Name: Petar Grozdanov Email: petar.grozdanov@ttuhsc.edu

The role of alternative polyadenylation in a TLR3-mediated mouse model of escalated alcohol consumption

Petar N Grozdanov[1], Brent R. Kisby[2], Clinton C. MacDonald[1], Igor Ponomarev[2]

[1]Cell Biology & Biochemistry, [2]Pharmacology & Neuroscience, TTUHSC SOM, Lubbock TX

Alternative polyadenylation (APA) produces mRNAs with different lengths of their 3' untranslated regions (UTR). APA is a widespread, but often overlooked mechanism to regulate gene expression at the posttranscriptional level. Longer APA transcripts have been shown to be enriched in neuronal processes, while shorter APA transcripts are often confined to the neuronal bodies. This differential localization of the transcripts may affect neuronal functions including neurogenesis and synaptic plasticity, key processes associated with the development of alcohol use disorder (AUD). The purpose of this study was to investigate the role of APA in AUD using a well-established neuroimmune mouse model of excessive ethanol consumption, in which repeated injection of a TLR3 agonist, Poly(I:C) (PIC) produces escalation of ethanol drinking in C57BL/6J male mice. We used RNA-Seq to determine the effects of chronic ethanol drinking or/and innate immune activation by PIC on gene expression and APA in frontal cortex. Our analysis identified hundreds of genes undergoing APA (e.g., Camk2a, Psd3, Ctnnd2, Grm5, Homer1*)* or differential expression (DE, e.g., B2m, C1qa, C1qb, Flt1, Sparc) after immune activation by PIC, suggesting that these genes may contribute to the PIC-induced escalation of ethanol drinking. Strikingly, the APA and DE genes did not overlap. Moreover, using previously published cell type-specific single nucleus RNA-Seq datasets, we were able to determine that the APA genes were primarily expressed in neurons, while the DE genes were expressed in microglia, endothelial and mural cells. Gene ontology functional group analyses revealed that the pathways associated with APA and DE genes were different: APA genes were involved in glutamate receptor signaling, cognition, and locomotor behavior, whereas the DE genes were involved in activation of the innate immune system and interferon alpha, beta, and gamma pathways. In summary, our study suggests that APA is an important molecular mechanism invoked in AUD that has the potential to regulate localized neuronal protein expression during AUD development. In addition, our study highlights new molecular targets for pharmacological interventions of AUD and other substance use disorders. Supported by AA027096 and AA028370.