Review Article

Dicer Functions in Aquatic Species

Yasuko Kitagishi, Naoko Okumura, Hitomi Yoshida, Chika Tateishi, Yuri Nishimura, and Satoru Matsuda

Department of Environmental Health Science, Nara Women’s University, Kita-Uoya Nishimachi, Nara 630-8506, Japan

Correspondence should be addressed to Satoru Matsuda, smatsuda@cc.nara-wu.ac.jp

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Dicer is an RNase III enzyme with two catalytic subunits, which catalyzes the cleavage of double-stranded RNA to small interfering RNAs and micro-RNAs, which are mainly involved in invasive nucleic acid defense and endogenous genes regulation. Dicer is abundantly expressed in embryos, indicating the importance of the protein in early embryonic development. In addition, Dicer is thought to be involved in defense mechanism against foreign nucleic acids such as viruses. This paper will mainly focus on the recent progress of Dicer-related research and discuss potential RNA interference pathways in aquatic species.

1. Introduction

In eukaryotes, small RNA-mediated RNA silencing called RNA interference (RNAi) is able to suppress gene expression. Dicer is the key enzyme of the RNAi pathway to cleave double-stranded RNA (dsRNA) into small RNAs categorized as small interfering RNAs (siRNAs) or micro-RNAs (miRNAs), which are mainly involved in invasive nucleic acid defense and endogenous genes regulation, respectively [1–3]. Then, Dicer is reported to participate in both the antiviral immune response and developmental regulation. For example, Drosophila harboring the Dicer mutant exhibited enhanced disease susceptibility to cricket paralysis virus [4, 5]. In addition, Caenorhabditis elegans harboring the Dicer mutant had developmental phenotype defects [6–8]. The meRNAi is a conserved eukaryotic gene silencing mechanism that works at both the transcriptional and the posttranscriptional levels [9]. We fortuitously cloned and sequenced the human Dicer, (initially designated as HERNA) for the first time [10]. Dicer belongs to the RNase III family with ATP dependent RNA helicase, PAZ (Piwi/Argonaute/Zwille), dsRNA binding, and RNase III domains (Figure 1), which is responsible for cleaving long dsRNAs into siRNAs or miRNAs when associated with other proteins like R2D2 in Drosophila or the transactivating response RNA-binding protein in Homo sapiens to recruiting Argonaute proteins. The PAZ domain binds the single stranded 3’ end of small RNA [11], and it might function in protein-protein interaction. Small RNAs includes PIWI-associated RNAs (piRNAs), short single-stranded RNAs arising from a Dicer-independent pathway, which are found in germ cells and associate with the PIWI subfamily of Argonaute proteins [12, 13]. Many zebrafish piRNAs are derived from repetitive sequences. Mutations in the Piwi homologue protein result either in loss of germ cells or in defects in meiosis and chromosome segregation in eggs. However, the Dicer knockout mouse eggs raise a question about overlapping functions of vertebrate miRNA, RNAi, and piRNA pathways.

Most vertebrates, Urochordata, and worms are reported to have only one Dicer-1 protein, which generates both miRNAs and siRNAs. While insects, fungi, and plants have more than one Dicer or Dicer-like proteins [14], Dicer enzymes in Drosophila melanogaster are classified into Dicer-1 and Dicer-2 in terms of their specialized functional activities. Dicer-1 can process loop pre-miRNA to mature miRNA, while Dicer-2 can process dsRNA precursors into siRNA molecules [15]. Recent studies have demonstrated that Dicer can function in an RNAi pathway independent manner. The Dicer-2 of Drosophila melanogaster participated in antiviral responses by mediating induction of antiviral gene Vago [16]. The Dicer-1 of Caenorhabditis elegans participates in fragmenting chromosomal DNA during apoptosis, and
undergoes a protease-mediated conversion from a ribonuclease to a deoxyribonuclease in addition to the processing of small RNAs [17].

Since the initial discovery in 1998 by Fire et al. [18], RNAi has taken the biological community by storm. Despite many advances, however, RNAi is still under development. A better understanding of the mechanism for Dicer pathway is a future goal for many scientists. This paper will mainly focus on the recent evidences of Dicer functions in aquatic species. We will also highlight the effects of RNAi in experimental models of the aquatic species.

2. Expression and Developmental Function of Dicer in Aquatic Species

Only one homolog of Dicer was identified from the sea urchin, suggesting that the sea urchin Dicer may mediate both miRNA and siRNA-silencing pathways, similar to humans. The Dicer mRNA accumulates asymmetrically in one periphery of the oocyte in punctate cytoplasmic structures [19, 20]. The asymmetric localization of Dicer mRNA is maintained throughout the development of blastula and gastrula stages. The Dicer transcript accumulation is enriched selectively in the presumptive oral ectoderm and endodermal epithelium. The transcript then decreases to undetectable levels in the larval pluteus stage. Knockdown of Dicer in sea urchin embryos results in anomalous morphogenesis, such as impairment of gastrulation and skeletogenesis at the mesenchyme blastula stage and later stages, suggesting that miRNA could be involved in the early development of sea urchin [20]. Similarly, the Dicer transcripts in rainbow trout are detectable throughout the embryonic stages. Peak expression of Dicer at the time of maternal mRNA degradation and initiation of embryonic genome activation could indicate its involvement in miRNA processing during the periods in the rainbow trout [21] (Figure 2).

During the developmental stages from fertilized egg to postlarva, shrimp (Litopenaeus vannamei) Dicer-1 is constitutively expressed at all developmental stages [22]. The highest expression is observed in fertilized eggs and followed a decrease from fertilized egg to nauplius stage.

Then, the higher levels of expression are detected at the late nauplius and postlarva stages. The shrimp Dicer-1 expression regularly increases at the upper phase of nauplius, zoea, and mysis stages than their prophase. The different expression in the larval stages might provide clues for understanding the early innate immunity in the process of shrimp larval development. The expression level of shrimp Dicer-1 mRNA varies significantly among different shrimp tissues. The expression in hemocyte is significantly higher than that in gill, muscle, brain, intestine, and pancreas. On the other hand, expression levels of shrimp Dicer-2 are about the same in most tissues, except in muscle, which has a lower expression level [23].

3. Immunological Function of Dicer in Aquatic Species

RNAi is a mechanism of posttranscriptional gene silencing that functions as a natural defensive response to viral infection in a variety of species (Figure 2). Knockdown of Dicer-1 in tiger shrimp (Penaeus monodon) resulted in increasing mortalities and higher viral loads, suggesting that the RNAi mechanism is active and has a powerful immunological function in shrimp [24]. Higher levels of Dicer-1 expression in lymphoid organs are consistent with a role in the natural defense response of shrimp. However, there is no correlation between levels of Dicer-1 expression and the viral genetic loads in shrimp lymphoid organ tissue during naturally acquired or persistent viral infections [25]. The Dicer-1 expression might be induced at an early stage of infection and recovers to normal levels later.

The white shrimp (Litopenaeus vannamei) Dicer-2 involves in the nonspecific antiviral immunity, and in some degree supporting the suggested relationship between nonspecific activation of antiviral immunity and induction of RNAi [23]. The Dicer-2 might be contributed to nonspecific activation of antiviral immunity in shrimp by enhancing RNAi potency and efficacy [26]. The shrimp Dicer-2 might
RNA silencing is now a well-known mechanism by which plants and invertebrates fight off viral infection; however, many plant and animal viruses possess proteins that suppress host RNA silencing mediated by siRNA or miRNA pathways. Striped jack nervous necrosis virus (SJNNV), which infects fish, has a bipartite genome of positive-strand RNAs. The Striped jack nervous necrosis virus (SJNNV), which infects teleost fish and poses a large threat to marine aquaculture. The control of viral diseases in shrimp remains a challenge for the shrimp aquacultural industry. As shrimps lack adaptive immunity and the typical interferon response, RNAi is thought to be an ancient and important immune mechanism against virus replication. As mentioned before, the original function of RNAi is thought to be involved in defense mechanism against foreign nucleic acids, such as viruses, and in endogenous transcriptional regulation [2]. Presently, RNAi is becoming attractive to develop as an important potential tool in viral disease prevention in shrimp [46]. RNAi technology shows considerable promise as a therapeutic approach and can be used as a strategy to protect shrimp against viral diseases. In order to use RNAi technology on an effective manner to protect shrimp against viral diseases, it would be essential that future studies focus on increasing the stability of siRNA, and the relationship between the siRNA and dsRNA.
between the expression of antiviral immune genes in the immune response and larval development could shed light on the further practical application. However, this seems to be expensive approach yet. Instead, making transgenic shrimp which express an anti-viral shRNA might be a feasible way to engineer shrimp to be resistant to the bad viruses. More research on the characterization of RNAi-related genes in immune response and larval development may be helpful for better understanding the antiviral mechanism and designing efficient strategies of viral disease control. And the further progress in understanding the mechanism of RNAi will definitely revolutionize therapeutic approaches for counteracting diseases in aquatic species.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

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