New design guidelines simplify development of targeted therapies for muscular dystrophy and other diseases

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Patients with DMD lack the protein dystrophin, which causes muscles to deteriorate and break down, leading to progressive difficulty with walking and general mobility. Credit: Huntstock/Thinkstock

The dystrophin protein offers critical support to muscle fibers. Mutations affecting dystrophin's expression cause the muscle-wasting disease muscular dystrophy. In Duchenne muscular dystrophy (DMD), these mutations take the form of small sequence changes that make much of the dystrophin gene (DMD) untranslatable, yielding nonfunctional protein or no protein at all.

Therapies based on a strategy known as 'exon skipping' could undo the damage from these mutations. Development of such treatments is set to

accelerate, thanks to research by a team led by Keng Boon Wee of the A*STAR Institute of High Performance Computing and Zacharias Pramono of the National Skin Centre in Singapore.

Proteins are translated from messenger RNA transcripts of genes; however, only certain RNA regions—known as exons—actually encode protein, and these are enzymatically spliced together prior to translation. Several clinical studies have demonstrated that small 'antisense oligonucleotide' (AON) molecules that bind mutated DMD exons can induce elimination of those defective exons during splicing, yielding shorter but largely functional versions of dystrophin. "We are cautiously optimistic that AON-induced exon skipping could be the first effective therapy for DMD patients," says Wee.

Unfortunately, DMD arises from many different mutations, and targeted AON design remains a time-consuming, trial-and-error process. To address this challenge, Wee and Pramono sought to define the characteristics of AONs that efficiently promote exon-skipping. They used computational analysis to zoom in on exonic sequences that coordinate splicing. They also identified regions of suitable length within dystrophin RNA transcripts that span these sequences and would be accessible to AONs in living cells.

The researchers thus derived a set of guidelines enabling them to effectively design AONs that targeted nine different exons affected in DMD patients. For each exon, at least one AON proved capable of boosting dystrophin expression to clinically relevant thresholds in cultured muscle cells (see image). "Our proposed set of factors resulted in a reasonable success rate of designing efficient AONs—61% versus 38% using semi-empirical methods," says Wee. Clinical studies have already demonstrated the promise of efficient exon skipping in treating DMD patients.

Wee notes that other diseases arising from abnormal RNA processing could also benefit from this approach. However, his team is also exploring this method as a general strategy to abort production of disease-causing proteins in cancer and other conditions. "In contrast to small-molecule inhibitor drugs that can target only about 10% of the human genome, this approach could downregulate most human genes," Wee says.

More information: Pramono, Z. A. D., Wee, K. B., Wang, J. L., Chen, Y. J., Xiong, Q. B. et al. A prospective study in the rational design of efficient antisense oligonucleotides for exon skipping in the DMD gene. *Human Gene Therapy* 23, 781–790 (2012). online.liebertpub.com/doi/abs/10.1089/hum.2011.205

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