Targeted Epigenome Editing with dCas9 Prevents Effects of Adolescent Alcohol Exposure

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Adolescent alcohol abuse is associated with increased risk of developing an alcohol use disorder (AUD) and co-morbid psychiatric disorders (anxiety, depression) in adulthood. Despite considerable advances in understanding the neurological and molecular mechanisms that underlie these changes, the cause of increased risk is still largely unknown. We recently identified that an enhancer region of the activity-regulated cytoskeleton-associated protein (*ARC* SARE) undergoes significant deleterious epigenetic remodeling in the postmortem amygdala in individuals who began drinking before the age of 21 (early onset)[1]. We observed increased EZH2 and H3K27me3 (EZH2's catalytic product) and decreased H3K27Ac associated at the *ARC* SARE site which is correlated with decreased ARC expression and silencing of this locus[1,2]. However, it is unclear whether epigenetic modifications at this locus contribute to adult behavioral phenotypes that arise from adolescent alcohol consumption.

Methods: Adolescent Sprague Dawley rats received saline (AIS) or intermittent ethanol (AIE, 2g/kg i.p 2 day on 2 day off) from PND 28 to 42 then allowed to mature until adulthood (PND > 92). We then infused dCas9-P300[3] lentivirus with 4 sgRNA viruses targeting the *Arc* SARE site into the central nucleus of the amygdala (CeA) and tested for anxiety-like behavior in light dark box (LDB). In a second cohort, we generated AIE animals, then infused EZH2 siRNA in adulthood into the CeA then evaluated animals in LDB, continuous two bottle choice (ethanol vs. water). Animals were then sacrificed for biochemical analysis using qPCR and chromatin immunoprecipitations[1,2].

Results: Transduction of dCas9-P300+ARC SARE sgRNAs into the CeA prevented AIE-induced anxiety-like behavior in LDB. We then evaluated the amygdala for mRNA expression of ARC and found that dCas9-P300+sgRNA transduction also prevented decreased *Arc* expression induced by AIE. Chromatin immunoprecipitation assays revealed that H3K27Ac was restored at the *Arc* SARE site. Next, we infused EZH2 into the CeA in adulthood which prevented both anxiety-like behavior and drinking caused by AIE. EZH2 infusion also prevented decreases in *Arc* expression and decreases in in H3K27Ac, increases in H3K27me3 and EZH2 at the *Arc* SARE.

Discussion: Our results indicate that AIE causes epigenetic remodeling at the *Arc* SARE which leads to behavioral changes and that this can be reversed by targeted epigenomic editing with a dCas9-P300 platform. Our studies demonstrate the use of a dCas9 platform to probe and modulate specific epigenomic changes. Further we identify EZH2 both in early onset human postmortem and reverse-translational model as a target for drug development for early onset AUDs.

References:

Bohnsack JP et al. *Transl. Psychiatry.* 2019 (1):34. Kyzar, EJ, Zhang H, Pandey SC, *Biol. Psychiatry* 2019. 85(11):901-914. Hilton IB et al. *Nat. Biotechnol.* 33(5):510-7.

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