

RESEARCH ARTICLE

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Deciphering targeting rules of splicing modulator compounds: case of TG003

Maki Sakuma¹, Kei Iida^{2*} and Masatoshi Hagiwara^{1,2*}

Abstract

Background: Recent advances in the development of small chemical compounds that can modulate RNA splicing brought excitement to the field of splicing-targeting therapy. Splicing-targeting therapy tries to ameliorate the disease by altering the exon combination of transcripts to reduce the undesired effect of genetic mutations. However, the knowledge and tools to understand factors contributing to splicing modulator compound sensitivity have been lacking. Our goal was to establish a method to characterize sequence features found in compound sensitive exons.

Results: Here we developed a comparative transcriptomic approach to explore features that make an exon sensitive to a chemical compound. In this study, we chose TG003, a potential drug for Duchenne muscular dystrophy, and performed RNA-sequencing on samples from human and mouse skeletal muscle cells, with and without TG003 treatments. We compared TG003 responsiveness between homologous exon pairs and identified 21 pairs in which human exons were skip-enhanced but not mouse exons. We compared the sequence features, splice site scores, number of splicing factor binding sites, and properties of branch sequence and polypyrimidine tracts, and found that polypyrimidine tracts were stronger (longer stretches and richer content of consecutive polypyrimidine) in the mouse TG003 insensitive exons. We also compared the features between TG003 skip-enhanced and insensitive exons within the species, and discovered that human TG003 skip-enhanced exons were shorter and had less splicing factor binding sites than the group of human TG003 insensitive exons. Mouse insensitive exons homologous to human TG003 skip-enhanced exons shared these properties. Our results suggested that these features are prerequisites for TG003 skip-enhanced exons and weak polypyrimidine tracts are defining features, which were supported by a decision tree analysis on all cassette exons in human.

Conclusions: In this study we established a comparative transcriptomic approach, which shed lights on how small chemical compounds modulate RNA splicing. The results described here was the first attempt to decipher the targeting rules of a splicing modulator compound. We expect that this approach would contribute to the precise understanding of the mechanism of TG003-induced splicing modulation, expand target diseases of splicing modulators in general, as well as the development of new splicing modulators.

Keywords: Comparative pharmacogenomics, Comparative transcriptomics, Virtual massive mutagenesis, RNA-targeting therapy, Personalized medicine, Splicing modulator, Polypyrimidine tract, Duchenne muscular dystrophy

Background

Mammalian gene expression requires the accurate excision of introns and ligation of exons from the pre-mRNA by splicing, and approximately 95 % multi-exon genes undergo alternative splicing in human [1]. Alternative

splicing contributes to proteomic diversity and organismal complexity because isoforms can have different functions or have non-functional forms to fine-tune the regulation and expression levels of one gene product.

Splicing has been a target of therapy for diseases [2–4]. There are genetic diseases with mutations located near splice sites that cause abnormal splicing such as familial dysautonomia. In this case, a mutation occurred 6 base downstream from exon 20 of IKBKAP gene inhibits inclusion of the exon. Attempts have been made to increase the inclusion of the exon by chemical compounds such as

*Correspondence: iida.kei.3@kyoto-u.ac.jp;
hagiwara.masatoshi.1@kyoto-u.ac.jp

¹ Medical Research Support Center, Kyoto University Graduate School of Medicine, Konoecho Yoshida Sakyo-ku, Kyoto 606-8501, Japan
Full list of author information is available at the end of the article

Deciphering Targeting Splicing Modulator Compounds

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