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A novel GATA-like zinc finger transcription factor involving in hematopoiesis of *Eriocheir sinensis*

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ABSTRACT

GATA transcription factor is a family of DNA-binding proteins that can recognize and bind to sequence of (A/T)GATA (A/G). In the present study, a GATA-like protein (named as *Es*GLP) was characterized from *Eriocheir sinensis*, including an 834 bp full length open reading frame of *Es*GLP, encoding a polypeptide of 277 amino acids. The deduced amino acid sequence of *Es*GLP contained one conserved GATA-type zinc finger of the form Cys-X2-Cys-X17-Cys-X2-Cys, with four cysteine sites. The *Es*GLP mRNA transcripts were mainly detected in the hematopoietic tissue, hepatopancreas and gonad. The recombinant *Es*GLP protein was prepared for the antibody production. The *Es*GLP protein was mainly distributed in the edge of lobules in the HPT and the cytoplasm of hemocytes. The mRNA transcripts of *Es*GLP in hemocytes were significantly decreased at 24 h (0.39-fold and 0.27-fold, $p < .05$) and 48 h (0.35-fold and 0.16-fold, $p < .05$) after LPS and *Aeromonas hydrophila* stimulation, respectively. However, one peak of *Es*GLP mRNA transcripts were recorded at 24 h (8.71-fold, $p < .05$) in HPT after *A. hydrophila* stimulation. The expression level of *Es*GLP mRNA in HPT was significantly up-regulated at 2 h, 2.5 h and 9 h (41.74-fold, 45.38-fold and 26.07-fold, $p < .05$) after exsanguination stimulation. When *Es*GLP gene expression was inhibited by the injection of double-stranded RNA, both the total hemocytes counts and the rate of EdU-positive hemocytes were significantly decreased (0.32-fold and 0.56-fold compared to that in control group, $p < .05$). All these results suggested that *Es*GLP was an important regulatory factor in *E. sinensis* which involved in the hemocytes generation and the immune response against invading pathogens.

1. Introduction

Hemocytes, the circulating surveillance immunocytes [1] in invertebrate, are central components in the immune system, which not only perform phagocytosis and encapsulation during the cellular immune responses [2], but also play key roles in humoral immune responses *via* synthesizing and secreting various immune factors [3]. In general, hemocytes have a lifespan of days to weeks, and thus they are continuously produced from a common set of hematopoietic progenitors or hematopoietic stem cells (HSCs), which reside in specialized hematopoietic organs [4]. The process of hemocyte production is called hematopoiesis, which has been demonstrated to be conserved among different species and occurs in two successive phases, primitive hematopoiesis and definitive hematopoiesis [5,6]. Certain conserved transcription factors, such as GATA3, Tal-1/SCL, Runx1, and ETS-family factor, are necessary and critical in hematopoiesis development by regulating the emergence of HSCs and the determination of cell fate [7–10]. Especially, the GATA transcription factors play an important role during hematopoiesis process in both vertebrates and invertebrates.

The GATA transcription factor is a family of DNA-binding proteins composed of one or two zinc finger structures of the form Cys-X2-Cys-X17-Cys-X2-Cys, which could bind to a recognition sequence (A/T)GATA(A/G) [11,12]. So far, six GATA factors have been identified and categorized as two subclasses in vertebrates, GATA1/2/3 and GATA4/5/6 [13]. The GATA1/2/3 are regulators of hematopoiesis and referred as hematopoietic GATA factors [14,15]. In the mouse, the complete loss of GATA1 expression led to embryonic lethality [16]. GATA2 was reported to be predominantly expressed in developing and adult HSCs as well as myeloid progenitors [17]. GATA3 is a master regulator of many immune processes, essentially contributing to T lymphocyte development and Th2 cell differentiation in peripheral organs [18]. The GATA4/5/6 are mainly involved in the formation of various mesoderm and endoderm-derived tissues. Moreover, there is

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another GATA like protein-1 (GLP-1) with two zinc fingers, but only one of them sharing high level homology to typical GATA zinc finger, which mainly participates in germ cell development in mice [19,20]. Recently, GATA transcription factors have also been identified in invertebrates, and they are reported to participate in the hematopoiesis. There are eleven, five, and two GATA genes annotated from the genomes of nematode *Caenorhabditis elegans* fruit fly *Drosophila melanogaster,* and sea urchin *Strongylocentrotus purpuratus,* respectively [21]. Most of inverberate GATAs harbor two conserved GATA-type zinc fingers, and they are also involved in the hemocytes proliferation, differentiation and maturation. For example, GATA1/2/3 orthologs in sea urchin *S. purpuratus* and scallop *Chlamys farreri* are reported to play essential roles in the hemocyte production and the regulation of immunocyte development [22,23]. The *Serpent* (*Srp*), an ortholog of GATA4/5/6, was reported to involve in the regulating of the hemocyte differentiation and lineage commitment in both two hematopoiesis phases in *D. melanogaster,* and specially played vital role during crystal cell development [24]. Moreover, there are some invertebrate GATA factors with only one GATA-type zinc finger, such as elt-2, elt-3, end-1, end-3, and med GATA factors. Elt-2 participated directly in the transcriptional regulation of the majority intestinal genes in *C. elegans*, and it could bind directly to TGATAA sites *in vitro* [25,26]. The med GATA factors participated in the endoderm specification in *C. elegans* [27]. Although many types of GATA transcription factors have been identified in invertebrate, their flexibly regulation mechanism in various physiological processes remain largely unknown.

Chinese mitten crab *Eriocheir sinensis*, an important commercial aquaculture species, has been achieved rapid development in the farming scale over the past few decades in China [28]. With the intensive culture and environmental deterioration, there are a growing number of serious infectious diseases in *E. sinensis* caused by bacteria, viruses, parasites, and other pathogens, which lead to the production reduction and enormous economic loss [29]. Because of the lacking of adaptive immunity, *E. sinensis* only depends on the innate immunity to fight pathogens [30]. As the main member of the innate immunity, hemocytes play important roles in immune response of *E. sineisis* [31]. Hematopoietic tissue (HPT) is the origin of hemocytes, which has been identified from *E. sinensis* [32]. However, there are few studies on the generation and differentiation of *E. sineisis* hemocytes. In the present study, a typical GATA zinc finger domain containing protein (named as *Es*GLP) was identified from *E. sinensis* and its mRNA expression pattern in hemocytes and HPT after pathogen challenge and exsanguination, as well as the change of hemocyte production after the interference of *Es*GLP expression were investigated to reveal its potential roles in hematopoiesis of crabs.

2. Materials and methods

2.1. Animals and challenges

Chinese mittrn crabs, *E. sinensis*, were collected from a commercial farm in Jiangsu Province, China, with the average weight of 20 g and cultured at 20 ± 2 °C in tanks for one week before processing.

One hundred and forty four crabs were used in the challenge experiment, and they were randomly divided into four groups. The crabs in the control group received an injection of 100 μL of 0.85% sterile saline (0.85 g saline in 100 mL ddH_2O) into the last walking legs. The crabs in treatment groups received an injection of 100 μL lipopolysaccharides (1 mg LPS in 1 mL 0.85% sterile saline) from *Eschenchia coli* 0111:B4 (Sigma), and 100 μL *Aeromonas hydrophila* (resuspended in 0.85% sterile saline at a concentration of 1×10^6 CFU mL^{-1}), respectively. Six crabs were sampled at 0, 3, 6, 12, 24 and 48 h post injection from each group to collect hemocytes and HPT. The samples from two crabs were mixed up at random as one replicate, and there were three replicates for each time point. The hemolymphs were drawn from crab legs with pre-cooled anticoagulant solution (450 mM NaCl, 100 mM glucose, 26 mM citric acid, 30 mM sodium citrate, 10 mM EDTA• 2Na, pH 4.6) and centrifuged at 800 *g*, 4 °C for 5 min to harvest the hemocytes. The hemocytes and HPT were stored in the Trizol reagent (Invitrogen, China) for total RNA extraction.

Another sixty-six crabs were used for the exsanguination experiment. Five hundred microliter of hemolymphs was drawn from each crab by syringe, and the HPT of six individuals were randomly sampled at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12 and 24 h after exsanguination, respectively. The samples from two crabs were mixed up as one replicate, and there were three replicates for each time point.

2.2. The cloning of EsGLP cDNA and sequence analysis

Two gene-specific primers, *Es*GLP-F and *Es*GLP-R (Table 1) designed on the basis of the GATA-like sequence from *E. sinensis* genome sequences (unpublished data in our laboratory), were used to amplify the full open reading frame (ORF) sequence of *Es*GLP by polymerase chain reaction (PCR) on a GeneAmp PCR System 9700 (Applied Biosystems, USA). After gel-purified with MinBEST Agarose Gel DNA Extraction Kit Ver. 4.0 (9762, Takara, China), the PCR products were cloned into the PMD19-T simple vector (6013, Takara, China), and then sequenced by M13-47 and RV-M primers (Table 1).

The homology searches for nucleotide and amino acid sequences were conducted by the BLAST algorithm at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.gov/blast).

Table 1
Primers used for qRT-PCR and procaryotic expression.

Designation	Sequence (5′ → 3′)
*Es*Glp-F	ATGGTGTTCGGACTCAAACCC
*Es*Glp-R	CTTTGTCATTAGTTCCTTGGCTTTT
M13-47	CGCCAGGGTTTTCCCAGTCACGAC
RV-M	GAGCGGATAACAATTTCACACAGG
*Es*Glp-qRT-F	GGAGGAACCTACTATGCCCAGA
*Es*Glp-qRT-R	GTTGAGTAAGGTGAGGTGCGAT
*Es*Actin-qRT-F	GCATCCACGAGACCACTTACA
*Es*Actin-qRT-R	CTCCTGCTTGCTGATCCACATC
*Es*GLP-NdeI-F	GGAATTCCATATGGTGTTCGGACTCAAACCC
*Es*GLP-XhoI-R	CCGCTCGAGCTTTGTCATTAGTTCCTTGGCTTTT
*Es*Glp-dsRNA-T7-F	TAATACGACTCACTATAGGATGGTGTTCGGACTCAAACCC
*Es*Glp-dsRNA-T7-R	TAATACGACTCACTATAGGGCTTTGTCATTAGTTCCTTGGCTTTT
EGFP-dsRNA-T7-F	TAATACGACTCACTATAGGAGCACCCAGTCCGCCCTGAGC
EGFP-dsRNA-T7-R	TAATACGACTCACTATAGGCGTCGCCGTCCAGCTC