Identification of Differential mRNA and lncRNA Expression in AcMNPV- Infected Sf9 Cells

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Short report

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Abstract

Autographa californica multiple Nucleopolyhedrovirus (AcMNPV) is the archetypal species of the *alphabaculovirus*. Sf9 are the ovarian cells of their host—*Spodoptera frugiperda*. In this study, a total of 3,463 pieces of time-series differentially expressed RNA are identified, including 1,200 mRNA and 2,263 lncRNA, with high-throughput sequencing technology using samples collected from Sf9 cells at different time points after AcMNPV infection. The result could provide a reference for the further study of the interaction and regulatory mechanism between the virus and the host.

1. Introduction

Baculoviruses are pathogenic microorganisms that infect insects and invertebrates, making them their host. The particle form of the virus is Baculoviridae (Vago et al., 1974). Its main hosts are *lepidoptera* (corn borer, etc.), *hymenoptera* (bees, etc.), and *diptera* (mosquitoes, etc.) (Slack et al., 2007). They are divided into four genera, namely, *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus*, according to the insect hosts from which they were isolated and their biological characteristics (Jehle et al., 2006). *Autographa californica multiple Nucleopolyhedrovirus* (AcMNPV) is the archetypal species of the *alphabaculovirus*. During the infection cycle, it produces two enveloped virion phenotypes with different structures and functions, which play different roles in virus pathogenesis: budded virus (BV) and occlusion-derived virus (ODV) (Rohrmann, 2013). The nucleocapsid structures of these viruses are similar, but the origin and composition of their envelope and their roles in the virus life cycle are different.

Spodoptera frugiperda belongs to *Lepidoptera* and *Noctuidae*. It is a major migratory insect from tropical and subtropical areas of America, and also one of the major hosts for AcMNPV and the ovarian cells for Sf9.

The baculovirus establishes a strict parasitic and adaptive relationship with its insect host during the long evolutionary process, and the information of the interaction is recorded and solidified in the viral genome. For example, polyhedrins need to be alkali-interpreted by releasing virions to initiate infection in the pH-alkaline larval midgut; the alkaline environment of the insect host's midgut and alkaline solubility of the viral polyhedrin protein crystal are the result of the long-term evolution of both.

Baculoviruses interact with their hosts at multiple levels, but the details remain unclear. The expression of baculovirus genes has a time sequence. In this process, the host will influence the expression of baculovirus genes. For example, the expression of early baculovirus genes depends on the host's transcription system and factors. After the baculovirus unshells and enters the nucleus, DNAs that have not yet been replicated are transcribed in the first place. The early transcription of the virus is inhibited by muscarine carnitine, so host RNA polymerase α mediates most early gene transcription, leading to the activation and expression of the late gene, thus making the expression of viral genes orderly (Tjia et al.,

1979). Host RNA polymerase is the most important host component in the early gene expression of the virus and plays a dominant role in the life cycle of the virus.

The baculovirus also has an effect on its host. For example, one of the characteristics of baculovirus overexpression in early gene expression to late gene expression is significant inhibition of host transcription. Some studies have shown that in Sf9 cells infected with AcNPV, host protein synthesis begins to decline between 6 and 10 hours after infection, and seems to stop completely after 24 hours. In addition, baculovirus can also inhibit the apoptosis of host cells and affect the cycle process of host cells. Bertin J et al. demonstrated that the P35 protein of baculovirus inhibits apoptosis induced by baculovirus infection (Bertin et al., 1996). One of the functions of AcMNPV's very early gene, IE2, has been shown to encode proteins that block cell cycles in a variety of cell lines.

The interaction between baculovirus and host is reflected at multiple levels, from simple physical adsorption, invasion to complex manipulation of host metabolic system, inhibition of host cell apoptosis, and inhibition of host hormone levels, etc. Many interesting and important host-virus interaction mechanisms have been identified from fundamental studies of the biochemistry and molecular biology of baculoviruses. These studies provide new strategies for biological pest control and eukaryotic expression vector systems.

A large part of functional genes of baculoviruses have been identified thus far, but the research on the relationship between the two mainly focuses on viruses, and there is less research on the function of host factors, so the work in this field is worthy of further development. In addition, many previous studies have carried out relevant findings from the perspective of host genes or viral gene functions, while from the perspective of bioinformatics, there is no relevant report on the changes in the expression level of host genes after baculovirus infection. In this study, after the baculovirus infecting Sf9 cells, infected cells were collected at different time points for sequencing, and the sequencing results were analyzed to show the expression of host genes at different time points. Our results provide a useful resource for further analysis of baculovirus-host interactions.

2. Material And Methodology

2.1 Cells and insects

Sf9 (*Spodoptera frugiperda* IPLB-Sf21-AE clonal isolate 9) insect cells were cultured in Grace's medium supplemented with 10% fetal bovine serum at 27°C. Groups of Sf9 cells were infected with AcMNPV at a multiplicity of infection (MOI) of 10, and subsequently, RNA samples at 6, 12, 24, 36, 48, 60 and 72 h post-infection (hpi), and mock-infected cells were extracted according to the manufacturer's protocol. The samples were sent to Beijing Genomics Institute Shenzhen Co. (BGI-Shenzhen) for sequencing using Solexa technology.

2.2 RNA isolation

Total RNA was isolated from approximately 10^7 infected cells harvested using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. Total RNA was dissolved in 50 μ L RNase-free H₂O. The RNA solutes were digested for 30 minutes at 37°C with RNase-free DNase I (TaKaRa, Japan) to remove any contaminating genomic DNA. RNA integrity was analyzed by gel electrophoresis using a 1% agarose gel alongside an RNA marker.

2.3 Raw data processing and transcriptome

Fastp (version 0.19.4) (Chen et al., 2018) was used to filter low-quality reads, cut adapters and quality control of raw FASTQ files to obtain clean reads. The clean reads of each sample were aligned against SLIVA (Quast et al., 2013) ribosomal RNA and AcMNPV genome (accession number NC_001623) with Bowtie2 (Langmead et al., 2012) to filter out ribosomal RNA and AcMNPV RNA.

Then, the unmapped reads of each sample were aligned against the *Spodoptera frugiperda* genome (NCBI, GCA_002213285.1) with HISAT2 (version 2.1.0) (Kim et al., 2015) and then subsequently assembled by StringTie (version 1.3.4d) (Pertea et al., 2015) separately. All the assemblies were merged into one transcriptome by TACO (Niknafs et al., 2017). Then, Salmon (version 0.11.2) (Patro et al., 2017) was applied to quantify the transcript expression of the transcriptome. To reduce noise, we include only transcripts with TPM >0.1 and read count >10.

BLASTP was used to compare the obtained sequences with the NR database, and the unmatched sequences were selected for subsequent analysis. PLEK (Li et al., 2014) was used to calculate the coding potential of the transcripts. Transcripts that had no coding potential, and harbored at least 2 exons, and had a length larger than 200 nt, were classified as lncRNAs. Transcripts that showed coding potential and/or had similar sequence with proteins were classified as mRNAs. R package EBSeqHMM (Leng et al., 2015) was used to identify transcripts that were changing over time; FDR < 0.01 was considered as its signature. The detailed flow is illustrated in Figure 1.

2.4 qRT-PCR analysis

SYBR green-based quantitative reverse transcription PCR (qRT-PCR) was performed to define the expression profile of miRNA and its target genes. We compared 57,104 RNAs with eggNOG and other databases to find the sequence of selected genes, and designed relevant primers based on the sequence information of selected genes. The mRNA and 5S rRNA were reversely transcribed by using a random primer (Takara) and included as a reference of the respective sample. The qRT-PCR results were analyzed by utilizing the $2^{-\Delta\Delta CT}$ method. The experiment was repeated three times and averaged to show changes in the expression of host genes at different time points after infection.

3. Results

3.1 Overview of RNA sequencing (RNA-seq)

After standardizing the raw data, a total of 24,230,852.75 clean reads that match the *Spodoptera frugiperda* genome were obtained through sequencing and filtering out ribosomal RNA and AcMNPV RNA of samples of the 8 time points.

3.2 Identification of lncRNA and mRNA

The obtained clean reads were *assembled* into 57,104 non-redundant RNA transcripts, including 19,982 lncRNA and 37,122 mRNA, as shown in Figures. 2 (A, B, and C). In general, GC content and length can be well differentiated.

3.3 Sequence expression differences of infected Sf9 cells

Aided by further EBSeqHMM analysis, it was found that 1,200 mRNA and 2,263 lncRNA showed differences in temporal expression after *AcMNPV* infection. Furthermore, we randomly selected 4 RNA, including 1 mRNA and 3 lncRNA, for further experimental validation.

As shown in Figure 3, the overall trend of the selected lncRNA and mRNA was basically the same whether the results were analyzed by EBSeqHMM or by qRT-PCR. After virus infection, XP_022825542.1, XP_022823371, XP_022827228.1, and XP_022814930.1 exhibit an overall downward trend in both the bioinformatics and qRT-PCR predictions. In contrast, XP_022827228.1 exhibit an overall upward trend in both the bioinformatics and qRT-PCR predictions.

The result indicates that among the obtained 19,982 lncRNA, 2,263 showed differences in temporal expression, accounting for 11.33%, while only 1,200 of the obtained 37,122 mRNA showed differences in temporal expression, accounting for 3.23%, implying that many non-coding RNA played a role in the interaction between viruses and hosts.

4. Discussion

Spodoptera frugiperda is a hybrid feeder with strong reproduction and migratory ability. Just take Africa as an example. In 2016, for the first time, *Spodoptera frugiperda* was found in Africa and rapidly swept across 44 countries in the south of the Sahara within two years (Goergen et al., 2016; Chapman et al., 2017). According to the Centre for Agriculture and Bioscience International (CABI), a survey of 12 African maize-producing countries shows that *Spodoptera frugiperda* is responsible for losses of between 8.3 million and 20.6 million tons, or \$2.5 billion to \$6.2 billion USD, of maize production each year. In addition, more than \$13 billion USD of crops are still at risk, causing major damage to agricultural production in Africa (Stokstad et al., 2017; Early et al., 2018). In 2017, the European Food Safety Authority (EFSA) listed *Spodoptera frugiperda* as a quarantine pest (Michael et al., 2017). The Food and Agriculture Organization (FAO) of the United Nations has also issued a global warning, putting it on the world's top 10 of blacklisted plant pests (FAO, 2018). The work on prevention and control has not stopped, and biological control, as an important method, has also attracted researchers' attention. Great progress in preventing and controlling *Spodoptera frugiperda* has been achieved by utilizing virus, bacteria, nematode

and other pathogenic microorganisms, such as *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SFMNPV) and *Beauveria bassiana* et. (Abrahams et al., 2017; Casmuz et al., 2010). When the pathogenic microorganisms, such as *B. bassiana*, *Metarhizium rileyi* and *C. elegans*, were mixed with insecticides, the prevention and control effect of *Spodoptera frugiperda* could be significantly improved.

AcMNPV, which is a parasite of *Spodoptera frugiperda*, and *Spodoptera frugiperda* itself have also been extensively studied. For example, in AcMNPV, researchers have not only studied its gene function, but also found its encoded miRNA and function successively. AcMNPV-miR-1 is considered to target and down-regulate the expression of viral gene odv-e25, accelerate polyhedra formation, and promote viral infection efficiency in *Trichoplusia ni* larvae (Zhu et al., 2016, 2013). AcMNPV-miR-3 is located on the opposite strand of the viral gene ac101 coding sequence in the AcMNPV genome, and it can be detected at 6 h post-infection and accumulated to a peak around 12 h post-infection in AcMNPV-infected Sf9 cells. Five viral genes (ac101, ac23, ac25, ac86, and ac98) were verified to be regulated by AcMNPV-miR-3 (Jiao et al., 2019).

Although researchers have conducted many studies of AcMNPV and its host, at present the understanding of the regulating mechanism of a series of virus life process in the host cells is relatively limited, including the temporal transcriptional regulation mechanism of host genes during infection, due to the lack of host genome sequence information. Kakumani et al. sequenced the genome of Sf21, which is the separated ovarian cell line of *Spodoptera frugiperda*, by using the second-generation sequencing technique, which was the first genome data of Sf21 (Kakumani et al., 2014). In 2017, scientists resequenced maize and rice strains of the same ovarian cell line (Sf21), yielding a genome of about 396 Mb (Gouin et al., 2017). In the same year, Nandaku-mar et al. also sequenced the genome of another cell line (Sf9) of *Spodoptera frugiperda*, which obtained a total of 451 Mb of genome data (Subhiksha et al., 2017). The assembly result and sequencing quality were better than the former two. In 2019, Liu et al. sequenced the entire genome of *Spodoptera frugiperda* that had invaded China and assembled the sequencing results to the chromosome level. The samples were collected from Yunnan and Guangdong provinces in China (Liu et al., 2017). This study obtained *Spodoptera frugiperda* genome size of about 536 Mb, with N50 reaching more than 14 Mb, and more than 80% of the genome data were assembled on the 31 chromosomes.

5. Conclusion

By high-throughput sequencing of RNA from Sf9 cells infected by AcMNPV, we found 1,200 mRNA and 2,263 lncRNA showing different temporal expression. Results from the experimental group are basically identical with those from the bioinformatic analysis. The overall trend is consistent, though there are differences at individual points in time, which may be associated with the variation in the repetition count of the experiment and sequencing analysis, and can be improved by more sequencing in further studies. The study is helpful to reveal the regulation mechanism of host-related virus life process and the interaction between AcMNPV and the host in the course of infection.

Abbreviations

AcMNPV, Sf9, lncRNA, mRNA, EBSeqHMM, RNA-Seq, BV, ODV, MOI, hpi, qRT-PCR, CABI, EFSA, FAO, SFMNPV

Declarations

-Ethics approval and consent to participate

The study design was approved by the appropriate ethics review board. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these. This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal.

-Consent to publicationNot applicable

- Availability of data and material

All the data are available to share upon request.

- Competing interests

There are no conflicts of interest to declare.

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- Authors' contributions

Tiejun Zhao and Mengqiu Chen performed the experiment and analysis. Tiejun Zhao, Riqiang Deng and Xunzhang Wang wrote the manuscript.

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Figures

Figure 1

LncRNA and mRNA sequences were separated and assembled by processing RNA-seq data through a series of analytical processes. First, the raw data were filtered to remove rRNA and viral RNA. The obtained clean data were then compared with the Sf9 genome. Sequences with TPM expression levels greater than 0.1 and read count greater than 10 were compared with the NR database. The unmatched sequences were further analyzed using PLEK to differentiate lncRNA and mRNA.

Figure 2

Isograph of GC content, length, and gene expression of lncRNA and mRNA. RNA meeting the requirement that its transcripts present in at least 2 samples, counts>10, TPM >0.1 for each sample, was used. lncRNA and mRNA can be distinguished from each other in all three aspects. A: GC percentage distribution comparison between lncRNA and protein coding RNA transcripts. B: Length distribution comparison between lncRNA and protein coding RNA transcripts. C: Cumulative gene expression (TPM) distribution of lncRNA and protein coding RNA at 0h. The horizontal axis is $\log_{10}$ (TPM) at 0h, and the vertical axis is the accumulation of its probability density.

Figure 3

Comparison between bioinformatics and qPCR results of lncRNA XP_022825542.1, XP_022823371, and XP_022827228.1, and mRNA XP_022814930.1, showing an overall downward trend for XP_022825542.1, XP_022823371, and XP_022814930.1, and an overall upward trend for XP_022827228.1, in both the bioinformatics and qRT-PCR predictions. The bioinformatics analysis was carried out by EBSeqHMM, and qPCR is the experimental methodology. After the cells were infected with the virus, cells at the time points

involved in the bioinformatics analysis were collected to extract RNA for reverse transcription, and then qPCR was performed.