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Targeting G-quadruplex DNA as cognitive function therapy for ATR-X syndrome

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Alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome is caused by mutations in *ATRX*, which encodes a chromatin-remodeling protein. Genome-wide analyses in mouse and human cells indicate that ATRX tends to bind to G-rich sequences with a high potential to form G-quadruplexes. Here, we report that *Atrx* mutation induces aberrant upregulation of Xlr3b expression in the mouse brain, an outcome associated with neuronal pathogenesis displayed by ATR-X model mice. We show that ATRX normally binds to G-quadruplexes in CpG islands of the imprinted *Xlr3b* gene, regulating its expression by recruiting DNA methyltransferases. Xlr3b binds to dendritic mRNAs, and its overexpression inhibits dendritic transport of the mRNA encoding CaMKII-α, promoting synaptic dysfunction. Notably, treatment with 5-ALA, which is converted into G-quadruplex-binding metabolites, reduces RNA polymerase II recruitment and represses *Xlr3b* transcription in ATR-X model mice. 5-ALA treatment also rescues decreased synaptic plasticity and cognitive deficits seen in ATR-X model mice. Our findings suggest a potential therapeutic strategy to target G-quadruplexes and decrease cognitive impairment associated with ATR-X syndrome.

Alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome (Online Mendelian Inheritance in Man (OMIM) entry 301040) is caused by *ATRX* mutations[1–3]. *ATRX* encodes the switch/sucrose nonfermentable (SWI/SNF)-like chromatin remodeling protein ATRX, which contains two signature motifs. One is a plant homeodomain designated the ATRX–DNMT3–DNMT3L domain, which binds to histone H3 tails, specifically at H3K4me0K9me2/3 (refs [4–6]). The other includes seven helicase subdomains that confer ATPase activity[7,8].

Genome-wide analysis that combines chromatin immunoprecipitation with next-generation sequencing (ChIP–seq) in both primary human erythroid cells and mouse embryonic stem cells (ESCs) shows ATRX enrichment at G-rich variable number tandem repeats, some of which form non-B DNA structures, including G-quadruplexes[9]. ATRX functions as a part of a histone chaperone complex that deposits the histone variant H3.3 onto pericentromeric heterochromatin and telomeres collaborating with death domain-associated protein (DAXX) in HeLa cells[10] and murine ESCs[11,12]. ATRX/H3.3 co-localization also occurs on the DNA-methylated allele of many imprinted genes and is associated with differentially methylated regions in mouse ESCs[13]. Some imprinted genes show upregulated expression in the forebrain of *Atrx* conditional knockout (cKO) mice[14,15], suggesting that ATRX silences the active allele. Moreover, expression of the autism-related gene *Nlgn4* significantly decreases in the forebrain of *Atrx* cKO mice[16]. Imprinting loss in neurons leads to various mental retardation syndromes, including Prader–Willi and Angelman syndromes[17]. However, the relationship between abnormal expression and cognitive dysfunction in ATR-X syndrome remains unclear.

Results

ATRX regulates *Xlr3b* expression in the mouse brain. $Atrx^{\Delta E2}$ mice, which are engineered to lack *Atrx* exon 2, show cognitive defects, among other phenotypes[18], and express a mutant protein that corresponds to a variant with an Arg37Stop (R37X) mutation in exon 2 seen in human ATR-X syndrome[19–21]. Moreover, $Atrx^{\Delta E2}$ mice show 80% reduction in ATRX protein levels[18,19], similar to outcomes seen in 27 individuals with ATR-X syndrome[8]. We used DNA microarrays to assess the transcriptional profiles at post-natal day 90 (P90) in the hippocampus of wild-type (WT) and $Atrx^{\Delta E2}$ mice. To identify differentially expressed genes, we used an algorithm combining the false discovery rate (FDR) and fold change in expression and identified 31 genes (8 upregulated and 23 downregulated) in WT versus $Atrx^{\Delta E2}$ samples. Among them was *Atrx* itself, which was downregulated in $Atrx^{\Delta E2}$ mice (see Supplementary Table 1 for a list of genes with an FDR < 0.05 and a $\log_2$ fold change of >0.5 or <−0.5). Among the genes that were markedly upregulated in $Atrx^{\Delta E2}$ mice was a member of the lymphocyte-regulated (Xlr) gene family, *Xlr3a*, and the imprinted gene *Xlr3b*[22–24] (FDR < 0.05 and $\log_2$ fold change > 0.5; Fig. 1a). *Xlr3a* and *Xlr3b* genes show 94% protein similarity, and DNA microarrays are limited in their ability to distinguish related factors. Thus, we carried out reverse transcription PCR (RT–PCR) analysis with a common forward (FW) primer and subtype-specific reverse (RV) primers (Supplementary Fig. 1a).

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