Gene 647 (2018) 150–156

Contents lists available at ScienceDirect

Gene

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

Research paper

Characterization of Selenoprotein P cDNA of the Antarctic toothfish *Dissostichus mawsoni* and its role under cold pressure

Yan Li[a,b], Juntao Luo[a,b], Qiongqiong Xu[a,b], Yanwen Hou[a,b], Penglei Jiang[a,b], Yutian Sun[a,b], Hanxu Lu[a,b], Bingshe Han[a,b,*], Junfang Zhang[a,c,*]

[a] Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources (Shanghai Ocean University), Ministry of Education, Shanghai 201306, China
[b] National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai 201306, China
[c] International Research Center for Marine Biosciences at Shanghai Ocean University, Ministry of Science and Technology, Shanghai 201306, China

ARTICLE INFO

Keywords:
Selenoprotein P
SECIS elements
Cold adaptation
ROS

ABSTRACT

Our previous study using comparative genome analysis revealed a significant gene copy number gain of *Dissostichus mawsoni selenoprotein P* (*Dm-SEPP*) during the evolutionary radiation of Antarctic notothenioids, suggesting that Dm-SEPP contribute to this process, but the detailed structure and function of this gene product remain unclear. In the present study, the *Dm-SEPP* cDNA was cloned and characterized. The *Dm-SEPP* cDNA contains 17 selenocysteines (Sec) encoded by TGA codons and 2 typical SECIS elements located in the 3′-UTR. Evolutionary analysis of the *Dm-SEPP* gene revealed that it's closely related to the *SEPP* gene of zebrafish (*Danio rerio*), showing 51% amino acid similarity. Over-expression of Dm-SEPP could protect mammalian cells under cold pressure, probably via eliminating ROS. Further study showed an increase of endogenous SEPP in zebrafish ZF4 cells under cold pressure, and knockdown of SEPP decreased cell viability, accompanied with increased ROS. Our results suggested a protective role of Dm-SEPP in cold adaptation in Antarctic notothenioids.

1. Introduction

Since Antarctica was detached from Gondwana, and the Antarctic Circumpolar Current was established to promote the geographic and thermal isolation of the Southern Ocean (Shevenell et al., 2004), climatic changes had become a major role in the extinction of the Antarctic marine fish fauna. Antarctic notothenioid fish had survived in the freezing habitats after undergoing an adaptive radiation, becoming the predominant Antarctic fish group today (Eastman, 2000), providing an excellent system to explore the mechanisms of adaptive evolution.

Genome-wide investigations of transcriptional and genomic changes associated with cold adaptation of Antarctic notothenioid revealed elevated copy number of some genes involved in various biological processes including protein biosynthesis, protein folding and degradation, anti-oxidative pressure and so on, which played an important role in adaptation to cold-induced stresses (Chen et al., 2008). Among them, some anti-ROS genes were obviously amplified, with a 22-fold increase of the gene copy number of *selenoprotein P*, suggesting anti-ROS pathway a major mechanism of cold adaptation in the Antarctic notothenioid *D. mawsoni.*

ROS (reactive oxygen species) is mainly produced from mitochondria within most mammalian cells (Wendelaar Bonga, 1997), which contributes to mitochondrial damage in a range of pathologies and plays an important role in many diseases (Nordberg and Arner, 2001). Selenoprotein P (SEPP), a glycoprotein that carries up to 50% of the selenium in plasma (Tujebajeva et al., 2000), is produced predominantly in the liver and transported to the brain via plasma (Burk and Hill, 2005). In addition, selenoproteins are a small class of proteins containing the 21st amino acid selenocysteine, some of which are capable of protecting cells from oxidative damage (Chen and Berry, 2003). Selenoprotein deficiency can result in many kinds of disorders and even death, SEPP deficiency causes regression in nerve cells, epilepsy and ataxia in mice, and dysplasia was observed in male mice (Kasaikina et al., 2012). SEPP play vital roles in the occurrence and development of many types of diseases, such as breast cancer, Kashin-Beck disease, carcinoma, chronic kidney disease, colorectal carcinoma and so on (Lobanov et al., 2008). SEPP usually serves as an antioxidant by protecting cells against oxidative injury, which may result in liver necrosis in rats (Atkinson et al., 2001). Recently SEPP of Nile tilapia, another fish model, has been successfully cloned, and its response to

Abbreviations: D. mawsoni, Dissostichus mawsoni; Dm-SEPP, D. mawsoni selenoprotein P; Em-SEPP, zebrafish selenoprotein P; Hm-SEPP, human selenoprotein P; cDNAs, complementary deoxynucleic acids; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; ROS, reactive oxygen species

* Corresponding authors at: Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources (Shanghai Ocean University), Ministry of Education, Shanghai 201306, China.
E-mail addresses: bs-han@shou.edu.cn (B. Han), jfzhang@shou.edu.cn (J. Zhang).

https://doi.org/10.1016/j.gene.2018.01.032
Received 21 November 2017; Received in revised form 3 January 2018; Accepted 9 January 2018
Available online 09 January 2018
0378-1119/ © 2018 Elsevier B.V. All rights reserved.

physiologically stressful conditions was investigated (Thummabancha et al., 2016). Selenoprotein P of *D. mawsoni* has never been characterized.

Selenoprotein P, a unique member of the selenoprotein family, contains multiple selenocysteine residues per protein molecule, its mRNAs in different mammalian species encode 10–12 selenocysteine residues (Kristina E. Hill et al., 1993). The selenocysteine residue, usually named Sec residue, is encoded by a single UGA codon, which typically functions as a stop codon. UGA codons are recoded to specify selenocysteine, rather than termination, with the presence of specific secondary structures in selenoprotein mRNAs, termed selenocysteine insertion sequence or SECIS elements. It has been reported that SECIS elements are located in the coding region just downstream of the UGA codons they serve in prokaryotes (Kromayer et al., 1996), while located in the 3′ untranslated region (UTR) in eukaryotes, and in one case in the 5′UTR in archaea (Rother et al., 2001). The mechanism of selenocysteine incorporation in eukaryotes is inherently different from that in prokaryotes (Berry et al., 2001).

Here, the cDNA encoding the Dm-SEPP was cloned and subjected to evolutionary analysis. Further studies showed Dm-SEPP protein could decrease ROS levels and protect cells under cold pressure. The present data indicated that amplification of the *Dm-SEPP* gene might contribute to cold adaptation in Antarctic notothenioid fish by its enhanced function of protecting cells under cold pressure.

2. Materials and methods

2.1. Cell culture and cold treatment

HEK-293T cells and ZF4 cells were obtained from the American Type Culture Collection (ATCC). HEK-293T cells were cultured at 37 °C, 5% CO_2, in complete medium DMEM (Gibco) containing 10% fetal bovine serum (10099141; Life Technologies), 1% penicillin–streptomycin-glutamine solution (SV30082.01; Hyclone). ZF4 cells were grown at 28 °C, 5% CO_2, in Dulbecco's modified Eagle's medium/F12 nutrient mix (SH30023.01B; Hyclone) supplemented with 10% fetal bovine serum (10099141; Life Technologies), 1% penicillin–streptomycin-glutamine solution (SV30082.01; Hyclone). For cold treatment, HEK-293T cells or ZF4 cells were seeded at 40–50% confluence and the next day moved into an incubator (Galaxy170R, CO170R-230-0000, Eppendorf) of 28 °C (HEK-293T) or 18 °C (ZF4), 5% CO2 for indicated times, ranging from 24 h to 30 days. All experiments were performed in triplicate. For cell viability assay, cells were prepared into a cell suspension and stained with 0.4% Trypan Blue solution (w/v) for 5 min, then counted with a hemocytometer.

2.2. Plasmid construction

To get the *Dm-SEPP* gene cDNA, a specific primer pair (Forward: 5′-ATGAGGGTGTGCCTCAGCCTGCT-3′; Reverse: 5′-CAGCTGCTCTC ATC-3′) was used to amplify the target cDNA from *D. mawsoni* liver cDNA library (Clone NO: G022C10) according the protocol reported previously (Chen et al., 2008). The cDNA containing ORF and 3′-UTR of the gene was cloned into pcDNA3.1(-) vector via the unique *XhoI* site and the neighboring *EcoRI* site. For convenient detection, a FLAG-tag encoding sequence (GATTATAAAGATGATGATGATAAA) was inserted before the stop codon (TAG) to express Dm-SEPP-FLAG fusion protein. For the expression of the *Dm-SEPP-FLAG*, codon optimization was performed by changing the codon-adaptation index from 0.22 to 1.0 without altering the amino acid sequence (Fig. S1).

2.3. Lentivirus production and cell transduction

DNA oligos targeting zebrafish *selenoprotein P*, set 1 (Forward: 5′-CCGGACATGTGGAGGAGGCTATAAACTCGAGTTTATAGCCTCCTCC ACATGTTTTTTG-3′; Reverse: 5′-AATTCAAAAAACATGTGGAGGAGGC TATAAACTCGAGTTTATAGCCTCCTCCACATGT-3′), and set 2 (Forward: 5′-CCGGGAGAAGCAGGGTTATCCAAATCTCGAGATTTGGAT AACCCTGCTTCTCTTTTTG-3′; Reverse: 5′-AATTCAAAAAGAGAAGCAG GGTTATCCAAATCTCGAGATTTGGATAACCCTGCTTCTC-3′) were synthesized by Shanghai Sangon Biotechnology (Shanghai, China). After annealing, the double-strand DNA was cloned into shRNA expressing vector pLKO.1-puro, 3 μg of packaging plasmids pCMV-VSVG: pCMV-dR 8.91 (1:5) and 5 μg of shRNA expressing vector were co-transfected into 2.5×10^6 HEK-293T cells using PolyFect transfection reagent (301105; Qiagen). 72 h later, the media containing lentivirus particles were collected and centrifuged at 1500 $\times g$ for 10 min. Then the supernatant was collected and used to infect ZF4 cells immediately in the presence of 10 μg/ml Hexadimethrine Bromide (a.k.a. polybrene, H9268; Sigma-Aldrich). 48 h after infection, cells were collected for analysis.

2.4. Immunoblot

Cells were washed twice with cold phosphate-buffered saline (PBS), drained, and 0.5 ml of lysis buffer (50 mM HEPES, 10 mM EDTA, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, pH 7.4), supplemented with protease inhibitor cocktail (Sigma–Aldrich) was added to 5×10^6 cells. The cells were then sonicated. After centrifugation at 4 °C and 15,000 $\times g$ for 10 min, protein aliquots containing 30 μg of protein were separated on denaturing and reducing Laemmli 12% polyacrylamide gels and transferred to nitrocellulose. The membrane was blocked in PBS containing 5% skimmed milk powder and 0.1% Tween 20 and incubated at 4 °C overnight with primary antibody and then for 1 h at 25 °C with horseradish peroxidase-conjugated secondary antibody. Antibody binding was visualized using Clarity Western ECL Substrate (1705060; Bio-Rad). Anti-actin antibody (A1987) and anti-FLAG antibody (F3165) were purchased from Sigma–Aldrich, and anti-selenoprotein P antibody (sc-376,858) was from Santa Cruz. β-Actin protein levels were used as a control to verify equal protein loading.

2.5. Measurement of intracellular reactive oxygen species

Intracellular ROS generation was measured using the fluorescent probe 2, 7-dichlorofluorescein diacetate (DCFH-DA) (D6883; Sigma-Aldrich). After treatment, cells were washed twice with PBS, and then incubated with 20 μM DCFH-DA for 30 min at room temperature. Cells were washed three times with PBS, and collected for flow cytometer assay. Fluorescence was measured using excitation wavelength of 488 nm and emission of 525 nm on the FlexStation 3 Multi-Mode Microplate Reader. ROS levels were expressed as median fluorescence intensity. All experiments were performed in triplicate and the student's *t*-test was performed using GraphPad Prism 5 software.

2.6. Characterization of the Dm-SEPP cDNA

The selenocysteine insertion sequence (SECIS) elements in the 3′UTR of the *Dm-SEPP* cDNA were predicted using the SECISearch3 (http://gladyshevlab.org/SelenoproteinPredictionServer/).
Organization of the *SEPP* mRNAs was made by IBS. To perform phylogenetic tree analysis, the open reading frame of the *Dm-SEPP* cDNA was aligned with other known *SEPP* genes from the GenBank database using DNAMAN software. A phylogenetic tree was constructed using the UPGMA method in the MEGA 5.05 program (http://www.megasoftware.net). The reliability of the obtained tree was assessed by bootstrapping using 1000 bootstrap replications.

2.7. Quantitative real-time PCR

Total RNA was isolated using TRIzol reagent (15596-018; Invitrogen). cDNA was produced from 1 μg of total RNA using

Download English Version:

https://daneshyari.com/en/article/8645616

Download Persian Version:

https://daneshyari.com/article/8645616

Daneshyari.com