Conserved role for PCBP1 in altered RNA splicing in the hippocampus after chronic alcohol exposure

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RNA splicing is a molecular mechanism that generates transcript and protein diversity. Evidence is accumulating that RNA splicing is altered by alcohol exposure and may contribute to alcohol-related behavioral phenotypes. We previously conducted RNA sequencing (RNA-Seq) of the hippocampus (HPC) from male rats undergoing withdrawal after chronic ethanol exposure and discovered increased expression of genes encoding RNA splicing factors. In this study, we examined the expression of splicing factors and alterations in RNA splicing in the HPC of both male and female rats during withdrawal from chronic ethanol exposure. Additionally, we analyzed postmortem HPC from human subjects diagnosed with alcohol use disorder (AUD). Using qPCR, we found elevated expression of splicing factors including Pcbp1, Snrpa, Alyref, and Sf3a2 in male rats during withdrawal from chronic ethanol exposure compared to controls. Interestingly, in female rats, the expression of these genes were significantly decreased during withdrawal in comparison to controls. In postmortem HPC from human subjects, PCBP1 expression was elevated in individuals diagnosed with AUD compared to controls independent of sex. To explore the consequences of altered expression of splicing factors, we analyzed splice junction differential expression (DE) in the RNA-Seq data from male rats, and identified 110 DE junctions between withdrawal and control conditions, corresponding to 53 unique annotated genes. Focusing on the gene encoding hyaluronan and proteoglycan link protein 2 (Hapln2), we discovered increased usage of a novel 3' alternative splice site in exon 4 during withdrawal. This newly identified splice site was conserved in human HAPLN2 and more frequently utilized in the HPC of AUD subjects compared to controls. We speculated that PCBP1 might play a role in the alternative splicing of HAPLN2 because we found a predicted PCBP1 binding motif adjacent to the canonical HAPLN2 exon 4 3' splice site. RNA immunoprecipitation with a PCBP1 antibody indicated enriched association of PCBP1 near this splice site in ethanol-withdrawn rats and AUD subjects. We propose that during ethanol withdrawal, PCBP1 binds to a cytosine-rich sequence near the canonical splice site, inhibiting its usage and leading to the increased utilization of the alternative site. In summary, our findings indicate a conserved role for the splicing factor PCBP1 in aberrant splicing of HAPLN2 after chronic ethanol exposure. As the HAPLN2 gene encodes an extracellular matrix protein involved in nerve conduction velocity, use of this alternative splice site is predicted to result in loss of protein function that could negatively impact hippocampal function in AUD.