Results in Immunology 3 (2013) 95–103

Contents lists available at ScienceDirect

Results in Immunology

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

Transcriptome analysis of neoplastic hemocytes in soft-shell clams *Mya arenaria*: Focus on cell cycle molecular mechanism

Ahmed Siah[a,b,*], Patty McKenna[b], Franck C.J. Berthe[c], Luis O.B. Afonso[d], Jean-Michel Danger[e]

[a] British Columbia Centre for Aquatic Health Sciences, BC CAHS, 871A Island Highway, Campbell River, BC, Canada V9W 2C2
[b] Department of Pathology & Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, Canada C1A 4P3
[c] Animal Health and Welfare Unit, European Food Safety Authority (EFSA), Largo N, Palli 5IA, I-43100, Parma,Italy
[d] School of Life & Environmental Sciences, Faculty of Science, Engineering and Built Environment, Deakin University, PO Box 423, Warrnambool, Victoria 3280, Australia
[e] Laboratory of Ecotoxicology, University of Le Havre, 25 rue Philippe Lebon, BP540, 76058 Le Havre, France

ARTICLE INFO

Article history:
Received 24 August 2013
Received in revised form 20 October 2013
Accepted 21 October 2013

Keywords:
Transcriptomic
Neoplasia
Tetraploidy
Mya arenaria
Cell cycle
Molecular mechanisms

ABSTRACT

In North America, a high mortality of soft-shell clams *Mya arenaria* was found to be related to the disease known as disseminated neoplasia (DN). Disseminated neoplasia is commonly recognized as a tetraploid disorder related to a disruption of the cell cycle. However, the molecular mechanisms by which hemocytes of clams are transformed in the course of DN remain by far unknown. This study aims at identifying the transcripts related to DN in soft shell clams' hemocytes using next generation of sequencing (Illumina HiSeq2000). This study mainly focuses on transcripts and molecular mechanisms involved in cell cycle. Using Illumina next generation of sequencing, more than 95,399,159 reads count with an average length of 45 bp was generated from three groups of hemocytes: (1) a healthy group with less than 10% of tetraploid cells; (2) an intermediate group with tetraploid hemocytes ranging between 10% and 50% and (3) a diseased group with more than 50% of tetraploid cells. After the reads were cleaned by removing the adapters, de novo assembly was performed on the sequences and more than 73,696 contigs were generated with a mean contig length estimated at 585 bp ranging from 189 bp to 14,773 bp. Once a Blastx search against NCBI Non Redundant database was performed and the duplicates removed, 18,378 annotated sequences matched known sequences, 3078 were hypothetical and 9002 were uncharacterized sequences. Fifty percent and 41% of known sequences match sequences from Mollusca and Gastropoda respectively. Among the bivalvia, 33%, 17%, 17% and 15% of the contigs match sequences from Ostreoida, Veneroida, Pectinoida and Mytiloida respectively. Gene ontology analysis showed that metabolic, cellular, transport, cell communication and cell cycle represent 33%, 15%, 9%, 8.5% and 7% respectively of the total biological process. Approximately 70% of the component process is related to intracellular process and 15% is linked to protein and ribonucleoprotein complex. Catalytic activities and binding molecular processes represent 39% and 33% of the total molecular functions. Interestingly, nucleic acid binding represents more than 18% of the total protein class. Transcripts involved in the molecular mechanisms of cell cycle are discussed providing new avenues for future investigations.

1. Introduction

Disseminated neoplasia (DN) is defined as a leukemia-like disease affecting hemocytes of soft-shell clams. This disease is mainly characterized by a high number of circulating hemocytes containing pleomorphic nuclei with a high nucleus–cytoplasm ratio [1,2]. These abnormal circulating cells lose their function of phagocytosis [3] and exhibit higher DNA contents and mitosis rates than normal cells resulting in the formation of tetraploid cells [4,5]. During normal cell cycle, chromosomes segregate only when an adequate kinetochore-microtubule attachment exists enabling cells to pass the spindle assembly checkpoint [6]. However, after prolonged arrest at the spindle assembly checkpoint that promotes cytokinesis failure, cells become tetraploid [7].

The etiology of DN in *Mya arenaria* remains unknown although several factors such as retrovirus infection or contamination seem to contribute to the development of the disease. Retroviral infection was shown as the main factor [8] of DN development; however, other studies did not succeed in detecting retroviral components in diseased clams [9]. In addition, studies stipulate that environmental contamination by pesticides could be the main cause of DN in soft shell clams collected from Prince Edward Island [10,11].

* Corresponding author at: British Columbia Centre for Aquatic Health Sciences, BC CAHS, 871A Island Highway, Campbell River, BCCanada V9W 2C2. Tel.: +1 250 286 6102; fax: +1 250 286 6103.
E-mail address: ahmed.siah@cahs-bc.ca (A. Siah).

2211-2839
http://dx.doi.org/10.1016/j.rinim.2013.10.001

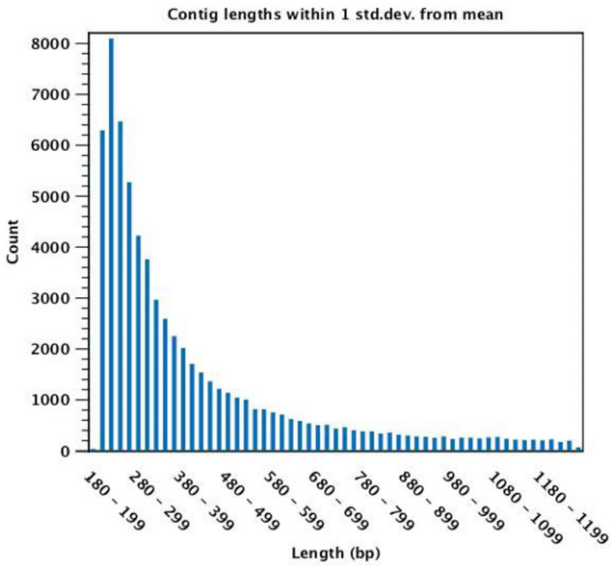

Fig. 1. Contigs length distribution after de novo *assembly*.

Many studies have investigated the molecular mechanisms involved in the development of the disease [12–18]. For instance, some studies have shown that the proliferation of hemocytes in clams is due to the sequestration of p53 by mortalin [13,14], whereas other studies demonstrated that p53's pathways were disrupted by the activation of the Mouse Double Minute 2 (MDM2) proto-oncogene [15]. Our recent investigation using subtractive suppressive hybridization techniques suggested the involvement of proto-oncogene such as RAS like protein members, c-myc and c-jun in the development of DN [17]. It was hypothesized that the proto-oncogenes were regulated by the presence of transposase whose gene expression was increased in tetraploid hemocytes [16]. Briefly, although several studies have been initiated, the molecular mechanisms by which hemocytes become neoplastic are still to be unravelled. Unfortunately, little is known on the transcriptome of soft shell clams. Only few sequences are available in GenBank represented mainly by Map53 (Acc# AF253323.1), Map73 (Acc# AF253324.1), and mortalin (Acc # EF576660).

The development of the new generation of sequencing technologies represents a great opportunity to increase the number of transcript sequences and would provide a basis platform for molecular mechanisms studies involved in DN in soft shell clams.

Thus, this study aims at increasing the numbers of transcript sequences in hemocytes of soft shell clams affected by DN. Our approach focused specifically on sequencing mRNA isolated from different groups of hemocytes characterized by their tetraploid status. The sequences generated in this study will constitute a baseline in unravelling the molecular pathway(s) involved in DN as well as a source of potential target transcripts which could be involved in hemocytes' immune system of soft shell clams, *M. arenaria*.

2. Materials and methods

2.1. Sampling

Approximately 5-cm-long specimens of *M. arenaria* were collected at low tide at a depth of 15–20 cm using a hand rake from May to November 2011 in North River (46°15′10″N, 63°10′42″W) (Charlottetown, Prince Edward Island, Canada). Clams were washed with seawater and transported to the Atlantic Veterinary College at the University of Prince Edward Island for further analysis.

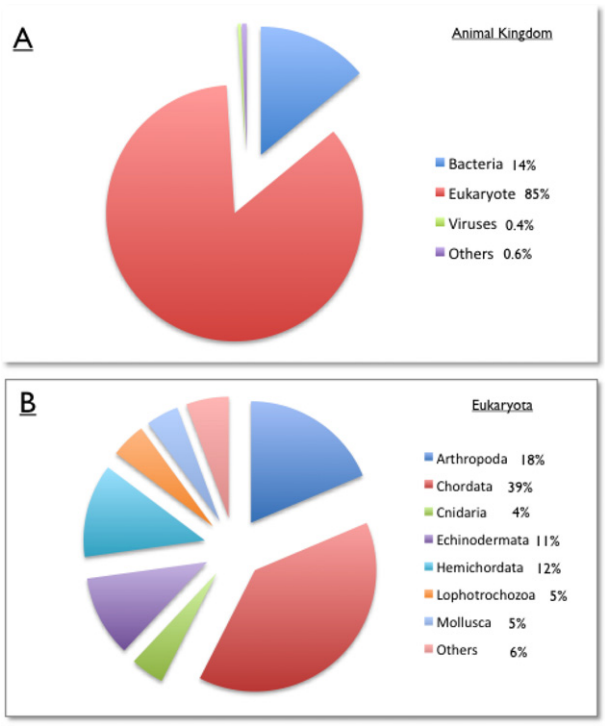

Fig. 2. Phylogenetic distribution of the best hit in animal kingdom (A) and eukaryota (B) based on BlastX search in the NCBI nr database.

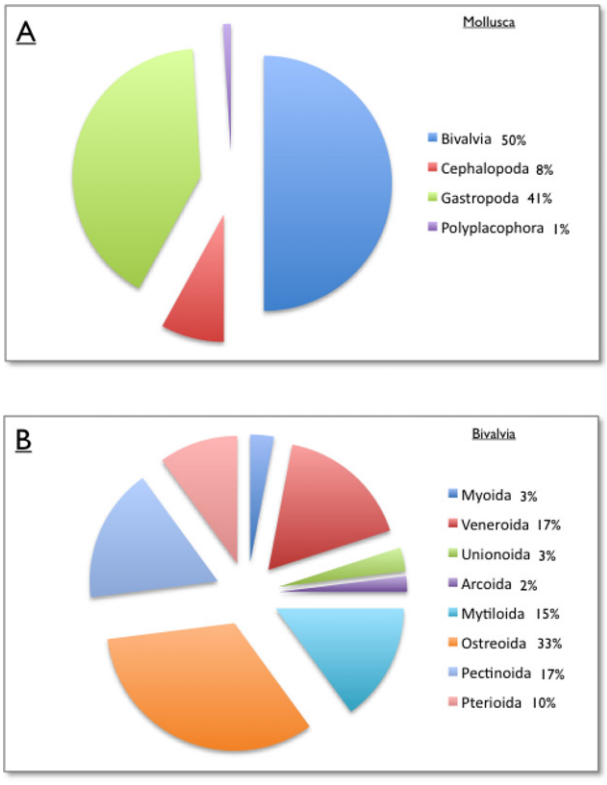

Fig. 3. Species distribution based on BlastX alignment in Bivalvia (A) and Mollusca (B) NCBI nr database.

2.2. Flow cytometry

Flow cytometric (FCM) analysis was used to assess the ploidy status of *M. arenaria's* haemocytes according to the methods described