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Differential lncRNA expression profiles reveal the potential roles of lncRNAs in antiviral immune response of *Crassostrea gigas*

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ABSTRACT

Long noncoding RNAs (lncRNAs) may play widespread roles in various biological processes. However, systematic profiles of lncRNAs in the biological responses of Pacific Oyster (*Crassostrea gigas*) to pathogen infection have not yet been demonstrated. Here, we have conducted an exhaustive comparative transcriptome analysis using a bioinformatics approach to exam the functions of lncRNAs response to Ostreid herpesvirus 1μVar (OsHV-1μVar) challenge. In total, 101 differentially expressed lncRNAs (DE-lncRNA) during OsHV-1μVar infections were identified. Compared with differentially expressed mRNAs (DE-mRNA), DE-lncRNAs are shorter in terms of overall length but longer in terms of exon length. These lncRNAs shared similar characteristics with previously reported invertebrate lncRNAs, such as relatively low GC content, low exon number and low sequence conservation, but low expression level were not observed. 20 DE-lncRNAs are typically co-expressed with their neighboring genes annotated as GO terms (GO: 0044237), indicating that these lncRNAs are involved in binding and cellular process functions in *cis* mode. The weighted gene co-expression network (WGCNA) analysis resulted in 15 modules. The highlighted blue module was specifically demonstrated a co-expression relationship between 14 DE-lncRNAs and 17 immune-related DE-mRNAs (IR-DE-mRNA). Three hub lncRNAs within this module were co-expressed with one hub IR-DE-mRNA involved in fibrinogen-related protein. It was speculated that lncRNAs is extensively involved in oyster antiviral innate immune system. The present study will facilitate subsequently experimental studies to unravel the function of lncRNAs in marine invertebrate response to pathogen infection.

1. Introduction

Long non-coding RNA (lncRNA) is commonly defined as ncRNAs that are at least 200 nucleotides (nt) in length but have little or no protein-coding capacity [1]. Based on the position and direction of transcription relative to protein-coding genes, lncRNA can be classified into long intergenic noncoding RNAs (lincRNAs), long noncoding natural antisense transcripts (lncNATs), long intronic noncoding RNAs and overlapping lncRNAs [2]. They have attracted considerable attention in recent years and emerged as a new class of regulatory transcripts in many biological processes, including transcriptional regulation, post-transcriptional control and epigenetic processes [3].

Oysters are important representative bivalve molluscs and widely distributed as sessile filter feeders in estuaries and intertidal zones. Besides, oysters have been regarded as potential biological models for marine molluscan studies, such as stress adaptation, development and innate immunity [4,5]. Because of their economic importance, oysters have been cultured worldwide. Global aquaculture production of oysters exceeded 5.3 million metric tons in 2015 [6]. The oyster is constantly exposed to harsh and dynamically changing environments, especially a wide range of microbial pathogens.

Herpes-like viruses were first reported by Farley et al., in 1972 in the eastern oyster, *Crassostrea virginica* [7]. After about 20 years, ostreid herpesvirus 1 (OsHV-1) were found in farmed Pacific Oysters (*Crassostrea gigas*) in France [8]. OsHV-1 is a DNA virus belonging to the *Malacoherpesviridae* family from the Herpesvirales order [9] and its genome was entirely sequenced [10]. In 2008, massive mortality events of spat and juvenile in *C. gigas*, often reaching 90–100% within a few days, were reported in almost all farming areas in France and throughout the world, in association with the infection of oysters with a newly described OsHV-1 variant called OsHV-1μVar [11,12]. To better understand genome-wide host-responses to viral infections, there have been a number of studies describing the relationship between oyster resistances and OsHV-1 infection at molecular and cellular levels [13–17].

In recent years, interest in oyster immunity has been increasing continuously. Unlike vertebrate immune system, that orchestrates innate and adaptive immune responses, oysters are generally believed to

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Abbreviations

DET	differentially expressed transcript
lncRNA	long noncoding RNA
lincRNA	long intergenic noncoding RNA
DE-lncRNA	differentially expressed lncRNA
IR-DE-lncRNA	immune-related DE-lncRNA
DE-mRNA	differentially expressed mRNA
IR-DE-mRNA	immune-related DE-mRNA
hpi	hour post-inoculation

solely rely on innate immunity to combat viral infection since there is as yet no clear evidence of adaptive antiviral immunity. Even though, like other marine invertebrates, oysters have evolved sophisticated host-defence mechanisms against diverse pathogens, such as immune recognition, signal transduction and effector synthesis [14,15,18,19].

However, few studies have focused on the systematic identification and characterization of non-coding RNAs (ncRNAs) associated with oyster immune and stress response. In vertebrates, more and more evidence suggests that lncRNAs were expressed in a highly lineage-specific manner and play an important role in immune regulation [20–22]. Concerning oyster ncRNA, Zhou et al. (2014) investigated the latent immunomodulation of miRNAs after bacteria challenge and heat stress and obtained 199 oyster miRNAs [16]. 55 differentially expressed miRNAs were considered of immune-responsive and essential regulators of immune response. The target protein-coding genes of 55 immune-responsive miRNAs were significantly enriched Gene Ontology (GO) terms associated with antioxidant, cell killing, death, immune system process, and response to stimulus. But there are few oyster examples focusing on lncRNAs and their biological functions.

In this study, a multiple RNA-seq datasets as previously described [13] was employed to address the protein-coding gene regulatory functions of lncRNAs during OsHV-1μVar infection. First, we identified the set of transcripts which were differentially expressed induced by OsHV-1μVar and profiled their expression time-dependent manner. Second, the systematic identification, characterization and expression pattern of differentially expressed lncRNAs during the antiviral immune response were investigated. Finally, we used the weighted gene co-expression network (WGCNA) approach to construct a functional lncRNA-mRNA regulatory network that is associated with immune response to OsHV-1μVar infection.

The results will provide new insights on the potential role of lncRNAs as candidates in the regulation of the antiviral immune response in oysters and help to improve the present annotation of the genome of *C. gigas*.

2. Methods

2.1. RNA-seq transcriptome data preparation

To assess the response of lncRNAs to viral infection in *C. gigas*, we retrieved publicly available RNA-seq data from a previous study [13] and downloaded from the NCBI Sequence Read Archive (SRA) website under the accession number SRP057827. Briefly, Juvenile oysters were injected with OsHV-1μVar (1.5×10^9 viral genomic units). Controls were injected with the same volume of filtered seawater (FSW). 33 individuals were selected for RNA-sequencing: 3 individuals prior to the injection, and 3 OsHV-1μVar-injected and 3 FSW-injected individuals each at 6, 12, 24, 48 and 120 h post-inoculation (hpi).

2.2. Transcriptome reconstruction

Each RNA-seq read was mapped to the *C. gigas* genome using HISAT2 v2.0.5 [23] with option –dta and default values for the remaining parameters. The mappable reads were assembled by StringTie v1.3.3 [24] with the following options: -G, -e and -B to generate files for analysis with R-package Ballgown v2.8.4 [25]. Then the individual

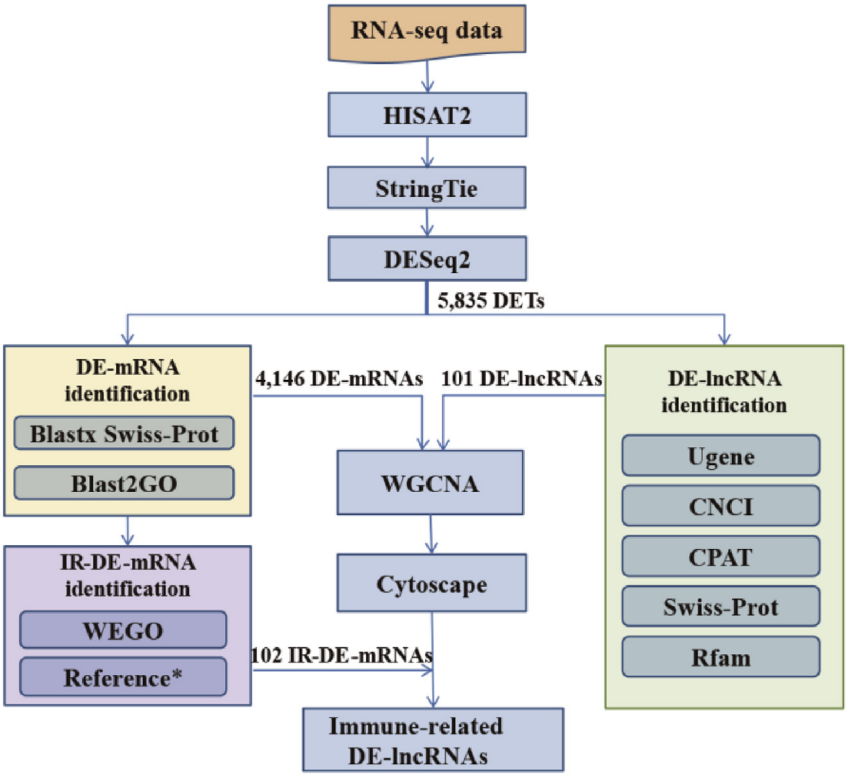

Fig. 1. Overview of the pipeline for the systematic profiles of DE-lncRNAs, DE-mRNAs, IR-DE- mRNAs and IR-DE-lncRNAs in response to OsHV-1μVar challenge. CNCI: Coding-Non-Coding Index; CPAT: Coding Potential Assessment Tool. Reference*: immune-related gene data set derived from Ref. [35].