CRISPR Dissection of Alcohol-responsive lncRNAs

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Introduction: Ethanol-induced changes in the brain transcriptome underlie the development and persistence of alcohol use disorder. While small non-coding RNAs such as miRNAs have received considerable attention as regulators of gene expression, long non-coding RNAs (lncRNAs) have been largely overlooked. lncRNAs are transcripts longer than 200 nucleotides that lack a functional open reading frame. lncRNA transcripts are surprisingly abundant. There are over 96,000 lncRNA loci in humans and ~87,000 in mice compared with only ~20,000 protein coding genes. lncRNAs are expressed in highly tissue- and temporal-specific patterns. Importantly, the brain expresses the greatest number of lncRNAs of any tissue in the body. This project tested the hypothesis that individual lncRNAs are key regulators of the brain transcriptome, behavioral sensitivity to ethanol, and ethanol drinking behavior.

Methods: To identify alcohol-responsive lncRNAs, gene expression analyses of brain tissue from mice genetically selected for high alcohol drinking, rats selected for alcohol preference, and humans with alcohol use disorder were conducted. The functional role of a subset of alcohol-responsive lncRNAs was investigated with a targeted CRISPR/Cas9 genetically engineered animal model screen. These lncRNA knockout mouse and rat lines were tested for molecular, cellular, and behavioral phenotypes that focused on ethanol responses.

Results: Individual knockout of three lncRNAs that were identified in HDID (High Drinking in the Dark) mice did not change any alcohol behavioral phenotype tested. Knockout of the LRAP (Long non-coding RNA for Alcohol Preference) lncRNA in rats resulted in dramatic alterations in the brain transcriptome that were accompanied by increased ethanol consumption and preference. Knockout of mouse homologues of three human alcohol-responsive lncRNAs (abbreviated TX1, TX2, TX3) each resulted in behavioral phenotypes. TX1 knockout mice had increased duration of loss of the righting reflex (LORR) in response to ethanol and ketamine, but not gaboxadol. Mechanistic studies revealed a functional interaction of the TX1 lncRNA with NMDA receptors, but not $GABA_A$ or glycine receptors. Electrophysiologic analysis revealed sexually divergent effects on NMDA receptor-mediated transmission in the hippocampus. TX2 and TX3 knockout mice had decreased duration of LORR in response to ethanol, gaboxadol and zolpidem, but not ketamine. In addition, TX2 knockout males drank less ethanol on a two-bottle free choice every other day drinking assay.

Discussion: CRISPR mutagenesis of alcohol-responsive lncRNAs revealed novel insight into the role of these non-coding RNAs in alcohol action. Alcohol-responsive lncRNAs appear to be key regulators of the brain transcriptome and alcohol-related behaviors including drinking. Focused whole animal CRISPR screens are a valuable approach for investigating the role of lncRNAs in alcohol use disorder.

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