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A scientific debate: The sword that cleaves chaos of DNAzyme catalysis research

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ABSTRACT

Therapeutic DNAzymes with RNA depleting effect are multifunctional building blocks for constructing biological materials. In a recent work published in *Nature Chemistry*, Holliger group and Chaput group had a wonderful debate base on previously reported approach for tailoring DNAzymes with high mRNA cleavage activity in mammalian cells. Their perspective pushes the catalytic mechanism investigation of DNAzymes to a new stage with more standardization and clarity.

DNA is traditionally considered as a biomolecule with genetic functions. From an alternative viewpoint, DNA is also an ingenious multifunctional component for biomaterials construction, ascribing to its inherent molecular recognition ability, sequence programmability and biocompatibility [1]. DNAzymes, as an oligonucleotide DNA sequence with metal cofactor, that facilitated the catalytic function, have the power of cleaving specified RNA sequence [2]. Therefore, it can be incorporated to construct RNA or metal responsive materials for disease treatment. DNAzymes-based therapy has been evolved for more than 20 years since the first demonstration of the *in vivo* activity of a 10–23 DNAzyme in 1999 [3]. High specificity and non-immunogenicity are now paving the way for DNAzymes to enter clinical trials [4]. To date, near 10 clinical trials concerning DNAzymes have been conducted, and some of the programs have demonstrated the safety and therapeutic effects in Phase II trials [5]. Nonetheless, like other oligonucleotide materials, DNAzymes still have the disadvantage of being easily degraded by enzymes, which restricts the rapid progression of DNAzymes in medicine.

At present, to further eliminate the weakness and re-accelerate the clinical progress of DNAzymes, the DNAzymes are combined with synergistic delivery system, as well as with other agents that can enhance their catalytic activity. For example, Qu et al. recently constructed smart nanomachines guided by a DNAzyme logic system for precise gene-chemodynamic therapy (CDT) [6]. Zhou et al. prepared photothermal-DNAzymes combined nanovaccine, which can promote antigen presentation and dendritic cell infiltration [7]. DNAzymes are also chemically modified to improve their tolerance to enzymatic degradation. With regard to the catalytic activity, the most representative work published by John C. Chaput group in *Nature Chemistry* [8], has reported a new type of 2′-fluoroarabino nucleic acid (FANA) and α-L-threofuranosyl nucleic acid (TNA) modified DNAzyme for efficient gene therapy in cells. FANA has a higher affinity for RNA and is more resistant to hydrolysis and nuclease degradation. Meanwhile, TNA is one of the few genetically man-made polymers that are highly resistant to nuclease digestion and capable of cross-pairing with DNA and RNA [7].

The Chaput's team modified all nucleotides within the substrate binding arm with FANA and capped each of the 5′ terminal and 3′ terminal with TNA residue (Fig. 1A). The results showed that these optimizations significantly enhanced the biological stability of DNAzymes, which cleaved target RNA 50 times faster than unmodified DNAzyme (Fig. 1B). This accomplishment represents an important advance towards the design of potent therapeutical DNAzymes [9].

However, now, as published in *Nature Chemistry* [10], Holliger et al. challenged the core claim of above-mentioned research. It was proposed that the above-mentioned silencing of mRNA was not entirely powered by the catalytic activity of the X10-23 DNAzyme, but to a large extent by RNase H mediated antisense effect (Fig. 1C). FANA could bind RNA and recruit RNase H. It was reported that the FANA/RNA complex, as short as 5 or 6 nt, can mediate enhanced degradation of RNA *in vivo*. Besides, Holliger group proved the core inverted X10-23 DNAzyme was not an appropriate negative control, since core sequence can cause impact on RNase H recruitment.

In addition, throughout the field of DNAzymes catalysis, for convenience, researchers generally quantitate DNAzymes activity on short, unstructured RNA substrates. However, clinically, DNAzymes will encounter long-chain mRNA with stable secondary structures. The complex mRNA secondary structures pose a challenge to the accessibility of DNAzymes to RNA. During the experiments, Holliger et al. found the catalytic efficiency for same X10-23 DNAzyme showed ~100-fold slower

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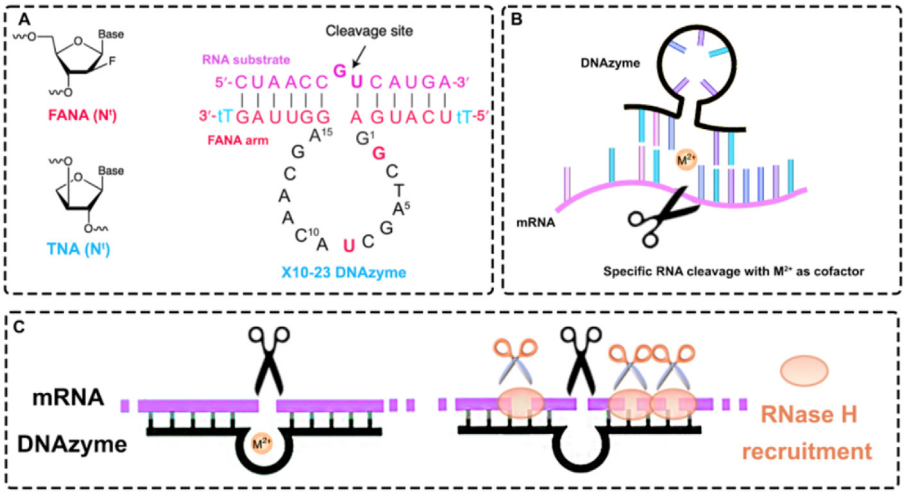

Fig. 1. Chemical structure of modified DNAzyme and the mechanism of action [8]. (A) FANA and TNA modified X10-23 MNAzyme. (B) X10-23 DNAzyme binds specific mRNA by Watson-Crick base pairing and silence RNA with M^{2+} as cofactor. (c) antisense mechanism mediated by RNase H.

than the results presented in ref8. They also proved that a substrate binding arm of FANA shorter than 8nt is insufficient to invade the long RNA with complex secondary structure. Therefore, they questioned the accessibility of the DNAzyme designed by Chaput's team, to the long mRNA.

What's more, they also proposed that for gene silencing analysis by using qPCR as the only reading would produce "false positive" data. Because in the process of qPCR reaction, DNAzyme modified by LNA will not be degraded and will continue to cleavage mRNA to cause fake positive results. In conclusion, Holliger et al. proposed three factors that need to be considered for catalytic analysis: 1) recruitment of RNase H; 2) complex secondary structure within real long-chain RNA substrates; and 3) potential artifact ascribed to persistent cleavage activity during treatment. Nonetheless, after these matters arising, Spitale and Chaput replied to the criticism immediately in *Nature Chemistry* [11]. They denied the RNase H-mediated antisense gene silencing effect and pointed out that the LNA would only recruit RNase H in using the "gapmer" approach. However, the authors support two of Taylor and Holliger's arguments, stating that they also used RNA substrates up to 103nt, and that the use of core inverted XNAzymes in the control group did give rise to misunderstandings.

In any case, we are grateful for this wonderful debate which have a more standardized and clearer driving force for the overall therapeutic DNAzymes-based catalytic study. Nowadays, the results from each laboratory are difficult to compare with, even for the same DNAzyme. For example, modification of the 3′ inverted dT was found to enhance the catalytic activity of the 10–23 DNAzyme [12], while another group did not observe this phenomenon [13]. As another example, the catalytic rate of the 2′-O-methyl monomers modified 10–23 DNAzyme was proved to be much higher than that of the LNA-modified DNAzyme [13], but this conclusion was subsequently overturned [8]. We speculate that the main reason for this confusion is that different laboratories use RNA substrates with different levels of secondary structures. Complex secondary structure will increase the difficulty of DNAzyme to approach the RNA sequence, but it can better simulate the real situation. Besides, RNase H mediated antisense effect as well as "false positive" which presented in this debate are also two important elements that need to be considered.

Therefore, in the future research work on DNAzymes, we expect scientists to pay attention to the requirements mentioned in the debate above. First, the researchers are expected to use long-chain RNA to simulate the real situation instead of using some short-chain RNA fragments for convenience. Second, in the catalytic experiment, the catalytic medium should be unified, including metal content and the addition of nuclease type. Finally, The RNase H mediated antisense effect should be fully considered in the performing of control group, and the silencing efficiency of target RNA (not only qPCR) should be verified by various means.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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