Fish and Shellfish Immunology 77 (2018) 244–251

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

CRISPR/Cas9-mediated deletion of *EcMIH* shortens metamorphosis time from mysis larva to postlarva of *Exopalaemon carinicauda*

Jiquan Zhang[a,b,c], Fengge Song[b,d], Yuying Sun[a,*], Kuijie Yu[b,c], Jianhai Xiang[b,c]

[a] College of Life Sciences, Hebei University, Baoding, Hebei, 071002, China
[b] CAS Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[c] Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266000, China
[d] University of Chinese Academy of Sciences, Beijing 100039, China

ARTICLE INFO

Keywords:
Exopalaemon carinicauda
CRISPR/Cas9
Molt-inhibiting hormone
Knock-out

ABSTRACT

The recently emerged CRISPR/Cas9 technology is the most flexible means to produce targeted mutations at the genomic loci in a variety of organisms. In Crustaceans, molt-inhibiting hormone (MIH) is an important negative-regulatory factor and plays a key role in suppressing the molting process. However, whether precise disruption of *MIH* in crustacean can be achieved and successfully used to improve the development and growth has not been proved. In this research, the complementary DNA (cDNA) and genomic DNA, including flanking regions of the *MIH* gene (*EcMIH*) of ridgetail white prawn *Exopalaemon carinicauda,* were cloned and sequenced. Sequence analysis revealed that *EcMIH* was composed of three exons and two introns. Analysis by RT-PCR showed that *EcMIH* mainly expressed in eyestalks. During different development periods, *EcMIH* was highest in juvenile stage and extremely low in others but adult prawns eyestalks. In addition, we applied CRISPR/Cas9 technology to generate *EcMIH* knock-out (KO) prawns and then analyzed the changes in their phenotypes. We efficiently generated 12 *EcMIH-KO* prawns out of 250 injected one-cell stage embryos and the mutant rate reached 4.8% after embryo injection with one sgRNA targeting the second exon of *EcMIH*. The *EcMIH*-KO prawns exhibited increased the body length and shortened the metamorphosis time of larvae from mysis larva to postlarva. Meanwhile, *EcMIH*-KO did not cause the health problems such as early stage death or deformity. In conclusion, we successfully obtained *EcMIH* gene and generated *EcMIH*-KO prawns using CRISPR/Cas9 technology. This study will certainly lead to a wide application prospect of *MIH* gene in prawns breeding.

1. Introduction

Targeted genome editing technologies have enabled a broad range of research and medical applications [1]. The recent development of genome engineering technologies are facilitating the translation of this genomic information into tangible benefits for biotechnology, agriculture, and human therapeutics [2]. Generally, Zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALENs), and clustered regularly interspaced short alindromic repeats (CRISPR)/CRISPR associated protein (Cas9) technology can be used to introduce targeted double-strand breaks (DSBs) in the genome. The resulting DSBs are repaired by nonhomologous end-joining (NHEJ) or homology-directed repair (HDR) pathway, thereby causing mutations. Compared with ZFNs and TALENs, CRISPR/Cas9 system possesses the advantages of simplicity and high efficiency [3]. CRISPR/Cas9 system acts as a bacterial defense system against invading viruses and plasmids in many different bacterial species [4]. At present, CRISPR/Cas9 technology has been extended into the genome editing of numerous organisms such as bacteria [5,6], yeast [7,8], mammals [9], viruses [10–12], fly fruit [13,14], zebrafish [15,16], prawns [17,18] and so on.

The Decapoda or decapods are an order of crustaceans, including many familiar groups, such as crayfish, crabs, lobsters, prawns, and shrimp. The order contains nearly 15,000 species in around 2700 genera. Most of the aquaculture species in crustacean belong to Decapoda and account for an indispensable part in the fisheries of the world. At present, major progress has been made in aquaculture genomics for some decapods including *Litopenaeus vannamei* [19], *Exopalaemon carinicauda* [20], *Neocaridina denticulate* [21], and *Eriocheir sinensis* [22]. Many functional genomic researches of decapods mainly focused on analysis at transcriptional or translational levels *in vitro* other than using loss-of-function approaches *in vivo*. At present, there is no model animal in crustaceans to be used in basic research. The ridgetail white prawn, *E. carinicauda*, one of the important commercial shrimp species naturally distributed in the coasts of China,

* Corresponding author.
E-mail address: sunyuying125@163.com (Y. Sun).

https://doi.org/10.1016/j.fsi.2018.04.002
Received 6 December 2017; Received in revised form 29 March 2018; Accepted 2 April 2018
Available online 03 April 2018

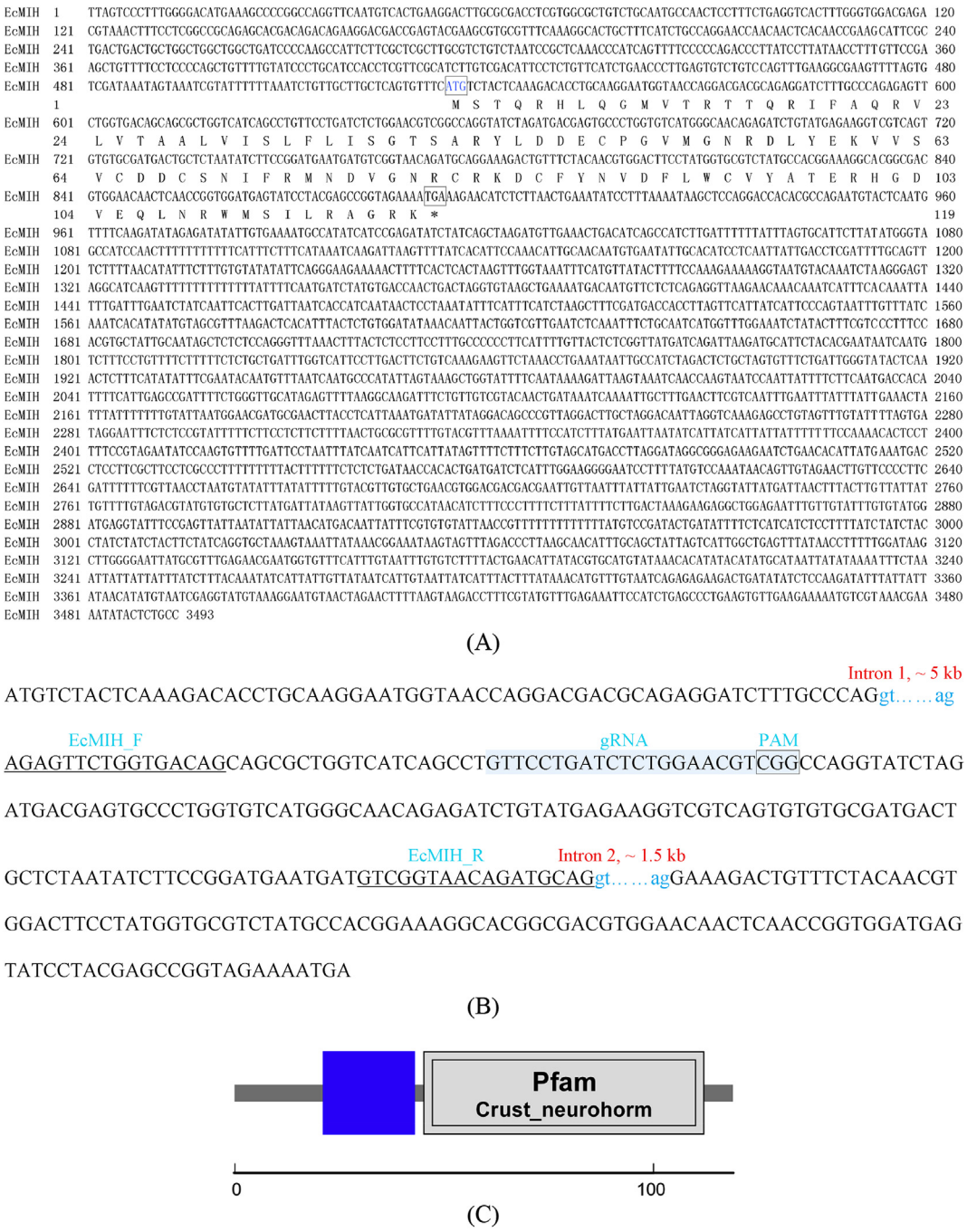

Fig. 1. Structure of the *EcMIH* gene. (A) Nucleotide sequence of *EcMIH*. The start codon (ATG) and stop codon (TGA) are in rectangles. (B) Genomic structure of *EcMIH* gene. The gRNA site was marked in grey and the PAM site was in rectangles. The primers used to amplify the CRISPR target fragment of *EcMIH* were underlined. (C) Schematic representation of EcMIH protein structure.

contributes to one third of the gross output of the polyculture ponds in eastern China [23]. In our laboratory, *E. carincauda* was used as an experimental animals in basic research and it could be maintained with reproductive capacity all the year round in the laboratory environment with an about 60-day reproduction cycle [24]. In our previous research, we succeeded in developing a high efficient microinjection method in *E. carinicauda* and deleting the interest gene using CRISPR/Cas9 technology [25]. In addition, we performed low-coverage sequencing and *de novo* assembly of the *E. carinicauda* genome which exhibited potential for the genomic and experimental research of decapods [20].

Recently, more and more researches were focused on the growth and reproduction of decapods. Molting is a crucial process for decapods which has a close relationship with growth [26]. In the complicated crustacean molting pathway, it was reported that molt-inhibiting hormone (MIH) was a negative-regulatory factor that suppressed its molting process [27,28]. Lots of researches focused on MIH have been reported such as *Carcinus maenas* [29], *Procambarus clarkia* [30], *Homarus americanus* [31], *Metapenaeus ensis* [28], and *Litopenaeus vannamei* [32] and so on. However, there is no any report about the precise disruption of *MIH* in decapods to clarify its function.

In this research, we cloned the full-length cDNA sequence of MIH from *E. carinicauda* (EcMIH) and obtained its genomic structure. Furthermore, we applied CRISPR/Cas9 technology to generate *EcMIH* knockout (KO) prawns and then analyzed the changes in their phenotypes.