A Transcriptome Analysis Suggests Apoptosis-Related Signaling Pathways in Hemocytes of Spodoptera litura After Parasitization by Microplitis bicoloratus

Ming Li1*, Zunyu Pang1*, Wei Xiao1, Xinyi Liu1, Yan Zhang1, Dongshuai Yu1, Minjun Yang2, Yang Yang1, Jiansheng Hu1, Kajjun Luo1**

1 School of Life Sciences, Yunnan University, Kunming, P. R. China; Key Laboratory for Animal Genetic Diversity and Evolution of High Education in Yunnan Province, Yunnan University, Kunming, P. R. China, 2 Shanghai—Ministry of Science and Technology Key Laboratory of Health and Disease Genomics, Chinese National Human Genome Center at Shanghai, Shanghai, P. R. China

Abstract

Microplitis bicoloratus parasitism induction of apoptotic DNA fragmentation of host Spodoptera litura hemocytes has been reported. However, how M. bicoloratus parasitism regulates the host signaling pathways to induce DNA fragmentation during apoptosis remains unclear. To address this question, we performed a new RNAseq-based comparative analysis of the hemocytes transcriptomes of non-parasitized and parasitized S. litura. We were able to assemble a total of more than 11.63 Gbp sequence, to yield 20,571 unigenes. At least six main protein families encoded by M. bicolorus bracovirus are expressed in the parasitized host hemocytes: Ankyrin-repeat, Ben domain, C-type lectin, Egf-like and Mucin-like, protein tyrosine phosphatase. The analysis indicated that during DNA fragmentation and cell death, 299 genes were up-regulated and 2,441 genes were down-regulated. Data on five signaling pathways related with cell death, the gap junctions, Ca+++, PI3K/Akt, NF-kB, ATM/p53 revealed that CypD, which is involved in forming a Permeability Transition Pore Complex (PTPC) to alter mitochondrial membrane permeabilization ( MMP), was dramatically up-regulated. The qRT-PCR also provided that the key genes for cell survival were down-regulated under M. bicoloratus parasitism, including those encoding Inx1, Inx2 and Inx3 of the gap junction signaling pathway, p110 subunit of the PI3K/Akt signaling pathway, and the p50 and p65 subunit of the NF-kB signaling pathway. These findings suggest that M. bicoloratus parasitism may regulate host mitochondria to trigger internucleosomal DNA fragmentation. This study will facilitate the identification of immunosuppression-related genes and also improves our understanding of molecular mechanisms underlying polydnavirus-parasitoid-host interaction.

Introduction

Polydnaviruses (PDVs) have a very special life cycle. Unlike many viruses, they are not always obligate intracellular parasites, replicating inside living host cells to produce virions that can transfer genes to other cells [1–4]. Rather, PDVs are obligate symbionts of many endoparasitic wasps in the families Braconidae (carrying bracovirus) and Ichneumonidae (carrying ichnovirus). Both viruses have similar life cycles, wherein viral DNAs are integrated into a wasp’s genome via Wasp Integration/Excision Motif (WIM) [5] and transmitted vertically to the wasp’s offspring.

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* Email: kajjun_luo@ynu.edu.cn
** These authors contributed equally to this work.

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elements, including AIF, EndoG and DFF40. Every element is regulated by different signaling pathways, defined as extrinsic and intrinsic pathways. Extrinsic apoptosis pathway is triggered by the ligand-induced oligomerization of specific cell surface receptors, and this process induces the intracellular assembly of the death-inducing signaling complex for the activation of a caspase cascade initiated from caspase 9 that results in activation of caspase 3 and further cascade activation of DFF (cleavage of DFF45 releases DFF40 into the nucleus). DFF, a heterodimeric protein comprising 45 kDa and 40 kDa subunits termed ICAD/DFF45 and CAD/DFF40 [7]. The DFF complex is localized in the cellular cytoplasm, resulting in the triggering of extrinsic apoptotic stress, and activated caspase 3 cleaves DFF45 and dissociates DFF40. Caspase 7 and Granzyme B also can cleave DFF45 but with a lower efficiency than caspase 3 [8]. Activated DFF40 translocates into the nucleus. In the nucleus, the activation of DFF40 is enhanced by interaction with the chromosomal protein Histone H1 and it cleaves chromosomal DNA at internucleosomal sites into fragments of ~200 bp. [9–11]. In contrast, the intrinsic pathway is also controlled by mitochondria, which collects and integrates pro- and anti-apoptotic signal stimuli from other organelles as well as from the extracellular microenvironment, such as DNA damage produced by Ataxia-Telangiectasia Mutated (ATM), endoplasmic reticulum (ER) stress and calcium overload. The intrinsic pathway can mediate caspase-independent and caspase-dependent apoptosis. Following intrinsic apoptotic stress triggering, EndoG is released from the mitochondrial intermembrane space and moves to the nucleus to produce nucleosomal DNA fragmentation, giving rise to 200–5,000 bp sized fragments in a caspase-independent manner. AIF is another endonuclease released from the mitochondrial intermembrane space. It is a flavoprotein that produces DNA fragments up to 5,000 bp in size, and it also does not require caspase activation [12]. Releasing cytochrome c can also mediate cell death via activation of caspase 8, which triggers a caspase-dependent apoptosis.

Numerous viruses are well known to modulate the mitochondrial apoptosis of infected host cells by altering Mitochondrial Membrane Permeabilization (MMP) in a direct and indirect manner with viral proteins. MMP regulation is performed via the Voltage-Dependent Anion Channel (VDAC) of the outer membrane (OM), the Adenine Nucleotide Translocase (ANT) of the inner membrane (IM), and cyclophilin D (CypD) of matrix proteins. Viral proapoptotic proteins are direct inducers of MMP. They include viral protein R (Vpr), which directly interacts with ANT and VDAC, thereby triggering MMP associated with mitochondrial membrane potential (∆Ψm) loss, mitochondrial intermembrane space (IMS) protein release, and caspase cascade activation. Viral proapoptotic proteins are also indirect MMP facilitators and promote apoptosis via both p53-dependent and -independent mechanisms [13]. The alteration of membrane permeability may release apoptotic-promoting factors from the mitochondria, such as AIF, EndoG, and Cyt c in the IMS, ultimately resulting in nuclear translocation. All of these signaling pathways involved in apoptotic DNA fragmentation are stimulated by intrinsic stress through the mitochondria via EndoG and AIF, in a process that is also called caspase-independent cell death, involving release of Cyt c, and extrinsic stress through caspase cascades via DFF40, which is also called caspase-dependent cell death [14].

After apoptotic stimulation, DFF40, EndoG and AIF migrate to the nucleus under the control of critical apoptosis-involved signaling pathways, including the gap junction signaling pathway, Ca²⁺ signaling pathway, PI3K/Akt signaling pathway, NF-κB signaling pathway, and ATM/p53 signaling pathway. The gap junction signaling pathway induces apoptosis via regulation of the permeability of the plasma membrane resulting in alteration of intracellular and extracellular communication via transmission of small molecules, such as apoptotic signaling ATP. Gap junction proteins are the target proteins of activated caspase 3 [15] and also Ca²⁺. The Ca²⁺ signaling pathway is involved in apoptosis via altering the permeability of the mitochondrial membrane to release apoptosis-inducing factors to trigger apoptotic caspase-dependent and -independent pathways [13]. Apoptotic caspase-dependent signaling pathways include the PI3K/Akt signaling pathway and NF-κB signaling pathway via regulation of caspase 3, and the apoptotic caspase-independent signaling pathways include regulation of the ATM/p53 signaling pathway by AIF expression [16]. The PI3K/Akt signaling pathway is crucial to many aspects of cell growth and survival, and its inhibition increases DNA fragmentation by the help of caspase 3 [17]. Baculoviruses inhibit cell apoptosis through activating the PI3K/Akt signaling pathway [18]. Nuclear Factor-κB (NF-κB) transcription factors regulate the expression of antimicrobial peptides (AMPs) and many genes involved in cell survival, such as c-IAP1/2, XIAP, and Bcl-XL. All NF-κB s are homo- or heterodimers of Rel proteins, such as p50/p65 subunits. p53 plays an important role in suppressing tumorigenesis through inducing genomic stability via DNA repair, cell cycle arrest and apoptosis. p53 promotes AIF activity and caspase-independent cell death by binding to a p53-responsive element (p53RE) in the AIF promoter, which ultimately results in efficient induction of large-scale DNA fragmentation (5 kb) [16].

In this paper, we aimed to clarify the mechanism of parasitism induction of host hemocyte apoptosis. To test the hypothesis that parasitism regulates host apoptotic signaling pathways to produce apoptotic DNA fragmentation involved in nuclear elements to the nucleus, resulting in internucleosomal DNA fragmentation from 5 kb to 200 bp, we sequenced the RNA from hemocytes of the Oriental Leafworm Moth Spodoptera litura parasitized by the wasp Microplitis bicoloratus and compared the transcriptome of hemocytes from non-parasitized controls. Using this transcription data, we obtained an overview on how M. bicoloratus parasitism regulates apoptosis signaling pathways during the immunosuppression and induced killing of host S. litura hemocytes. Furthermore, we proposed M. bicoloratus bracovirus products to regulate mitochondrial permeability to trigger internucleosomal DNA fragmentation and block a set of key genes in the cell survival signaling pathway.

Results

Transcription sequencing and analysis

Gene expression profiling of S. litura hemocytes, both non-parasitized and parasitized, was achieved via sequencing with an Illumina Hiseq 2000 (Table S1). A million paired-end sequences (Table S2) from four samples, M1 and M2 from S. litura hemocytes parasitized by M. bicoloratus and samples S1 and S2 from non-parasitized S. litura hemocytes, were assembled into 3 different transcriptomes, M (M1+M2), S (S1+S2) and All (M1+M2+S1+S2), using Trinity. This gave a large number of EST cluster contigs: 15,208 (M), 15,206 (S) and 20,571 (All) (Table S3). A comparison of the transcriptome pattern of the average M and average S transcriptomes indicated that 299 consensus genes were up-regulated, and 2,441 genes were down-regulated, under M. bicoloratus parasitism in host hemocytes.
Table 1. Transcription of *M. bicoloratus* bracovirus genes during development of parasitoid *M. bicoloratus* in host hemocytes.

| Protein Family | Protein | Consensus ID | Length | NCBI_E_value | NCBI_ID | Function | Species |
|----------------|---------|--------------|--------|---------------|---------|----------|---------|
| Ankyrin-repeat | MbANK1  | comp576933_c0_seq1 | 207    | 1.00E-14      | ref|YP_239402.1| viral ankyrin 1 | [Microplitis demolitor bracovirus] |
|                | MbANK1  | comp119151_c0_seq1 | 558    | 3.00E-58      | ref|YP_239402.1| viral ankyrin 1 | [Microplitis demolitor bracovirus] |
|                | MbANK1  | comp26305_c0_seq1 | 561    | 6.00E-40      | ref|YP_239402.1| viral ankyrin 1 | [Microplitis demolitor bracovirus] |
|                | MbANK2  | comp728608_c0_seq1 | 225    | 6.00E-35      | ref|YP_239372.1| viral ankyrin 2 | [Microplitis demolitor bracovirus] |
|                | MbANK3  | comp18368_c0_seq1 | 525    | 1.00E-30      | ref|YP_239406.1| viral ankyrin; | [Microplitis demolitor bracovirus] |
| Ben domain     | MbBEN1  | comp20976_c0_seq1 | 1053   | 4.00E-54      | ref|YP_239364.1| hypothetical protein | [Microplitis demolitor bracovirus] |
|                | MbBEN1  | comp20957_c0_seq1 | 1572   | 1.00E-115     | ref|YP_239364.1| hypothetical protein | [Microplitis demolitor bracovirus] |
|                | MbBEN2  | comp9824_c0_seq1  | 2046   | 1.00E-166     | ref|YP_184800.1| CcBV_9.1 | [Microplitis demolitor bracovirus] |
|                | MbBEN3  | comp177162_c0_seq1| 618    | 2.00E-19      | ref|YP_184814.1| CcBV_12.2 | [Cotesia congregata bracovirus] |
|                | MbBEN4  | comp252441_c0_seq1| 237    | 2.00E-34      | gb|AEE09539.1| DUF-like 1 protein | [Cotesia congregata bracovirus] |
|                | MbCLECT1| comp19781_c0_seq1 | 666    | 1.00E-34      | ref|YP_184818.1| CcBV_2-13.1 | [Cotesia congregata bracovirus] |
|                | MbCLECT2| comp37160_c0_seq1 | 474    | 1.00E-43      | gb|AE09593.1| lectin | [Cotesia vestalis bracovirus] |
|                | MbCLECT3| comp375850_c0_seq1| 333    | 1.00E-31      | gb|AAS10157.1| lectin | [Cytoplaus Polydnavirus] |
| C-type lectin  | MbCRP1  | comp22262_c0_seq1 | 561    | 8.00E-67      | gb|ABB922678.1| CRP1, egf 1.5 | [Microplitis bicoloratus bracovirus] |
|                | MbGlc1.8| comp118173_c0_seq1| 153    | 5.69E-14      | ref|YP_239419.1| Glc1.8 | [Microplitis demolitor bracovirus] |
|                | MbGlc1.8| comp85587_c0_seq1 | 126    | 1.46E-54      | ref|YP_239404.1| Glc1.8 | [Microplitis demolitor bracovirus] |
|                | MbPTP1  | comp360492_c0_seq1| 444    | 7.00E-35      | ref|YP_239404.1| PTP 1 | [Microplitis demolitor bracovirus] |
|                | MbPTP2  | comp330407_c0_seq1| 417    | 7.00E-49      | ref|YP_239404.1| PTP 1 | [Microplitis demolitor bracovirus] |
|                | MbPTP2  | comp207973_c0_seq1| 375    | 4.00E-64      | ref|YP_239382.1| PTP 2 | [Microplitis demolitor bracovirus] |
|                | MbPTP2  | comp130820_c0_seq1| 618    | 1.00E-106     | ref|YP_239382.1| PTP 2 | [Microplitis demolitor bracovirus] |
|                | MbPTP3  | comp556935_c0_seq1| 354    | 4.00E-59      | ref|YP_239383.1| PTP 3 | [Microplitis demolitor bracovirus] |
|                | MbPTP4  | comp188579_c0_seq1| 177    | 3.00E-15      | ref|YP_239386.1| PTP 4 | [Microplitis demolitor bracovirus] |
|                | MbPTP4  | comp498102_c0_seq1| 330    | 2.00E-33      | ref|YP_239386.1| PTP 4 | [Microplitis demolitor bracovirus] |
|                | MbPTP4  | comp584871_c0_seq1| 201    | 9.00E-20      | ref|YP_239386.1| PTP 4 | [Microplitis demolitor bracovirus] |
|                | MbPTP5  | comp279111_c0_seq1| 249    | 2.00E-11      | ref|YP_239381.1| PTP | [Microplitis demolitor bracovirus] |
|                | MbPTP5  | comp279367_c0_seq1| 285    | 7.00E-35      | ref|YP_239381.1| PTP | [Microplitis demolitor bracovirus] |
|                | MbPTP5  | comp56541_c0_seq1 | 315    | 6.00E-38      | ref|YP_239381.1| PTP | [Microplitis demolitor bracovirus] |
|                | MbPTP5  | comp283025_c0_seq1| 420    | 1.00E-41      | ref|YP_239381.1| PTP | [Microplitis demolitor bracovirus] |
|                | MbPTP6  | comp767898_c0_seq1| 264    | 8.00E-42      | ref|YP_239390.1| PTP | [Microplitis demolitor bracovirus] |
|                | MbPTP   | comp96210_c0_seq9 | 252    | 5.90E-37      | gb|ACE75309.1| PTP | [Glyptapanteles indensis bracovirus] |
Table 2. The differential expression of genes regulated by *M. bicoloratus* bracovirus in the host gap junction signaling pathway.

| A_ID               | Function                                      | read_M | RPKM_M | read_S | RPKM_S | log2(Fold_change) | p-value          | Result | S_ID          | M-ID          |
|--------------------|-----------------------------------------------|--------|--------|--------|--------|-------------------|------------------|--------|---------------|---------------|
| comp95316_c0_seq3  | adenylate cyclase 8                           | 414    | 1.538851011 | 5365   | 20.60652119 | −3.746951184 | 0.0000008 | down | comp59135_c1_seq10 | comp18779_c0_seq1 |
| comp96543_c0_seq4  | classical protein kinase C                    | 1502   | 6.301173776  | 3740   | 16.2542916  | −1.367229143 | 4.6108E-232 | down | comp30329_c0_seq1 | comp20807_c0_seq1 |
| comp97909_c0_seq6  | guanine nucleotide-binding protein G(s) subunit alpha | 41     | 0.622358814  | 430    | 6.762398457  | −3.441716531 | 3.85048E-83 | down | comp59076_c0_seq5 | / |
| comp88846_c0_seq1  | gap junction                                   | 628    | 3.260154915  | 1623   | 8.73E+00    | −1.420902221 | 2.19E-107  | down | comp57555_c2_seq1 | comp19421_c1_seq1 |
| comp60335_c0_seq1  | gap junction                                   | 1808   | 16.23951848  | 11125  | 1.04E+02    | −2.672414439 | 0.0000008 | down | comp45671_c0_seq1 | comp10397_c0_seq1 |
| comp99381_c0_seq1  | gap junction                                   | 3994   | 22.25932132  | 36919  | 213.1714802  | −3.259532924 | 0.0000008 | down | comp59804_c0_seq1 | comp30941_c0_seq1 |
| comp121018_c0_seq1 | gap junction                                   | 36     | 0.410377626  | 935    | 1.10E+01    | −4.749973239 | 7.01E-217  | down | comp59264_c0_seq1 | comp10397_c0_seq1 |
| comp96275_c0_seq13 | inositol 1,4,5-triphosphate receptor type 1   | 2068   | 3.72067313   | 5214   | 9.718904704 | −1.38523083   | 0.0000008 | down | comp59099_c0_seq4 | comp94669_c0_seq1 |
| comp106866_c0_seq1 | protein kinase A                              | 2326   | 9.047590091  | 7695   | 31.01038396 | −1.77145916  | 0.0000008 | down | comp65026_c0_seq1 | comp17984_c0_seq1 |
| comp97791_c0_seq2  | phosphatidylinositol phospholipase C, beta     | 366    | 2.438775601  | 738    | 5.09474476  | −1.062852854 | 8.52689E-32 | down | comp55943_c2_seq1 | comp8084_c0_seq1 |
| comp95574_c0_seq5  | protein kinase, cGMP-dependent                | 1795   | 5.701522537  | 4620   | 15.20350361 | −1.1324E-301 | 0.0000008 | down | comp58204_c1_seq7 | comp16873_c0_seq1 |
| comp63482_c0_seq3  | tubulin alpha                                  | 20547  | 224.0520545  | 53712  | 606.8022006 | −1.437392362 | 0.0000008 | down | comp41562_c0_seq1 | comp14668_c0_seq1 |
| comp94424_c0_seq3  | adenylate cyclase 1                            | 53     | 0.61819881   | 151    | 1.824754972 | −1.561559971 | 1.42371E-12 | down | comp29410_c0_seq1 | comp12872_c0_seq1 |
| comp94556_c0_seq4  | adenylate cyclase 5                            | 23     | 0.17008429   | 341    | 2.61255705  | −3.94114659  | 7.22672E-73 | down | comp55791_c2_seq2 | / |
| comp96534_c0_seq1  | adenylate cyclase 9                            | 17     | 0.46329195   | 29     | 0.81808247  | −0.82159384  | 0.06997849 | down | comp55534_c1_seq1 | comp4228_c0_seq1 |
| comp76441_c0_seq1  | cyclin-dependent kinase 1                     | 1207   | 7.41056731   | 2282   | 14.5160537 | 0.969948801 | 1.51194E-82 | down | comp27910_c0_seq1 | comp16516_c0_seq1 |
| comp93202_c0_seq1  | epidermal growth factor receptor               | 6514   | 18.3968606  | 6149   | 17.99184785 | 0.032116227  | 0.238280263 | down | comp72419_c0_seq1 | comp33128_c0_seq1 |
| comp83895_c0_seq2  | guanine nucleotide-binding protein G(q) subunit alpha | 4274   | 19.02930474  | 7980   | 36.8100670  | −0.951877524 | 5.7673E-277 | down | comp55254_c0_seq5 | comp17311_c0_seq3 |
| comp103695_c0_seq1 | growth factor receptor-binding protein 2       | 4553   | 31.55832529  | 7467   | 5.62135235  | −0.764786958 | 6.7036E-179 | down | comp59056_c4_seq1 | comp101386_c0_seq1 |
| comp12119_c0_seq1  | GTPase KRas                                    | 2904   | 11.39420805  | 3605   | 14.6544025  | −0.363033492 | 2.01055E-23 | down | comp46714_c0_seq1 | comp156998_c0_seq1 |
| comp81191_c0_seq1  | mitogen-activated protein kinase kinase 1      | 1619   | 6.495897191  | 2927   | 12.16719112 | −0.905395446 | 3.63546E-94 | down | comp174662_c0_seq1 | comp20958_c0_seq1 |
M. bicoloratus bracovirus genes transcribed in the hemocytes of parasitized host

It is well known that polydnaviruses manipulate host cell physiology [19]. Bracoviruses encode at least 20 gene families identified from 5 species of bracoviruses, *Cotesia congregata* bracovirus (CcBV) [20], *Microplitis demolitor* bracovirus (MdBV) [21], *Glyptapanteles indiensis* bracovirus (GiBV) [22], *Glyptapanteles flavicosta* bracovirus (GfBV) [5], and *Cotesia vestalis* bracovirus (CvBV) [23]. In the present study, genes belonging to at least 6 conserved gene families were found to be expressed in the host hemocytes parasitized by *M. bicoloratus* including 1) Ankyrin-repeat, 2) BEN domain, 3) C-type lectin, 4) Epidermal growth factor-like (EGF-like), 5) Mucin-like, and 6) protein tyrosine phosphatases (PTPs) (Table 1). Some of the proteins encoded by these genes are likely to be involved in regulating host cell death.

Gap junction signaling pathway regulation by *M. bicoloratus* parasitism

Gap junction proteins form gap junction channels connecting cells for cell-cell communication and form hemichannels facilitating extracellular and intracellular communication including between ER and mitochondria to exchange small molecular, such as ATP and Ca2⁺, to trigger apoptosis [24]. In the insect circulating hemocytes, gap junction proteins form hemichannels to allow communication between the cell and environment. Under lipopolysaccharide (LPS) immunochallenge, hemichannel dye uptake decreases [25]. Typically, the decrease of the transcription level of hemichannel components and the decrease in opening of hemichannels on the cell surface result in the decrease of dye uptake. Gap junction proteins, Spli-Inx2 and Inx3, have been characterized and functioned [26] and in this study, Spli-Inx1 and Inx4 also were detected from hemocytes (Fig. S1 and Table S4). Comparisons with S and M transcriptome data indicated that all 26 elements of the gap junction signaling pathway existed in the hemocytes. During immune challenge by *M. bicoloratus* parasitization, 2 genes (Spli-GNAS, ADCY5) were not expressed in the parasitized host hemocytes. To determine the differential expression of genes, all transcriptome were assembled into a combined pool, and S1, S2, M1, and M2 were mapped using this pool to obtain reads and the RPM values of S and M. Furthermore, the analysis obtained the fold change and p-value between parasitized and non-parasitized. These analyses indicated that 12 genes (ADCY8, CPKC, GNAS, INX1, INX2, INX3, INX4, ITPR1, PKA, PLCB, PRKG, and TUBA) were down-regulated (Table 2).

The qRT-PCR results indicate that the parasitization down-regulated 3 key molecules, Inx1, Inx2, Inx3, on the cell membrane, not Inx4 (Fig. 1). These molecules are involved in forming hemichannels and gap junctions, suggesting that there might be disruptions of intracellular between ER, mitochondria and extracellular molecular exchanges.

Ca²⁺ signaling pathway regulation by *M. bicoloratus* parasitism with respect to apoptosis

Calcium ions (Ca²⁺) control every aspect of cells as cellular messengers. Ca²⁺ ions also can become death signals when delivered at physiologically aberrant conditions. Mitochondria eventually decide whether Ca²⁺ signals are life or death signals via regulation of the mitochondrial membrane proteins Bcl-2 and Bax/Bak [27]. Comparisons of the transcription data from the S and M pools indicate that all 31 elements of the Ca²⁺ signaling pathway existed in the examined hemocytes. Under *M. bicoloratus* parasitism, 3 genes (Spli-ANT, CypD, PLCG2) increased in

| Table 2. Cont. |
|-----------------|-----------------|
| M/S            | log2(Fold_change) | normalized p-value | Result | S_ID | M_ID |
| A_ID            | Function         | read_S RPKM_S      | read_M RPKM_M |
| comp2361_c0_seq1 | mitogen-activated protein kinase 1/3 74 | 11123.21362 | 11141.63189 |
| comp23021_c0_seq1 | son of sevenless 74 | 40.93760357 | 20.40394E-10 |
| comp56420_c1_seq1 | tight junction protein 1 71 | 0.57122537 | 71 |
| comp58674_c2_seq3 | tubulin beta 63513 | 102657 922.7626088 |
| S_ID             | M_ID             |
| comp84123_c0_seq1 | comp84123_c0_seq1 |
| comp90223_c0_seq1 | comp90223_c0_seq1 |
| comp96251_c0_seq1 | comp96251_c0_seq1 |
| comp97251_c0_seq1 | comp97251_c0_seq1 |
| comp97925_c2_seq1 | comp97925_c2_seq1 |
| comp100372_c0_seq1 | comp100372_c0_seq1 |
| comp106968_c0_seq1 | comp106968_c0_seq1 |
| comp118372_c0_seq1 | comp118372_c0_seq1 |
| comp118372_c0_seq1 | comp118372_c0_seq1 |
| comp151101_c0_seq1 | comp151101_c0_seq1 |
| comp151101_c0_seq1 | comp151101_c0_seq1 |
| comp151101_c0_seq1 | comp151101_c0_seq1 |
| comp2361_c0_seq1 | comp2361_c0_seq1 |
| comp23021_c0_seq1 | comp23021_c0_seq1 |
| comp56420_c1_seq1 | comp56420_c1_seq1 |
| comp58674_c2_seq1 | comp58674_c2_seq1 |
| comp90223_c0_seq1 | comp90223_c0_seq1 |
| comp96251_c0_seq1 | comp96251_c0_seq1 |
| comp97251_c0_seq1 | comp97251_c0_seq1 |
| comp97925_c2_seq1 | comp97925_c2_seq1 |
| comp100372_c0_seq1 | comp100372_c0_seq1 |
| comp106968_c0_seq1 | comp106968_c0_seq1 |
| comp118372_c0_seq1 | comp118372_c0_seq1 |
| doi:10.1371/journal.pone.0110967.t002 | |
expression, and 1 gene (Spli-PDE1) was not expressed in the parasitized hemocytes. The other 13 genes (ADCY8, ATP2A, ATP2B, CPK2, GNAS, ITPR1, ORAI1, PHKA_B, PKA, PLCB, VDAC1, VDAC2 and VDAC3) had been down-regulated (Table 3). The qRT-PCR results indicate that the parasitism up-regulated a key molecule, CypD, in the mitochondria (Fig. 1). This molecule is involved in forming a permeability transition pore complex (PTPC), suggesting that the M. bicoloratus alters Ca\textsuperscript{2+} signaling pathway to promote apoptosis.

**PI3K/Akt signaling pathway regulation by M. bicoloratus parasitism**

The PI3K/Akt signaling pathway is involved in multiple different pathways, including cell survival, apoptosis, cell cycle, and DNA repair, through different downstream molecules. A comparison of the transcription data from the S and M pools revealed that all 65 elements of the PI3K/Akt signaling pathway existed in the hemocytes. Under immune challenge, 4 genes (ATF4, RP-S6e, EIF4EBP1, and GNB1) were expressed in the parasitized hemocytes, and 7 genes (COL1AS, FGFR2, G6PC, p85, PPP2R3, THBS2S, and TSC1) were not expressed in the parasitized host hemocytes (Table 4). Another 19 genes (COL4A, CREB3, HSP90B, IRS1, ITGB1, LAMA3.5, LAMB1, LAMC1, PDK1, PPP2C, PPP2R2, PPP2R5, PTEN, PTK2, RAC1, STK11, TSC2 and YWHAE) were down-regulated, (Table 4). The qRT-PCR results indicated that the parasitism down-regulated a key molecule, the p110 subunit, in the PI3K/Akt signaling pathway, suggesting that the disruption of cell survival signaling pathway by the parasitism may promote cell apoptosis (Fig. 1).

**NF-κB signaling pathway regulation by M. bicoloratus parasitism**

The NF-κB signaling pathway regulates gene expression via regulation of nuclear transcription factor. Comparison of the transcription data from the S and M pools indicates that all 18 elements of NF-κB signaling pathway existed in the hemocytes. Under M. bicoloratus parasitism, 1 gene (Spli-PLCG2) was expressed in the parasitized host hemocytes, and 5 genes (Spli-CSNK2A, MYD88, P50, P65 and XIAP) were down-regulated (Table 5). The qRT-PCR results indicate that the parasitism down-regulated two key molecules, the p50 (Relish) and p65 (Dorsal) subunits in the NF-κB signaling pathway, suggesting the disruption of the cell survival signaling pathway (Fig. 1).

**ATM/p53 signaling pathway regulation by M. bicoloratus parasitism**

The ATM/p53 signaling pathway plays an important role in cell cycle control and apoptosis. In normal cells, the p53 protein level is low. DNA damage and stress signaling may trigger an increase of p53 protein levels, which has three major functions: cell cycle arrest, DNA repair and apoptosis. The cell cycle arrest prevents replication of proteins involved in DNA repair. Apoptosis avoids proliferation of cells containing abnormal DNA. p53 is a transcriptional activator that regulates the expression of MDM2. A comparison of the transcription data from the S and M pools indicate that all 21 elements of the ATM/p53 signaling pathway existed in the hemocytes. Under M. bicoloratus parasitism, 1 gene (Spli-SESN), was expressed in the parasitized host hemocytes, and 1 gene (CYC) was not expressed in the parasitized host hemocytes. Another 3 genes (Spli-PPM1D, PTEN, and TSC2) were down-regulated (Table 6). The qRT-PCR results indicate that the parasitism increased expression of a key molecule, p53, in the ATM/p53 signaling pathway (Fig. 1).

**Discussion**

M. bicoloratus parasitism regulated host hemocyte apoptosis, resulting in DNA fragmentation. In this study, we examined the impacts of both the apoptotic caspase-dependent and -independent signaling pathways on the host hemocytes based on transcriptome data. Our results demonstrated that bracovirus proteins are expressed in the host hemocytes, suggesting their roles in DNA fragmentation by regulating key signaling pathways,
Table 3. The differential expression of genes regulated by *M. bicoloratus* bracovirus in the host Ca²⁺ signaling pathway.

| Gene family | A_ID | Function | read_M | RPKM_M | read_S | RPKM_S | log2(Fold_change) | normalized p-value | Result | S_ID | M-ID |
|-------------|------|----------|--------|--------|--------|--------|-------------------|-------------------|--------|------|------|
| ANT         | comp95003_c0_seq1 | mitochondrial adenine nucleotide translocator | 482 | 5.819038231 | 0.5 | 0.006253879 | 9.86181365 | 7.35681E-80 | up | / | comp11118_c0_seq1 |
| CypD        | comp93813_c0_seq1 | peptidyl-prolyl isomerase F (cyclophilin D) | 448 | 6.696320619 | 0.5 | 0.007742898 | 9.756279236 | 2.02443E-75 | up | / | comp11549_c0_seq1 |
| ADCY8       | comp95316_c0_seq3 | adenylate cyclase 8 | 414 | 1.538851011 | 5365 | 20.66052119 | -3.746951184 | 0 | down | comp58820_c1_seq2 | comp18779_c1_seq1 |
| ATP2A       | comp23165_c0_seq2 | Ca²⁺ transporting ATPase | 4330 | 11.26751338 | 12042 | 32.46890454 | -1.526171778 | 0 | down | comp45209_c0_seq2 | comp20999_c0_seq2 |
| ATP2B       | comp102625_c0_seq1 | Ca²⁺ transporting ATPase | 6998 | 16.2324803 | 17020 | 40.90211972 | -1.33292153 | 0 | down | comp61676_c0_seq1 | comp19993_c0_seq2 |
| CPKC        | comp96543_c0_seq4 | classical protein kinase C | 1502 | 6.301173776 | 3740 | 16.2542916 | -1.36729143 | 4.6108E-232 | down | comp30329_c0_seq1 | comp28070_c0_seq1 |
| GNAS        | comp97983_c1_seq2 | guanine nucleotide-binding protein G(s) subunit alpha | 149 | 2.139786276 | 363 | 5.400899151 | -1.335732903 | 7.99287E-23 | down | comp58416_c0_seq4 | comp20437_c1_seq1 |
| ITPR1       | comp96275_c0_seq13 | inositol 1,4,5-triphosphate receptor type 1 | 2068 | 3.72067313 | 5214 | 9.718904704 | -1.385230083 | 0 | down | comp59099_c0_seq4 | comp94669_c0_seq1 |
| ORAI1       | comp97905_c0_seq1 | calcium release-activated calcium channel protein 1 | 238 | 1.972431074 | 588 | 5.048676205 | -1.355390267 | 4.8930E-37 | down | comp57934_c0_seq2 | comp71014_c0_seq1 |
| PHKA_B      | comp92577_c0_seq1 | phosphorylase kinase alpha/beta subunit | 1191 | 3.39676742 | 3490 | 10.32111467 | -1.602129309 | 6.6379E-274 | down | comp58502_c0_seq5 | comp101238_c0_seq1 |
| PKA         | comp106866_c0_seq1 | protein kinase A | 2326 | 9.047590091 | 7695 | 31.01038396 | -1.77145916 | 0 | down | comp65026_c0_seq1 | comp7984_c0_seq1 |
| PLCB        | comp97791_c0_seq2 | Phosphatidylinositol phospholipase C, beta | 366 | 2.438775601 | 738 | 5.09474476 | -1.062828254 | 8.52689E-32 | down | comp55982_c0_seq1 | comp8276_c0_seq1 |
| VDAC1       | comp90986_c0_seq1 | voltage-dependent anion channel protein 1 | 4 | 0.179365731 | 42 | 1.95E+00 | -3.44393109 | 3.25E-09 | down | comp56820_c0_seq2 | comp79085_c0_seq1 |
| VDAC2       | comp99405_c0_seq1 | voltage-dependent anion channel protein 2 | 5522 | 67.03373209 | 10842 | 1.36583388 | -1.02443805 | 0 | down | comp60098_c0_seq1 | comp29398_c0_seq1 |
| VDAC3       | comp89185_c0_seq1 | voltage-dependent anion channel protein 3 | 2 | 0.093999153 | 26 | 1.27E+00 | -3.751515404 | 1.70E-06 | down | comp60098_c0_seq1 | comp29398_c0_seq1 |
| ADCY1       | comp94424_c0_seq3 | adenylate cyclase 1 | 53 | 0.61819881 | 151 | 1.824754792 | -1.561559971 | 1.4237E-12 | down | comp48930_c1_seq2 | comp28727_c0_seq1 |
| ADCY9       | comp69334_c0_seq1 | adenylate cyclase 9 | 17 | 0.46329195 | 29 | 0.8180247 | -0.82159384 | 0.069978649 | down | comp55334_c1_seq1 | comp4228_c0_seq1 |
| CALM        | comp23241_c0_seq1 | calmodulin | 27610 | 270.0746841 | 42753 | 433.2707575 | -0.681910456 | 0 | down | comp45080_c1_seq2 | comp65530_c0_seq1 |
| CAMK2       | comp97973_c0_seq1 | calcium/calmodulin-dependent protein kinase II | 944 | 3.291051665 | 1737 | 6.273903548 | -0.930814675 | 5.2731E-59 | down | comp57321_c0_seq1 | comp19479_c0_seq1 |
| Gene family | A_ID | Function | read_M | RPKM_M | read_S | RPKM_S | log2(Fold_change) normalized | p-value | Result | S_ID | M-ID |
|-------------|------|----------|--------|--------|--------|--------|-----------------------------|---------|--------|------|------|
| EGFR        | comp93202_c0_seq1 | epidermal growth factor receptor | 6514   | 18.3986606 | 6149   | 17.99184785 | 0.032116227 | 0.238280263 | comp72419_c0_seq1 | comp33128_c0_seq1 |
| GNAQ        | comp83895_c0_seq2 | guanine nucleotide-binding protein G(q) subunit alpha | 4274   | 19.02930474 | 7980   | 36.81006701 | -0.95187524 | 5.7673E-277 | comp50512_c0_seq1 | comp166552_c0_seq1 |
| ITPK        | comp30903_c0_seq1 | 1D-myoinositol-triphosphate 3-kinase | 962    | 11.05221153 | 1777   | 21.1512899 | -0.936410568 | 6.2508E-61 | comp5786_c0_seq2 | comp37996_c0_seq1 |
| MYLK        | comp95483_c0_seq1 | myosin-light-chain kinase | 65     | 1.078904349 | 198    | 3.40494919 | -1.658064893 | 1.8630E-17 | comp46122_c0_seq1 | comp19788_c0_seq1 |
| PDE1        | comp96257_c0_seq5 | calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase | 8      | 0.058446689 | 531    | 4.01921305 | -6.103643737 | 1.0771E-127 | comp58443_c0_seq1 | / |
| PHKG        | comp97075_c0_seq1 | phosphorylase kinase gamma subunit | 350    | 2.19800775  | 633    | 4.11996628 | -0.905926263 | 2.4877E-21 | comp56788_c0_seq1 | comp57668_c0_seq1 |
| PLCG1       | comp95371_c0_seq1 | phosphatidylinositol phospholipase C gamma-1 | 155    | 1.337532688 | 289    | 2.58373252 | -0.949876963 | 3.7148E-11 | comp54883_c1_seq1 | comp1243_c0_seq1 |
| PLCG2       | comp94580_c0_seq1 | phosphatidylinositol phospholipase C gamma-2 | 110    | 1.770661702 | 159    | 2.651644792 | -0.582596928 | 0.0015832 | / | comp78811_c0_seq1 |
| PPP3C       | comp108295_c0_seq1 | serine/threonine-protein phosphatase 2B catalytic subunit | 2433   | 13.2733981 | 4265   | 24.106511 | -0.860885107 | 1.4074E-125 | comp38261_c0_seq1 | comp20495_c0_seq2 |
| PPP3R       | comp109656_c0_seq1 | serine/threonine-protein phosphatase 2B regulatory subunit | 1743   | 13.13252377 | 2743   | 21.4117348 | -0.705257739 | 7.2469E-58 | comp42185_c0_seq1 | comp35682_c0_seq1 |
| SPHK        | comp92166_c0_seq3 | sphingosine kinase | 76     | 0.79803821  | 134    | 1.457774018 | -0.869237637 | 3.5239E-05 | comp52416_c0_seq1 | comp8718_c0_seq1 |
| STIM1       | comp94633_c0_seq1 | stromal interaction molecule 1 | 1970   | 8.552321068 | 2535   | 11.40173775 | -0.414865803 | 3.0316E-21 | comp55152_c1_seq2 | comp19536_c0_seq1 |

doi:10.1371/journal.pone.0110967.t003
Table 4. The differential expression of genes regulated by *M. bicoloratus* bracovirus in the host PI3K/Akt signaling pathway.

| Gene family | A_ID         | Function                                                                 | read_M | RPKM_M | read_S | RPKM_S | log2(Fold_change) normalized | p-value       | Result | S_ID | M-ID |
|-------------|--------------|---------------------------------------------------------------------------|--------|--------|--------|--------|-------------------------------|---------------|--------|------|------|
| ATF4        | comp93717_c0_seq1 | CREB2; cyclic AMP-dependent transcription factor ATF-4                   | 391    | 5.031431973 | 0.5    | 0.006665921 | 9.559949111 | 8.92893E-68 | up    | /    | comp42406_c0_seq1 |
| PEPCK       | comp109757_c0_seq1 | phosphoenolpyruvate carboxykinase (GTP)                                  | 3834   | 27.16382297 | 574    | 4.213334979 | 2.688652009 | 0      | up    | comp58069_c0_seq1 | comp30301_c0_seq1 |
| RP-S6e      | comp24289_c0_seq1 | small subunit ribosomal protein 56e                                     | 1353   | 27.7189041   | 0.5    | 0.010612644 | 11.35087044 | 8.601E-176 | up    | /    | comp11154_c0_seq1 |
| COL4A       | comp23243_c0_seq1 | type IV, alpha                                                           | 33659  | 235.0626028  | 160548 | 1.161.15892 | -2.305016159 | 0      | down  | comp45047_c0_seq1 | comp10045_c0_seq2 |
| CREB3       | comp101801_c0_seq1 | cyclic AMP-responsive element-binding protein 3                          | 3995   | 24.04913606  | 11685  | 72.87636897 | 1.599466012 | 0      | down  | comp28212_c0_seq1 | comp9969_c0_seq1 |
| GSK3B       | comp23136_c0_seq1 | glycogen synthase kinase 3 beta                                         | 1248   | 13.91068523  | 2965   | 34.2400086  | 1.299489856 | 1.9348E-170 | down  | comp47318_c0_seq1 | comp20759_c0_seq2 |
| IRS1        | comp97702_c0_seq2 | insulin receptor substrate 1                                            | 3701   | 23.9880501   | 9865   | 66.2426184  | 1.465479601 | 0      | down  | comp28148_c0_seq1 | comp19950_c0_seq1 |
| ITGB1       | comp107868_c0_seq1 | integrin beta 1                                                          | 2897   | 11.30115561  | 8616   | 34.8213249  | 1.62354251  | 0      | down  | comp46010_c0_seq2 | comp20980_c0_seq2 |
| LAMA3_5     | comp99575_c0_seq1 | laminin, alpha 3/5                                                       | 27851  | 42.99292765  | 112433 | 179.8147605 | 2.064340192 | 0      | down  | comp59989_c0_seq1 | comp17635_c0_seq1 |
| LAM1B       | comp95243_c0_seq1 | laminin, beta 1                                                          | 18108  | 49.24188678  | 51196  | 144.2366222 | 1.590479569 | 0      | down  | comp60102_c0_seq1 | comp10145_c0_seq2 |
| LAMC1       | comp100060_c0_seq1 | laminin, gamma 1                                                         | 15999  | 40.61005237  | 52368  | 137.716847  | 1.761779459 | 0      | down  | comp60624_c0_seq1 | comp10076_c0_seq1 |
| PDPK1       | comp89371_c0_seq4 | 3-phosphoinositide-dependent protein kinase-1                            | 1184   | 5.484101314  | 2310   | 11.0851368  | 1.015299457 | 5.1312E-90 | down  | comp57389_c0_seq3 | comp19669_c0_seq2 |
| PPP2C       | comp99514_c0_seq1 | serine/threonine-protein phosphatase 2A catalytic subunit                | 4007   | 52.05795373  | 8123   | 109.3350776 | 1.07056582 | 0      | down  | comp134046_c0_seq1 | comp24560_c0_seq1 |
| PPP2R2      | comp95673_c0_seq2 | serine/threonine-protein phosphatase 2A regulatory subunit B             | 362    | 3.27661055   | 761    | 7.136352468 | 1.122982442 | 1.0852E-35 | down  | comp56391_c0_seq2 | comp21423_c0_seq2 |
| PPP2R5      | comp25110_c0_seq1 | serine/threonine-protein phosphatase 2A regulatory subunit B'            | 414    | 4.316267161  | 1061   | 11.4603703  | 1.408797609 | 9.8914E-70 | down  | comp226319_c0_seq1 | comp18379_c0_seq1 |
| PTEN        | comp97411_c0_seq4 | PTEN                                                                      | 233    | 1.759294286  | 911    | 7.12649954  | 2.201896785 | 5.8123E-99 | down  | comp59211_c2_seq7 | comp62639_c0_seq1 |
| PTK2        | comp89387_c1_seq2 | focal adhesion kinase 1                                                 | 1443   | 7.251236554  | 3747   | 19.5076073  | 1.427740364 | 3.4416E-248 | down  | comp58747_c0_seq2 | comp20857_c0_seq2 |
| RAC1        | comp102261_c0_seq1 | Ras-related C3 botulinum toxin kinase 1                                 | 4703   | 22.53778311  | 13189  | 65.4822111  | 1.538758631 | 0      | down  | comp61307_c0_seq1 | comp211969_c0_seq1 |
| Gene family | A_ID | Function | read_M | RPKM_M | read_S | RPKM_S | log2(Fold_change) |
|-------------|------|----------|--------|--------|--------|--------|------------------|
| STK11       | comp68494_c0_seq3 | serine/threonine-protein kinase 11 | 275   | 2.752794104 | 643 | 6.668487203 | 1.276462805 | 6.66465E-37 |
| TSC2        | comp93326_c0_seq1 | tuberous sclerosis 2 | 42   | 1.079341152 | 227 | 6.043807401 | 2.48530675 | 1.25293E-32 |
| YWHAE       | comp94021_c0_seq1 | 14–3-3 protein epsilon | 55   | 1.03509774 | 357 | 6.960848804 | 2.749496236 | 2.16594E-56 |
| AKT         | comp103304_c0_seq1 | RAC serine/threonine-protein kinase | 4590 | 25.54845119 | 8713 | 50.24541632 | 0.975751075 | 0 |
| ATF2        | comp63925_c0_seq1 | CREBP1; cyclic AMP-dependent transcription factor ATF-2 | 33   | 1.162462472 | 51 | 1.861274773 | 6.93106908 | 0.041341344 |
| BRCA1       | comp95658_c0_seq1 | breast cancer type 1 susceptibility protein | 355 | 2.744122154 | 366 | 2.931105739 | 0.09510031 | 0.409730291 |
| CCND2       | comp92629_c0_seq2 | cyclin D2 | 1830 | 9.174971528 | 2968 | 15.41675056 | 0.74823129 | 2.87139E-69 |
| CONE        | comp86772_c0_seq2 | cyclin E | 48   | 0.642601979 | 99 | 1.373128968 | 0.095469805 | 1.62283E-05 |
| CDC37       | comp104439_c0_seq1 | cell division cycle protein 37 | 1645 | 17.75049078 | 2932 | 0.884873205 | 8.69703E-91 |
| CDK4        | comp93505_c0_seq2 | cyclin-dependent kinase 4 | 91   | 1.026690365 | 248 | 2.898846262 | 1.49747356 | 9.30914E-19 |
| COL1A5      | comp140925_c0_seq1 | type I/II/III/IV/V/VII alpha | 0.5 | 0.035296871 | 59 | 3.415126435 | 6.393718735 | 8.23218E-15 |
| EGFR        | comp93202_c0_seq1 | epidermal growth factor receptor | 6514 | 18.3968606 | 6149 | 17.99184785 | 0.032116227 | 0.238280263 |
| EIF4B       | comp103484_c0_seq1 | translation initiation factor 4B | 3735 | 23.01200087 | 6307 | 40.25890145 | 0.80921376 | 7.4266E-166 |
| EIF4E       | comp108936_c0_seq1 | translation initiation factor 4E | 671 | 7.903316174 | 1192 | 14.4950751 | 0.8807525 | 2.69219E-37 |
| EIF4EBP1    | comp88188_c0_seq1 | eukaryotic translation initiation factor 4E binding protein 1 | 78   | 2.176304201 | 0.5 | 0.01445341 | 7.23432653 | 3.10204E-18 |
| FGFR2       | comp86525_c0_seq1 | fibroblast growth factor receptor 2 | 9    | 0.147300349 | 80 | 1.35652114 | 3.203078779 | 1.50516E-15 |
| FRAP        | comp89229_c0_seq3 | RKB12-rapamycin complex-associated protein | 501 | 1.205543964 | 1616 | 4.028672805 | 1.74060375 | 3.1793E-143 |
| G6PC        | comp92782_c0_seq1 | glucose-6-phosphatase | 2    | 0.039148868 | 87 | 1.764346079 | 5.490419182 | 5.9857E-22 |
| GBL         | comp96734_c0_seq1 | G protein beta subunit-like | 166  | 2.709305209 | 307 | 5.191149549 | 0.9381311 | 1.47018E-11 |
| Gene family | A_ID          | Function                                                                 | read_M | RPKM_M | read_S | RPKM_S | log2(Fold_change) normalized | p-value | Result | S_ID                          | M-ID        |
|-------------|---------------|---------------------------------------------------------------------------|--------|--------|--------|--------|-------------------------------|---------|--------|--------------------------------|-------------|
| M/S         |               |                                                                           |        |        |        |        |                               |         |        |                                 |             |
| GNB1        | comp81476_c0_seq1 | guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1           | 7596   | 24.34298724 | 15821 | 52.52888221 | -1.109604667 | 0 | / | comp20574_c0_seq1              |             |
| GNB5        | comp90181_c0_seq2 | guanine nucleotide-binding protein subunit beta-5                          | 337    | 4.024274883 | 558   | 6.903466944 | -0.77859216 | 6.67432E-15 | comp45009_c0_seq1 | comp17866_c0_seq1 |
| GNG13       | comp95223_c0_seq1 | guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-13         | 7200   | 46.4612554 | 8333  | 55.7102541 | -0.261914759 | 6.81673E-29 | comp61890_c0_seq1 | comp24329_c0_seq1 |
| GRB2        | comp103695_c0_seq1 | growth factor receptor-binding protein 2                                   | 4553   | 31.55832529 | 7467  | 53.62135235 | -0.764786958 | 6.7036E-179 | comp59056_c4_seq1 | comp101386_c0_seq1 |
| GYS         | comp95471_c0_seq2 | glycerol(3R)-phosphate synthase                                             | 96     | 0.657627746 | 591   | 4.194471817 | -2.673127505 | 7.1693E-90 | comp55945_c1_seq1 | comp231427_c0_seq1 |
| HSP90A      | comp233248_c1_seq1 | molecular chaperone HtpG                                                    | 30676  | 217.778149 | 38447 | 282.7825966 | 0.376836341 | 1.7963E-255 | comp55995_c0_seq2 | comp15914_c0_seq1 |
| IKBKB       | comp46046_c0_seq1 | inhibitor of nuclear factor kappa-B kinase subunit beta                     | 628    | 4.03761507 | 887   | 5.908346682 | 0.549245231 | 6.14389E-13 | comp46039_c0_seq1 | comp19169_c0_seq1 |
| INSR        | comp97941_c0_seq7 | insulin receptor                                                            | 381    | 2.063839032 | 405   | 2.27290447 | 0.139206596 | 0.201218987 | comp55149_c2_seq1 | comp9154_c1_seq1 |
| JAK2        | comp98009_c1_seq1 | Janus kinase 2                                                              | 407    | 1.908290622 | 691   | 3.356629968 | 0.814732602 | 1.51786E-19 | comp93258_c0_seq1 | comp16460_c1_seq1 |
| KRAS        | comp112119_c0_seq1 | GTPase KRas                                                                 | 2904   | 11.39420805 | 3605  | 14.6544025 | -0.363031492 | 2.0105E-23 | comp37119_c0_seq1 | comp46714_c0_seq1 |
| MAP2K1      | comp81191_c0_seq1 | mitogen-activated protein kinase kinase 1                                   | 1619   | 6.495897191 | 2927  | 12.6179112 | -0.905395446 | 3.63546E-94 | comp174562_c0_seq1 | comp20958_c0_seq1 |
| MAPK1.3     | comp23316_c1_seq1 | mitogen-activated protein kinase kinase 1/3                                 | 5679   | 21.44919695 | 7831  | 30.64302995 | -0.514635322 | 3.1947E-93 | comp28328_c0_seq1 | comp9963_c0_seq1 |
| MYB         | comp93622_c1_seq4 | myb proto-oncogene protein                                                  | 1093   | 4.733328627 | 1565  | 7.021601066 | -0.568949492 | 2.9113E-23 | comp58816_c0_seq6 | comp9456_c0_seq2 |
| MYC         | comp63425_c0_seq2 | Muc proto-oncogene protein                                                  | 2255   | 7.977064372 | 3204  | 11.74260281 | -0.5578224 | 8.8295E-45 | comp47218_c0_seq2 | comp20721_c0_seq2 |
| P110        | comp97931_c0_seq1 | phosphatidylinositol-4, 5-bisphosphate 3-kinase, PIK3C                      | 165    | 1.192085437 | 623   | 4.663229594 | -1.967841825 | 6.9635E-66 | comp56297_c1_seq1 | comp85699_c0_seq1 |
| P85         | comp27492_c0_seq1 | phosphoinositide-3-kinase, regulatory subunit, PIK3R                        | 0.5    | 0.039061874 | 15    | 1.214086421 | -4.957966281 | 0.000103715 | comp39459_c0_seq1 | /             |
| PKN         | comp67156_c0_seq1 | protein kinase N                                                            | 778    | 3.949050183 | 1366  | 7.183549165 | -0.863191109 | 3.1830E-41 | comp5044_c0_seq1 | comp19192_c0_seq1 |
| PPP2R1      | comp101848_c0_seq1 | serine/threonine-protein phosphatase 2A regulatory subunit A               | 4788   | 35.95160432 | 8113  | 63.11330498 | -0.811888022 | 2.797E-215 | comp28215_c0_seq1 | comp10104_c0_seq1 |
| Gene family | A_ID          | Function                                      | read_M | RPKM_M  | read_S | RPKM_S  | log2(Fold change) | normalized p-value | Result | S_ID          | M-ID          |
|-------------|---------------|-----------------------------------------------|--------|---------|--------|---------|-------------------|-------------------|--------|---------------|---------------|
| PPP2R3      | comp207060_c0_seq1 | serine/threonine-protein phosphatase 2A regulatory subunit B | 17     | 0.429343833 | 42     | 1.098957536 | −1.355930267 | 0.000936344 |       |               |               |
| PRKAA       | comp88394_c0_seq1 | AMPK, S^5^-AMP-activated protein kinase, catalytic alpha subunit | 935    | 5.517046633 | 1081   | 6.608395875 | −0.260403939 | 9.36228E-05 |       |               |               |
| RAPTOR      | comp96751_c0_seq3 | regulatory associated protein of mTOR | 186    | 1.325822604 | 550    | 4.061724643 | −1.615204683 | 1.12036E-44 |       |               |               |
| RHEB        | comp94584_c0_seq1 | Ras homolog enriched in brain | 150    | 2.527973388 | 197    | 3.43971752  | −0.444308815 | 0.006072962 |       |               |               |
| RPS6KB      | comp110869_c0_seq1 | p70 ribosomal S6 kinase | 1391   | 8.265982996 | 2672   | 16.4505016  | −0.992873274 | 3.286E-100 |       |               |               |
| SOS         | comp96783_c0_seq11 | son of sevenless | 74     | 1.123281762 | 195    | 3.06669068  | −1.448952634 | 2.40343E-14 |       |               |               |
| THBS2S      | comp28902_c0_seq1 | thrombospondin 2/3/4/5 | 31     | 0.720784511 | 15     | 0.361335244 | 0.996230029 | 0.029739095 |       |               |               |
| TSC1        | comp87058_c0_seq1 | tuberous sclerosis 1 | 15     | 0.611757829 | 46     | 1.93665035  | −1.667747046 | 5.20854E-05 |       |               |               |
| YWHA_B2_Z   | comp63845_c0_seq2 | 14-3-3 protein beta/theta/zeta | 25941  | 59.75452622 | 34171  | 81.54871003 | −0.448614059 | 0       |       |               |               |

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Table 5. The differential expression of genes regulated by *M. bicoloratus* bracovirus in the host NF-κB signaling pathway.

| Gene family | A_ID | Function | read_M RPKM_M | read_S RPKM_S | log2(Fold_change) normalized | p-value | Result | S_ID | M-ID |
|-------------|------|----------|---------------|---------------|------------------------------|---------|--------|------|------|
| CSNK2A      | comp67611_c0_seq1 | casein kinase II subunit alpha | 1490 10.76489273 2927 21.90894546 | -1.025186101 1.5073E-115 | 2 | down | comp56988_c0_seq2 | comp10015_c0_seq2 |
| MYD88       | comp68137_c0_seq1 | myeloid differentiation primary response protein MyD88 | 577 6.026390147 1241 13.42853162 | -1.159935577 4.14962E-60 | 2 | down | comp76362_c0_seq1 | comp49638_c0_seq1 |
| P50         | comp97501_c11_seq1 | nuclear factor kappa-B p105/100, Relish 1A | 756 8.18783011 1588 1.78E+01 | -1.121828459 4.83E-73 | 2 | down | comp57569_c0_seq1 | comp46759_c0_seq1 |
| P65         | comp89974_c0_seq4 | nuclear factor kappa-B/Rel, Dorsal 1A | 725 6.340266258 2092 1.90E+01 | -1.579905637 2.35E-161 | 2 | down | comp58671_c0_seq4 | comp19997_c0_seq5 |
| XIAP        | comp66362_c0_seq1 | E3 ubiquitin-protein ligase XIAP, Bcl-2 | 1252 4.419168216 1858 6.794495692 | -0.620591624 8.2147E-32 | 2 | down | comp57322_c0_seq1 | comp52055_c0_seq1 |
| ATM         | comp98156_c1_seq4 | ataxia telangiectasia mutated family protein | 659 2.224231496 928 3.245023613 | -0.54922026 2.64245E-13 | 2 | down | comp56187_c0_seq2 | comp18276_c0_seq1 |
| BIRC2_3     | comp98152_c0_seq6 | baculoviral IAP repeat-containing protein 2/3 | 1252 4.419168216 1858 6.794495692 | -0.620591624 8.2147E-32 | 2 | down | comp57322_c0_seq1 | comp52055_c0_seq1 |
| CSNK2B      | comp102869_c0_seq1 | casein kinase II subunit beta | 2230 21.59701753 3660 36.72360578 | -0.765875624 1.66408E-88 | 2 | down | comp55287_c0_seq1 | comp20218_c0_seq1 |
| IKB         | comp108698_c0_seq1 | inhibitor of nuclear factor kappa-B, Cactus | 3403 15.84566756 3613 1.74E+01 | -0.137465789 0.000117519 | 2 | down | comp46039_c0_seq1 | comp19169_c0_seq1 |
| IKB         | comp46046_c0_seq1 | inhibitor of nuclear factor kappa-B kinase subunit beta | 628 4.037631507 887 5.908346682 | -0.459245231 6.14389E-13 | 2 | down | comp54039_c0_seq1 | comp19169_c0_seq1 |
| IRAK4       | comp50303_c0_seq1 | interleukin-1 receptor-associated kinase 4 | 486 4.085524166 723 6.29687569 | -0.624115019 3.09261E-13 | 2 | down | comp55403_c1_seq1 | comp50008_c0_seq1 |
| MALT1       | comp86328_c0_seq1 | MAL T1 | 110 1.62564556 120 1.87906574 | -0.176065568 0.38601433 | 2 | down | comp29501_c0_seq1 | comp69294_c0_seq1 |
| MAP3K7      | comp97891_c0_seq2 | mitogen-activated protein kinase kinase 7 | 541 3.421954775 876 5.740588546 | -0.746377962 3.47298E-21 | 2 | down | comp47143_c1_seq1 | comp18892_c0_seq2 |
| MAP3K7P1    | comp96428_c0_seq2 | TAK1-binding protein 1 | 226 2.447686633 387 4.342434795 | -0.82708648 9.53466E-12 | 2 | down | comp27058_c0_seq1 | comp60240_c0_seq1 |
| PLCG1       | comp95371_c0_seq1 | phosphatidylinositol phospholipase C, gamma-1 | 155 1.337538268 289 2.58373252 | -0.498785963 3.71489E-11 | 2 | down | comp54883_c1_seq1 | comp81243_c0_seq1 |
| PLCG2       | comp94580_c0_seq1 | phosphatidylinositol phospholipase C, gamma-2 | 110 1.770661702 159 2.65164792 | -0.582598928 0.00156832 | 2 | down | comp78811_c0_seq1 | comp81243_c0_seq1 |
| TRAF6       | comp91031_c0_seq1 | TNF receptor-associated factor 6 | 38 0.903867364 54 1.330730718 | -0.38035675 0.079258626 | 2 | down | comp52687_c0_seq1 | comp130761_c0_seq1 |
| UBE2i       | comp106025_c0_seq1 | ubiquitin-conjugating enzyme E2 i | 1630 22.0296837 1997 27.9384991 | -0.344038054 2.46942E-12 | 2 | down | comp57828_c0_seq1 | comp16034_c0_seq1 |
Table 6. The differential expression of genes regulated by *M. bicoloratus* bracovirus in the host ATM/p53 signaling pathway.

| Gene family | A_ID          | Function                                      | read_M | RPKM_M | read_S | RPKM_S | log2(Fold_change) | p-value | Result | S_ID     | M_ID     |
|-------------|---------------|-----------------------------------------------|--------|--------|--------|--------|-------------------|---------|--------|-----------|----------|
| PPM1D       | comp23378_c0_seq1 | protein phosphatase 1D                        | 436    | 5.195890813 | 968    | 11.95154904 | −1.201754598 | 4.38033E−50 | down | comp45971_c0_seq1 | comp9608_c0_seq1 |
| PTEN        | comp97411_c0_seq4 | PTEN                                          | 233    | 1.759294286 | 911    | 7.126499544 | −2.018196785 | 5.8123E−99 | down | comp59211_c2_seq7 | comp62639_c0_seq1 |
| TSC2        | comp93326_c0_seq1 | tuberous sclerosis 2                          | 42     | 1.079341152 | 227    | 6.043807401 | −2.485306765 | 1.25293E−32 | down | comp31311_c0_seq1 | comp12675_c0_seq1 |
| ATM         | comp98156_c1_seq4 | ataxia telangiectasia mutated family protein  | 659    | 2.224231496 | 928    | 3.245023613 | −0.544922026 | 2.64245E−13 | comp56187_c0_seq2 | comp18276_c0_seq1 |
| ATR         | comp85208_c0_seq1 | serine/threonine-protein kinase ATR           | 62     | 1.180743424 | 98     | 1.933593758 | −0.71158922  | 0.00305328 | comp53816_c1_seq1 | comp15900_c0_seq1 |
| CCBN        | comp86097_c0_seq2 | cyclin B                                      | 984    | 4.996128296 | 1852   | 9.742149268 | −0.963429564 | 1.7509E−66 | comp57367_c0_seq1 | comp19132_c0_seq2 |
| CCND2       | comp92629_c0_seq2 | cyclin D2                                     | 1830   | 9.174971528 | 2968   | 15.41675056 | −0.748723129 | 2.87139E−69 | comp55005_c1_seq1 | comp20586_c0_seq2 |
| CCNE        | comp86772_c0_seq2 | cyclin E                                      | 48     | 0.642601979 | 99     | 1.373128968 | −1.095469805 | 1.6228E−05 | comp29156_c0_seq1 | comp130315_c0_seq1 |
| CCNG2       | comp87700_c0_seq1 | cyclin G2                                     | 2216   | 12.141316031| 3124   | 18.1300477 | −0.456125285 | 3.78105E−42 | comp38929_c0_seq2 | comp21305_c1_seq2 |
| CDK1        | comp76441_c0_seq1 | cyclin-dependent kinase 1                     | 1207   | 7.410567531 | 2282   | 14.51560537 | −0.999948801 | 1.51194E−82 | comp27910_c0_seq1 | comp16516_c0_seq1 |
| CDK4        | comp93950_c0_seq2 | cyclin-dependent kinase 4                     | 91     | 1.026690365 | 248    | 2.898456262 | −1.497473756 | 9.3091E−19 | comp46659_c0_seq1 | comp104770_c0_seq1 |
| CHK2        | comp93714_c0_seq1 | serine/threonine-protein kinase Chk2          | 116    | 1.042448685 | 212    | 1.973821482 | −0.921015425 | 3.8821E−08 | comp29305_c0_seq1 | comp185379_c0_seq1 |
| CYC         | comp93023_c0_seq1 | cytochrome c                                  | 1      | 0.030570159 | 77     | 2.438730115 | −6.317862227 | 1.8734E−19 | comp96131_c0_seq1 | / |
| E2F4        | comp94889_c0_seq2 | etoposide-induced 2.4 mRNA                    | 143    | 1.901385289 | 220    | 3.030624197 | −0.672564063 | 2.1863E−05 | comp87761_c0_seq1 | comp67955_c0_seq1 |
| GADD45      | comp87685_c0_seq2 | growth arrest and DNA-damage-inducible protein | 933    | 10.69806017 | 999    | 11.86763536 | −0.149681283 | 0.02904024 | comp58271_c0_seq2 | comp19840_c0_seq1 |
| P53         | comp93894_c0_seq1 | p53                                           | 1569   | 1.061581334 | 2957   | 1.963101    | −0.884869043 | 1.49E−91 | comp27951_c0_seq1 | comp20024_c0_seq1 |
| RCHY1       | comp94610_c0_seq1 | RING finger and CHY zinc finger domain-containing protein 1 | 80 | 1.340450829 | 140    | 2.430487591 | −0.885830608 | 2.8702E−05 | comp30489_c0_seq1 | comp128630_c0_seq1 |
| RFWD2       | comp93760_c0_seq1 | E3 ubiquitin-protein ligase RFWD2             | 122    | 1.853497561 | 146    | 2.298054677 | −0.310162907 | 0.094260381 | comp99768_c0_seq1 | comp10686_c1_seq1 |
| RRM2        | comp10097_c0_seq1 | ribonucleoside-diphosphate reductase subunit M2 | 4699   | 44.24117718 | 6338   | 61.8281522 | −0.482749578 | 1.8154E−67 | comp61643_c0_seq1 | comp10097_c0_seq1 |
| SESN        | comp313996_c0_seq1 | sestrin                                       | 12     | 0.967587612 | 0.5    | 0.041769028 | 4.53386815 | 0.000805877 | /     | comp150310_c0_seq1 |
| SIAH1       | comp38385_c0_seq1 | E3 ubiquitin-protein ligase SIAH1             | 886    | 4.980482149 | 1456   | 8.479580246 | −0.767707437 | 4.4008E−36 | comp189698_c0_seq1 | comp20596_c1_seq1 |

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resulting in the triggering of caspase-dependent and -independent pathways. First, we found that \textit{M. bicoloratus} parasitism regulated genes involved in forming the PTPC, which control mitochondrial apoptosis. Following \textit{M. bicoloratus} parasitization, Spli-CypD was dramatically up-regulated (Table 3, Fig. 1). PTPC, which is a large multiprotein structure assembled at the contact sites between outer membrane (OM) and inner membrane (IM) of mitochondria, regulates MMP. PTPC activation provokes a sudden increase in the IM permeability to solutes of low molecular weight, causing the unregulated entry of water and osmotic swelling of the mitochondrial matrix. Numerous studies suggest that the PTPC is assembled by ANT (in the IM), VDAC (in the OM) and mitochondrial matrix protein cyclophilin D (Cyp D) [13]. According to our data, under \textit{M. bicoloratus} parasitism, PTPC can form in the context of host hemocytes. Some DNA viral proteins may be direct inducers of MMP, and some may be indirect MMP facilitators, resulting in the activation of the mitochondrial apoptosis pathway [13]. This suggests an inducing condition of PTPC. \textit{M. bicoloratus} parasitism may promote cell death via regulation of PTPC formation to release factors involved in DNA fragmentation from mitochondria into nuclei to cleave DNA.

PTPC formed suddenly during immunochallenge, AIF, EndoG, and Cyt c in the mitochondria were released from the intermembranous space into the cytoplasm. EndoG and AIF directly move into the nucleus to digest DNA [28,29]. In mammals, the endonuclease DFF40 initiates DNA fragmentation. A recent report found that in \textit{Caenorhabditis elegans}, there is an unexpected connection between Dicer and DNA degradation during cell death [30]. The Dicer-family RNase III enzymes include helicase, PAZ, RNaseIII, and dsRNA-binding domains [30]. CED-3 cleaves DCR-1, the \textit{C. elegans} Dicer orthologue, as a candidate, at a specific position to yield a short isoform termed tDCR-1, which lacks the helicase and PAZ domain, and gains the capacity to cleave DNA into fragments [31]. Once DNA suffers double-strand breaks, the ATM signaling pathway activates and interacts with many different proteins to induce cell cycle arrest, increase DNA repair, and inhibit apoptosis, which involves the p53 signaling pathway, NF-kB signaling pathway and PI3K/Akt signaling pathway via the activation of IKKβ and p53 [32]. Typically, the activated ATM signaling pathway should inhibit host cell apoptosis for cell survival [33,34].

At this point, we wish to examine how the parasitism inhibited the ATM-triggered DNA repair and cell survival signaling pathways. During DNA damage in the host hemocytes, ATM is expressed (Table 6). The ATM signaling pathway is responsible for DNA repair via activation of the related cell survival signaling pathway [35]. DNA damage may activate protein kinases, such as ATM, to phosphorylate p53 at one of these three residues, which thereby increases the p53 level. After the DNA damage is repaired, the ATM kinase is no longer active. p53 will be quickly dephosphorylated and destroyed by the accumulated MDM2 [36]. p53 is conserved across eukaryotic organisms, and the decrease of transcriptional levels of genes regulated by p53 leads to a subdued resistance to pathogens infections. In \textit{C. elegans}, p53/ CEP-1 are inhibited by the nucleolar protein NOL-6, a nucleolar RNA-associated protein, causing innate immune suppression [37].

It is well known that PI3K/Akt signaling pathway regulates cell survival and apoptosis. PI3K is composed of heterodimers of inhibitory adaptor/regulatory (p85) and a catalytic (p110) subunits. p85 binds and integrates signals from various cellular proteins, including transmembrane tyrosine kinase-linked receptors and intracellular proteins, providing an integration point for activation of p110. Akt, which contains a PH domain in the N-terminal region, is the primary downstream mediator of the effects of PI3K. The PH domain of Akt interacts with 3'-phosphoinositides, contributing to recruitment of Akt to the plasma membrane. Recruitment to the membrane results in a conformation change, contributing to exposure of two crucial phosphorylation sites, serine 473 and threonine 308, for activation. An unexpected finding is that p85 was not expressed under \textit{M. bicoloratus} parasitism (Table 4). HSV-1, herpes simplex virus, induces the phosphorylation of Akt during infection of oral epithelial cells, leading to anti-apoptosis, and inhibition of HSV-1-induced PI3K activity increases DNA fragmentation [17]. Insect baculovirus AcMNPV activates PI3K/Akt signaling pathway anti-apoptosis to replicate itself in the host cell via enhancing phosphorylation of Ser 473 of Akt [18]. In our laboratory, overexpression of the gap junction proteins Im2 and Imx3 caused dramatic apoptosis in S09 and Spli221 cells but no phosphorylation of Akt in Hi5 cell lines, which reveals an anti-apoptosis function [26].

NF-kB signaling pathway regulates cell survival and apoptosis. In innate immunosuppression in invertebrates, it is well known that PDI protein vankyrins, which lack the phosphorylation and ubiquitination domains, function as IkB mimics via completion for the NF-kB site with IkB [38]. This results in retention of NF-kB in the cytoplasm, which inhibits immune gene expression for products such as antimicrobial peptides (AMPs) [39]. Three vankyrin genes were expressed in the host hemocytes (Table 1). NF-kB is constituted of p50 and p65 subunits. Normally, the p50/p65 complex is released from IkB and translocated to the nucleus to activate the transcription of genes involved in cell survival. During the immunochallenge, p50 and p65 were down-regulated by \textit{M. bicoloratus} parasitism (Table 5, Fig. 1) suggesting that \textit{M. bicoloratus} blocked the critical signaling pathway to promote cell apoptosis.

Ca²⁺ overload from the ER to mitochondria is required for initiation of programmed cell death. An unexpected result concerns Ca²⁺ loading between the endoplasmic reticulum and mitochondria. Previously, we proposed that innexin hemichannels on the ER can be Ca²⁺ channels, providing a pannexin 3-like function in the mammal to deliver Ca²⁺[24,31]. In such a case, inx genes should be up-regulated to produce more hemichannels, but 5 inx genes were been down-regulated, only inx4 was up-regulated (Table 2, Fig. 1). This suggests a disruption in hemichannel activation under \textit{M. bicoloratus} parasitism.

In Table 1 and Fig. 1, we show six types of gene transcriptions in the parasitized host hemocytes related to the Ankyrin-repeat, PTP, C-type lectin, Ben domain, Murin-like and EGF-like families. Recent research indicates that C-type lectin (SIGN-R1) enhances uptake and the processing of circulating apoptotic cells in the spleen [40]. CpBV-lectin encoded by \textit{C. phletae} bacovirus is secreted into plasma and binds to the surface of parasitoid eggs to induce host immunosuppression via inhibition of host hemocyte non-self recognition [41]. In our research system, considering the interaction between \textit{M. bicoloratus} bacovirus proteins and apoptosis, whether MbBV-lectin provides a relative contribution to apoptotic cell clearance, similar to SIGN-R1, requires further examination. However, it is reasonable to indicate that most important genes displayed less transcription in the host hemocytes during apoptosis. The Ben domain-containing proteins are well known to be involved in the transcriptional repression through its interaction with histone deacetylase, and overexpression causes cell cycle arrest [42]. The ankyrin-repeat protein family acts as inhibitors of nuclear transcription factors via binding of NF-kB homodimers [39]. Protein tyrosine phosphatases are the largest.
family encoded by bracovirus, and PTPs are well known as a regulator of apoptosis in human [43], such as PTP-1B regulation of the PI3K/Akt cascade to influence the nuclear localization of FOXO1, a transcription factor that regulates the expression of several pro-apoptotic genes [44], and SHP-1 that disrupts anti-apoptotic pathways through the regulation of the p85 subunit of PI3K [45], and TC-PTP also regulates p53 expression during apoptosis [46]. PTP-H2 from MbBV is a functional tyrosine phosphatase [47] and induces apoptosis of Sf21 cells [48]. MbCrp (egf-like) disrupts the cytoskeleton of host hemocytes [49].

In conclusion, our findings demonstrated that M. bicoloratus parasitism could regulate critical signaling pathways of host hemocytes to promote apoptosis to suppress host cellular immunity. Bracovirus may regulate proteins to form a PTPC structure that altered mitochondrial permeability, resulting in the release of DNA fragmentation elements, causing DNA damage and keeping ATM expression. This might have implications for better understanding of the mechanism of innate immunosuppression via the apoptosis pathway. However, analysis of the bracovirus proteins regulation of the critical signaling pathway may involve three levels in the cell, as a ligand binding to receptor on the cell surface, as a mini-protein to compete with scaffold proteins, as a nuclear factor to promote gene expression, as a host translation inhibitory factor to inhibit host protein translation or utilization of an RNAi mechanism [50] to inhibit gene expression on the mRNA level. The proteins responsible for specific signaling molecules in host hemocytes remain to elucidated.

Materials and Methods

Insect rearing and experimental animals

The S. litura colony was reared on an artificial diet (formulated according to [51]) at 27±1°C, RH 60–80%, and under a 12:12 h photoperiod regimen. The parasitoid M. bicoloratus colony was maintained on S. litura larvae reared in the laboratory according to established methods [52]. Adults were also provided with honey as a dietary supplement.

Isolation of hemocytes from larvae of S. litura

Hemocytes were collected 5 days post-parasitization from parasitized S. litura larvae (more than 1,000) (when immature parasitoids in the host developed to the second larvae [52], approximately 21% hemocytes underwent apoptosis [1]) and named ‘M’ (parasitized by M. bicoloratus) in this paper. The fourth instar S. litura larvae were used to collect hemocytes to serve as the control group, named ‘S’ (non-parasitized S. litura hemocytes) in this paper.

Total RNA extraction

Total RNA was isolated from hemocytes using an RNeasy Plus Universal Mini Kit (QIAGEN, Maryland, USA), which is specific for genome DNA elimination, according to the manufacturer’s instructions. The concentration of each RNA sample was determined by measuring OD at A260/A280 using the NanoDrop 2000. Samples with an A260/A280 ratio 2.0, concentration 2000 and running 1 x TBE agarose gel. High quality samples (with an A260/A280 ratio >2.0, A260/A230>2.0, concentration> 500 ng/μl) showing 28S and 18S RNA bands clearly were stored at –80°C until use. RNA was prepared from at least two biological replicates and used for independent library preparations.

Transcription mRNA sequencing, assemble, gene predicted

Sequencing libraries were prepared using a RNA-Seq sample preparation kit from Illumina following the manufacturer’s instructions. The transcription sequences were sequenced using an Illumina Hiseq2000, and the total base number was more than 26.3 Gb per sample. There were two replications for the M1, M2, S1, and S2 pools. RNA-seq de novo assembly was performed using Trinity [53]. GetORF in EBOSS were used to find protein from contigs [54].

Gene Ontology (GO) and KEGG data

GO Slim test were assigned to the NR-annotated transcripts using a local Blast2GO pipeline b2gpipe [55] with access to a local GO MySQL database (version of April 2013). The Kyoto Encyclopedia of Genes and Genomes (KEGG) was used for analysis of molecular networks [56].

Definition of up- or down-regulated genes based on fold change

Clean reads were mapping to assembled contigs, to get RPM value based on reads number [57]. Statistical analysis of data was performed using DESeq [58]. Transcript abundances for each gene were expressed as a weighted mean of counts from each replicate normalized to the overall library size (known as ‘base mean’). p-values (adjusted for false discovery rate) were generated for each gene in pair-wise comparisons between different conditions. In our analyses, we used an adjusted p-value of 0.001 as a criteria for identifying significant differences in gene expression.

Total RNA isolation, cDNA synthesis and qRT-PCR

Total RNA was isolated from hemocytes of parasitized S. litura larvae 5 days post-parasitization using RNAiso Plus (TaKaRa, Dalian, China), according to manufacturer’s instructions, including DNase treatment. The concentration and purity of each RNA sample was determined by measuring OD at A260/A280 using NanoDrop 2000. Samples with an A260/A280 ratio >2.0 were used to synthesize cDNA using Oligo d (T) 18 primers following manufacturer’s instruction (TaKaRa, Dalian, China). All cDNA samples were stored at −80°C for preservation. qRT-PCR was performed using SYBR PCR Kit (Takara, Dalian, China) with the ABI 7500 system following the cycling parameters: 50°C, 2 min; 95°C, 10 min; 95°C, 5 sec, 60°C, 34 sec, 40 cycles; 95°C, 15 sec; 60°C, 1 min; 95°C, 30 sec; 60°C, 15 sec. The 2-ΔΔCT method was used to get the relative mRNA levels [59]. 18S rDNA gene was used as the housekeeping genes for normalization. Three replications have been carried out for per sample.

GenBank accession numbers

The whole RNA-Seq project was deposited into DDBJ/DRA/GenBank under the accession DRA001149.

Supporting Information

Figure S1 Completed ORF and short qRT-PCR products. (TIF)
Table S1 Sample information. (XLS)
Table S2 Sequencing output and quality. (XLSX)
Table S3 EST cluster contigs. (XLSX)

Table S4 Primers of completed ORF and short qRT-PCR. (XLSX)

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