Transcriptomic analysis of bovine monocytes in response to non-cytopathic bovine viral diarrhea virus infection

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Research article

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Abstract

Background: Monocytes are significant players in the detection of invading pathogens, particularly in pathogen defense. Bovine Viral Diarrhea Virus (BVDV) can cause a persistent infection and immune suppression if animals are infected with a non-cytopathic (ncp) biotype. However, its exact role in ncp BVDV-infected bovine monocytes remains poorly understood. To explore the immune suppression mechanisms of ncp BVDV, we used a transcriptomics approach to find genes with differential expression patterns in monocytes during infection with ncp BVDV over time.

Results: Bovine monocytes were sampled at 2 and 24 h post-infection (hpi) to represent the early and late stages of an ncp biotype strain of bovine viral diarrhea virus infection. Compared with the non-infected cells, 9959 and 7977 differentially expressed gene (DEGs) were identified at 2 and 24 h hpi, respectively. These DEGs were associated with signal transduction, immune response, apoptotic process, cellular process, binding and cellular component. The differential expression profiles of select the type I interferon signaling pathway, interferon (IFN)-stimulated genes (ISGs), and genes involved in the innate immune response, including IRF7, DDX3X, TLR13, DDX58(RIG-I), MVAS, TLR9, TRAF6, IRF1, IFIT1, STAT1, ISG20, TRIM25, MX1, NLRX1, CYLD, SIKE1 and ZAP70 were confirmed by real-time quantitative PCR and consistent with the RNA-seq data. These results indicated that infection with ncp BVDV could activate type I interferon signaling pathway in bovine monocytes and induces weak ISGs responses, which extends our present understanding how the virus modulates the immune response and leads to better understanding behind the immunopathogenesis of ncp BVDV.

Conclusion: Our transcriptome analysis provides useful initial data towards better understanding of the infection mechanisms used by ncp BVDV, while highlighting the potential molecular relationships occurring between the virus and the host's immune response.

Background

Bovine viral diarrhea virus (BVDV) is a small positive-sense single-stranded enveloped RNA virus that is part of the Pestivirus genus of the Flaviviridae family and a viral pathogen of cattle. BVDV infection is found in cattle of all ages and breeds worldwide, with a huge economic impact due to production and reproductive losses[1, 2]. BVDV can also be categorized under two biotypes based on their growth characteristics in cell cultures. The rare cytopathic (CP) biotype will damage tissue cultures and the much more common noncytopathic (ncp) will not[3, 4]. The pathogenesis of the disease caused by BVDV is complex and involves persistent infection (PI) and immune suppression with the ncp biotype during early gestation, followed by an acute infection by a cp biotype[5].

In recent years, the understanding that the vertebrate innate immune system represents the first defense against pathogens has emerged. The synthesis and secretion of type I interferons (IFN-I) is the first step in the induction of cellular innate immune responses [6] to viral infections or double-stranded RNA (dsRNA). dsRNA is a common pathogen-associated molecular pattern (PAMP) that is recognized by a
variety of pattern recognition receptors (PRRs). Currently identified PRRs can be divided into the Toll-like receptors (TLRs), the retinoic acid-inducible gene I (RIG-I) receptor-like receptors (RLRs), and the NOD-like receptors (NLR). All three PRRSs recognize the viral PAMPs [7]. Each of the three receptors cooperates with their adaptor proteins to deliver information in the presence of viral PAMPs and interferons. The Toll-like receptors affect the Total Recordable Injury Frequency (TRIF) adaptor protein, the Cyclic GMP-AMP synthase (cGAS) receptor effects the Stimulator of interferon genes (STING) adaptor protein, and the RIG-I receptor interacts with the Mitochondrial antiviral-signaling (MAVS) adaptor protein. These connecting molecules recruit and activate downstream signaling pathways to produce corresponding inflammatory cytokines, chemokines, and Type I (IFN-α/β) and III (IFN-γ) interferons [8, 9]. Much research has demonstrated that evading innate immunity is vital to viral persistence because it evades host immunity [10].

Previous studies have revealed many crucial discrepancies in the results of ncp and cp biotypes in variety of cell types. ncp BVDV does not induce IFN synthesis in cultured cells in vitro, suggesting that this could be a defense mechanism to evade innate host immunity that might be critical for establishing persistent infections [11, 12, 13]. However, the molecular mechanisms by which BVDV evades host cell immunity remains unclear. RNA-seq has recently been developed for transcriptomics analysis of BVDV infection in MDBK cells. Research was performed with microarrays that showed gene expression changes in host cells infected with ncp BVDV compared with cp BVDV [14, 15]. However, it was reported that a large number of genes related to BVDV invasion and replication mechanisms still need to be characterized to better understand the interaction between BVDV pathogenetic mechanisms and host immune defenses. Although ncp BVDV is thought to play an important role in BVDV-infected bovine monocytes [16–19], little is known about how BVDV disturbs host cell gene expression profiles. During this research, we showed that BVDV infected bovine monocyte gene expression profiles, which provided meaningful information from genetic analyses that could not be discerned using biochemical methods.

Materials And Methods

Animals

Nine conventionally reared, healthy BVDV-free cows from the Shihezi University Animal Experimental Station were used. All animal experiments were approved by the Shihezi State University Institutional Animal Care and Use Committee. All animals were confirmed to be free of BVDV, infectious bovine rhinotracheitis virus, bovine parainfluenza virus, and Mycoplasma bovis infection using enzyme linked immunosorbent assay (ELISA) kits (Idexx Labs Inc., USA) and by reverse transcription PCR (RT-PCR) or PCR for viral nucleic acid detection [18]. As we expected, all animals were BVDV mRNA negative.

Cell preparation

Blood samples (150 ml) were obtained by jugular puncture into blood collection tubes (16 x 100 mm, Tyco Healthcare). Bovine PBMCs were isolated as previously reported elsewhere [16, 17]. Briefly, PBMCs
were separated from the blood on a Histopaque gradient (1.077 g/ml, Amersham Biosciences) and resuspended in RPMI–1640 supplemented with 10% FBS, 1% Glutamax–1 (Invitrogen), 5 x 10⁻⁵ M 2-Mercaptoethanol, and 100 IU/ml gentamicin (Invitrogen). For monocyte isolation, 40 ml of the PBMC suspensions (5 x 10⁸ cells) were added to Petri dishes (150 x 25 mm, BD Biosciences) for 2 hours at 37°C. Non-adherent cells were removed, and adherent cells were washed two times with PBS. The yield of recovered adherent cells was 20–30% of the initial PBMC. Adherent cells were then incubated with a CD14 monoclonal antibody (MM61A, VMRD) followed by incubation with mouse anti-IgG1 microbeads (Miltenyi Biotech, Auburn, CA). Using magnetic bead separation technology, CD14⁺ monocytes were selected using the manufacturer’s instructions (Miltenyi Biotech), yielding a final monocyte concentration that was 2–3% of the original PBMC population[18,19].

**BVDV stocks and infection**

The ncp BVDV strain was named BVDV-LC (GenBank accession NO. MK102095, identified and kept in our lab), and was classified as genotype 1q and non-cytopathic (ncp). For titration of virus stock, four replicates of 10-fold serially diluted virus (starting from 1/10) were inoculated on MDBK cell monolayer in 96-well culture plates. After 48 h incubation, the culture plates were fixed at 4 °C for 30 min with ice cold absolute ethyl alcohol and subjected to immunofluorescence staining with fluorescein isothiocyanate (FITC)-conjugated polyclonal anti-BVDV (VMRD, USA) antibody (diluted 1:1000). The fluorescence signals were observed under a fluorescence microscopy (ZEISS) and viral titers was expressed as the 50% tissue culture infective dose (TCID₅₀)/mL by Reed-Muench method.

For infection of bovine monocytes, virus dilutions were made in DMEM 4Mm L-glutamine, 4.5 g/l glucose, 1.5 g/l sodium bicarbonate and 10% horse serum. 5x10⁶ monocytes were added to each well of a 6 well tissue culture plate and adsorbed with ncp BVDV biotypes at the same MOI of 0.1 for 2 h. After infection 2 h and 24 h, at least 10⁷ cells were pooled in one tube. Uninfected control (mock) cells were treated similarly but were dosed with media and no viral inoculum. All data were determined using triplicate monocyte cultures.

**RNA extraction**

Cells were harvested and prepared for RNA extraction 2 hours and 24 hours after infection. The monocytes were rinsed three times with ice-cold PBS, and whole RNA was extracted by DNase digestion using the RNeasy mini kit following the manufacturer’s instructions (Qiagen). RNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher) and stored at −80°C. For RNA-seq analysis, the RNA quality was assessed with an Agilent Bioanalyzer (Agilent Technologies).

**RNA sequencing and analysis**
The RNA elimination was performed using Ribo-Zero rRNA removal kit (Epicenter, USA) following the manufacturer’s instructions. Illumina sequencing libraries were prepared using the TruSeq Stranded mRNA Sample Preparation Kit (Illumina, USA) according to the manufacturer’s protocol. RNA sequencing was performed on the Illumina HiSeq 4000 platform at the Novogene Bioinformatics Institute (Beijing, China).

The sequence data of the reference genome was checked against the NCBI database. The filtered mass reads were aligned with the reference genomic sequence using Bowtie 2. Relative transcript abundance was metered as fragments per kilobase per million mapped reads (FPKM). Genes were clustered into functionally relevant groups using the eggNOG (Genetic Evolution Genealogy: Unsupervised Orthologous Group) database and the metabolic pathways were analyzed using the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. Visualization of mapping outcomes and differentially expressed gene (DEG) analysis was performed using the CLRNASEqTM program (ChunLab, Korea).

Infection and control time points were performed in triplicate using purified monocytes, and three biological replicates were generated. ncp BVDV expression and infected monocyte gene expression were contrasted with gene expression from the inoculum and uninfected monocytes. The fold change in gene expression was calculated from three different sample types.

**Real-Time Quantitative Reverse Transcription PCR (qRT-PCR)**

cDNA was synthesized from 2 μg of total RNA using a reverse transcription kit (Fisher Scientific, USA) and by following the manufacturer’s instructions. Gene expression was quantified using a LightCycler 480 PCR platform (Roche Applied Science, Indianapolis, IN, USA). The qRT-PCR procedure began with an initial step of 10 minutes at 95 °C followed by 45 cycles at 95 °C for 30 seconds, 58 °C for 30 seconds, and 72 °C for 30 seconds. Gene transcription levels were quantified relative to GAPDH gene expression using the relative cycle threshold (ΔCT) method. Primers were made using Primer 5.0 software (Table 1).

**Statistical analysis**

All data are shown as means ± SE. Differences were evaluated using ANOVA followed by the Student’s t-test. Statistical significance was defined as P < 0.05.

**Results**

**RNA sequence analysis of ncp BVDV-infected monocytes**

The results show that more differential expression, either up- or down-regulated was seen at 2 h post-infection (hpi), the expression of 9959 genes was significantly changed compared with those of the controls, of which 4968 genes were upregulated, and 4991 genes were down-regulated (Fig.1A, Additional file 1:Table S1). At 24 hpi, the expression of 7977 genes was significantly different from that of the
controls, with 4184 genes up-regulated and 3793 genes down-regulated (Fig.1A, Additional file 2:Table S2). These significantly altered genes were involved in signal transduction, immune response, apoptotic process, cellular process, binding and cellular component, with most of the differential genes being involved in signal transduction.

The DEGs were filtered to determine which genes were present at both of the time points (2 hpi and 24 hpi). At 2 hpi time point, 9959 genes responded to ncp BVDV infection, and at 24 hpi time point, 7977 genes responded; however, only 5709 genes were common at both time points (Figure 1A). Two libraries were packaged and analyzed against the non-redundant NCBI database using the BLAST program. Next, we mapped and annotated the genetic libraries using the Gene ontology (GO) database. To identify pathways involved in immune activation after ncp BVDV infection of bovine monocytes, transcript records were further analyzed. The GO terms classify the function of BVDV-infected cell transcripts, offering 4014 transcripts involved in molecular functions, 4476 involved in biological processes, both and 3341 involved in discretely cellular processes (Figure. 1B). Analysis revealed that differentially expressed genes are involved in a variety of biological processes, including immune responses and immune system procedures, cell death, macromolecular localization, apoptosis, cell death regulators, antigen processing and presentation, protein localization, and others (Figure. 1C, Additional file 3:Table S3, Additional file 4:Table S4).

To further define DEGs function, KEGG pathway/enrichment analysis was performed. DEGs were significantly enriched in 20 pathways Fig.1D and E 126 and 113 DEGs show Ribosome pathway enrichment in 2 hpi and 24 hpi, respectively. This pathway was mainly responsible for gene expression regulation and protein translation. Many significantly DEGs were involved in immune responses such as with the TNF signaling, B cell receptor signaling pathway, Fc epsilon RI signaling pathway, T cell receptor signaling pathway and NF-κB signaling pathway (Fig.1D and E, Additional file 5:Table S5, Additional file 6:Table S6). These DEGs, which include TRIM25, TRAF6, TRAF3, IL−1β, CD14, BCL2, TICAM1/2, TNIP3, TNFSF13B, ZAP70, TRAF2, TRAF2, TNFSF11, PIDD1, CARD11, LYN, BLNK, BCAP, BCL−10, CD81, CD19, MEK1/2, AP1, BCL10, Ikκα, Ikκβ, PD−1, CD45, LCK, CBL, ZAP70, LAT, etc, play an important role in activating or inhibiting innate and adaptive immunity (Table 2, Additional file 1:Table S1, Additional file 2:Table S2). These DGEs were further analyzed (GO: 0006955) (Figure.2A), A list of genes that were differentially expressed between 2 hpi and 24 hpi is shown in Table 2. Most of the upregulated genes were related to the immune system. Upregulated genes included IL6 (3.1 fold), TNFSF13B (2.5 fold), CD14 (2.8 fold), IFI27 (5.7 fold), RNF125 (1.8 fold) and MAPK12 (2.4 fold), and downregulated genes included IRF7 (1.08 fold), TLR13 (2.2 fold), ZAP70 (2.9 fold), TLR8 (1.5 fold), TLR9 (1.5 fold), IFITM1 (1.8 fold), and IGHG2 (2.9 fold). Some genes (eg, IFI30, JUN, CD40, LTBP2, IKBKB, CCL5, and MAPK11) were up-regulated at 2 hpi and down-regulated at 24 hpi. Other genes, such as EIF2AK2, DHX58, DDX3X, IFI6, TIRAP, STING, and IFIT1 were downregulated at 2 hpi and upregulated at 24 hpi (Table 2).

Through careful examination of the information set (Figure. 2B and Table 2), several ISGs [20] mainly produced by type I interferons, were differentially expressed in ncp BVDV-infected cells. Figure.2A and B show stratified heat maps of all of the up or down regulated genes in ncp BVDV-infected cells, and they
were all associated with immune responses and type 1 IFN signaling. Overall, the RNA-seq analysis indicated that ncp BVDV infection resulted in different expression of genes related to inherited immunity, type I IFNs, stimulatory cytokines, and cell signaling pathways, leading to the creation of antiviral responses in monocytes.

Partial validation of the DEGs involved in the type 1 IFN pathway by qRT-PCR

Sequences found using RNA-seq analysis were validated with qRT-PCR to assess if mRNA expression was upregulated or downregulated in relation to the genes and to the immune responses. The genes were identified if they were recognized as being involved in type I IFN responses, were reported to be related to innate antiviral immune responses [21, 22], and if they were common to both infected groups. Using GAPDH as an internal control, the transcription levels of 31 DEGs were measured (between the 2 and 24 hour time points) with qRT-PCR with the primers listed in Table 1. In conclusion, the RNA-seq and qRT-PCR results from bovine monocytes at 2 and 24 hpi were fairly consistent (Figure. 3). IRF7, TLR13, ZAP70, IFITM1 were all down-regulated 2 hours and 24 hpi with ncp BVDV virus. DDX3X, IFIT1, STING were down-regulated 2 hpi and up-regulated 24 hpi. The expression of IKBKB, TRAF1, IRF5 was up-regulated 2 hpi and down-regulated 24 hpi. EF2AK2, IFI27, IFI30, MX1, MX2 and other genes were up-regulated sharply 2 hpi compared with 24 hpi.

Discussion

The complex and unique nature of BVDV continues to challenge infectious disease researchers, veterinarians, and the cattle industry. The different genotypes and biotypes of BVDV ability to induce persistently infection, as well as its ability to interfere both innate and adaptive immunity of the host, make it difficult for prevention and control[5]. Although Mais[18] evaluated the effect of cp and ncp BVDV infection of bovine monocytes to determine their role in viral immune suppression and uncontrolled inflammation by using proteomics, this study was the first to analyze transcriptionally diversified gene expression after in vitro ncp BVDV infection of bovine monocytes. In this study, using RNA-seq, we demonstrated that most of the DEGs between ncp BVDV infected monocytes for 2 and 24 hours, involved immune responses, suggesting that ncp BVDV infection plays an important role in bovine monocytes. We observed increased levels in a variety of antiviral genes such as IFI27, IFI30, MX1, TRIM25, DDX41, ISG20, STAT1, TRAF6, CD14, CD40, IL1B, IL6, MAPK12, MAPK13, TNFSF13B, and TBKBP1 (Table 2), It seems to play a fundamental role in ncp BVDV infection in monocytes. The expression levels of the IFI27, IFI30, ISG20, STAT1, TRAF6 and TRIM25 genes obtained from the RNA-seq data were confirmed by the real-time PCR mRNA expression data (Table 1 and Figure. 3). These proteins might be upregulated in bovine monocytes because of the ncp BVDV infection. However, we observed that type I IFN genes were reduced such as IRF7, DDX3X, DDX58, TLR13, TLR9, STING, and IFIT1, which was possibly caused by the ncp BVDV viral particles[12, 13]. On the other hand, upregulated DEGs, such as NLRX1, CYLD, SIKE1, ATG12, and RNF125, were shown to interfere with host immune responses in previous documents [23–26].
The DEAD-box proteins are characterized by the conserved sequence Asp-Glu-Ala-Asp (DEAD) is a presumptive RNA unwindase involved in many cellular processes, such as RNA combination and RNA secondary framework changes. This gene codes proteins involved in the RNA helicase-DEAD box protein motif and the caspase employment domain (CARD). It is related to the viral double-stranded (ds) RNA identification and coordination of immune responses [27, 28, 29].

Our research showed that DHX16, DHX34, DHX37, DHX58 (Table 2) were not significantly DEGs after ncp BVDV infection, but DHX58 expression was increased (Figure. 3). In other studies, viral dsRNA stimulation did not induce high expression of DEAD-box proteins, and viral dsRNA was shown to be degraded by some proteases, such as BVDV Erns protein [30, 31]. We found that DHX16, DHX34, DHX37, DHX58 DDX3X, DDX10, DDX51, DDX54, and DDX55 were differentially expressed after the ncp BVDV infection of monocytes. Post-downregulation was not significant (Table 2), but DHX41 expression was significantly increased, suggesting that DDX41 plays an important role in RNA binding and secondary structure changes [32, 33].

Toll-like receptors (TLRs) are one of the most essential innate immune receptors that identify many types of PAMPs from different pathogens and activate downstream immune responses. TLRs are grouped into two groups: TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11, which are concerned with membrane proteins that detect microbial membrane components, while TLR3, TLR7, TLR8, and TLR9 are concerned with nucleic acid recognition of proteins, cells, intracytoplasmic molecules, and microorganisms [34, 35]. Nevertheless, studies showed that infections and administrative mechanisms restrict the TLR13 signaling pathway in mollusks. In this study, TLR8, TLR9, and TLR13 expression in BVDV infected bovine monocytes were downregulated (Table 2 and Figure. 3), which may be the reason for the decreased type 1 IFN expression that was seen [36- 40].

This study found common groups of genes related to important cellular processes at 2 hpi. For example, inhibition of NF-κB activation was induced by upregulated IKKE and SIKE1, negative regulators of the NF-κB signaling pathway, and by downregulated p65/RelA-NF-κB [26]. These inhibitory actions could lead to blocked or delayed antiviral responses (Table 2). These results are interesting because they show that infected living cells exhibit strong immunosuppressive phenotypes. In fact, the results also show downregulation of vital immune response genes, for example, the PIDD1, IKBKG, IKBKB, INFGR2, PRKCQ and EIF2AK2 genes (Table 2). Moreover, the expression of IKKA and SIKE1 transcripts indicates the blocking of NF-κB in infected cells, which were support by the qRT-PCR results (Figure. 3 and Table 2).

Our research demonstrates that there is antiviral regulation into bovine monocytes upon activation, as shown by upregulation of the NLRX1, CYLD, SIKE1, ATG12, and RNF125 genes (Table 2 and Figure. 3). These findings are vital for the understanding of the molecular mechanisms by which ncp BVDV evades innate immunity. NLRX1 is involved in antiviral signaling. By inhibiting the virus-induced RLH (RIG-like helicase)-MAVS interaction, NLRX is a negative regulator of the MAVS-mediated antiviral response [23]. RNF125 mediates DDX58/RIG-I ubiquitination inducing DDX58/RIG-I degradation of Lys–181 [24]. SIKE1, IKK-epsilon, and TBK1 genes are physiological inhibitors that act on viral and TLR3-triggered IRF3 to
inhibit TLR3-mediated interferon-stimulated response elements (ISRE) and IFN-β promoters. Activation of the ISRE and IFN-β promoters can be achieved by disrupting the interaction between IKBKE or TBK1 and TICAM1/TRIF, IRF3, and DDX58/RIG-I, which does not inhibit the NF-κB activation pathway [26].

Type I IFNs are key mediators of host immune responses to viral infections. These IFNs induce the expression of many ISGs of which some could be antiviral. Besides, they regulate innate and adaptive immunity by activating immature DCs, strengthening NK cell responses, and boosting the survival and effector functions of T and B lymphocytes [41]. In this research, a significant decrease in ZAP70, TNFSF11, V-TCR, and CARD11 was regulated in ncp BVDV-infected bovine monocytes. ZAP70 regulates T cell activation by modulating TCR expression on T cell surfaces [42, 43]. CARD11 is involved in the costimulatory signals necessary for T cell receptor (TCR)-mediated T cell activation. CARD11 connects with the DPP4 and guides T cell multiplication and NF-κB activation in a T cell receptor/CD3-dependent manner [44]. TNFSF11 is a necessary regulator of T cell and dendritic cell interactions and might regulate T cell-dependent immune responses [45]. Most serine/threonine kinase-expressing genes are increased upon ncp BVDV infection of monocytes, which is quite interesting because only MAP2K6 is significantly reduced. MAP2K6 plays a vital role in regulating cytokine to cytokine responses, particularly as it relates to the induction of IL-6 production. IL-6 is increased 2 hpi and is decreased by 24 hpi (Table 2 and Figure. 2) [46, 47].

Conclusions

In conclusion, the lack of knowledge on how BVDV evades host immune responses causes improper control of this disease. Our studies have identified IRF7, DDX3X, TLR13, TLR9, STING, and IFIT1 as key downregulatory molecules in ncp BVDV infected monocytes. Conversely, NLRX1, CYLD, SIKE1, ATG12, and RNF125 act as upregulatory molecules. There also appears to be negative regulation of IFN production. On the basis of previously reported studies and current information, we feel that the underlying role of these genes are to defend against ncp BVDV infection. More research is needed to clarify the mechanism by which ncp BVDV upregulates and downregulates these various molecules and to determine the role of these genes in the pathogenesis of BVDV, which will thus enhance our understanding of BVDV replication and pathogenesis.

Abbreviations

BVDV: Bovine Viral Diarrhea Virus; ncp: non-cytopathic; ISGs: interferon stimulated genes; IRF7: interferon regulatory factor 7; DDX3X: DEAD-Box Helicase 3 X-Linked; TLR13: Toll-like receptor TLR 13; TRAF6: TNF receptor-associated factor 6; IFIT1: Interferon-induced protein with tetratricopeptide repeats 1; STAT1: Signal transducer and Activator of transcription 1; ISG20: interferon-stimulated gene 20 kDa; TRIM25: Tripartite Motif Containing 25; MX1: MX Dynamin Like GTPase 1; NLRX1: the nucleotide-binding oligomerization domain, leucine rich repeat containing X1; CYLD: Cylindromatosis, turban tumor syndrome; SIKE1: Suppressor Of IKBKE 1; ZAP70: Zeta chain of T cell receptor-associated protein kinase 70; PAMP: pathogen-associated molecular pattern; PRRs: pattern recognition receptors; TLRs: Toll-like
receptors; RIG-I: retinoic acid-inducible gene I; NLR: NOD-like receptors; TRIF: Total Recordable Injury Frequency; cGAS: Cyclic GMP-AMP synthase; STING: Stimulator of interferon genes; MAVS: Mitochondrial antiviral-signaling; PBMC: peripheral blood mononuclear cells; TCID50: The 50% tissue culture infectious dose; mock: Uninfected control; FPKM: fragments per kilobase per million mapped reads; DEG: differentially expressed gene; GO: Gene ontology; NF-κB: nuclear factor kappa-B; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; qRT-PCR: real-time quantitative reverse transcription-polymerase chain reaction;

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors’ contributions

YH and CC contributed to the study conception and design. YH, JY and XH wrote the manuscript. CC, CX and FZ checked and revised it. YH, JY, XM, XH, XZ and TG performed the experiments and analysed the data. All authors read and approved the final version of the manuscript.

Ethics approval

This study was carried out in accordance with the recommendations of National Standards for Laboratory Animals of the People's Republic of China (GB149258–2010). The protocol was approved by the Shihezi State University Institutional Animal Care and Use Committee. Cattles used for the study were handled in accordance with good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.
Consent for publication

Not applicable

Conflicts of interest

The authors have no conflicts of interest to declare.

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Tables

| Table 1. Primers used for real-time PCR. |
|---|
| **Name** | **5′Sequence** | **3′Sequence** |
| IRF7 | CGCGCGTCCTCTCGAGGCGAG | CCCCTGGGCTACGAGCAACAA |
| DHX58 | TGCTGCTCTGCTGTTGTTGGA | AAGCAGACAGCTTTCCTTCT |
| DDX3X | GCTGGCCTAGGATGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| MAVS | ATGGGCCATCTGAGGAAAGC | CTGGGGTAGGCGCCGCGTA |
| IKKKB | GCCATGATCACTGCTCATGCG | TGTGCCCAACTCCGCTG |
| TRAF6 | TGGCCATAGAAAGATGACGAGA | GCAGCTGAGCAGAGCACCT |
| TRAF1 | CTTGCAACAGCCTGCTGCTGCT | TGGCCGAGATCCACGAGGAG |
| IRF5 | GCTGGCAAGGTGAGGAGGAGG | TGGAGAAGCTGGACATG |
| IRF9 | CTTGATGCTGCTGCTGCTGCT | TGGCCGAGATCCACGAGGAG |
| IRF1 | GCCAGCTGACGACCTGCTGCT | GCCAGCTGACGACCTGCT |
| STAT1 | CAGCGAGATGACGACCTGCTGCT | GCCAGCTGACGACCTGCT |
| EIF2AK2 | GATGGTTCGTAGTCTCTGCTGCT | CTGGGAGACAGGAGGAGAGC |
| TRIM25 | GGGGCGAGGAGTGTTGCTGCTGCT | AAGCAGACAGCTTTCCTTCT |
| TLR13 | GCTGGGCTAGGATGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| TLR9 | CTGGGCTAGGATGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| IFIT27 | TTTGCCACTGAGGACGACGAC | AGGAGGAAGCAGTATGCT |
| IFI30 | CTTACCTTGTGTCGCTGCTGCT | GCTGGGCTAGGCGCGGCCGT |
| ISG20 | GGGCAGGAAGTGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| ZAP70 | AGCGCAAGATCAGGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| SHK1 | ACCAGCTGACGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| RNF125 | GACACTCCTGCTGCTGCTGCT | ACTAAACGGCAAGTGGCAAG |
| CYLD | GAGGCTACAGGATGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| NLRX1 | GACCCAGATGCCTGTGCTGCT | TGGGAGACAGGAGGAGAGC |
| STING | CGGTGCTGCTGCTGCTGCTGCTG | CTGGGCTAGGCGCGGCCGT |
| IFITM1 | CTTGAGGAGGAGGAGGAGGAGG | TGGGAGACAGGAGGAGAGC |
| IFITM3 | GCTTAAAGGAGGAGGAGGAGG | TGGGAGACAGGAGGAGAGC |
| IFIH1 | ACTGAGGAGGAGGAGGAGGAGG | TGGGAGACAGGAGGAGAGC |
| IFI6 | AAAAGCATCAGGAGAAACAGC | CATGAGGAGGAGGAGAGC |
| MX1 | CTTGCGTGGGCTGCTGCTGCTGCT | CTGGGCTAGGCGCGGCCGT |
| MX2 | CTTGCGTGGGCTGCTGCTGCTGCT | CTGGGCTAGGCGCGGCCGT |
| IFIT1 | CCTTAAACAGGACGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| GAPDH | GATTGTAGGACGACGACGAC | CTGGGCTAGGCGCGGCCGT |
| Gene ID   | Genename           | Protein                                                                 | log2Fold Change 2 h | log2Fold Change 24 h | regulation |
|----------|--------------------|--------------------------------------------------------------------------|---------------------|---------------------|------------|
| 10012559 | IRF7               | Interferon regulatory factor 7                                           | -1.0805             | -0.3302             | Down       |
| 100137737| PIDD1              | p53-induced death domain-containing protein 1                           | -1.4506             | -0.6936             | Down       |
| 100138357| TLR13              | Toll-like receptor 13                                                    | -2.2337             | -1.4470             | Down       |
| 100300510| V-TCR              | Viral T-cell receptor beta chain-like T17T-22                            | -2.4117             | -0.8672             | Down       |
| 101902461| IGHM               | Interferon-induced protein with tetratricopeptide repeats 1, IFIT-1     | -2.0308             | -0.6729             | Down/UP    |
| 101904342| Unkwon             |                                                                         | -2.0912             | -0.5966             | Down       |
| 101905399| MP2K1              | Dual specificity mitogen-activated protein kinase kinase 1, MAP kinase 1 | 1.6315              | 0.8817              | Up         |
| 107131142| TN13B              | Tumor necrosis factor ligand superfamily member 13B                     | 2.5355              | 0.6563              | Up         |
| 280826   | IL6                | Interleukin-6                                                            | 3.1302              | 1.7687              | Up         |
| 280831   | JUN                | Transcription factor AP-1 (Activator protein 1, AP1)                     | 0.2522              | -0.4913             | Up/Down    |
| 280872   | MX1                | Interferon-induced GTP-binding protein Mx1                               | 0.2973              | 1.3411              | Up         |
| 280991   | AKT3               | RAC-gamma serine/threonine-protein kinase                               | -0.3475             | -0.1926             | Down       |
| 281048   | CD14               | Monocyte differentiation antigen CD14                                    | 2.7904              | 1.6051              | Up         |
| 281073   | IKKA               | Inhibitor of nuclear factor kappa-B kinase subunit alpha                 | 0.3704              | 0.4059              | Up         |
| 281251   | IL1B               | Multifunctional fusion protein (Includes: Interleukin-1; Interleukin-1 beta) | 2.1884              | 0.9302              | Up/Down    |
| 281346   | PLAU               | Urokinase-type plasminogen activator                                     | 1.2409              | 0.9751              | Up         |
| 281408   | RAC1               | Ras-related C3 botulin toxin substrate 1                                | 1.1334              | 0.4546              | Up         |
| 281499   | SPP1               | Osteopontin,OPN                                                          | -2.8820             | -1.3743             | Down       |
| 281534   | TLR2               | Toll-like receptor 2                                                     | 0.3926              | 0.4348              | Up         |
| 281850   | IGHG1              | Immunoglobulin heavy constant gamma 1                                    | -2.9087             | -0.6335             | Down       |
| 281854   | IKBKB              | Inhibitor of nuclear factor kappa-B kinase subunit beta                  | 0.4542              | -0.5201             | Up/Down    |
| 533223   | IKKE               | Inhibitor of nuclear factor kappa-B kinase epsilon subunit homolog 1, IKBKE | 1.6054              | 0.4558              | UP         |
| 281855   | IKBKG              | NF-kappa-B essential modulator, NEMO                                      | -0.6517             | -0.3850             | Down       |
| 281857   | IL12B              | Interleukin-12 subunit beta, IL-12B                                     | -0.7333             | -1.8455             | Down       |
| 281987   | PLCG1              | 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1        | -1.9601             | -0.4078             | Down       |
| 282023   | PTGS2              | Prostaglandin G/H synthase 2,cox-2                                      | -1.5531             | -0.4333             | Down       |
| 282152   | BCL2L1             | Bcl-2-like protein 1, Bcl2-L-1 (Apoptosis regulator Bcl-X)              | 2.0242              | 1.7502              | Up         |
| 282170   | CCL3               | C-C motif chemokine 3, SCYA3                                             | -0.8464             | -0.4205             | Down       |
| 282258   | IFNAR2             | Interferon alpha/beta receptor 2                                         | -0.4874             | -0.9526             | Down       |
| 282307   | PIK3R1             | Phosphatidylinositol 3-kinase regulatory subunit alpha, PI3K regulatory subunit alpha | -0.8549             | -0.5501             | Down       |
| 282527   | TNFRSF1A           | Tumor necrosis factor receptor superfamily member 1A, TNFR1             | -0.2498             | -0.4574             | Down       |
| 282602   | TLR9               | Toll-like receptor 9                                                     | -1.4592             | -1.0121             | Down       |
| 286849   | CD40               | CD40 ligand, CD40-L                                                     | 1.5229              | -0.4701             | Up/Down    |
| 286883   | MAP2K6             | Dual specificity mitogen-activated protein kinase kinase 6, MAP kinase kinase 6 | -1.2655             | -0.7933             | Down       |
| 327712   | CCL5               | C-C motif chemokine 5, SCYA5                                             | 1.2276              | -0.8678             | Up/Down    |
| 347700   | EIF2AK2            | Interferon-induced, double-stranded RNA-activated protein kinase, PKR   | -0.4040             | 1.2915              | Up/Down    |
| 404109   | IGHG2              | Immunoglobulin heavy constant gamma                                       | -2.9326             | -0.4829             | Down       |
| Gene Symbol | Description | Fold Change | P-Value | Regulation |
|-------------|-------------|-------------|---------|------------|
| 407131      | CD80        | T-lymphocyte activation antigen CD80 | -0.3815 | 0.2950 | Down/Up |
| 407237      | TLR6        | Toll-like receptor 6 | 1.0333 | 0.9866 | Up |
| 414347      | CCL4        | C-C motif chemokine 4, SCY4A | -0.5452 | -1.5640 | Down |
| 444881      | MYD88       | Myeloid differentiation primary response protein MyD88 | 0.3157 | 0.7378 | Up |
| 493720      | FADD        | FAS-associated death domain protein | 0.8561 | 0.4406 | Up |
| 497199      | CFLAR       | CASP8 and FADD-like apoptosis regulator | 0.6936 | 0.6236 | Up |
| 504507      | TNFSF13B    | Tumor necrosis factor ligand superfamily member 13B | 2.8432 | 0.8164 | Up |
| 504509      | ZAP70       | Tyrosine-protein kinase ZAP-70 | -2.9472 | -0.8753 | Down |
| 504653      | LTBR        | Tumor necrosis factor receptor superfamily member 3 | 1.4498 | 0.6032 | Up |
| 504727      | RIPK1       | Receptor-interacting serine/threonine-protein kinase 1 | 0.5676 | 0.6115 | Up |
| 505901      | PRKCQ       | Protein kinase C type | -1.6829 | -0.5520 | Down |
| 506604      | ISG20       | Interferon-stimulated gene 20 kDa protein | 1.0151 | 1.3866 | Up |
| 506848      | SIKE1       | Suppressor of IKBKE 1 | 0.2907 | 0.6086 | Up |
| 507484      | DDX41       | Probable ATP-dependent RNA helicase DDX41 | 1.2221 | 1.864 | Up |
| 508233      | RELA        | Bifunctional (p)ppGpp synthase/hydrolase RelA | 1.0480 | 0.4738 | Up |
| 508581      | DDX54       | Probable ATP-dependent RNA helicase DDX54 | -0.3170 | -0.4606 | Down |
| 508728      | DDX56       | Probable ATP-dependent RNA helicase DDX56 | 0.7844 | 0.9073 | Up |
| 508378      | DHX58       | Probable ATP-dependent RNA helicase DHX58 | -0.4027 | 0.7525 | Down/Up |
| 506405      | DHX16       | Probable ATP-dependent RNA helicase DHX16 | 0.3052 | 0.2765 | Up |
| 506965      | DHX34       | Probable ATP-dependent RNA helicase DHX34 | 0.5960 | 0.4518 | Up |
| 508289      | LCK         | Tyrosine-protein kinase Lck | -2.1462 | -0.9103 | Down |
| 509855      | IRF9        | HUMAN | -0.4370 | 0.3477 | Down |
| 510093      | DDX3X       | ATP-dependent RNA helicase DDX3X | -0.3125 | 0.3726 | Down/Up |
| 510393      | BLNK        | B-cell linker protein | -0.8093 | -0.6531 | Down |
| 510427      | TICAM1      | TIR domain-containing adapter molecule 1 | 1.8071 | 0.7284 | Up |
| 515383      | IFI30       | Gamma-interferon-inducible lysosomal thiol reductase, GILT | 1.2038 | 0.3533 | Up/Down |
| 510814      | STAT1       | Signal transducer and activator of transcription 1-alpha/beta | 0.6806 | 1.0941 | Up |
| 510923      | TRIM25      | E3 ubiquitin/ISG15 ligase TRIM25 | 0.7529 | 1.0850 | Up |
| 512373      | TKFC        | Triokinase/FMN cyclase, DAK | -0.8394 | -0.9184 | Down |
| 512913      | IF16        | Interferon alpha-inducible protein 6 | -0.6431 | 0.4839 | Down/Up |
| 512943      | MAPK12      | Mitogen-activated protein kinase 12 | 2.3863 | 1.0708 | Up |
| 513038      | CTSK        | Cathepsin K | 1.3518 | 0.6320 | Up |
| 513836      | TNFSF11     | Tumor necrosis factor ligand superfamily member 11 | -6.6088 | -2.5936 | Down |
| 514735      | LAT         | Linker for activation of T-cells family member 1 | -1.6198 | -0.6212 | Down |
| 514889      | IFNGR2      | Interferon gamma receptor 2, IFN-gamma receptor 2, IFN-gamma-R2 | 0.3675 | -0.2189 | Up/Down |
| 515377      | CARD11      | Caspase recruitment domain-containing protein 11 | -1.5137 | -0.7376 | Down |
| 515515      | SYK         | Tyrosine-protein kinase SYK | 0.6282 | 0.5178 | Up |
| 515640      | IL1R1       | Interleukin-1 receptor type 1 | 0.8671 | 0.5319 | Up |
| 517948      | PIK3CB      | Phosphatidylinositol 4,5-bisphosphate 3- | 1.2348 | 0.7218 | Up |
| Gene ID   | Gene Name                  | Description                                                                 | Fold Change 1 | Fold Change 2 | Regulation |
|-----------|----------------------------|------------------------------------------------------------------------------|---------------|---------------|------------|
| 519541    | RNF125                     | Kinase catalytic subunit beta isoform E3 ubiquitin-protein ligase RNF125     | 1.8224        | 1.2301        | Up         |
| 523962    | MAP3K1                     | Mitogen-activated protein kinase kinase 1                                   | 0.4868        | 0.4092        | Up         |
| 526469    | MAP2K4                     | Dual specificity mitogen-activated protein kinase kinase 4, MAP kinase kinase 4 | 0.8372        | 0.6697        | Up         |
| 529757    | LTBP2                      | Latent-transforming growth factor beta-binding protein 2                    | 2.1305        | -0.7872       | Up/Down    |
| 530884    | TNFRSF11A                  | Tumor necrosis factor receptor superfamily member 11A                      | 0.9452        | 0.7040        | Up         |
| 531079    | TIRAP                      | Toll/interleukin-1 receptor domain-containing adapter protein               | -1.0706       | 0.4089        | Down/Up    |
| 531391    | MAPK3                      | Mitogen-activated protein kinase 3, MAP kinase 3                            | 0.6463        | 0.7910        | Up         |
| 532262    | TLR8                       | Toll-like receptor 8                                                        | -1.4958       | -0.5007       | Down       |
| 533141    | TBK1                       | Serine/threonine-protein kinase TBK1                                        | 0.9298        | 0.5684        | Up         |
| 533199    | MAP2K1                     | Dual specificity mitogen-activated protein kinase kinase 1, MAP kinase kinase 1, MEK1 | 1.7738        | 1.1024        | Up         |
| 533216    | IKBKE                      | Inhibitor of nuclear factor kappa-B kinase subunit epsilon                  | 1.5211        | 0.4558        | Up         |
| 533661    | TMEM173                    | Stimulator of interferon genes protein, ERIS, MITA, STING                   | -0.7046       | 0.2969        | Down/Up    |
| 533692    | IRAK4                      | Interleukin-1 receptor-associated kinase 4                                  | 0.8041        | 0.4558        | Up         |
| 534125    | MAPK9                      | Mitogen-activated protein kinase 9                                           | 0.4329        | 0.5851        | Up         |
| 534492    | MAPK14                     | Mitogen-activated protein kinase 14                                         | 0.9916        | 1.0225        | Up         |
| 534996    | LYN                        | Tyrosine-protein kinase Lyn                                                 | 1.1763        | 0.4651        | Up         |
| 535327    | MAPK13                     | Mitogen-activated protein kinase 13                                          | 2.3265        | 2.0249        | Up         |
| 535470    | PIN1                       | Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1                      | 0.4819        | 0.2186        | Up         |
| 535622    | MAP3K8                     | Mitogen-activated protein kinase kinase 8                                   | 0.8805        | 0.2926        | Up         |
| 536421    | CYLD                       | Ubiquitin carboxyl-terminal hydrolase CYLD                                  | 1.5235        | 0.9096        | Up         |
| 539124    | TRAF6                      | TNF receptor-associated factor 6                                            | 1.0078        | 0.5226        | Up         |
| 539974    | NLRX1                      | NLR family member X1                                                        | 2.3460        | 0.6370        | Up         |
| 540203    | TAB2                       | TGF-beta-activated kinase 1 and MAP3K7-binding protein 2                    | 0.5059        | 0.5130        | Up         |
| 540824    | BCL10                      | B-cell lymphoma/leukemia 10                                                 | 0.5599        | 0.2978        | Up         |
| 540944    | TRAF1                      | TNF receptor-associated factor 1                                            | 0.4740        | -0.4845       | Up/Down    |
| 613464    | IFITM1                     | Interferon-induced transmembrane protein 1                                 | -1.8299       | -1.4268       | Down       |
| 613753    | LY96                       | Lymphocyte antigen 96                                                       | -0.8608       | -0.2878       | Down       |
| 613845    | IRF2BPL                    | Interferon regulatory factor 2-binding protein-like                          | 1.2784        | 1.3427        | Up         |
| 614838    | PIK3R5                     | Phosphoinositide 3-kinase regulatory subunit 5, PI3-kinase regulatory subunit 5 | 0.6818        | -0.1916       | Up/Down    |
| 615340    | IRF5                       | Interferon regulatory factor 5                                              | 0.3004        | -0.8504       | Up/Down    |
| 615930    | IFITM3                     | Interferon-induced transmembrane protein 3                                 | 1.2038        | -0.3533       | Up/Down    |
| 617420    | IFI127                     | Interferon alpha-inducible protein 27                                       | 2.5072        | 5.7561        | Up         |
| 618508    | MAVS                       | Mitochondrial antiviral-signaling protein, Ips1, Visa                       | 0.6098        | 0.6317        | Up         |
| 618906    | MAPK11                     | Mitogen-activated protein kinase 11, MAP kinase 11                          | 1.3666        | -0.4751       | Up/Down    |
| 767903    | ATG12                      | Ubiquitin-like protein ATG12                                                 | 0.6840        | 0.5125        | Up         |
| 767932    | AZI2                       | 5-azacytidine-induced protein 2, NAP1, TBKBP2                              | 0.7515        | 0.4546        | Up         |
| 767939    | IFRD2                      | Interferon-related developmental regulator 2                               | -0.4901       | -0.9573       | Down       |
| 785603    | TBKBP1                     | TANK-binding kinase 1-binding protein 1, TBK1-binding protein 1             | 2.1172        | 1.6489        | Up         |
| 787278    | MAP2K7                     | Dual specificity mitogen-activated protein kinase 7, MAP kinase             | -0.5272       | -0.3009       | Down       |
Additional Files Legends

Additional file 1: Table S1. Differentially expressed genes between mock and ncp BVDV infected samples at 2 hpi. (XLS 1601 kb)

Additional file 2: Table S2. Differentially expressed genes between mock and ncp BVDV infected samples at 24 hpi. (XLS 1281 kb)

Additional file 3: Table S3. GO enrichment results of the DEGs between mock and ncp BVDV infected samples at 2 hpi. (XLS 2632 kb)

Additional file 4: Table S4. GO enrichment results of the DEGs between mock and ncp BVDV infected samples at 24 hpi. (XLS 2147 kb)

Additional file 5: Table S5. KEGG pathway enrichment result of the DEGs between mock and ncp BVDV infected samples at 2 hpi. (XLS 549 kb)

Additional file 6: Table S6. KEGG pathway enrichment result of the DEGs between mock and ncp BVDV infected samples at 24 hpi. (XLS 469 kb)

Figures
Figure 1

Gene ontology (GO) and KEGG analysis of DEGs at 2 hpi and 24 hpi. (A) A Venn diagram depicting the distribution of differentially expressed transcripts from ncp BVDV infected bovine monocytes (BIC) at 2h and 24h. (B) GO assignments are shown for the differentially expressed genes (DEGs) in BIC. Molecular functions, Biological processes, and Cellular components (C) GO analysis of DEGs between 2 and 24 h...
after infection. (D) KEGG database analysis of signal pathways involved in DEGs 2 h after infection (E) KEGG database analysis of signal pathways involved in DEGs 24 h after infection.

**Figure 2**

DEGs profiling of cellular genes involved in the immune responses and the type I interferon pathway in bovine monocytes after infection with ncp BVDV. Heat maps show genes related to the immune responses (left: GO: 0006955) and the type I interferon signaling pathway (right: GO: 0060337) that were
either upregulated or downregulated upon ncp BVDV infection of bovine monocytes. The color coding represents a normalized expression of genes in monocytes infected with ncp BVDV (see color key). Gene upregulation is denoted in red and gene downregulation is denoted in blue.

Figure 3

Partial validation of the DEGs involved in the type 1 IFN pathway by qRT-PCR. Bovine monocytes were isolated as described in the materials and methods section. The monocytes were infected with ncp BVDV and harvested 2h and 24h post-infection. qRT-PCR was performed on extracted RNA to amplify the selected genes using specific primers. Relative mRNA expression of the indicated genes is shown. Values are expressed as the fold change in with ncp BVDV of the 2h and 24h time points post-infection compared with mock-infected cells. Expression was normalized with GAPDH expression (the housekeeping gene). The results represent the mean and standard deviation (SD) of three samples from one experiment (P < 0.05).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile5TableS5.xls.xls
- Additionalfile4TableS4.xls.xls
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- Additionalfile2TableS2.xls.xls
- Additionalfile1TableS1.xls