. 471-83. 11. Caillet-Boudin, M.L., et al., *Regulation of human MAPT gene expression.* Mol Neurodegener, 2015. **10**: p. 28. 12. Niblock, M. and J.M. Gallo, *Tau alternative splicing in familial and sporadic tauopathies.* Biochem Soc Trans, 2012. **40**(4): p. 677-80. 13. Kar, A., et al., *Tau alternative splicing andfrontotemporal dementia.* Alzheimer Dis Assoc Disord, 2005. **19 Suppl 1**: p. S29-36. 14. Qian, W. and F. Liu, *Regulation of alternative splicing of tau exon 10.* Neurosci Bull, 2014. **30**(2): p. 367-77. 15. Qian, W., et al., *Regulation of the alternative splicing of tau exon 10 by SC35 and DyrklA*. Nucleic Acids Res, 2011. **39**(14): p. 6161-71. 16. Jiang, Z., et al., *Mutations in tau gene exon 10 associated with FTDP-17 alter the activity of an exonic splicing enhancer to interact with Tra2 beta*. J Biol Chem, 2003. **278**(21): p. 18997-9007. 17. Kar, A., et al., *RBM4 interacts with an intronic element and stimulates tau exon 10 inclusion*. J Biol Chem, 2006. **281**(34): p. 24479-88. 18. Wu, J.Y., et al., *SRp54 (SFRS11), a regulator for tau exon 10 alternative splicing identified by an expression cloning strategy.* Mol Cell Biol, 2006. **26**(18): p. 6739-47. 19. Gu, J., et al., *Transactive response DNA-binding protein 43 (TDP-43) regulates alternative splicing of tau exon 10: Implications for the pathogenesis of tauopathies*. J Biol Chem, 2017. **292**(25): p. 10600-10612.

P3-200

RNA BINDING DICTATES TDP43-MEDIATED TOXICITY IN MODELS OF ALS AND FTD

Brittany Flores[1], Xingli Li[1], Jose Martinez[1], Weisheng Wu[1], Asim Beg[1], Sami Barmada[2], [1]*University of Michigan, Ann Arbor, MI, USA;* [2]*Michigan Alzheimer's Disease Center, Ann Arbor, MI, USA.* *Contact e-mail: bnflores@umich.edu*

Background: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are distinct disorders, yet these conditions share key pathologic and genetic features, suggesting conserved disease mechanisms[1,2]. The majority of individuals with ALS and FTD exhibit neuronal cytoplasmic inclusions rich in the RNA binding protein TDP43[3], and changes in TDP43 localization and levels are strongly predictive of neurons loss in ALS/FTD[4,5]. Previous investigations suggest that TDP43's ability to bind RNA is essential for neurodegeneration upon TDP43 accumulation, but the mechanism by which TDP43 deposition leads to neuron loss in ALS and FTD remains unclear. **Methods:** We manipulated the RNA binding properties of TDP43 by targeting an intramolecular salt bridge that is essential for maintaining TDP43 structure and activity. We then examined the impact of these modifications on TDP43 RNA binding *in vitro* and *in vivo*. We also investigated the localization, stability and toxicity of RNA binding-deficient variants of TDP43 in rodent primary neurons. We confirmed our results

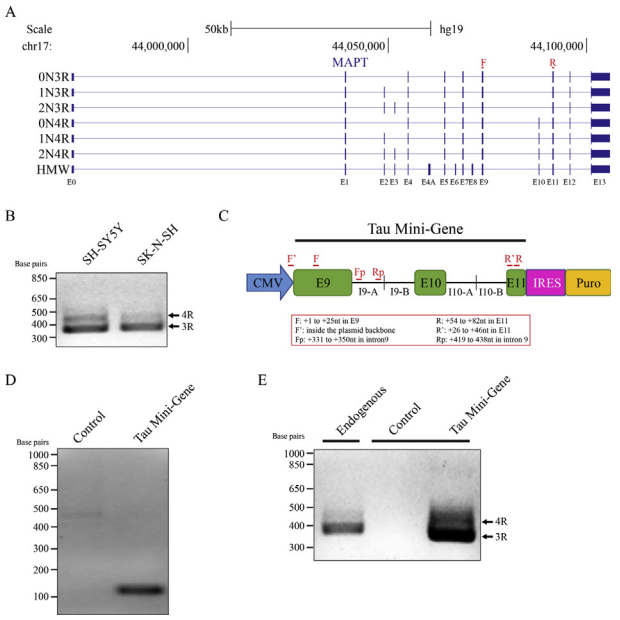

Figure 1. Expression of Tau isoforms. (A) Alternative splicing of exon 10 (E10) gives rise to Tau with either 3 or 4 micro- tubule-binding domain repeats, termed 3R and 4R, respectively. Alternative splicing of exons 2 and 3 (E2 and E3) towards the N-terminus gives rise to additional Tau isoforms (0N, 1N, and 2N) found in CNS. Other exons are alternatively spliced in high molecular weight Tau isoforms (HMW) in peripheral tissues, but are found at low frequency in the CNS. Chromosome map adapted from UCSC Genome Browser. (B) Endogenous Tau 3R and 4R isoforms in the SH-SY5Y and SK-N-SH neuroblastoma cell lines measured by RT-PCR, using primers F and R as shown in (A) and (C). (C) Construct of Tau mini- gene expressed in the neuroblastoma cell lines. I9-A: +1 to +990nt in intron 9; I9-B: +12645 to +13645nt in intron 9; I10-A: +1 to +1000nt in intron 10; I10-B: +2840 to +3840nt in intron 10. (D) Detection of the pre-mRNA of the Tau mini-gene product in SK-N-SH cells after transfection using RT-PCR, with primers Fp and Rp as shown in (C). The endogenous Tau pre-mRNA was not detectable in the transfection control. (E) Using RT-PCR, the ratio between Tau 3R and 4R isoforms expressed from the mini-gene (right, with primers F' and R' as shown in panel C) is similar to that of endogenous Tau (left, with primers F and R as shown in panel C) in SK-N-SH cells.

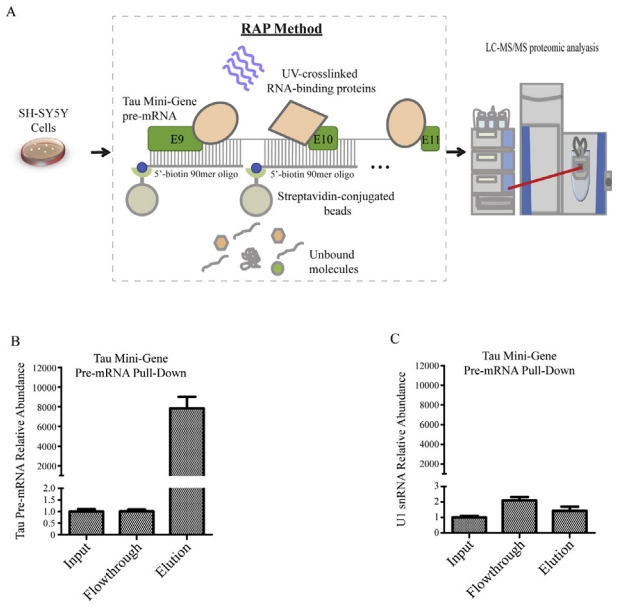

Figure 2. Identification of proteins binding to the Tau mini-gene pre-mRNA using the RAP proteomic method. (A) Identification of Tau specific RBP candidates by performing the RAP enrichment procedure in the SH-SY5Y cell line that stably express the Tau mini-gene cassette, followed by proteomic analysis. (B-C) Specific enrichment of the Tau mini-gene pre-mRNA in SK-N-SH cells stably expressing the Tau mini-gene cassette after transfection. (B) The Tau mini-gene pre-mRNA abundance was measured by qRT-PCR using the primers Fp and Rp as shown in Figure 1C. (C)TheUl snRNA abudance through the RAP process was measured by qRT-PCR. GAPDH was used as the internal standard in both (B) and (C).

by modifying the endogenous TDP43 ortholog (TDP-1) in *Caenorhabditis elegans*. Lastly, to identify the RNA misprocessing events most closely linked to neuron loss in TDP43 mediated disease, we sequenced RNA from human cell lines overexpressing TDP43 and RNA binding-deficient TDP43 variants. **Results:** Mutations affecting the TDP43 salt bridge reduce the cooperativity and affinity of nucleic acid binding by TDP43, and eliminate recognition of its native RNA targets. These same mutations dramatically destabilize the protein, impair nuclear localization and largely eliminate TDP43 toxicity upon overexpression in primary neurons. Worms harboring analogous mutations in TDP-1 demonstrate phenotypes similar to knockout strains, confirming the necessity of the salt bridge for TDP43 function. High-throughput RNA sequencing and splicing analyses indicated that TDP43 accumulation predominantly affects transcripts encoding components of the ribosome and oxidative phosphorylation pathways, thereby impairing protein synthesis and energy production. **Conclusions:** Our results suggest that an intramolecular salt bridge is critical for TDP43's RNA binding properties. Moreover, selective disruption of the salt bridge significantly and effectively reduced TDP43-dependent toxicity in ALS/FTD models. These studies therefore uncover fundamental mechanisms contributing to neurodegeneration in ALS and FTD that may serve as valid targets for therapy development.