Molecular Therapy
Nucleic Acids
Editorial

DNA/RNA heteroduplex oligonucleotides: An unanticipated twist in the delivery of ASOs

Delivery of RNA oligonucleotide therapeutics into tissues and cells remains the rate-limiting problem to solve before these therapeutic platforms can be utilized to treat wide-spread human disease.[1–4] Typical antisense oligonucleotides (ASOs) are single-stranded RNAs that sterically block splicing or are, in the case of Gapmer ASOs, a combination of RNA on the ends with an intervening DNA segment in the middle that activates RNase H to cleave the target mRNA.[1,2] In contrast, siRNAs are double-stranded RNAs that induce RNA interference (RNAi) responses to knock down the target mRNA.[1,2] Compared with native RNA, ASOs are heavily modified and have a full phosphorothioate backbone, where one of the non-bridging oxygen atoms is replaced with a more "hydrophobic" sulfur atom, which results in both a significant increase in metabolic stability and an enhanced escape from endosomes.[1,2,4] All of the ASO's 2′ hydroxyls have been replaced with 2′ O-methyl (OMe), 2′ methoxyethyl (MOE) or 2′–4′ locked nucleic acids (LNAs).[1,2] ASOs need a targeting domain to concentrate the ASO on the diseased cell type of interest. N-acetylgalactosamine (GalNAc), cholesterol, and anti-transferrin receptor monoclonal antibodies (mAbs) are all examples of ASO targeting domains that are being tested in the clinic for delivery of ASOs and siRNAs.[1–4]

To obtain optimal ASO activity, the conjugated targeting domain needs to be separated or cleaved from the ASO in the cell. While there are several approaches to address this issue, Prof. Takanori Yokota's laboratory at the Tokyo Medical and Dental University approached it in an entirely unique manner that paid off in spades. Back in 2015, they designed a prodrug-like double-stranded DNA/RNA heteroduplex oligonucleotide (HDO) that contains a Gapmer ASO that is hybridized to a complementary RNA (cRNA) oligonucleotide that the targeting domain is conjugated to, which is similar to siRNA designs.[5] The cRNA contains a central stretch of unmodified phosphodiester RNA nucleotides that are opposite the DNA gap of the hybridized ASO. Once inside the cell, the duplex is recognized by RNase H, and the cRNA strand is selectively cleaved, resulting in release of the fragments along with the conjugated targeting domain, and thereby activating the ASO. While this is a straightforward approach to activate the ASO, what was completely unexpected is that it also resulted in a significant increase in ASO delivery and activity compared with the same active ASO delivered as a single strand. Mechanistically, the HDO appears to result in a more rapid and efficient shuttling of the ASO into the nucleus compared with the same ASO as a single strand.[6]

The Yokota lab has now published a series of studies investigating HDOs in preclinical models.[5,7–12] In this issue of *Molecular Therapy: Nucleic Acids*, Kaburagi et al. utilized the Yokota lab's previously published tocopherol-conjugated HDO (Toc-HDO) to investigate the ability of systemically administered Toc-HDOs to efficiently enter dorsal root ganglia (DRG) neurons in the peripheral nervous system. The DRG lacks a sufficient neurovascular barrier, enabling access to the interstitium surrounding DRG neurons. Impressively, intravenously (i.v.) administration of Toc-HDO resulted in a 2-fold higher target gene knock down in murine DRG neurons from the cervical to lumbar cord and in the sciatic nerve compared with the same single-stranded ASO. However, there are a couple of caveats when thinking of converting these observations into the clinical setting. First, for ASOs, this was an exceedingly high dose of 50 mg/kg (see Figure 6). Second, the *MALAT1* target gene is the ASO field's go-to target gene because it is by far the best ASO and responsive target gene identified. As such, it is not surprising that it shows the best activity here. Third, having said that, a single 50 mg/kg dose of Tco-HDO targeting *Scarb1* showed a ∼70% knockdown (see Figure 6). Lastly, because the ASO does not have the hydrophobic Toc lipid conjugated to it, the TocHDO to ASO comparison here is apples to oranges. However, this group has previously published the direct comparison and observed a significant improvement in HDO delivery and activity.[9] Overall, the HDO technology defies the field. The collective results from the Yokota lab place a solid foundation for further improvements as HDOs move toward clinical development for a variety of indications, including DRG pathophysiology, neuropathic pain, and peripheral nerve diseases.

ACKNOWLEDGMENTS

R.L.S. was supported by the NCI CBIO training grant (T32CA067754-25).

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https://doi.org/10.1016/j.omtn.2022.06.013

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