Effect of intronic sequences on splicing regulation of Spinal Muscular Atrophy gene

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive, neurodegenerative disease that affects infants. It results from the loss of function of the Survival Motor Neuron 1 (SMN1) gene. Survival Motor Neuron 2 (SMN2), a nearly identical copy of SMN1, fails to compensate for the loss of SMN1 due to predominant skipping of exon 7 during pre-mRNA splicing (Prior, 2008). This skipping of SMN2 exon 7 results in a shortened mRNA that makes an unstable, truncated protein. Currently, researchers aim to develop therapeutic strategies that will correct SMN2 exon 7 splicing. However, successful strategies cannot be developed until the mechanism of SMN2 pre-mRNA splicing is fully understood. Thus, in this study we screened for novel cis-acting regulatory elements that modify and control the splicing of SMN2 pre-mRNA to test the following hypothesis: if there remain critical unknown cis-elements that regulate the splicing of SMN2 pre-mRNA, then by finding these elements we can gain a better understanding of the mechanism behind SMN2 pre-mRNA splicing (Figure 1).

Materials and Methods

Within a reporter system previously established (Singh et al., 2004), called a “minigene”, that contains SMN2 DNA from exon 6 to exon 8 (introns included), I created three different 10bp deletions within intron 7 to see their effect on SMN2 pre-mRNA splicing (Figure 2).

Results

As shown in Figure 4 lane 4, mutant ∆426-435 showed the greatest inclusion. The other two mutants shown in lane 2 and 3 exhibited little inclusion similar to wild type in lane 5. Lanes 6 and 7 show previously generated mutants (Singh et al., 2013). Branch point is deleted in mutant ∆421-440 (lane 7); thus, no splicing occurs. Lane 8 is a negative control, no plasmid was used during transfection.

Conclusion

From these results, we propose that, due to the deletion in mutant ∆426-435, an alternative branch point in intron 7 was used during pre-mRNA splicing. This novel branch point shift results in more inclusion of exon 7 in the final spliced products. Overall, a novel RNA area that appears to have regulatory activity in splicing of SMN2 was found. Further characterization of this area is needed.

References


