

Novel oligonucleotide based on DNA/RNA heteroduplex structures: Opening a new horizon for human gene therapy

A brand-new oligonucleotide drug, heteroduplex oligonucleotide

Two major types of RNA targeting oligonucleotide drugs are currently being developed as therapeutic platforms for the reduction of target gene expression: short interfering RNA (siRNA) and RNase H dependent antisense oligonucleotides

(ASO). Although the design of oligonucleotides has progressed considerably, methods that further increase the potency of oligonucleotide drugs and improve their safety and tolerability are highly desirable, as with any medical products. The insufficient delivery and poor cellular uptake of oligonucleotides, and their inefficient access to target RNA, are major impediments to their use in *in vivo* gene silencing.

I developed a novel short DNA/RNA

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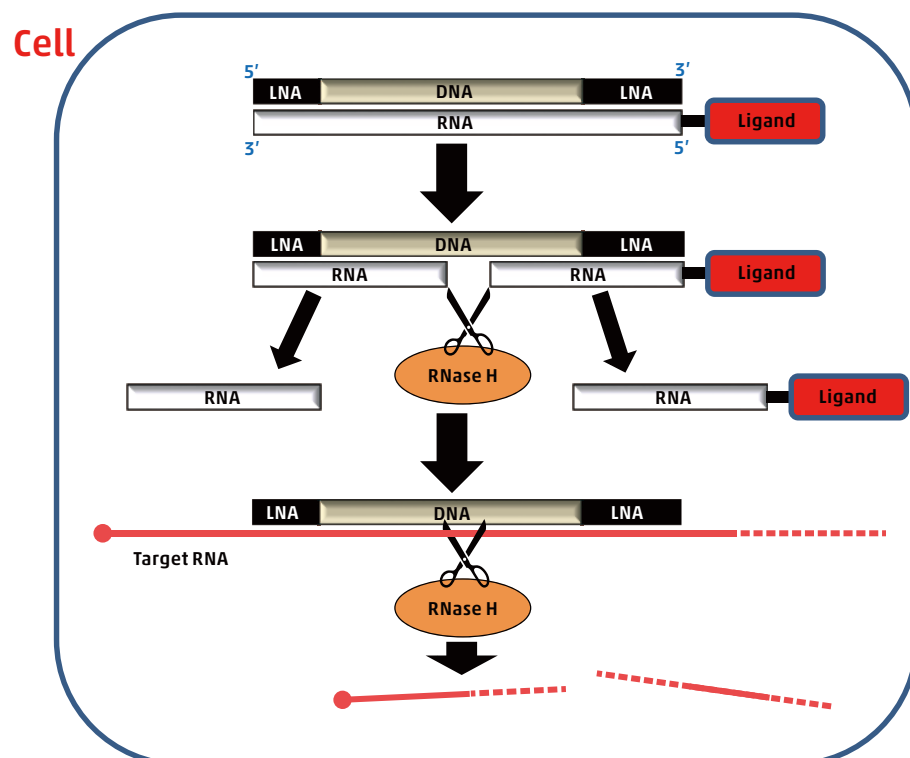
Dr. Yokota received his MD from TMDU. His postdoctoral research was performed in several institutes including Tokyo Metropolitan Neurological Hospital and Sanford-Burnham Medical Research Institute in the United States. He returned to TMDU as a Junior Associate Professor of Neurology in 2000, became an Associate Professor of Neurology and Neurological Science in 2004, and assumed his present post in 2009.

heteroduplex oligonucleotide (HDO). This HDO has a structure different from the double-stranded RNA used for siRNA and from the single-stranded DNA used for ASO, and also different functional molecular mechanisms from siRNA or ASO in cells. HDO is composed of DNA/locked nucleic acid (LNA) gapmer as ASO and its complementary RNA (cRNA). When α -tocopherol (vitamin E) is conjugated to cRNA of HDO, α -tocopherol can improve the delivery of HDO to the liver, and the gapmer DNA strand is activated by its release from HDO due to cleavage of cRNA by cellular nuclease (Fig.1).

Unique structure enables high efficacy with less adverse effect

When α -tocopherol (Toc) as a drug delivery moiety is conjugated to ASO directly without any linker (Fig.2), its silencing effect is reduced because conjugated lipid interferes with the mechanisms of ASO. On the other hand, Toc-HDO is significantly more potent at reducing the target messenger RNA compared to the parent ASO (Fig.2). I measured the Effective Dose 50 (ED₅₀)

Fig. 1. Molecular mechanism of HDO in cells



–the dose required for a 50% reduction of the target gene. Toc-HDO targeting *Apolipoprotein B (ApoB)* mRNA (ED₅₀, 0.038 mg/kg) was 22.2 times more potent than the parent ASO (ED₅₀, 0.841 mg/kg) in mouse liver. In addition to lowering *ApoB* mRNA, the Toc-HDO reduced serum low-density lipoprotein (LDL)-cholesterol. Moreover, the pharmacological effects lasted more than one month at a 0.75 mg/kg of Toc-HDO injection only, not at ASO injection.

A significant improvement in activity was also observed when targeting another gene in the liver. In addition, the Toc-HDO using another chemically modified nucleic acid instead of LNA in the wing portion of the DNA strand showed a similarly enhanced potency. This HDO technique can be applied to any ASOs that have been previously reported. Furthermore, the high potency of the suppression of the target messenger RNA was observed not only in rodents but also in non-human primates.

Mipomersen, the first oligonucleotide drug, was approved by the U.S. FDA, but not by the EU, due to liver toxicity. A reduction in liver dysfunction was observed when using high potency Toc-HDO, probably because much smaller doses of nucleotide were administered, than when using the parent ASO with the same silencing effect. These results suggest that DNA/RNA heteroduplex structures can be the basis for a novel class of oligonucleotide drugs, opening a new horizon for human gene therapy.

From lab to clinical use

To commercialize this highly promising HDO technology, a start-up company named Rena Therapeutics Inc. was established in January, 2015 as the fourth venture company launched by TMDU. Its mission is to address unmet medical needs by creating a novel class of nucleic acid medicine with unique and effective drug delivery systems and chemical

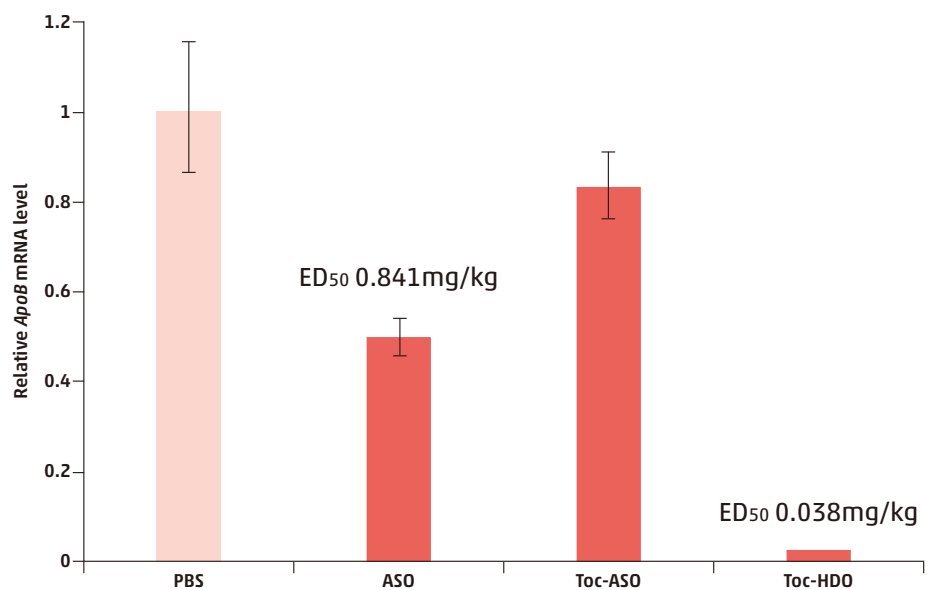


Fig. 2. High efficacy of the Toc-HDO

When Toc-HDO that targets mouse *Apolipoprotein B (ApoB)* mRNA was intravenously injected into mice, it produced a greater reduction of *ApoB* mRNA compared with an equivalent dose of the parent ASO without α -tocopherol, or with α -tocopherol that is directly conjugated to ASO.

modifications. On April 20, 2015, six million Japanese yen Series A financing from Innovation Network Corporation of Japan, DBJ Capital Co., Ltd. and KSP Inc. was completed and Rena's research and development operations kicked into gear. The company name "RENA" is an acronym for Renaissance of Nucleic Acid, reflecting the company's mission to revive nucleic acid medicine through research and development of this HDO technology, especially pursuing practical clinical applications.

TMDU and co-patent holder Osaka University granted the exclusive license of the HDO patent family to Rena, and Rena will make this HDO technology applicable to nucleic acid medicine as quickly as possible through cooperation with biotech and pharmaceutical companies. Its revenue model comprises three parts: 1. The research and development of HDO seeds and pipelines up to the preclinical or early clinical stage, and licensing to pharmaceutical companies; 2. The creation of co-development project

(s) of HDO technology with partner companies as collaborative research or alliances; and, 3. The licensing and sub-licensing of the HDO patent family and related Rena patents to outside companies.

Although Rena's first important role will be to cross a deep "Death Valley" where many obstacles are expected, this HDO technology has such strong competitive advantages for drug delivery that we see more than enough potential to cross through the valley. For the next few years, Rena plans to focus on developing delivery modifications of HDO technology to develop it for drug pipelines. Through these research, clinical and business development activities, TMDU and Rena hope to increase HDO technology's potential further so as to enter clinical trials as early as possible.

DNA/RNA heteroduplex oligonucleotide for highly efficient gene silencing, *Nature Communications*, 6:7969 doi: 10.1038/ncomms8969.