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Research Paper

Upregulation of Haploinsufficient Gene Expression in the Brain by Targeting a Long Non-coding RNA Improves Seizure Phenotype in a Model of Dravet Syndrome

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ABSTRACT

Dravet syndrome is a devastating genetic brain disorder caused by heterozygous loss-of-function mutation in the voltage-gated sodium channel gene SCN1A. There are currently no treatments, but the upregulation of SCN1A healthy allele represents an appealing therapeutic strategy. In this study we identified a novel, evolutionary conserved mechanism controlling the expression of SCN1A that is mediated by an antisense non-coding RNA (SCN1ANAT). Using oligonucleotide-based compounds (AntagoNATs) targeting SCN1ANAT we were able to induce specific upregulation of SCN1A both *in vitro* and *in vivo*, in the brain of Dravet knock-in mouse model and a non-human primate. AntagoNAT-mediated upregulation of Scn1a in postnatal Dravet mice led to significant improvements in seizure phenotype and excitability of hippocampal interneurons. These results further elucidate the pathophysiology of Dravet syndrome and outline a possible new approach for the treatment of this and other genetic disorders with similar etiology.

1. Introduction

Currently there is no treatment for many genetic disorders associated with loss-of-function mutations in one of the copies of a single gene (haploinsufficiency). Dravet syndrome (DS) is one of such disorders. DS is caused by heterozygous mutations in the SCN1A gene coding for the pore-forming alpha subunit of the voltage-gated sodium channel $Na_v1.1$. Clinically, DS is characterized by seizure onset in the first year of life, febrile seizures, prolonged seizures resistant to anticonvulsants, progressive psychomotor retardation and high incidence of sudden unexpected death (Dravet, 2011). Importantly, in most studied DS cases no mutant protein is produced and the characteristics of the Nav1.1-mediated sodium current are not significantly altered. However, the amplitude of the sodium current and SCN1A mRNA and protein levels are diminished (Sugawara et al., 2003; Vanoye et al., 2006; Ohmori et al., 2006; Bechi et al., 2011). Although significant insights into DS disease mechanism have been achieved in recent years, it is still not clear if major manifestations of the disease are caused by disturbances in embryonic development or by persistent SCN1A deficiency in later life. It is also not known if increasing SCN1A expression after birth, when most genetic diseases are diagnosed, would alter the disease phenotype. Arguably the therapeutically required increase in SCN1A should not be very high, because just doubling the expression in haploinsufficient cells would restore the normal levels of the protein. In addition, excessive sodium currents, for example in cases of genomic duplications of sodium channel genes, also lead to seizures (Goeggel Simonetti et al., 2012; Yoshitomi et al., 2015).

To explore the effects of postnatal upregulation of SCN1A expression in Dravet syndrome, we took advantage of a novel long non-coding RNA(lncRNA)-based mechanism of gene regulation which, as we

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show below, controls the expression of SCN1A mRNA. This mechanism is mediated by a lncRNA from the natural antisense transcript (NAT) class, which we named SCN1ANAT. Similar to other NATs (Katayama et al., 2005; Derrien et al., 2012), SCN1ANAT is a multi-exonic lncRNA transcribed from the opposite strand of the SCN1A gene (Fig. 1). SCN1ANAT shares small overlaps with the SCN1A coding sequences in both human and mouse genomes. NATs are known to function as fine modulators of on-going transcription, affecting a single gene or a small subset of related genes (Wahlestedt, 2013; Nakagawa and Kageyama, 2014; Zhao et al., 2010; Davidovich et al., 2013; Yu et al., 2015). The mechanisms of this gene-specific regulation likely involve tethering/scaffolding of general-purpose epigenetic complexes at a particular gene locus (Magistri et al., 2012; Khalil et al., 2009; Peschansky and Wahlestedt, 2014). Methylation and other modifications of histones and DNA deposited by these complexes trigger chromatin compaction and transcriptional inhibition. Depleting NAT molecules or blocking their interaction with epigenetic complexes and DNA, or in essence inhibiting the inhibitor, leads to upregulation of their target protein coding genes (Katayama et al., 2005; Meng et al., 2015; Chung et al., 2011; Halley et al., 2014; Modarresi et al., 2012; Matsui et al., 2013; Yamanaka et al., 2015). Several epigenetic protein complexes have been shown to depend on NATs for their specificity (Nakagawa and Kageyama, 2014, Khalil et al., 2009; Kotake et al., 2011; Zhao et al., 2010). For example, interfering with BDNF NAT function using synthetic oligonucleotide-based compounds (AntagoNATs) resulted in reduced levels of methylated lysine 27 in histone 3 in BDNF locus, reduced binding of a PRC2 component at the BDNF promoter, and increased expression of biologically active BDNF protein (Modarresi et al., 2012). Furthermore, blocking APOA1 NAT caused significant changes in histone H3 methylation levels and affected expression of several genes in the APOA1 cluster (Halley et al., 2014). In another example, a NAT from Lrp1 locus was shown to directly inhibit the activity of Hmgb2, a protein known to enhance the transcription of Lrp1. Oligonucleotides targeting Lrp1 NAT lifted its inhibitory interaction with Hmgb2 protein and increased Lrp1 expression (Yamanaka et al., 2015). Such DNA-level mechanisms enable NATs, frequently present in very low copy numbers, to efficiently control transcription at a given locus. As a consequence of their mechanism of action, the inhibitory activity of NATs is highly specific: it is limited to particular genes and engages only in cell populations that normally express their target coding genes (Magistri et al., 2012, Halley et al., 2014; Modarresi et al., 2012). Overall, NAT-mediated regulation is likely more suited to cases of haploinsufficiency than viral gene transfer, which often induces significant overexpression, is only partially responsive to endogenous controls, and has multiple technical and regulatory problems in the clinic. NAT-mediated regulation is present in many gene loci and is potentially applicable to the treatment of multiple genetic disorders

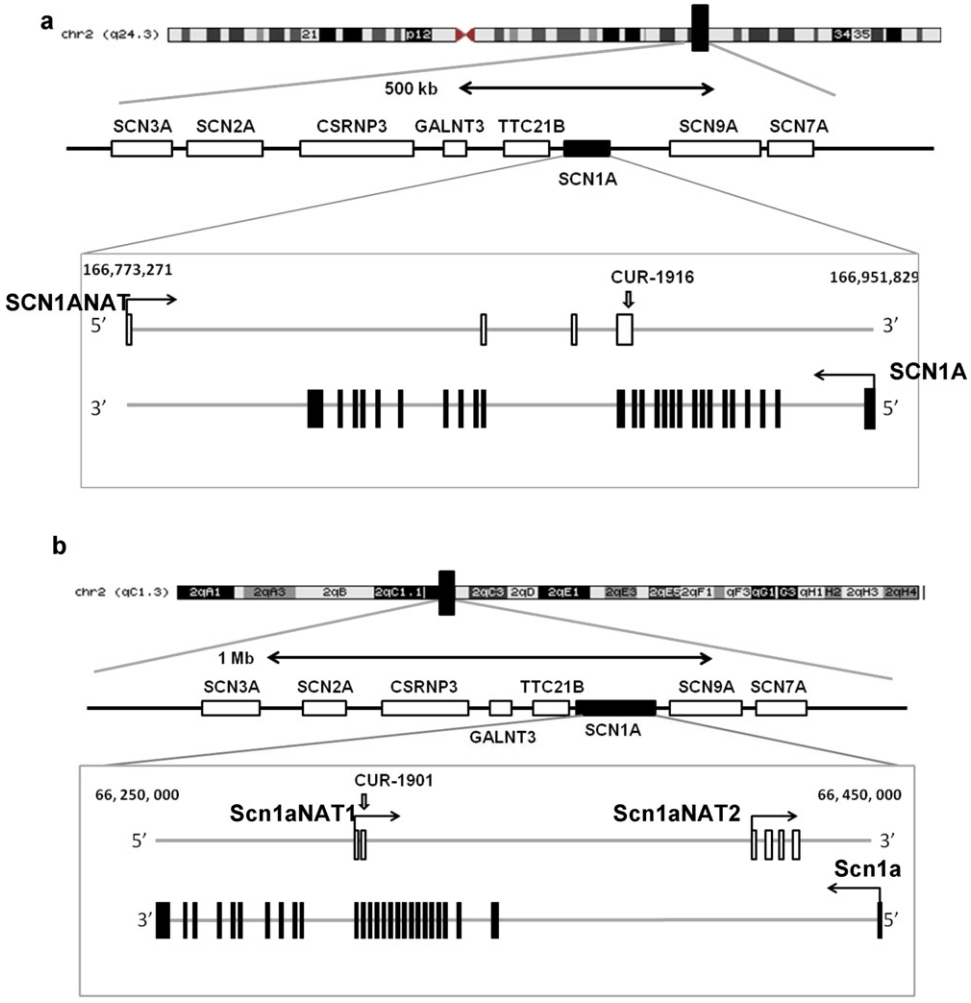

Fig. 1. SCN1A and SCN1ANAT coding regions are localized on opposite chromosomal strands in human and mouse genomes. (a) Human chromosome 2. (b) Mouse chromosome 2. In the insets: empty boxes — SCN1ANAT exons; filled boxes — SCN1A exons; grey lines — complementary chromosomal strands; angled arrows — direction of transcription; CUR-1916, CUR-1901 — positions of sequences complementary to respective AntagoNATs.