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Review Article

A Brief Overview of NgAgo-gDNA system for Genome Editing: from Reality to Sophistication -

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ABSTRACT

Background and aims: It has been reported that *Natronobacterium Gregoryi* Argonaute (NgAgo) protein is a type of endonucleases which is guided by an ssDNA to target ssDNA and RNA. In fact, it requires only a single, 24 base paired, and 5' phosphorylated ssDNAs guide DNA (gDNA) for DNA targeting. In this review, we aim to introduce the NgAgo / gDNA system and report different studies on investigating its reproducibility based on Dr. Han's protocol.

Methods: This review was prepared using the databases of Science Direct, Pub-Med, Scopus, Web of Science, reference lists check and hand searching using keywords such as "gene editing", "CRISPR-Cas","NgAgo", "challenge", "tool editing". The selected papers were fully reviewed and required information for the review was extracted and summarized.

Results: The NgAgo-gDNA system was introduced by Chinese scientists from the Hebei University for the first time. These scientists claimed that these proteins have several advantages over the CRISPR-Cas9 system especially in the flexibility and efficiency of gene editing. While the declaration of NgAgo has enraptured numerous specialists, resulting worries over the reproducibility of the tests which alert us against drawing such big claims by a researcher from an article. After publishing his article in Nature Biotechnology, many scientists criticized the fact that they were not able to confirm and repeat the results of Dr. Han's experiment. However there are reports in gene knockdown activity and gene down regulation achieved by this system due to the DNA-dependent RNA cleavage activity of NgAgo not DNA editing.

Conclusion: NgAgo/gDNA system does not provide genome editing activity like ZNF, TALEN and CRISPR-Cas system. Despite all of these reports, the genome editing ability of NgAgo-gDNA is probable in the future under a specific condition that is yet to be known. Thus, more trial and error is required to design and develop a new gene editing tool over the recent well-known genome engineering toolkits such as ZNFs, TALEN and CRISPR-Cas system.

INTRODUCTION

Argonautes (AGOs) are considered as highly specialized binding proteins which take part in several cellular processes including transcriptional regulation and splicing, and gene silencing process through their effect on mRNA translation and decay. However, in eukaryotes, Argonaute proteins are mainly known by their specific role in RNA interference (RNAi) process and RNAi-mediated gene silencing due to accommodate the small RNA component such as siRNA (short-interfering RNA), miRNA (microRNA) and piRNA (PIWI-associated RNA) [1]. As we know, in the siRNA pathway the target strand of siRNA is loaded to the RISC complex (RNA-induced silencing complex) and directs the complex to detect and cleave the target mRNA with perfect complementarity. Specially, the AGO2 (protein argonaute-2) as RISC catalytic core in both animals and plants which leads to endonucleolytic cleavage of the target mRNA [2,3]. In eubacteria and archaea, AGOs are complexed with RNA or DNA guides and eliminate the foreign mobile genetic elements such as plasmids and phages [4-6]. In addition, structural studies have revealed four specific domains for these proteins as follows: amino-terminal (N), PAZ (PIWI–ARGONAUTE–ZWILLE), MID (middle) and PIWI domains [1]. It has been shown in some studies that TtAgo (argonaute derived from *Thermus thermophilus*) and AaAgo (argonaute derived from *Aquifex aeolicus*) could target single-stranded (ss) DNA and RNA and catalyze their targeted cleavage by using a ssDNA oligo as a guide [7,8]. However, since the *T.thermophilus* live in high-temperature habitants, their argonautes are only efficient at 65°C or higher temperature [6]. In one study published in Nature biotechnology, Dr. Chunyu Han and his colleagues claimed that they have reached to an Argonaute protein derived from *Natronobacterium gregoryi*, which is efficient at 37°C. They showed, when the NgAgo is associated with a 5' phosphorylated single-stranded guide DNA, it can cleave the plasmid in vitro. They further demonstrated that co-transfection of the plasmid encoding NgAgo and gDNA to the human 293Tcells leads to the cleavage of eGFP (enhanced Green Fluorescence Protein) plasmid and a significant reduction in eGFP protein was obtained. It is noteworthy that subsequent studies determined the optimal length of 24 base pair for gDNA. More importantly, they claimed that human endogenous genome modification can be also achieved by using NgAgo with a Nuclear Localization Signal (NLS) which is even more effective than the CRISPR-Cas9 system [9]. In table 1 is mentioned list of gene editing tools.

ADVANTAGES

Dr. Han detected some NgAgo-gDNA system advantages over the CRISPR-Cas9 system as follows: (1) The NgAgo utilizes ssDNA to cleave the DNA, while the Cas9-system recruit single guide RNA (sgRNA) to create a Double-Strand Break (DSB) in the target DNA sequence. (2) The mammalian cells have a very low level of ssDNA which can reduce the off-target effect (3). NgAgo seems to be more specific than Cas9, as it completely loses activity when there are three mismatches between gDNA and a target sequence. (4) NgAgo is a smaller protein, which is easier to express and (5) unlike the CRISPR-

Table 1: List of gene editing tools.

Name of Technology	ZFNs	TALENs	CRISPR/Cas
Target -efficient	Limiting factor	average	good
Designed componenet	protein	protein	RNA
Essential component	Zinc finger proteins + FokI endonuclease	TALE proteins + FokI endonuclease	Guide RNA + Cas9 protein
advantages	High specificity	High specificity	Recruited to DNA via sgRNA
	Low immunity	Low cytotoxic effects	Double strand cleavage
		Easy ex vivo delivery	Affordable
limitations	Difficult & laborious engineering process.	Time consuming	Higher off-target effects
	Difficult to predict cytotoxic off-target effects.	Off-target effects	Requirement for a PAM sequence adjacent to targer site
		Pair of ZFNs required to target specific DNA sequence	
Discovery and Development	since the early 1990s	since 2009	Since 2012

Cas9 system, the NgAgo does not require a PAM (Protospacer Adjacent Motif) and can target GC-rich genomic loci with high efficiency.

THE USEFUL FEATURES OF NGAGO

Dr.Han's study introduced some useful features of NgAgo for genome editing which are mentioned as follows: First, A single nucleotide mismatch at each position of the gDNA disrupted the cleavage efficiency of NgAgo, and mismatches at three positions completely blocked DNA cleavage. Second, 5' phosphorylated ssDNAs are scarce in mammalian cells, which minimizes inaccurate cellular oligonucleotides. Third, NgAgo acts as a faithful guide for gDNA, once it loaded with gDNA, it cannot replace gDNA with other free ssDNA at 37°C. All of these properties can minimize the off-target effects of NgAgo-gDNA system. Finally, NgAgo-gDNA system is easy to design and synthesize and to adjust their concentration, while it is relatively difficult for the Cas9-sgRNA system [10].

DISCUSSION

When AGOs are complexed with DNA and RNA guides, they can mediate the cleavage of target nucleic acid regions which their sequence is complementary with the guide. In eukaryotes, the Argonaute proteins suppress gene expression through RNAi process and in eubacteria and archaea, AGOs act as a defense system by taking part in eliminating foreign genetic elements [5,6]. Dr. Han et al. claimed that argonaute derived from *Natronobacterium gregoryi* (NgAgo) is a DNA-guided DNase which efficient at 37°C, thus provides genome editing activity. After publishing this novel method with advantages over the CRISPR-Cas9 system, many researchers were excited to design and apply specific NgAgo/gDNA system to modify their genes of interest. For instances, researchers failed to detect any genome editing by NgAgo/gDNA system in various eukaryotic cells [11-14]. Nevertheless, there are reports related to the gene knockdown activity of NgAgo/gDNA system. As Qi et al. demonstrated that unlike the detection of any mutation in zebrafish embryo genome, the NgAgo-based fab11a gene knockdown was accomplished through blocking the gene transcription. Since the fab11a has a key role in eye development from early stages, its knockout induces eye developmental defects in zebra fish [11]. Ye et al. further showed that NgAgo is a DNA-guided RNase rather than a DNase that mediates degradation of RNA molecules such as mRNA or lncRNA (long non-coding RNA) in a targeted manner [4]. A Similar result was obtained by Wu et al. who utilized NgAgo/gDNA system to target critical region of viral genome to inhibit HBV (hepatitis B virus) replication. No DNA editing activity was detected confirmed by T7E1 and Sanger sequencing. However the ability of this system in inhibiting the HBV replication was associated with the pregenomic RNA degradation and lowering its half-life time [15].

Taken together these results did not confirm the reproducibility of Dr. Han's experiment and researchers failed to confirm the DNA-guided genome editing activity of NgAgo/gDNA system. Despite all of these reports against the NgAgo-gDNA system efficiency, with attention to the uncertainties in the experimental research field, the improvement of NgAgo-gDNA is likely to be foreseeable in the future. Thus, more trial and error is required to design and develop a new gene editing tool over the recent well-known genome engineering toolkits such as ZNFs, TALEN and CRISPR-Cas system. We cannot also eliminate the possibility that NgAgo may exhibit genome editing activity under specific conditions that we have not yet found.

CONCLUSION

The above statements are a brief mention of the benefits of the NgAgo technique, which was quickly introduced in scientific societies, but the reality was different. After this, laboratories and researchers have tried to make their research using this attractive technique, but there were great disadvantages and the most important of them was the lack of reproducibility which is reflected in the researchers' findings. Finally, it seems that the results of researchers rejected this technique because of non-reproducibility. However, it would be better to postpone judgment and allow further research. These current studies decisively throw down *the earlier suggestions that NgAgo is a genome editing tool.*

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