Journal of Proteomics 148 (2016) 57–64

Contents lists available at ScienceDirect

Journal of Proteomics

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

Combined proteomics and transcriptomics identifies sting-related toxins of jellyfish *Cyanea nozakii*

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ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form 16 June 2016
Accepted 22 July 2016
Available online 25 July 2016

Keywords:
Jellyfish
Cyanea nozakii
Toxin
Venom
Transcriptomics
Proteomics

ABSTRACT

Jellyfish sting has become a worldwide issue of critical concern to human health and safety in coastal areas in recent decades. *Cyanea nozakii* is one of the dominant blooming species and dangerous stingers in China. However, it remains unclear how many and what types of toxins are present in the venom. So, we used a combined transcriptomics and proteomics approach to investigate the venom composition of jellyfish *C. nozakii*. In total 4,608,524 Illumina valid reads were obtained to *de novo* assemble to 40,434 unigenes in the transcriptomics analysis. And, a total of 311,635 MS/MS spectra with 12,247 unique MS/MS spectra were generated to 1556 homologous proteins in the proteomics analysis. 174 potential toxin proteins were identified, with 27 proteins homology to the toxins from venomous animals, including phospholipase A2, zinc metalloproteinase-disintegrin agkistin, serine protease inhibitor, plancitoxin-1, alpha-latrocrustotoxin-Lt1a, *etc.* This study described the transcriptomics and venom proteomics of jellyfish *C. nozakii* for the first time. Our findings provide a comprehensive understanding of the venom composition of *C. nozakii*. Furthermore, the results may also be very helpful for the discovery of novel bioactive proteins, as well as the development of effective treatments for jellyfish sting in the future.

Biological significance: Jellyfish *Cyanea nozakii* is one of the most dangerous stingers in the coast of china. Hundreds of thousands of people would be stung every year and victims suffered a severe pain, itch, swelling, inflammation, wheal and even more serious consequence. However, it remains unclear how many and what types of toxins are present as well as the relationship between the clinical symptoms and toxins. Our combined transcriptomics and proteomics findings can provide a comprehensive understanding of the venom composition of *C. nozakii* and will also be helpful for the development of effective treatments for jellyfish sting in the future.

1. Introduction

Globally, jellyfish populations have been often increasing significantly in coastal areas from late spring into the early fall in recent decades [1–4]. Jellyfish blooms can interfere with fisheries, power plants, marine ecosystem [5,6] and, in particular, tourism, as tourists are frequently stung by jellyfish while swimming. Jellyfish sting has become an issue of critical concern to human health and safety in coastal areas during the high season. Hundreds of thousands of victims got stung by jellyfish every year [7–10] and even many deaths worldwide [11–13]. Jellyfish is a member of the phylum *Cnidaria*, which all have stinging cells, cnidocyte, and the stinging organelle nematocysts. The nematocyst discharges the tubule to inject the venom into the body for predation or defense [14]. The venom is a mixture of polypeptides and enzymes that are toxic or antigenic to man [15], including lethal toxins [16,17], hemolysins [18–22], cytotoxins [23,24], neurotoxins [25,26], myotoxins [27], and cardiotoxins [28–31] *etc.*

Currently, *Cyanea nozakii* was one of the three dominant jellyfish species, which often bloomed in Chinese seas [10]. It is a large and dangerous jellyfish with a bell of 400–600 mm in diameter and tentacles can be up to >2 m in length at maturity [32]. So, this jellyfish is much easier to sting its predators or preys as a result of its long tentacles. A huge number of victims got stung by this jellyfish in China every year. Those victims suffered a severe pain, itch, swelling, inflammation, wheal and even more serious consequence (Fig. 1). However, it is unclear how many and what types of toxins are present in the venom from *C. nozakii*. In addition, it is also very difficult to isolate and identify all toxins individually. Nowadays, omics technologies are mature and convenient methods for analyzing all of the components. Furthermore, omics analysis has also been successfully used in many venomous animals to exploit toxins and bioactive molecules [33–43]. Therefore, we used a combined transcriptomics and proteomics approach with experimental verifications to investigate the toxins in the venom from the jellyfish *C. nozakii* in the present study. The results of our study will be helpful for gaining a better understanding of the major components in

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http://dx.doi.org/10.1016/j.jprot.2016.07.023
1874-3919/

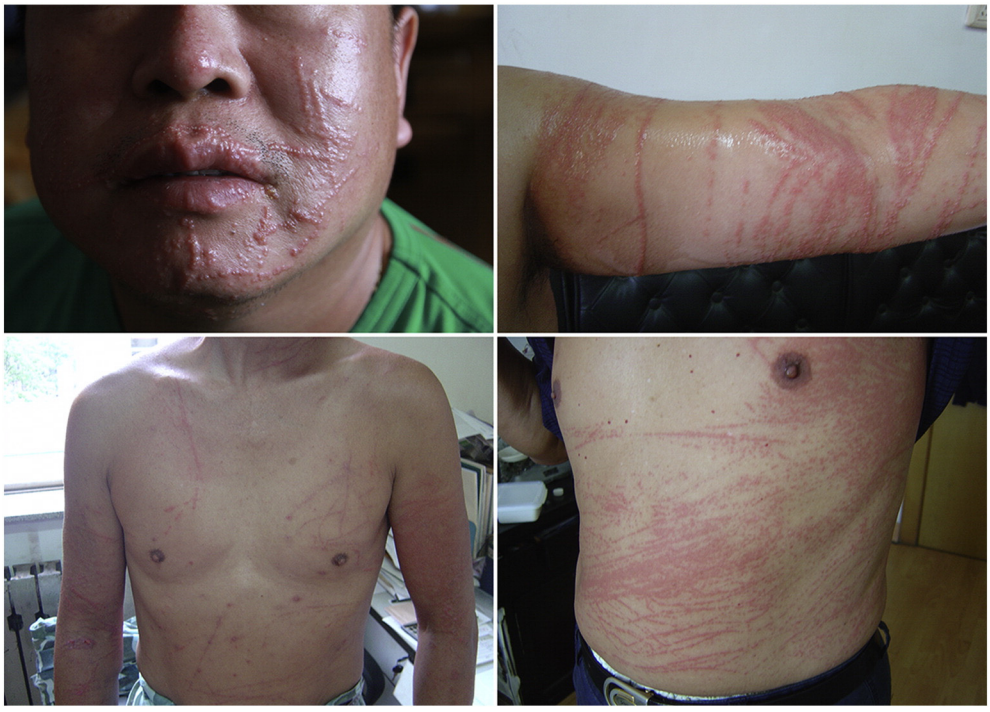

Fig. 1. The external symptoms of victims caused by the sting of the jellyfish *C. nozakii*. The victims were stung in No. 1 bath beach of Qingdao, China.

venom and will also provide a good reference developing drugs to treat the stings by this jellyfish in the future.

2. Material and methods

2.1. Jellyfish samples

Jellyfish *Cyanea nozakii* Kishinouye was collected from the coast of Qingdao, China, in August 2014. Fresh tentacles were then excised manually from the living jellyfish as soon as possible and were snap-frozen immediately in liquid nitrogen and stored at −80 °C for use.

2.2. Transcriptomics analysis of the tentacle

A cDNA library was constructed as previously described [38]. Total RNA of *C. nozakii* Kishinouye tentacles was extracted with TRIZOL following the manufacturer's procedure. The quantity and purity of the total RNA for transcriptomics analysis was analyzed by using a Bioanalyzer 2100 and RNA 6000 Nano LabChip Kit. The RNA was then used to construct cDNA libraries. PolyA mRNA was purified from approximately 10 μg of total RNA using polyT oligo-conjugated magnetic beads. Then, the mRNA was fragmented into small pieces using divalent cations at elevated temperature. The cleaved RNA fragments were then reverse-transcribed to generate the cDNA library according to the protocol provided with the mRNA-seq sample preparation kit.

2.3. Sequencing and de novo assembly of unigenes

RNA-seq for *C. nozakii* tentacle tissue was performed according to our previously described method [38]. The constructed cDNA library was paired-end sequenced using an Illumina HiSeq 2500 sequence platform following the manufacturer's recommended protocol. Before assembly, low-quality reads, including sequencing adapters, sequencing primers and nucleotides with a Q quality score < 20, were removed. The raw sequence reads were submitted to the NCBI Short Read Archive database under accession number SRP060482. The reads were then *de novo* assembled using Trinity under the default parameters, and the assembly quality was evaluated by length distribution analysis using common Perl scripts for the average length, max length, GC%, N_{50} number and contig number for different length intervals. We also scanned the best candidate coding sequence (CDS) for each contig and obtained ratios of long-CDS with transcripts to the corresponding length contigs and the longest obtained sequences were defined as unigenes.

2.4. Annotation and analysis of unigenes

Unigene annotations were analyzed according to the protein sequence similarity. All the unigene sequences were submitted to the protein databases Nr, Swiss-Prot, COG and KEGG for homolog and annotation comparison using BLASTx with an e-value <0.0001. Protein function was predicted according to the annotations of the most similar proteins in the databases. The GO functional annotation was obtained from the Swiss-Prot annotation, including molecular function, cellular component and biological process. All of the unigenes were also aligned to the COG database for prediction of their possible functions. The KEGG pathway database contains networks of molecular interactions in cells and variants of these pathways.

2.5. Proteomics analysis using LC-MS/MS

The jellyfish tentacles were sonicated in cold lysis buffer (1 mM PMSF, 2 mM EDTA, 10 mM DTT, 7 M Urea, 2 M Thiourea, 4% CHAPS, 40 mM Tris-HCl, pH 8.5). The supernatant was mixed with 5× cold acetone, 10% (v/v) TCA, and centrifuged after incubation at −20 °C for 2 h. The precipitate was then sonicated in cold lysis buffer. After centrifugation, 10 mM DTT and 55 mM IAM (final concentration) was added into the supernatant to reduce disulfide bonds and block the cysteine of the venom proteins. The supernatant was mixed with 5× cold acetone at −20 °C for 2 h. After centrifugation, the precipitate was dissolved and sonicated in 0.5 M TEAB. Finally, after centrifugation, the supernatant was quantified using a Bradford method and then kept at −80 °C for further analysis.

The iTRAQ proteome analysis of the venom proteins was according to Leong et al. [44]. Briefly, 100 μg jellyfish *C. nozakii* and *N. nomurai* venom proteins were digested separately with same quantity Trypsin Gold (protein: trypsin = 30: 1) at 37 °C for 16 h. After freeze drying,