Characterization of a complex regulatory element of Spinal Muscular Atrophy genes

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Spinal muscular atrophy (SMA)

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SMN2 gene, Spinal Muscular Atrophy, Regulatory Element, RNA Splicing, Exons

Research Abstract

DESCRIPTION (provided by applicant): Humans have two nearly identical copies of Survival Motor Neuron (SMN) gene, SMN1 and SMN2. Low SMN levels due to deletion and/or mutation

of SMN1 lead to spinal muscular atrophy (SMA), a major genetic cause of infant mortality. SMN2 fails to compensate for the loss of SMN1 due to a C to T mutation at the 6th position (C6U in transcript) in exon 7. C6U weakens the 32-splice site and triggers SMN2 exon 7 skipping, resulting in synthesis of a truncated protein (SMN¿7), which is unstable. It is known that strategies aimed at correction of SMN2 exon 7 splicing hold the promise for a cure. This proposal emanates from our recent discovery of a unique RNA structure formed by a long-distance interaction (LDI) as a regulator of SMN2 exon 7 splicing (Singh et al., Nucleic Acids Res., 2013, doi:10.1093/nar/gkt609). We call this structure internal stem through LDI-1 (ISTL1). Employing the SHAPE (Selective 22-Hydroxyl Acylation analyzed by Primer Extension) method, we confirmed the formation and functional significance of ISTL1. We showed that an antisense oligonucleotide (ASO)-mediated sequestration of the 32 strand of ISTL1 fully corrects SMN2 exon 7 splicing and restores high levels of SMN and Gemin2, an SMN-interacting protein, in SMA patient cells. Our results also revealed that the 32 strand of ISTL1 is located within a large inhibitory region that we termed intronic splicing silencer N2 (ISS-N2). To continue with our lead, here we propose to characterize additional (novel) intronic cis-elements and their cognate transacting factors in regulation of SMN2 exon 7 splicing. A part of the proposal is aimed at validating the therapeutic efficacy of ISS-N2- targeting ASOs. In Aim 1, we will use overlapping deletions, ASO-based strategies and SHAPE analyses to determine the significance of novel cis-elements within SMN2 intron 7. We will validate our findings in different cell types including motor neuron-like NSC34 cells. We will examine the effect of a larger structural context on the accessibility of the splice sites of SMN2 exon 7. We will determine whether SMN2-specific mutations lead to a structural difference between SMN1 and SMN2 pre-mRNAs, particularly at the splice sites of SMN exon 7. In addition, we will evaluate whether critical cis-elements within intron 6 affect the structural context of intron 7 and potentially lead to the remodeling of the 52 slice site of exon 7. In Aim 2, we will employ over-expression and siRNA-based strategies to identify splicing factors that assist ISTL1 formation and/or use ISTL1 as a site for self-recruitment. We will use a biotinylated oligonucleotide as a trapping device to capture novel RNA-protein complexes that are deposited on SMN2 intron 7 and are critical for the inhibitory effect of LDI. We have previously shown that TIA1 stimulates SMN2 exon 7 splicing by binding to intron 7. We also demonstrated critical role of the Q-rich domain of TIA1 in regulation of SMN2 exon 7 splicing. Recently, a point mutation within Q-rich domain of TIA1 has been shown to cause Welander distal myopathy as well as promote SMN2 exon 7 skipping. Based on these findings and our preliminary results, we will examine the role of another Q-rich domain containing splicing regulator, SFPQ, in SMN2 exon 7 splicing. We will employ UV-crosslinking, footprinting, in vitro binding and SHAPE-based approaches to characterize RNA-protein interactions. In Aim 3, we will perform in vivo studies in a mild as well as in a severe mouse model of SMA to determine the therapeutic efficacy of an ISS-N2 targeting lead phosphorodiamidate morpholino oligomer (PMO). We will design our experimental plan based on several successful in vivo studies reported recently and will employ rigorous criteria of sample-size estimation, randomization and blinding. A successful outcome will lead to the development of a novel ASO-based therapy for SMA.

Lay Summary

PUBLIC HEALTH RELEVANCE: This proposal is aimed at understanding the molecular mechanism of aberrant RNA processing in Spinal muscular atrophy (SMA), a leading genetic cause of infant mortality. Findings will uncover the role of novel regulatory sequences and proteins factors that modulate aberrant RNA processing of SMA gene and will also validate the

efficacy of a novel target for a mechanism-based therapy of SMA.

Further information available at:

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