Argonaute proteins: Structural features, functions and emerging roles

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Abstract

Argonaute proteins are highly conserved in almost all organisms. They not only involve in the biogenesis of small regulatory RNAs, but also regulate gene expression and defend against foreign pathogen invasion via small RNA-mediated gene silencing pathways. As a key player in these pathways, the abnormal expression and/or mis-modifications of Argonaute proteins lead to the disorder of small RNA biogenesis and functions, thus influencing multiply biological processes and disease development, especially cancer. In this review, we focus on the post-translational modifications and novel functions of Argonaute proteins in alternative splicing, host defense and genome editing.

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Abbreviations: AKT3, AKT serine/threonine kinase 3; CCR4-NOT, carbon catabolite repressor 4-negative on TATA; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (cas9); DGCR8, DiGeorge syndrome critical region gene 8; EGFR, epidermal growth factor receptor; GW182 protein, glycine/tryptophan repeats-containing protein with molecular weight of 182 kDa; H3K9, histone H3 lysine 9; Hsp70/90, heat shock proteins 70/90; JEV, Japanese encephalitis virus; KRAS, Kirsten rat sarcoma oncogene; PIWI, P-element-induced wimpy testis; PAZ, PIWI-argonaute-zwille; P4H, prolyl 4-hydroxylase; PAM, protospacer adjacent motif; RISCs, small RNA-induced silencing complexes; TRBP, the transactivating response (TAR) RNA-binding protein; TRIM71/LIN41, tripartite motif-containing 71, known as Lin41; WSSV, white spot syndrome virus.

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Introduction

The gene regulation mediated by small nucleic acid sequences, including small interfering RNA (siRNA), microRNA (miRNA), P-element-induced wimpy testis (PIWI)-interacting RNA (piRNA) and small interfering DNA (siDNA), has demonstrated to be the fundamental principle in regulating many biological processes, including cell proliferation, differentiation and homeostasis [1–5]. The key protein effector element in those processes is the Argonaute protein family. Argonaute proteins are found in almost all eukaryotes, bacteria and archaea (Table 1) [6].

Eukaryotic Argonaute proteins (eAgos) and prokaryotic Argonaute proteins (pAgos) have very low sequence homology, but their overall architecture and functions are extremely conserved in almost all organisms [7,8]. Both eAgos and pAgos with catalytic activity have the similar catalytic cycle, mainly including guide binding, target recognition and annealing, and target cleavage and release [9]. However, the structural differences of Argonaute proteins render them to have distinct functions. For example, almost all eAgos and most pAgos guide small RNA to regulate gene silencing [10–12], while a small part of pAgos utilize DNA as a guide [9,13–15]. However, the amount of Argonaute proteins in each organism is considerably different. For instance, there exist 8 different Argonaute proteins in human beings, including 4 Ago proteins and 4 PIWI proteins [16], while there are 27 Argonaute proteins in Caenorhabditis elegans [17].

Argonaute proteins constitute a main component of the small RNA-induced silencing complexes (RISCs) with small RNAs and/or additional proteins by direct or indirect binding, including Dicer, glycine/tryptophan (GW) repeats-containing 182 protein, heat shock proteins 70/90 (Hsp70/Hsp90) and so on [18–21]. These complexes not only involve in small RNA biogenesis by Drosha/DiGeorge syndrome critical region gene 8 (DGC8)-dependent or -independent pathways, but also provide anchor sites for small RNA to regulate gene expression in cytoplasm [2,12]. In the process of target recognition, Ago proteins can bypass secondary structure and protein barriers to diffuse along the substrate and effectively scan a canonical target through loose protein-nucleic acid interactions and intersegmental transfer [22]. Meanwhile, it also was reported that Argonaute proteins were able to be localized into the nucleus, where engaged in remodeling chromatins through histone modification, DNA methylation, alternative splicing of precur- sor mRNA, and double-strand DNA break repair processes [3,23–25]. In addition, Argonaute proteins are also closely associated with some human cancer [26–28]. These findings are expanding our understanding of the role of Argonaute proteins beyond gene silencing.

Evolutionary and structural characters of Argonaute proteins

According to the domain architecture, eAgos are divided into four classes: Ago-like family, PIWI-like family, WAGO family and Trypanosoma Ago family, while pAgos are grouped into three, including long pAgos, short pAgos and PIWI-RE proteins [7,29]. Of these categories, long pAgos contain the very similar domains to eAgos, including N-terminal (aminio-terminal), PAZ (PIWI-Arnaugene-Zwille), MID (Middle) and PIWI domains (Fig. 1) [30]. Their overall structure is a two-lobe scaffold, with one lobe comprising the N-PAZ domains and the other consisting of the MID-PIWI domains. The biled configuration of Argonaute proteins is connected by Linker 1 (L1) and Linker 2 (L2), and they occur structural rearrangement when binding to small RNA molecules. Unlike long pAgos, short pAgos only consist of the MID and PIWI domains.

Structural and biochemical studies have revealed that the N-terminal domain is required for deriving duplex small RNAs unwinding and loading a guide strand to assemble mature RISCs [31]. The PAZ domain can non-specifically anchor the 3’-end overhang of small RNA or siDNA into the specific binding pocket of Argonaute proteins (Fig. 1) [32–34]. The insertion of siRNA/miRNA 3’-hydroxy or methylated piRNA 3’ ends into the PAZ domain is essential for protecting small RNA from degradation [34,35]. To functionally coordinate with the PAZ domain, the MID domain, being located between the PAZ and PIWI domains, provides an insertion site for the 5’-phosphorylated terminal base of guide RNA through a highly conserved tyrosine in the nucleotide binding pocket [36]. The interaction of the 5’-end nucleotide binding pocket in the MID domain with the phosphorylated small RNA is more critical for guide binding and cleavage activity than the one with the 3’-hydroxyl end nucleotide binding pocket in the PAZ [37]. In some Argonaute proteins, which are structurally similar to ribonuclease H (RNase H), the PIWI domain contains a slicer active site that cleaves target RNA complementary with small RNA [38]. In the cleavage-compatible conformation, several highly conserved amino acid residues, such as DXD/DEXD (X stands for D or H), form a slicer activity center with an Mg2+ cation [15,39]. Surprisingly, only a part of Argonaute protein members have cleavage activity. For example, only Ago2 possesses catalytic and slicing activity in mammals [40]. However, it has been recently demonstrated that human Ago3 also has cleavage activity through selecting the guide RNA with bearing 5’- and 3’-flanking sequences in the guide-target complementary region [41]. Thus, it is possible that the structural difference of Argonaute proteins may be closely related to small RNA types they bind to and also to binding manners.

Table 1

| Argonaute proteins | Types | Host | Guide | Classify | Function | Reference |
|--------------------|-------|------|-------|----------|----------|-----------|
| Ago1-4             | Ago-like family | Homo sapiens | miRNA-guided RNA | eAgos | Interference and/or cleavage target gene | [16,41] |
| PIWI-4             | PIWI-like family | Homo sapiens | piRNA-guided RNA | eAgos | Transposon silencing and infertility | [57,109] |
| Ago1-10            | Ago-like family | Arabidopsis thaliana | miRNA/siRNA-guided RNA | eAgos | Interference and/or cleavage target gene | [110] |
| Ago1-27            | Ago-like family | Caenorhabditis elegans | miRNA/siRNA-guided RNA | eAgos | Interference, cleavage and silencing | [17] |
| PIAGO              | Long Ago | Thermus thermophilus | siRNA-guided DNA/RNA | pAgos | Host defence, cleavage, gene-editing | [72] |
| MJAGO              | Long Ago | Methanocaldococcus | siRNA-guided DNA | pAgos | Silencing, cleavage and gene-editing | [15] |
| NpAGO              | Long Ago | Natronobacterium gregoryi | siRNA-guided DNA | pAgos | Silencing, interference | [87] |
| MoAGO              | Long Ago | Marinotoga piezophila | siRNA-guided RNA/DNA | pAgos | Cleavage ssRNA | [111] |
| TpAGO              | Long Ago | Thermotoga profundana | siRNA/miRNA-guided DNA | pAgos | Cleavage ssDNA | [111] |
| AIAGO              | Long Ago | Archeoglobulus fulgidus | siRNA-guided mRNA | pAgos | Cleavage mRNA | [112] |
| AIAGO              | Long Ago | Aquifex aeolicus | siRNA-guided RNA | pAgos | Cleavage, interference | [113] |
| RIAGO              | Long Ago | Rhodobacter sphaeroides | siRNA-guided DNA | pAgos | Host defence and target cleavage | [5] |

Note: miRNA: microRNA; eAgos: eukaryotic Argonaute proteins; siRNA: small interfering RNA; piRNA: P-element-induced wimpy testis (PIWI)-interacting RNA; pAgos: prokaryotic Argonaute proteins; siDNA: single-stranded DNA.
In the small RNA-mediated gene silencing pathway, the binding process of the guide strand to Argonaute protein is linked to the biogenesis of the guide strand itself. When pre-miRNAs are exported to the cytoplasm, they are cut by Dicer, a kind of RNase III enzyme, to produce a short double-stranded RNA duplex (guide strand and passenger strand). This duplex together with Dicer is directly embedded into an Argonaute protein to recognize and select the guide strand through the thermodynamic asymmetry rule, followed by cleavage and ejection of passenger strand [42]. However, the mechanism how to eject the passenger strand is still elusive.

Mechanisms of RISC assembly

In the small RNA-mediated gene silencing pathway, the binding process of the guide strand to Argonaute protein is linked to the biogenesis of the guide strand itself. When pre-miRNAs are exported to the cytoplasm, they are cut by Dicer, a kind of RNase III enzyme, to produce a short double-stranded RNA duplex (guide strand and passenger strand). This duplex together with Dicer is directly embedded into an Argonaute protein to recognize and select the guide strand through the thermodynamic asymmetry rule, followed by cleavage and ejection of passenger strand [42]. However, the mechanism how to eject the passenger strand is still elusive.

Together with small RNA, Argonaute proteins compose the core of RISC to repress translation or to enhance the degradation of target mRNA. Previous studies have demonstrated that Argonaute
proteins divide the guide-strand RNAs into five distinct function regions: 5’ anchor into the MTD domain (g1), the seed sequence of miRNAs (g2-g8), the central region (g9-g12), 3’ complementary sequence (g13-g16), and 3’ tail region into the PAZ pocket (g17-g21) [43,44]. In addition to Argonaute proteins and small RNA, the assembly of RISC also requires the involvement of other key proteins, such as transactivating response RNA-binding protein (TRBP) and Hsp70/Hsp90. TRBP is a dsRNA-binding protein in human and senses the asymmetry and location of RNA to select and load the correct guide strand as R2d2 does in Drosophila melanogaster [45]. The Hsp70/Hsp90 multi-chaperone systems play a major role in protein function and structural activation, being involved in the conformation change of Argonaute proteins in an open and active status by hydrolyzing ATP that allows them to accommodate the RNA duplex [21]. Therefore, it is likely that Dicer, Hsp70/Hsp90 system, Argonaute and dsRNA-binding protein consist of a minimal RISC-loading complex.

Post-translational modification of Argonaute proteins

Like most proteins, Argonaute proteins also undergo post-translational modifications in multiple amino acid residues, mainly including phosphorylation, ubiquitylation and hydroxylation, which affect the functions of Argonaute proteins (Fig. 1).

Phosphorylation

Phosphorylation is the most basic and common mechanism for regulating protein activity and functions based on the signaling cascade effect. Several studies have reported that phosphorylated Argonaute proteins possess the ability to regulate its localization, binding to small RNA and gene silencing activity [46–48]. For example, human Ago2 protein contains three main phosphorylation regions, including Ser387 and Tyr393 in the L2 region [49,50], Tyr529 in the MID domain [47] and a Ser824–832 cluster in the PIWI domain [48]. It is noted that hyper-phosphorylated Ago2 protein in humans does not affect miRNA binding, localization or cleavage activity, but hyper-phosphorylation is essential for Argonaute proteins to execute these functions in C. elegans [51,52]. Although these studies are contradictory, it is clear that the phosphorylation of Argonaute proteins directly affects their localization and functions.

Ubiquitylation

In addition to phosphorylation, Argonaute proteins can also be ubiquitylated. This modification may play a more important role in the degradation of Argonaute proteins than that in their localization, turnover and regulation. In Drosophila, a RING-type E3 ubiquitin ligase can preferentially recognize and eliminate the dysfunctional and empty Argonaute proteins by selective ubiquitylation at Lys514 to control the quality of Argonaute proteins and miRNA-mediated silencing [53]. It was also reported that the tripartite motif-containing 71, known as Lin41 (TRIM71/LIN41) in C. elegans and mouse acted as E3 ubiquitin ligase for the ubiquitination of Argonaute proteins [54,55], but these findings were refuted in a subsequent study [56]. Moreover, in male sterile germ cell lines, deficient mutations in human and mouse PIWI proteins prevent their ubiquitination and degradation, and eventually lead to azoospermia via disrupting histone retention during spermiogenesis [57,58]. Together, these studies reveal that the ubiquitin–proteasome system mainly regulates the abundance of Argonaute proteins.

Hydroxylation

The hydroxylation modification in proteins is quite essential for their biological roles, which may alter the interactions between proteins and their downstream signaling events. The modification not only occurs at proline residues, but also at lysine, aspartate and histidine residues [59]. Although studies on the hydroxylation in Argonaute proteins are scarce, it is likely to play a role in their stability and activity. In mouse and human, the hydroxylation of Ago2 at Pro390 increases its stability [60]. Meanwhile, it was also reported that hypoxia-induced hydroxylation modification in Ago2 enhanced its endonuclease activity and the association with Hsp90 [61].

The biological functions and associated diseases of Argonaute proteins

As a key player in the small RNA-mediated gene silencing network, Argonaute proteins not only regulate the biogenesis of small RNA, mRNA translation and decay, but also involve in small RNA-guided host defense against exogenous nucleic acids, genome editing and even disease development (Fig. 1).

Biogenesis of Argonaute protein-associated small RNA

It has been known that small RNA exerts the functions in the developmental processes by associating with Argonaute proteins. Of them, miRNAs regulate their target mRNA expression as a post-transcriptional regulator [62]. Generally, most miRNAs are processed from primary transcripts (pri-miRNAs) to form precursor transcripts (pre-miRNAs) in the nucleus by Drosha, which were exported to the cytoplasm via Exportin 5 and further processed into dsRNAs by Dicer. When dsRNAs are successfully loaded into Argonaute proteins in an ATP-dependent manner, the duplexes will be unwound following wedged by the N-terminal domain of Argonaute protein. Subsequently, one guide strand is selected based on the unstably paired 5’ end and thermodynamic asymmetry rule, whereas the passenger strand is cleaved and/or discarded [63,64]. This process is referred as the canonical pathway. Additionally, there are also other biogenesis pathways of Argonaute protein–associated small RNA, termed as non-canonical pathways. Those small RNA species bypass the canonical Drosha and Dicer processing, such as piRNAs and agotrons [65,66].

piRNAs

The biogenesis of piRNAs is realized by the loading of PIWI proteins with piRNAs via the ping-pong cycle [67]. Briefly, antisense pri-piRNAs are cleaved by the nuclease 2uc to generate pre-piRNAs with U-preference at the 5’ end. The piRNAs are loaded into PIWI proteins to form cleavage-active piRNA complexes, which recognize, bind and cleave sense pre-piRNAs under the guiding of piRNAs to generate mature sense piRNAs. Subsequent steps, the sense piRNAs are loaded into other PIWI proteins to cleave antisense pre-piRNAs in the same way. In the cycle, sense and/or antisense piRNAs with PIWI proteins silence transposon activity to maintain genome integrity and other mobile genetic elements in germinal cells by targeting the transposon RNA via a miRNA-like pairing rule [68]. Although the ping-pong cycle model provides a reasonable explanation for the generation and functions of piRNAs, the detailed mechanism still remains unclear, such as piRNA initiation and specific target selection.

Agotrons

Agotrons are transcribed from small gene introns with a size of 80–100 nt and exported to the cytoplasm possibly via Exportin 5
[2]. Afterwards, although the agotrons have a complex secondary structure, they are loaded into Argonaute proteins to regulate mRNA expression in a seed-dependent manner [69]. Agotrons are conserved in mammals and act as a miRNA-like regulator with lower off-target or as an Argonaute protein protector by preventing proteasome-mediated degradation due to the high stability after miRNA loading [70]. However, the mechanism and biological functions still need to be further explored.

**Argonaute protein-mediated gene regulation**

In addition to orchestrating small RNA generation, Argonaute proteins have also been reported to be involved in gene regulation by directly binding and cleaving the targeted RNA or indirectly silencing the translation of the targeted RNA together with additional silencing proteins [71,72]. For the small RNA that is perfectly complementary with their target genes, mRNA targets will be directly cleaved if Argonaute proteins possess catalytic activity [73], but this phenomenon mostly occurs in plants [74]. In mammals, Argonaute proteins interact with several auxiliary factors to silence the targeted mRNA through the “seed sequence” complementary to miRNAs [62].

The GW protein family containing many glycine-tryptophan (GW) repeats harbors multiple binding sites for Argonaute proteins, and the affinity with Argonaute is increased upon miRNA binding [75]. Meanwhile, it was also demonstrated that a human GW182 protein recruited up to three human Argonaute proteins based on the multiple repeat binding sites in the GW motifs [76]. The GW182 protein is thought to act as a flexible structural scaffold to bridge the interactions between Argonaute proteins and downstream effectors [77]. These effectors mainly include the deadenylase complexes (such as poly A binding protein cytoplasmic [78,79], carbon catabolite repressor 4-negative on TATA (CCR4-NOT) and poly A Nuclease 2/3 [80]) and the decapping complexes (such as decapping 1/2, maternal expression at 31B and human pluripotency-associated transcripts [81]).

In addition, Ago1 and Ago2 are also present in the nucleus in mammals, and are involved into chromatin modification and alternative splicing [23]. The process mainly implicates the methylation of histone H3 lysine 9 (H3K9) and recruitment of the spliceosome complex to regulate gene expression, but the details of this mechanism are still largely unknown. Therefore, more studies will be required to explore the model for Argonaute protein-mediated chromatin modification and alternative splicing.

**Defense mechanisms of Argonaute proteins**

The survival of healthy cells depends not only on the integrity and precise replication mechanism of genetic information, but also on the defense mechanisms that prevent the infiltration and invasion of foreign nucleic acids. A number of studies have demonstrated that Argonaute proteins play a crucial role in defense against viral infection and transposon insertion into the genome [5,68,82–84]. For instance, during HIV infection, Ago2 has been shown to bind preferentially to unspliced HIV pre-mRNA to regulate HIV-1 multiply-spliced transcripts and the production of viral particles in a Dicer-independent manner [82]. When Ago1 and 3 are double-knocked out, the susceptibility and mortality of mice to influenza A infection are enhanced [85]. Similarly, the inhibition of Ago1 using isoform-specific siRNAs resulted in greater load of white spot syndrome virus (WSSV) in Marsupenaeus japonicus shrimp [86]. It was also reported that Ago2 was involved in significant inhibition of Japanese encephalitis virus infection in Aedes aegypti (JEV) [84]. Moreover, the NgAgo-siDNA complex efficiently suppressed HBV replication by accelerating pre-viral RNA decay [87]. These studies provide compelling evidence that Argonaute proteins are closely involved in host antiviral infection.

Transposons have the ability to randomly insert or extract themselves into the genome [88]. Although transposon symbiosis may cause DNA damages, living organisms have evolved an elaborate Argonaute-dominating mechanism to reduce the mobility of transposable elements and preserve genome integrity, namely the pRNA-dependent transposon silencing mechanism [66,68,89].

**Argonaute proteins as a novel genome-editing tool**

The recently developed clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR–Cas9) complexes are the most extensively used tool for editing genomes, and can cleave genomic dsDNA sequences based on RNA–DNA hybridization [90,91]. However, the application of CRISPR-Cas9 is greatly limited due to the high tolerance to guide-target mismatches, relatively easy-to-form RNA-directed secondary structures and the requirement of a protospacer adjacent motif (PAM).

In view of the above-mentioned various characteristics of Argonaute proteins, more and more researchers have begun to speculate whether Argonaute proteins have DNA-editing functions. At present, a growing number of studies have been demonstrated that some pAgos not only bind siDNA to mediate DNA-guided DNA silencing, but also cleave the siDNA cognate single-stranded target DNA in vitro, such as pAgos from Thermus thermophilus (TtAgo), Methanocaldococcus jannaschii (MjAgo) and Pyrococcus furiosus (PfAgo) [15,72,92]. However, TtAgo requires the minimum reaction temperature above 65 °C, MjAgo is most active around 85 °C and PfAgo most active between 87 °C and 99 °C. Therefore, the activity of these three pAgos is obviously limited in mammalian cells. Interestingly, the gDNA-Natronobacterium gregoryi Argonaute system (gDNA/NgAgo) was used as a genetic tool to knock out a specific gene fabp11a, eventually causing eye developmental defects in zebrafish [93]. It was also reported that the NgAgo-gDNA system was able to efficiently inhibit HBV replication through gene silencing but not DNA editing [87]. Nevertheless, a recent study showed that NgAgo accelerated homologous arm-guided gene editing rather than relying on its enzymatic activity and gDNA, with the editing efficiency of 80–100% in Pasteurella multocida and Escherichia coli. The principle where NgAgo system works is that the interaction of PiWI-like domain with recombinase A (RecA) enhances RecA-guided DNA strand exchange [94]. Recently, a great progress has been made from two Argonaute proteins from human gastrointestinal bacteria Clostridium perfringens (CpAgo) and Intestinibacter bartletti (IbAgo), and they are demonstrated to cleave single- and double-stranded DNA sequences at 37 °C in a DNA guide-dependent manner [95]. The discovery raises exciting possibility for developing CpAgo- or IbAgo-based novel tools to accurately edit genomic DNA.

Taken together, it is becoming clear that Argonaute proteins contain nucleic acid-guided nuclease activity, but there are still no characterized Argonaute proteins that show robust genome editing ability.

**Argonaute proteins in diseases**

A large amount of data has demonstrated that Argonaute proteins are involved in the development of diseases through a miRNA-dependent or -independent way, such as tumorgenesis [96] and infertility [57]. Argonaute proteins, especially human Ago2, have been found to be overexpressed in most cancer, including colon cancer [28], ovarian carcinoma [97], gastric cancer [98,99] and Gliomas [100]. The upregulated Ago2 eventually leads to the disbalance of small RNA and facilitates oncogenic miRNAs to repress or activate their target genes by a feed-back regulatory
loop in special tissues [97,98]. Moreover, accumulating studies have reported that more than one-third of human cancers exist a Kirsten rat sarcoma oncogene (KRAS)-oncogenic mutation [101–103] and the mutant KRAS directly interacts with Ago2 to promote cellular transformation and specifically potentiate the KRAS-mediated tumorigenesis [103]. It also was found that Ago2 was post-translationally modified by some tumor factors, such as AKT serine/threonine kinase 3 (AKT3), epidermal growth factor receptor (EGFR) and prolly 4-hydroxylase (P4H), to promote cancer cell growth, angiogenesis and metastasis [26]. However, there is an inconsistency that the expression of Ago2 is strongly reduced in melanoma and breast cancer [96,104,105]. This discrepancy may be due to the differences in cancer types and miRNAs expression patterns. In addition to a role of Ago2 in cancer, PIWI proteins also play a key role in tumorgenesis. A number of studies have demonstrated that PIWI proteins are not only involved in cancer cell apoptosis and proliferation, but also in cancer metastasis and invasion [106–108]. It was also reported that the mutations in human PIWI protein caused male infertility [57,109]. However, whether Argonaute proteins are dysfunctional in these disorders still requires further exploration.

Concluding remarks

Argonaute proteins play an important role in post-translational gene silencing and the biogenesis of small RNA. Accumulating studies have revealed that Argonaute proteins also implicate several other cellular processes, such as host defense, gene regulation and genome editing. Although considerable breakthroughs have been made in the structure and functions, there are still many issues that need to be further addressed. For example, most eAgo trend to target RNA using RNA guides, while pAgo seem to target DNA based on either DNA or RNA guides. What is the basis of the preferance and specificity of Argonaute proteins for their guide strands and target genes? Next, it is known that Argonaute proteins without small RNA loading are extremely unstable, and whether these loaded small RNAs protect Argonaute proteins from degradation and why. Furthermore, what are the functions and mechanism of short pAgo?

For Argonaute, another emerging field is the use in genome editing, which has rapidly grown at an unprecedented rate in the past few years. Although some pAgo have been demonstrated to selectively cleave target DNA based on the complementary guide strand, their applications are greatly restricted due to high working temperature required, such as TiAgo and PfAgo. Fortunately, CpAgo and IbAgo give us a new hope for the development of Argonaute-based genome editing tools though a long way to go in clinic applications.

In conclusion, a comprehensive understanding the functions of Argonaute proteins will help us better reveal and explore the causes of disease development. Meanwhile, it is expected to discover more Argonaute proteins with genome-editing functions in future, which will bring the gospel to the treatment of human genetic diseases.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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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Author contributions

Yadong Zheng had the idea for the article. Jin’en Wu and Yang Jing performed the literature search and data analysis. Jin’en Wu drafted the work. Yadong Zheng and William C. Cho critically revised the work.

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