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Characterization of the lipidomic profile of BmN cells in response to *Bombyx mori* cytoplasmic polyhedrosis virus infection

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**Keywords:** BmCPV, Lipids, Infection, Metabolism

**Abstract**

*Bombyx mori* cytoplasmic polyhedrosis virus (BmCPV) that belongs to the genus Cypovirus in the family of Reoviridae is one of the problematic pathogens in sericulture. In our previous study, we have found that lipid-related constituents in the host cellular membrane are associated with the BmCPV life cycle. It is important to note that the lipids not only affect the cellular biological processes, they also impact the virus life cycle. However, the intracellular lipid homeostasis in BmN cells after BmCPV infection remains unclear. Here, the lipid metabolism in BmCPV-infected BmN cells was studied by lipidomics analysis. Our results revealed that the intracellular lipid homeostasis was disturbed in BmN cells upon BmCPV infection. Major lipids constituents in cellular membrane were found to be significantly induced upon BmCPV infection, which included triglycerides, phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phospholipids, glucose ceramide, mono-etherphosphatidylcholin, ceramide, ceramide phosphoethanolamine and cardiolipin. Further analysis of the pathways related to these altered lipids (such as PE and PC) showed that glycerophospholipid metabolism was one of the most enriched pathways. These results suggested that BmCPV may manipulate the lipid metabolism of cells for their own interest. The findings may facilitate a better understanding of the roles of lipid metabolic changes during virus infection in future studies.

1. Introduction

As one of the members of Cypovirus genus of Reoviridae family, *Bombyx mori* cytoplasmic polyhedrosis virus (BmCPV) is one of the most problematic pathogens in sericulture. The genome of BmCPV comprises ten dsRNA linear segments that are assembled as part of the virion structure (Cao et al., 2012). Among different tissue types in silkworms, the midgut is one of the most susceptible organs specifically infected by BmCPV. Silkworms can be infected by BmCPV at any instar stage. BmCPV-infected silkworms show symptoms such as translucent appearance, decreased movement ability and lowered body size. Such symptoms aggravate gradually in line with the infection process. At the event of death, the midgut tissues turn milky in appearance.

The host response of silkworms to BmCPV infection has been comprehensively explored, including the expression pattern analysis of genes (Kolliopoulou et al., 2015; Guo et al., 2015), proteins (Gao et al., 2017) and non-coding RNAs (Hu et al., 2018). In addition, the function of BmCPV genes (He et al., 2017), viral encoded non-coding RNAs (Guo et al., 2019; Zografidis et al., 2015) and viral peptide (Hu et al., 2019a) have also been identified. Our previous studies have found alterations in a large number of genes related to important signaling pathways, including those associated with innate immunity, development and metabolism following BmCPV infection (Guo et al., 2015; Hu et al., 2018). Although there has been many studies that focus on the pathogenesis of BmCPV, the exact mechanism of BmCPV infection, as well as the interactions between virus and host factors remain unclear. As
non-living entities, the life cycles of viruses depend on the host cells that provide essential materials and energy for their replication (Buck et al., 2017; Sanchez and Lagunoff, 2015). Lipid is a vital component of cellular and organelle membranes that plays crucial roles in the regulation of many biological processes including virus-host interaction. An increasing number of studies in recent years have demonstrated changes of lipid metabolism in host cells after virus infection. It has been shown that infection by some single-stranded RNA (ssRNA) viruses can alter the lipid metabolism and other biological processes of the host cells to facilitate the completion of the virus life cycle (Sanchez and Lagunoff, 2015). Free fatty acids (FFAs), which can be used directly by the body for energy metabolism have been shown to be associated with the life cycle of various RNA viruses, including influenza A virus (IAV) (Lim-suwat et al., 2020), classical swine fever virus (CSFV) (Ma et al., 2019), Middle East respiratory syndrome coronavirus (MERS-CoV) (Yan et al., 2019a), hepatitis C virus (HCV) (Hofmann et al., 2018), zika virus (ZIKV) (Queiroz et al., 2019) and ebola virus (Kyle et al., 2019). Furthermore, some lipids are produced in the cells and others are imported from extracellular environment (Lim-suwat et al., 2020). Some lipid-related constituents in the cell membrane are indispensable for virus entry and replication. Viral receptor ICAM-5 plays important roles in the replication of Enterovirus D68 (Jiang et al., 2020). Apolipoprotein A-I binding protein is an intrinsic factor that suppresses human immunodeficiency virus (HIV) replication (Dubrovsky et al., 2020). Zebrafish C-reactive protein-like protein inhibits spring viraemia of carp rhadovirus replication by causing alterations of cholesterol ratios in the host cellular membranes (Bello-Perez et al., 2020). Lipids have also been shown to play important roles in the replication of coxsackievirus B3 (Wang et al., 2020). An increased level fatty acid biosynthesis coupled with accumulation of free fatty acids that are associated with virus replication has been observed in host cells following CSFV infection (Ma et al., 2019). Phosphorylated 5′-adenosine monophosphate-activated protein kinase induced by the infection of porcine reproductive and respiratory syndrome virus can inactivate the fatty acid biosynthesis pathway, playing an antagonistic role in the virus replication (Long et al., 2019). Upon enterovirus A71 and coxsackievirus A16 infection, disturbed lipid homeostasis in the infected cells has been shown to be related to virus replication (Yan et al., 2019b). Fatty acid synthase and stearoyl-CoA desaturase required for fatty acid metabolism have been demonstrated to be required for chikungunya virus infection (Bakhache et al., 2019). Cholesterol can also affect hemagglutinin fusion activity and hence the virus assembly of influenza viruses (Hu et al., 2019b). ZIKV-triggered lipid metabolism that has been found in patient serum samples may also be associated with virus replication (Queiroz et al., 2019). Host cell lipid response has been observed to be significantly altered upon human coronavirus 229E infection (Yan et al., 2019a). The increased levels of polyunsaturated fatty acids that have been found upon HCV infection are essential for viral progeny production (Hofmann et al., 2018). Our previous study has shown that ganglioside GM2 and cholesterol in the cellular membrane are required for BmCPV attachment and entry (Guo et al., 2015). Analysis of differential expressed circRNAs has revealed changes in the metabolism of fatty acids upon BmCPV infection (Hu et al., 2018). All these reports have demonstrated that lipid-related metabolisms play vital roles in the life cycles of RNA viruses. It is worth noting that the virus-induced changes of cellular metabolomic activity generally facilitate the progress of virus infection (Diop et al., 2018; Tian et al., 2019). To date, the lipid metabolism in cell infected by double-stranded RNA (dsRNA) viruses, such as, BmCPV remains unknown. In this study, silkworm ovary-derived BmN cells, which are normally unsusceptible of but infectable by BmCPV were used as the model to investigate the lipid metabolomics of BmCPV-infected cells. We found that the expression of multiple lipid classes was changed in BmN cells upon BmCPV infection. An understanding of the post-infection cellular metabolism changes may provide some insights on the mechanism of viral pathogenesis in order to contribute to the development of novel therapeutic strategies for viral diseases.

2. Materials and methods

2.1. Cell culture and virus

BmN cells were cultured at 26 °C in complete TC-100 medium supplemented with 10% fetal bovine serum. The virion solution of BmCPV-SZ strain used for infecting BmN cells was stored in our lab.

2.2. Virus infection

BmN cells (1 × 10⁶) were seeded in T25 flasks and cultured in complete medium overnight. Out of 16 flasks of prepared cell culture, 8 flasks were used for virus infection, while the other 8 flasks without virus infection were used as controls. Cells were infected with 5 μl BmCPV solution (10⁶ polyhedron/1 mL lysis buffer) (Zhang et al., 2019) and incubated at 4 °C for 30 min before washing with phosphate buffer saline (PBS) buffer followed by further incubation at 26 °C in fresh complete medium. All samples were collected at 48 h post-infection.

2.3. Sample preparation

BmN cells with (CASE) and without (CON) BmCPV infection were collected by centrifugation and the precipitates were washed with PBS buffer at 4 °C. To evaluate BmCPV infection in BmN cells, a proportion of the cell precipitates were used for total protein extraction followed by western blotting to detect BmCPV structural protein VP7 using anti-mouse VP7 primary antibody (He et al., 2017) and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG secondary antibody (Abcam). The expression level of vp1 gene was detected from CASE samples using real-time PCR with the primer pair qvp1-F (5′-GGTCTCGAC GTGAATACCGA-3′) and qvp1-R: 5′-TGGTCGTCCTCAGACTGAC-3′. Tif4a gene was used as the internal reference with the primer pair qTif4a-F (5′-GAATTGGACCTGGGACACT-3′) and qTif4a-R (5′-CTGACTGGGCTTGGACGATA-3′). For lipid metabolomic analysis, methanol: water (1:1) solution was added to each sample and Lyso PC 17:0 (0.3 mg/mL) dissolved in methanol was used as internal standard. Trichloromethane (600 μl) was added to each sample Before 1 × 10⁶ of BmN cells were used for cell lysis with an ultrasonic homogenizer set at 500 W for 6 min (6 s power on, 4 s power off). After centrifugation, 600 μL of cell lysis solution drawn from the lower part of the mixture was dried Before 600 μL of methanol:trichloromethane (1:2) was added for extraction by ultrasonication. After that, 300 μl of solution was drawn from the lower part of the extract and dried before reconstitution with 300 μL of isopropanol:methanol (1:1). Following centrifugation, 200 μL of the supernatant was used for LC-MS analysis.

2.4. Chromatographic mass spectrometry

Metabolic profiling was analyzed using a Nexera UPLC (Shimadzu, Kyoto, Japan) system fitted with Q-Exact quadrupole-Orbitrap mass spectrometer equipped with heated electrospray ionization (HESI) source (Thermo Fisher Scientific, Waltham, MA, USA). For sample preparation, acetonitrile:water (6:4) was used as Solvent A, while acetonitrile:isopropanol (1:9) as Solvent B. Both solvents contained 0.1% formic acid and 10 mM/L ammonium formate. Samples were run on an ACQUITY UPLC BEH C18 column in both positive and negative modes. With the mass range of the instrument set at m/z 120–1800, the precursor and product ions with accurate m/z on the mass spectrometry profile were matched against LipidBlast database.

2.5. Data preprocessing and statistical analysis

Raw data generated from LC-MS were analyzed by progenosis QI software (version 2.3, Waters Corporation, Milford, USA). Positive and
negative data were combined and imported into SIMCA software package (version 14.1, Umetrics, Umeå, Sweden). Metabolic alterations among the experimental groups were shown with Principle component analysis (PCA) and (orthogonal) partial least-squares-discriminant analysis (O) PLS-DA. Variable importance in the projection (VIP) scores from the OPLS-DA model was used to rank the contribution of each variable, where variables with VIP > 1 were considered to be significant. To avoid overfitting, default 7-round cross-validation was applied to exclude from the model in each round. Differential metabolites were selected based two statistically significant thresholds of VIP scores and p values. The p values were acquired from two-tailed Student’s t-test with normalized peak areas.

3. Results

3.1. Lipidomics analysis in BmN cells infected with BmCPV

To study the effects of lipid metabolism in vitro after BmCPV infection, BmCPV-infected BmN cells were collected and analyzed by western blotting and real-time PCR to evaluate the virus multiplication. The results showed that BmCPV structural protein VP7 was identified in BmCPV-infected BmN cells at 48 h post infection (hpi). Furthermore, the expression level of vp1 was also detected in the same cell pools (Fig. 1A). Thus, BmN cells were successfully infected by BmCPV.

Lipidomic analysis using UHPLC-QTOF-MS were performed with lipids isolated from CASE and CON at 48 hpi (Fig. 1B). To validate the reliability of UHPLC-QTOF-MS in BmN cells, both the positive (+) or negative ion mode (−) were featured in the base peak ion chromatograms (matching similarity >80%) to display the distribution of metabolites. The results revealed that metabolite retention in samples within the same group were not significantly changed and that the signal representing each substance was detected with disparity (Fig. 2). Therefore, we concluded that the data from UHPLC-QTOF-MS analysis are suitable and reliable, which can be used in the following study.

3.2. BmCPV infection alters intracellular lipid homeostasis

To truly display the alterations of intracellular lipid homeostasis between CASE and CON, SIMCA software package was applied to integrate the positive and negative ion data for principle component analysis (PCA). A significant trend of lipid distribution was found between CASE and CON in the PCA score chart (Fig. 3A and B). To show the altered metabolites between CASE and CON, OPLS-DA model was applied. The main quality parameters (R2X = 0.953, R2Y = 0.934, Q2 = 0.91) from the OPLS-DA model showed that the two groups (CASE and CON) were effectively distinguished from each other (Fig. 3C). With 7-fold cross-validated parameters and 200 response permutation test, the OPLS-DA model in the multivariate analysis accompanied by data comparison from the two groups were considered satisfactory. To assess the quality of the current model, R2Y and Q2 were used as the parameters. As shown in Fig. 3D and E, our analysis (R2Y > 0.5 and Q2 > 0.5) indicated that our established model was suitable and reliable. These results demonstrated that the established model is reliable, and can be used to identify differential lipids between CASE and CON.

3.3. Identification of differential lipids profile

To screen the significant differential lipids between CASE and CON, univariate and multivariate analyses were performed based on the lipid mass spectrum. In univariate analysis, a total of 345 differential lipids were detected between CASE and CON. A total of 194 and 151 differential lipids were respectively identified in the positive and negative mode (Supplementary Table 1).

To further investigate the significant differential lipids from the data obtained from univariate analysis, parameters, where VIP > 1 and p value < 0.05 were applied in the screening process by combining multidimensional and one-dimensional analysis. A total of 34 significant differential lipids were detected between CASE and CON, where, 24 and 10 lipids were respectively identified in the positive and negative model (Table 1). These results indicated that BmCPV infection can markedly alter the intracellular lipid homeostasis in BmN cells.

3.4. Identification of differential lipids in BmCPV-infected BmN cells

To further characterize the differential lipids, volcano plots were applied to visualize the differential metabolites (Fig. 4A). Meanwhile, the correlation between identified lipids was shown using Hierarchical Clustering Analysis (HCA), which indicated that the differential lipids identified from CASE were markedly distinguished from those identified from CON (Fig. 4B).

A total of 34 significantly differential lipids were identified between CASE and CON, which can be clustered into 11 classes, including 3 triacylglycerides (TG), 10 phosphatidylcholine (PC), 4 phosphatidyethanolamine (PE), 4 sphingomyelin (SM), 4 phospholipids (PS), 2 monoetherphosphatidylcholin (MePC), 1 ceramide (Cer), 3 ceramide phosphoethanolamine (CerPE), 1 glucoside ceramide (Hex1cer) and 2 cardiolipin (CL) (Table 1).

As shown in Fig. 5, upon BmCPV infection, except PC (18:1/18:1),

![Fig. 1](image-url) Identification of BmCPV components. (A) Detection of structural protein VP7 by western blotting and vp1 gene by real-time PCR. (B) Flow chart of lipidomic analysis using UHPLC-QTOF-MS.
Fig. 2. Typical base peak ion (BPI) chromatograms under positive (A) or negative (B) ion mode. Low quality samples were removed from the analysis. CPV2-CPV8, CASE, n = 7; CON1–CON8, CON, n = 5.

Fig. 3. Sample score diagrams of BmCPV-uninfected BmN cells (CON) and BmCPV-infected BmN cells (CASE). (A) PCA score chart of CON and CASE. (B) PLS-DA score chart of CON and CASE. (C) OPLS-DA score chart of CON and CASE. (D–E) Validation plot of the OPLS-DA model obtained from 200 permutation tests. All samples were mixed in equal amounts. QC, quality control.
Table 1
Differential expressed metabolic classified into 11 categories.

| Lipid Group | Class | Fatty Acid | VIP | P value | log2(FC) |
|-------------|-------|------------|-----|---------|----------|
| PS(35:1)   | PS(35:1)-H | PS (35:1) | 1.13005 | 6.5E-06 | 2.73869 |
| MePC(33:1) | MePC(33:1)-Na | MePC (33:1) | 1.5893 | 2.5E-05 | 1.54273 |
| PC(34:4)   | PC(34:4)-H | PC (34:4) | 1.6075 | 4E-07 | 2.05284 |
| CL(70:2)   | CL(70:2)-2H | CL (70:2) | 1.74391 | 1.3E-06 | 3.05962 |
| CL(78:1)   | CL(78:1)-2H | CL (78:1) | 1.00694 | 0.00017 | 2.52006 |
| PS(39:1)   | PS(39:1)-H | PS (39:1) | 2.25766 | 8.3E-06 | 1.4497 |
| MePC(38:2) | MePC(38:2)-H4 | MePC (38:2) | 1.29956 | 1.3E-06 | 1.80126 |
| SM(d34:1)  | SM(d34:1)-Na | SM (d34:1) | 1.37159 | 7.3E-06 | 2.90571 |
| Hex1Cert(d14:1/22:0) | Hex1Cert(d36:1)-H | Hex1Cert (d14:1/22:0) | 1.22963 | 1.2E-05 | 2.28268 |
| PC(16:1/16:1) | PC(32:2)-HCOO | PC (16:1/16:1) | 2.05835 | 2.2E-06 | 2.03429 |
| PE(18:5/16:1) | PE(34:1)-H | PE (18:5/16:1) | 2.28767 | 6.3E-07 | 1.99762 |
| TG(16:1/16:1/18:1) | TG(50:3)+NH4 | TG (16:1/16:1/18:1) | 1.17639 | 0.00496 | 0.90632 |
| PC(16:0/16:1) | PC(32:1)-HCOO | PC (16:0/16:1) | 1.38262 | 9.4E-06 | 2.13527 |
| PC(18:1/18:1) | PC(36:2)-Na | PC (18:1/18:1) | 1.50651 | 0.00528 | 0.67001 |
| TG(16:1/18:1/18:1) | TG(52:1)+NH4 | TG (16:1/18:1/18:1) | 1.22963 | 1.2E-05 | 2.28268 |
| PE(16:1/18:1) | PE(34:2)-H | PE (16:1/18:1) | 2.50912 | 3.2E-06 | 2.57009 |
| TG(16:0/18:1/18:1) | TG(52:1)+NH4 | TG (16:0/18:1/18:1) | 1.129956 | 1.3E-06 | 1.80126 |
| SM(d38:1)  | SM(d38:1)-H | SM (d38:1) | 1.01517 | 3E-07 | 2.19507 |
| SM(d38:4)  | SM(d38:4)-H | SM (d38:4) | 1.19202 | 9.4E-07 | 1.84312 |
| CerPE(d36:2) | CerPE(d36:2)-H | CerPE (d36:2) | 1.04533 | 1.8E-07 | 2.255 |
| PC(35:1)   | PC(35:1)-H | PC (35:1) | 1.08274 | 1.4E-06 | 2.13527 |
| PC(38:5)   | PC(38:5)-H | PC (38:5) | 1.28944 | 5.9E-05 | 1.91246 |
| PS(38:3)   | PS(38:3)-H | PS (38:3) | 1.00837 | 7.4E-06 | 1.63198 |
| CerPE(d34:1) | CerPE(d34:1)-H | CerPE (d34:1) | 2.63867 | 1.5E-07 | 2.13403 |
| Cer(m36:3) | Cer(m36:3)-H | Cer (m36:3) | 1.82975 | 1.1E-05 | 2.18173 |

Fig. 4. Volcano plots and Hierarchical Clustering analysis of identified metabolites. (A) Volcano plots of all identified metabolites in CASE. Red: Upregulation; blue: Downregulation; gray: Not significant. (B) Hierarchical Clustering map of significant differential levels of metabolites in CASE. Low quality samples were removed from the analysis. FC, fold change; CPV2-CPV8,CASE, n = 7; CON1–CON8, CON, n = 5. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
most of the altered lipids were observed to be upregulated, which included PS (35:1), MePC (33:1), PC (34:4) and CL (70:2). Our results suggested that the metabolisms of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and phospholipids were significantly changed in BmCPV-infected BmN cells.

3.5. Kyoto encyclopedia of genes and genomes (KEGG) analysis

To understand the potential regulatory mechanisms of significant differential lipids associated with the metabolic pathway upon BmCPV infection, several pathway maps in the KEGG database were constructed based on the pathway information. As shown in Fig. 6, upon BmCPV infection, pathways related to glycerophospholipid metabolism, glycosphingolipid (GPI)-anchor biosynthesis, autophagy, linoleic acid metabolism, alpha-linolenic acid metabolism and arachidonic acid metabolism were found to be most significantly affected. These results suggested that these metabolic pathways may be applied for intracellular signaling upon virus infection.

4. Discussion

In our previous study, multiple components in membrane lipid rafts, including proteins, lipids and glycans have been shown to play important roles in the virus cell entry (Zhu et al., 2018). It is worth noting that the movement of lipid rafts in cellular membrane is induced upon virus infection, a process that includes the recruitment and interaction of potential receptors with the virions during cell attachment (Suzuki and Suzuki, 2006; Wang et al., 2009). The global lipidomic profiles upon BmCPV infection in BmN cells remain unknown. In this study, lipidomic analysis was applied to identify the cellular lipid changes upon BmCPV infection. A total of 34 lipids, including categories of TG, PC, PE, SM, PS, MePC, Cer, CerPE, Hex1Cer, CL and Cer were significantly changed upon BmCPV infection. These results indicated that global lipidomic homeostasis in BmN cells is significantly changed under BmCPV infection.

In virus life cycle, the first step involves successful attachment of the virion particles on the membrane of infected cells, where the presence lipid rafts enriched in protein, cholesterol and sphingolipids have been reported to play important roles in the cell entry of many viruses (Suzuki and Suzuki, 2006; Wang et al., 2009). Cellular lipids have also been reported to play crucial roles in the replication of a number of viruses, including enveloped viruses and no-enveloped viruses (Suzuki and Suzuki, 2006; Wang et al., 2009; Hu et al., 2017). During virus infection, the host cellular metabolism, including the homeostasis of nucleotide anabolism, amino acid and lipids, which can limit the host cell growth and proliferation, is hijacked by the infecting virus (Liu et al., 2019; Ghazal et al., 2000). In our previous study, cellular membrane constituents, such as ganglioside GM2 and cholesterol have been found to be required for BmCPV attachment and entry (Zhu et al., 2018). In addition, fatty acid metabolism has been observed to be significantly changed by the differentially expressed circRNAs in the midgut of silkworms infected by BmCPV (Hu et al., 2018). These KEGG enrichment analysis of differential proteins from the midgut of silkworms infected by BmCPV have shown that oxidative phosphorylation and steroid hormone biosynthesis are the most effected pathways at certain time (Gao et al., 2017). These results have suggested that the consistency of lipids present in cellular membrane or in cells are associated with BmCPV infection. In this study, whilst the levels of cholesterol were found to be remain unchanged, the levels of a number of lipids, including phosphatidylethanolamine, sphingomyelin, phosphatidylserine and phosphatidylycholine were observed to be increased.
Glycerophospholipids are the primary components of the membrane. The PS lipids were reported to play vital roles in some viruses’ replication (Adu-Gyamfi et al., 2015; Nanbo et al., 2018; Soni and Stabelin, 2014). The phospholipids metabolism was also found to be related to the lipid regulation, lipoprotein, whole-body energy metabolism and metabolic disorders (van der Veen et al., 2017). Therefore, we speculated that the disorder of phospholipids metabolism may be associated with the BmCPV entry. Most of the glycerophospholipids were identified with unsaturated fatty acids, and it is noted that the condensation of phospholipid films was negative correlated with unsaturated fatty acids under BmCPV with unsaturated fatty acids, and it is noted that the condensation of phospholipid films was negative correlated with unsaturated fatty acids (Hac-Wydro et al., 2009). Therefore, we speculated that the increased level of glycerophospholipids with unsaturated fatty acids under BmCPV infection may be related to the geometry of phospholipid monolayers in cellular membrane. PE derived from PS was validated to contribute to the formation of neurite in cells (Ikemoto and Okuyama, 2000). The increased PEs were identified in BmCPV infected BmN cells, indicating that these PEs may be affecting the chemical signal materials transport among the BmN cells.

A study has reported that the levels of sphingomyelin species were decreased during acute HCV infection (Diamond et al., 2010). Whilst decreased levels of sphingomyelin have been identified in BmCPV-infected BmN cells, the levels of other species with different long aliphatic chains have been unaltered. Meanwhile, Cer(m36:3) which constitutes the corresponding precursors and reconversion products have been found to be increased as well.

In addition, several types of Cer with different length of fatty acids have been reported to be associated with apoptosis and autophagy (Kyle et al., 2019). Data from these reports have been consistent with our predicted results by KEGG analysis that shows enrichment of the altered lipids in the pathway of autophagy. Taken together, autophagy is induced via lipid metabolic changes during the process of BmCPV infection. However the exact mechanism of how autophagy is induced by BmCPV infection requires further studies.

The level of TGs was found to be significantly increased in CASE, which indicated a higher level of lipolysis in BmN cells as a result of virus infection. Notably, the adipose tissue is an important energy reservoir in animals, which can be mobilized in response to adaptations of environmental stimuli, such as hormonal and energetic signals (Kyle et al., 2019; Hodson et al., 2008). During BmCPV infection, most silkworms stop consuming mulberries. A study has found that, under starvation condition, the activity of lipolysis is increased in response to virus infection, and that TGs were broken down to FFAs, glycerol and diglyceride(DG) (Kyle et al., 2019). Normally, as FFAs are toxic to cells, they are recycled back into TGs. Studies have shown that, upon NDV infection, oxidative stresses are increased, and that the activities of antioxidants are decreased in the brain and liver of chickens (Subbaia et al., 2011; Rehman et al., 2018). Our findings suggested that BmCPV-infected BmN cells may exhibit similarities to those of chickens, that cells can undergo excessive fatty acid β-oxidation and oxidative stress.

Through lipid pathways analysis, we found that glycerophospholipid metabolism, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, autophagy, linoleic acid metabolism, alpha-linolenic acid metabolism and arachidonic acid metabolism are the most enriched pathways and may be associated with BmCPV infection. Glycerophospholipid metabolism is involved in the biosynthesis of PC and PE. An increasing number of studies have shown that disorders of glycerophospholipid metabolism in cells can lead to onset of diseases (Bello-Perez et al., 2020; Wang et al., 2020). In this study, the metabolism of glycerophospholipids, especially PC(38:2) and PE(36:1) were found to be the most enriched pathway during BmCPV infection, indicating that glycerophospholipid metabolism may be crucial for the pathogenesis of BmCPV. Although there has been no reports about other related pathways, the potential roles of tglycerophospholipid metabolism in BmCPV infection requires further exploration. Collectively, we demonstrated that intracellular lipid homeostasis is disturbed upon BmCPV infection. A total of 34 significant differential lipids are detected in BmN cells upon BmCPV infection, where 24 and 10 lipids are respectively identified in the positive and negative models. All the identified differential lipids are clustered into TG, PC, PE, SM, PS, MePC, Cer, CerPE, Hax1cer and CL categories. These data suggested that some specific cellular lipids are required for virus infection. Further analysis of the pathways related to the altered lipids (such as PE and PC) indicates that glycerophospholipid metabolism is one of the most enriched pathways amongst other the top 6 pathways identified by KEGG. The lipidomic and transcriptomics profiles can be used to uncover the mechanism of interaction between silkworms and BmCPV in the future.
Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

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