Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
31 (3): 411-417, 2025
DOI: 10.9775/kvfd.2025.33698

Research Article

Transcriptomics Analysis Identifies Critical Genes Involved in the Infection of Crandell-Reese Feline Kidney Cell Lines by Feline Panleukopenia Virus

Shengnan SONG [1,#] Pan CHEN [1,#] Chuangfu CHEN [1 (*)] Jia GUO [1 (*)]

# These authors contributed equally to this work

[1] State International Joint Research Center for Animal Health Breeding, College of Animal Science and Technology, Shihezi University, Shihezi 832003, CHINA

(*) **Corresponding author:**
Chuangfu CHEN & Jia GUO
Phone: +86-0993-2057971 (C.C)
86-0993-2058839 (JG)
E-mail: chenchuangfu_ccf@163.com (CC),
453202616@qq.com (JG)

How to cite this article?
Song S, Chen P, Chen C, Guo J: Transcriptomics analysis identifies critical genes involved in the infection of crandell-reese feline kidney cell lines by feline panleukopenia virus. *Kafkas Univ Vet Fak Derg,* 31 (3): 411-417, 2025.
DOI: 10.9775/kvfd.2025.33698

Article ID: KVFD-2025-33698
Received: 17.01.2025
Accepted: 13.05.2025
Published Online: 27.05.2025

Abstract

Feline panleukopenia virus (FPV) is an extremely contagious pathogen that induces severe vomiting, diarrhea, and dehydration in cats, often resulting in high mortality rates and substantial economic losses. Host responses are crucial for viral entry, replication, assembly, and disease progression, despite so much is not known, especially regarding the interactions between hosts and viruses. Herein, we employed the FPV strain FPV-XJ-04, which was previously isolated and characterized by our team in Xinjiang, China. To perform a comparative transcriptomic analysis of the gene expression profile in Crandell-Reese feline kidney (CRFK) cells following infection with the FPV-XJ-04 strain. Following infection of CRFK cells with the FPV-XJ-04 strain, the differentially expressed genes in CRFK cells were predicted to be involved in several key signaling pathways, including the TNF signaling pathway, IL-17 signaling pathway, cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway, MAPK signaling pathway, and RIG-I-like receptor signaling pathway. In addition, the immune response was significantly enriched in the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Furthermore, we highlight 11 key genes in the TNF signaling pathway that are associated with host defense response to the invasion of FPV-XJ-04 strain. This research provides valuable insights into the gene transcription processes within immune cells, elucidating the pathways critical to the early stages of infection pathogenesis.

Keywords: Feline Panleukopenia Virus, Host-virus interactions, Immune response, Comparative transcriptomic analysis, TNF signaling pathway

Introduction

Parvoviruses, which belong to the family *Parvoviridae*, constitute a group of small, non-enveloped viruses with single-stranded DNA genomes. These viruses possess a linear DNA genome approximately 4.5-5 kb in length, characterized by hairpin structures formed by inverted terminal repeats (ITRs) at each end of the genome [1,2]. The remaining portion of the viral genome consists of two major open reading frames (ORFs) that encode both non-structural proteins (NS1 and NS2) and structural proteins (VP1 and VP2) within the same mRNA through alternative splicing [3,4]. Parvoviruses are currently widespread globally and capable of naturally infecting a diverse range of hosts [5-8]. Due to their rapid evolution and efficient transmission, the range of potential hosts for parvoviruses is continually expanding, posing significant threats to numerous endangered wild animal species and domestic pets [9].

Carnivore protoparvovirus 1 belongs to the *Protoparvovirus* genus within the *Parvoviridae* family and is characterized as a distinct viral species [1]. Notable members of this species include feline panleukopenia virus (FPV) and canine parvovirus (CPV) [10-12]. FPV is one of the deadliest viral pathogens in pets and can cause diarrhea, vomiting, and feline panleukopenia [13,14]. FPV has a wider host range and higher pathogenicity. In addition to domestic cats, FPV have been reported to infect monkeys [15], tigers [16], lions [17], and linsangs [18], causing significant economic losses. In recent years, FPV has emerged as a significant threat to companion animals, economically valuable species, and

This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

wild fauna in China. Consequently, there is an urgent need for scientific research to curb the dissemination of FPV and to elucidate its molecular pathogenesis.

Host responses are crucial for viral entry, replication, assembly, and pathogenesis [19-21]. Despite significant progress, many aspects remain to be elucidated, especially regarding the intricate interactions between hosts and viruses. The non-structural protein 1 (NS1) of human parvovirus B19 can interact with AP-1 and AP-2 on the TNF-α promoter of the host to regulate the expression of TNF-α [22]; the NS1 protein of B19 can activate relevant factors in the endothelial cell inflammation signaling pathway, thereby affecting the host cell inflammation response [23]. The NS1 protein also plays a significant role in the host cell apoptosis process, and studies have shown that the B19 virus NS1 protein induces red blood cells to arrest in the G1 phase, leading to apoptosis [24]. Other interactions between the virus and the immune system include immune evasion and suppression of potent innate immune responses. In the context of FPV infection, it has been observed that the NS2 protein of FPV interacts with the host cell's TANK-binding kinase 1 (TBK1) molecule, thereby antagonizing the binding of TBK1 to stimulator of interferon genes (STING). This interaction reduces the phosphorylation of downstream STING and interferon regulatory factor 3 (IRF3) molecules, ultimately preventing the activation of IFN-I transcription [25]. Insight into these modulations will further elucidate the pathogenesis of these viruses.

Few studies have investigated cellular host responses to FPV infection, especially using clinical isolates. Transcriptome studies based on next-generation sequencing have the potential to elucidate cellular responses following viral infection, thereby providing further insight into viruses' pathogenesis. Analyzing the transcriptome of infected cells can provide valuable information on virus replication and host-pathogen interactions [26]. Therefore, the present work was conducted to investigate the role of mRNA in the immune defense of host cells against FPV infection. We used RNA-seq to profile mRNA expression in two groups of feline kidney cells: FPV-infected and uninfected, and identified many differentially expressed transcripts. The primary objective of this study was to identify the differentially expressed transcripts and determine mRNA expression patterns during FPV-XJ-04 infection of CRFK cells, elucidating the associated pathways and biological processes.

Material and Methods

Ethical Statement

This study did not involve any human participants, animal experiments, or the use of personal data. Therefore, ethical approval was not required. All procedures were carried out in accordance with the relevant laws and regulations.

Viruses and Cell Culture

Crandell-Reese Feline Kidney (CRFK) cell lines were purchased from Pricella Biotechnology Co., Ltd. (Wuhan, China) and the cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, Carlsbad, CA) containing 5% fetal bovine serum and 1% penicillin-streptomycin at 37ºC with 5% CO_2. FPV strain (FPV-XJ-04) was originally isolated from feces of infected cats in a pet hospital of Xinjiang, China. Initially, the fecal samples were confirmed positive for FPV using a colloidal gold strip test. Subsequently, the fecal material was suspended in cell culture medium, centrifuged, and the resulting supernatant was filtered through a 0.22 μm filter membrane. The filtered supernatant was then inoculated into CRFK cells for blind passage up to the fifth generation, during which cytopathic effects (CPE) became evident. Viral DNA was extracted from individual fecal samples utilizing the TIANamp Virus DNA Kit (TIANGEN, China). The extracted DNA served as the template for detecting FPV using a set of universal primers (FPV-F: 5′-TAACTCCTCTGACTCCGGAC-3′; FPV-R: 5′-ACCACCGTCTGGTTGAACTG-3′). These primers amplify a 750 bp fragment within the FPV genome, specifically covering nucleotides 2,062 to 2,819 [27]. The supernatant from the FPV positive sample was sterilized using a 0.22 μm filter membrane. Subsequently, the filtered supernatant was inoculated into CRFK cells and incubated at 37ºC in a 5% CO_2 atmosphere. The CPE-positive cell cultures were subjected to centrifugation at 28.000 × *g* for 12 min. Subsequently, the samples were negatively stained with 0.5% phosphotungstic acid and examined using transmission electron microscopy (TEM). To further characterize the harvested virus, an indirect immunofluorescence assay (IFA) was performed. In this assay, murine polyclonal antibodies specific to FPV were employed as the primary antibody, while FITC-conjugated goat anti-mouse IgG (H&L) served as the secondary antibody. Finally, the cells were washed, mounted on glass slides, and examined under a fluorescence microscope.

Growth Characterization of the Isolated Virus

To evaluate the viral growth kinetics, CRFK cells were infected with the isolated parvovirus at a multiplicity of infection (MOI) of 0.01. Supernatants from the cell culture medium were harvested at 12 h intervals from 0 to 72 h post-infection (hpi). Viral titers ($TCID_{50}$/mL) were determined using endpoint dilution assays with CRFK cells. Each experiment was performed in triplicate independently, and the results are expressed as the mean ± standard deviation.

RNA-seq and Data Analysis

In the investigation of host transcriptomic responses, CRFK cells were infected with FPV-XJ-04. and CRFK

Table 1. Primers of qRT-PCR used for this work

Primer	Sequence
GAPDH-F	TGGAAAGCCCATCACCATC
GAPDH-R	ACTCCACAACATACTCAGCACCA
TNFAIP3-F	CTACCAACGGGATCATTCAC
TNFAIP3-R	TTCTCGACACCAGTTCAGCT
NFKB1-F	CAATCCAGAAATATTTCAACCA
NFKB1-R	TTCACTAGACGCACCCGG
IL6-F	CTCCTGGTGGTGGCTACT
IL6 -R	CAGAGATTTTGCCGAGGA
IFNB1-F	TTGCCTCAAGGACAGGAT
IFNB1-R	ATCCCGTGCTAGAGGTGC
MAP3K8-F	GTGAAGAGCCAGCGGTTT
MAP3K8-R	ACGGAGGACAACCAAGGC
MAPK8-F	ATGAGCAGAAGCAAGCGT
MAPK8-R	CGGCTCAGCTTCTTGATT
TRAF1-F	GTCTGTAAGCCCAGGAAGCC
TRAF1-R	TTGGGCTCCCCTTGTAGG
TNF-F	CAGGGCTCCGGAAGGTG
TNF-R	TGCAGGCCATGTGGGAG
TRAF3-F	CGCTGAAGCTGCACCCT
TRAF3-R	CTCCGTCTGCTTCGGGT
MAP3K14-F	CAGGCGATGGGCAAGAA
MAP3K14-R	AGATGGCGGCCAGTCCT
IRF1-F	TCCAACCAAATCCCAGG
IRF1-R	CTTTTCCCCTGCTTTGT
NFKBIA-F	GAGCACGCCCAGGACTG
NFKBIA-R	CCGCAGCTCCTTCACCA

cells were cultured in six-well plates (Corning, USA) and divided into two groups, each containing three replicates, for the construction of mRNA libraries. The first group was inoculated with FPV-XJ-04 at a concentration of 1×10^6 $TCID_{50}$, while the second group served as an uninfected control. At 24 h post-infection, total RNA from both groups was isolated using Trizol reagent (Invitrogen, CA, USA) according the manufacturer's instructions. The RNA quality was evaluated via the Bioanalyzer (Agilent, Santa Clara, CA, United States) and sent (1 µg per sample) for mRNA sequencing to Novogene, Inc. (Sacramento, CA, United States). Then, the high-quality clean reads were compared with the specified reference genome by using Bowtie software. The Padj ≤0.05 and the absolute value of $\log_2$ ratio ≥2 were used to identify DEGs. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were used to analyze the pathways.

Quantitative Real-time PCR

To validate the findings from the RNA-seq experiment, a set of 12 genes associated with the TNF signaling pathway were selected for further analysis through quantitative real-time PCR (qRT-PCR). Total RNA was extracted from both uninfected and FPV-infected CRFK cells, cDNA synthesis was performed using 1.0 µg of the extracted total RNA, in accordance with the protocol provided by the reverse transcription kit manufacturer (RIBOBIO, China). The primers are listed in *Table 1*. The qRT-PCR conditions included a pre-incubation step at 95ºC for 5 min, followed by 40 amplification cycles (95ºC for 30 sec, 58ºC for 30 sec, and 72ºC for 30 sec). Each sample was analyzed in triplicate using a 20 µL reaction mixture containing 2× SYBR Premix Ex Taq II (Takara, USA). The reactions were performed on a Roche Light Cycler 480 II system (Basel, Switzerland), with each reaction containing 100 nM of each primer and 1 µg of cDNA template. All experiments were conducted in triplicate.

Statistical Analyses

When comparisons were made, a Student's *t*-test was performed and *P* value <0.05 was considered statistically significant. The data was presented as "mean ± SD".

Result

Characterization of the FPV-XJ-04 Strain

The PCR analysis using the extracted viral DNA demonstrated a prominent and bright band at 750 bp *(Fig. 1-A)*, indicative of a positive result. To further verify the infection status, fecal samples were inoculated into CRFK cells in an effort to the virus. As shown in *Fig. 1-B*, after five blind passages in CRFK cells, CPE induced by parvovirus became evident, manifesting as cell rounding, pyknosis, disruption of the monolayer, and eventually complete necrosis. By the tenth passage, the viral titer of the nonclonal virus population, as measured by endpoint dilution assay, had increased to 1×10^6 $TCID_{50}$/mL. TEM analysis of negatively stained and purified cell supernatant showed spherical particles with an average diameter of about 20 nm, which is consistent with the characteristic morphology of parvoviruses *(Fig. 1-C)*. The growth kinetics of the resulting clonal virus, named FPV-XJ-04, were subsequently analyzed. The results showed that viral replication initiated steadily and reached its peak at 60 h post-infection *(Fig. 1-D)*.

Identification and Characterization of mRNAs in FPV-XJ-04 Infected CRFK Cells by RNA-seq

To investigate the cellular host responses to FPV-XJ-04 infection, we conducted a transcript profiling experiment using RNA-seq technology. Gene expression profiles from both infected and control samples were compared, with

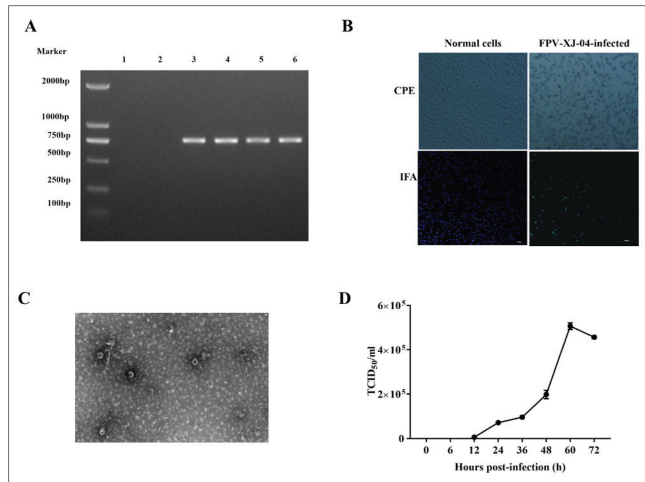

Fig 1. Identification and characterization the FPV-XJ-04 strain. **(A)** PCR products were analyzed by 1.5% agarose gel. Marker, DL 2000 DNA marker; lane 1, 2, nucleotide-free water (negative control), lane 3-6, the FPV-XJ-04 strain, **(B)** The cytopathic effect (CPE) and IFA identification, **(C)** Morphology of FPV-XJ-04 particles exhibited with negative-stained transmission electron microscopy, **(D)** Growth curve of FPV-XJ-04 as measured by end-point dilution. Each datapoint shows averages of three independent replicates, and standard deviations are indicated as error bars

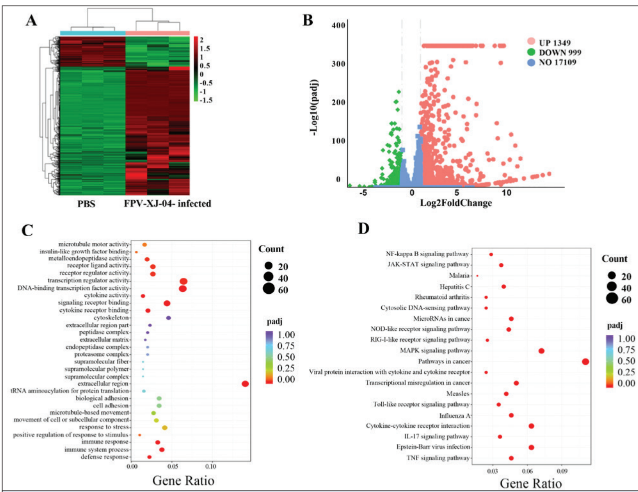

Fig 2. Expression of differentially expressed genes (DEGs) between the CRFK cells infected with FPV-XJ-04 and control group. **(A)** The heatmap shows the expression levels of DEGs between the the CRFK cells infected with FPV-XJ-04 and control group, **(B)** Volcano plot of expressed genes between CRFK cells infected with FPV-XJ-04 and control group. The red, green, and blue denote upregulated, downregulated, and non-regulated genes, respectively, **(C, D)** GO and KEGG analyses of the differentially expressed genes (DEGs) between the CRFK cells infected with FPV-XJ-04 and control group. The rich factor represents the ratio of upregulated genes differentially expressed gene numbers annotated in this pathway term to all gene numbers annotated with this pathway term. A greater rich factor indicates a greater degree of pathway enrichment

expression levels analyzed using the Illumina HiSeq™ 2000 platform. Cluster analysis was applied based on expression patterns to group the samples, revealing potential relationships among them *(Fig. 2-A)*. Our comparative transcriptomic analysis identified 2,348 differentially expressed genes (DEGs) with Padj ≤0.05 and fold change ≥1, of which 1,349 genes were upregulated and 999 genes were downregulated.

Clustering and Functional Enrichment Analysis of Differentially Expressed Genes

The Volcano Plot is presented in *Fig. 2-B*. To investigate the distribution of candidate target genes and elucidate their diverse functions, we employed GO and KEGG pathway analyses to identify the enriched target genes. We performed a GO enrichment analysis to explore the host cell' biological processes in responding to FPV. The defense response, immune system process, positive regulation of response to stimulus, cell adhesion, biological adhesion, cytokine receptor binding, signaling receptor binding, and cytokine activity were the dominant groups in all three DEG sets *(Fig. 2-C)*. Based on KEGG pathway enrichment analysis, most upregulated genes involved TNF signaling pathway, Cytokine-cytokine receptor interaction, IL-17 signaling pathway, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, RIG-I-like receptor signaling pathway, MAPK signaling pathway, and NF-kappa B signaling pathway *(Fig. 2-D)*. In the GO and KEGG analysis, immune signaling is the main pathway involved in the FPV infection process *(Fig. 2-C,D)*.

Validation of Selected mRNAs

Based on the above results, we hypothesize that immune-related genes and signaling pathways are likely involved in the FPV infection process *(Fig. 3-A,B)*. To validate this hypothesis, total RNA isolated from FPV-infected and uninfected CRFK cells was subjected to qRT-PCR analysis to confirm the differentially expressed mRNA candidates *(Fig. 3-C)*. Previous studies have indicated that the TNF signaling pathway play a central role in virus infection process. The gene expression changes related to

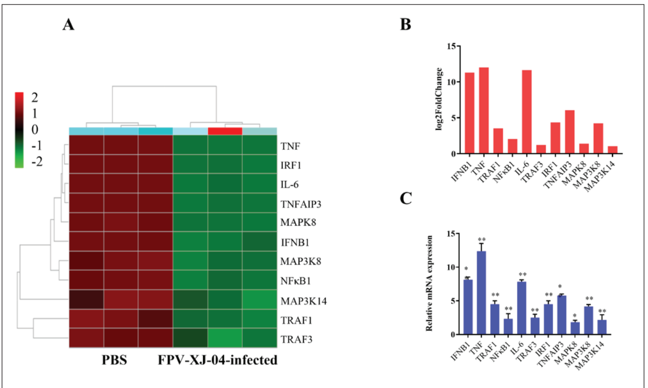

Fig 3. Differentially expressed genes (DEGs) were evaluated by quantitative reverse transcription PCR (qRT-PCR) assays between the CRFK cells infected with FPV-XJ-04 and control group. **(A)** The heatmap shows the expression levels of 11 key genes in the TNF signaling pathway between the CRFK cells infected with FPV-XJ-04 and control group, **(B, C)** The 11 key genes in the TNF signaling pathway expression levels were further detected by qRT-PCR. The results at each time point are expressed as the means ± standard deviations from at least three independent experiments

TNF signaling pathway were assessed to explore whether this pathway can respond to FPV infection process. Specifically, the major upregulated genes involved in the TNF signaling pathway included TNFAIP3, NFKB1, IL6, IFNB1, MAP3K8, MAPK8, TRAF1, TNF, TRAF3, MAP3K14, IRF1. The expressions of these genes using qRT-PCR were in good agreement with the RNA-seq results *(Fig. 3)*.

Discussion

Messenger RNA (mRNA) plays a pivotal role in the regulation of gene expression by participating in the entire process of protein synthesis, thereby serving as a critical link between DNA and proteins. Proper mRNA synthesis and degradation are indispensable for maintaining cellular functions and organismal homeostasis. Moreover, the roles of mRNA in the host's antiviral response have been increasingly elucidated through ongoing research [28]. Recent research indicates that, CRFK cells which are permissive for productive replication of virus, showed induction of a large network of immunological and virally induced pathways, other interaction between viruses and hosts involves a complex interplay between the host's antiviral response and the virus's immune evasion strategies [29].

The isolation and characterization of FPV-XJ-04 were systematically validated through molecular, cytopathic, morphological, and kinetic analyses. The PCR detection of a 750 bp amplicon strongly indicated FPV DNA, targeting conserved regions of the viral genome nucleotides 2,062 to 2,819 [27]. Successful virus isolation in CRFK cells was confirmed by progressive CPE, including cell rounding and necrosis, alongside a rising viral titer, reflecting efficient *in vitro* adaptation. TEM visualization of ~20 nm spherical particles provided definitive morphological evidence consistent with parvoviruses, excluding other pathogens. Growth kinetics show that the virus reaches its replication peak at 60 h after infection, which might be slightly delayed due to unique virus-host interactions or replication kinetics. The gradual titer increase aligns with the lytic nature of autonomous parvoviruses, which depend on host cell cycles.

Furthermore, we identified distinct expression profiles between FPV-XJ-04 infected CRFK cells by RNA sequencing and comparative transcriptomic analysis. RNA-seq analysis identified 2,348 DEGs (1,349 upregulated, 999 downregulated) in FPV-XJ-04-infected cells, reflecting extensive transcriptional reprogramming. Upregulated genes were enriched in immune pathways (e.g., TLR, RIG-I, cytokine signaling), aligning with GO/KEGG findings and indicating a robust antiviral response driven by innate immunity and inflammation. Downregulation of genes may reflect viral evasion via suppression of non-essential processes or metabolic rewiring. The clear clustering of infected samples and control samples highlights the systemic interaction between the host and the pathogen. Future studies should prioritize functional validation of key DEGs (e.g., cytokines, immune receptors) to elucidate their roles in viral control or pathogenesis. To further investigate the biological functions of these genes, the DEGs were mapped to the GO and KEGG pathways. The associated cellular processes discussed below could be classified into several main sections: TNF signaling pathway, IL-17 signaling pathway, and MAPK signaling pathway. We summarized the above analyses and formed a gene change model of FPV-XJ-04 infected CRFK cells.

Transcriptome analysis indicated that FPV infection significantly activated the host's TNF-α and IL-17 signaling pathways. The TNF-α signaling pathway and the IL-17 signaling pathway are associated with multiple immune and inflammatory pathways [30-32]. TNF-α is a major pyrogenic cytokine produced by immune cells during inflammatory responses and the acute phase of infection, and is closely related to the cytokine storm [33]. During viral infections, the expression level of TNF-α is correlated with the severity of the disease. In addition, TNF-α has been reported to stimulate the replication of human polyomavirus in neural cells [34]. Mumps virus infection disrupts the blood-testis barrier by inducing TNF-α [35]. In our research, we found that the genes related to the TNF signaling pathway, including TNFAIP3, NFKB1, IL6, IFNB1, MAP3K8, MAPK8, TRAF1, TNF, TRAF3, MAP3K14, IRF1, and NFKBI, were significantly upregulated after the infection of CRFK cells. Whether the production of TNF-α can stimulate viral replication has not been confirmed, but the induction of TNF-α production by FIPV has been reported [29].

The IL-17 family is considered highly relevant to infectious diseases occurring in epithelial sites [36]. Research on the role of IL-17 in regulating viral infections is also ongoing, where it plays multiple key roles. The cytokine IL-17 may have completely opposite effects in different circumstances. During systemic viral infections, excessive production of IL-17 can lead to liver damage and death. The level of IL-17 during viral infection is closely related to the severity of the disease, and at this time, IL-17 plays a pathological damage role in this disease. This phenomenon has been reported in viruses such as influenza virus, dengue virus, and respiratory syncytial virus [37-39]. It has been reported that IL-17 plays an important role in virus-induced acute lung injury during the H1N1 pandemic. Treating H1N1-infected mice with IL-17 monoclonal antibodies significantly improved the acute lung injury caused by the virus [40]. At the same time, the induction of IL-17 has been reported to antagonize the broad-spectrum antiviral response of monocyte chemoattractant protein-induced protein 1 (MCPIP1) in PRRSV [41]. These results suggest

that FPV infection triggers a host immune storm by activating the classical pro-inflammatory pathway, and its dynamic balance may determine the infection outcome (protective immunity or pathological damage). It is necessary to further analyse its spatiotemporal regulatory mechanism and its relationship with viral replication.

The RNA-seq and qRT-PCR analyses collectively demonstrate significant activation of the MAPK signaling pathway during FPV infection. Key upstream regulators (e.g., MAP3K8, MAPK8) and downstream effectors (e.g., NF-κB, IL-6) within this pathway were markedly upregulated, suggesting its critical role in amplifying pro-inflammatory responses via cytokine production (e.g., TNF, IL-6) and immune cell activation [42]. MAPK signaling likely intersects with TNF and IL-17 pathways to form a synergistic network [43], driving NF-κB-mediated transcriptional activation and exacerbating inflammatory cascades [44]. While this hyperactivation may enhance antiviral defenses, sustained MAPK activity could also contribute to tissue damage or viral immune evasion. Further studies should delineate whether MAPK signaling primarily facilitates host resistance or is exploited by FPV to promote replication, potentially identifying therapeutic targets to modulate this pathway during infection.

Overall, this research shows a series of genes involved in FPV infection and provides a scientific reference for exploring some genes which may aggravate or inhibit virus replication.

Declarations

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Acknowledgments: None.

Funding Support: This work was supported by Tianchi Talent - Young Doctoral Program, Scientific Research Startup Project for High-level Talents of Shihezi University (RCZK202460), Youth Innovation Talent Program of Shihezi University (CXPY202323).

Conflict of Interest: The authors declare no conflict of interest.

Declaration of Generative Artifical Intelligence (AI): The authors have declared that the article, tables, and figure were not written/created by AI and AI-assisted technologies.

Author Contributions: Conceptualization: S. Song, P. Chen, C. Chen, J. Guo; methodology: S. Song, P. Chen; data curation: S. Song, P. Chen; writing-original draft preparation: S. Song, P. Chen, C. Chen, J. Guo; writing- review and editing: all authors. All authors have read and agreed to the published version of the manuscript.

References

1. Cotmore SF, Agbandje-McKenna M, Canuti M, Chiorini JA, Eis-Hubinger AM, Hughes J, Mietzsch M, Modha S, Ogliastro M, Pénzes JJ, Pintel DJ, Qiu J, Soderlund-Venermo M, Tattersall P, Tijssen P, ICTV Report Consortium: ICTV virus taxonomy profile: Parvoviridae. *J Gen Virol,* 100, 367-368, 2019. DOI: 10.1099/jgv.0.001212

2. Pénzes JJ, de Souza WM, Agbandje-McKenna M, Gifford RJ: An ancient lineage of highly divergent parvoviruses infects both vertebrate and invertebrate hosts. *Viruses,* 11:525, 2019. DOI: 10.3390/v11060525

3. Reed AP, Jones EV, Miller TJ: Nucleotide sequence and genome organization of canine parvovirus. *J Virol,* 62, 266-276,1988. DOI: 10.1128/JVI.62.1.266-276.1988

4. Chung HC, Kim SJ, Nguyen VG, Shin S, Kim JY, Lim SK, Park YH, Park BK: New genotype classification and molecular characterization of canine and feline parvoviruses. *J Vet Sci,* 21:e43, 2020. DOI: 10.4142/jvs.2020.21.e43

5. Stucker KM, Pagan I, Cifuente JO, Kaelber JT, Lillie TD, Hafenstein S, Holmes EC, Parrish CR: The role of evolutionary intermediates in the host adaptation of canine parvovirus. *J Virol,* 86, 1514-1521, 2012. DOI: 10.1128/JVI.06222-11

6. Jager MC, Tomlinson JE, Lopez-Astacio RA, Parrish CR, Van de Walle GR: Small but mighty: Old and new parvoviruses of veterinary significance. *Virol J,* 18 (1):210, 2021. DOI: 10.1186/s12985-021-01677-y

7. François S, Filloux D, Roumagnac P, Bigot D, Gayral P, Martin DP, Froissart R, Ogliastro M: Discovery of parvovirus-related sequences in an unexpected broad range of animals. *Sci Rep,* 6:30880, 2016. DOI: 10.1038/srep30880

8. Calatayud O, Esperón F, Cleaveland S, Biek R, Keyyu J, Eblate E, Neves E, Lembo T, Lankester F: Carnivore Parvovirus ecology in the serengeti ecosystem: Vaccine strains circulating and new host species identified. *J Virol,* 93 (13):e02220-18, 2019. DOI: 10.1128/JVI.02220-18

9. Capozza P, Martella V, Buonavoglia C, Decaro N: Emerging Parvoviruses in domestic cats. *Viruses,* 13 (6):1077, 2021. DOI: 10.3390/v13061077

10. Parrish CR: Emergence, natural history, and variation of canine, mink, and feline parvoviruses. *Adv Virus Res,* 38, 403-450, 1990. DOI: 10.1016/s0065-3527(08)60867-2

11. Stuetzer B, Hartmann K: Feline parvovirus infection and associated diseases. *Vet J,* 201 (2): 150-155, 2014. DOI: 10.1016/j.tvjl.2014.05.027

12. Parrish CR, Aquadro CF, Carmichael LE: Canine host range and a specific epitope map along with variant sequences in the capsid protein gene of canine parvovirus and related feline, mink, and raccoon parvoviruses. *Virology,* 166 (2): 293-307, 1988. DOI: 10.1016/0042-6822(88)90500-4

13. Franzo G, Tucciarone CM, Cecchinato M, Drigo M: Canine parvovirus type 2 (CPV-2) and Feline panleukopenia virus (FPV) codon bias analysis reveals a progressive adaptation to the new niche after the host jump. *Mol Phylogenet Evol,* 114, 82-92, 2017. DOI: 10.1016/j.ympev.2017.05.019

14. Barrs VR: Feline Panleukopenia: A re-emergent disease. *Vet Clin North Am Small Anim Pract,* 49 (4): 651-670, 2019. DOI: 10.1016/j.cvsm.2019.02.006

15. Yang S, Wang S, Feng H, Zeng L, Xia Z, Zhang R, Zou X, Wang C, Liu Q, Xia X: Isolation and characterization of feline panleukopenia virus from a diarrheic monkey. *Vet Microbiol,* 143 (2-4): 155-159, 2010. DOI: 10.1016/j.vetmic.2009.11.023

16. Duarte MD, Barros SC, Henriques M, Fernandes TL, Bernardino R, Monteiro M, Fevereiro M: Fatal infection with feline panleukopenia virus in two captive wild carnivores *(Panthera tigris* and *Panthera leo). J Zoo Wildl Med,* 40 (2): 354-359, 2009. DOI: 10.1638/2008-0015.1

17. Foley JE, Swift P, Fleer KA, Torres S, Girard YA, Johnson CK: Risk factors for exposure to feline pathogens in California mountain lions *(Puma concolor). J Wildl Dis,* 49 (2): 279-293, 2013. DOI: 10.7589/2012-08-206

18. Inthong N, Sutacha K, Kaewmongkol S, Sinsiri R, Sribuarod K, Sirinarumitr K, Sirinarumitr T: Feline panleukopenia virus as the cause of diarrhea in a banded linsang *(Prionodon linsang)* in Thailand. *J Vet Med Sci,* 81 (12): 1763-1768, 2019. DOI: 10.1292/jvms.19-0238

19. Yu F, Zhu Y, Li S, Hao L, Li N, Ye F, Jiang Z, Hu X: Dysfunction and regulatory interplay of T and B cells in chronic hepatitis B: Immunotherapy and emerging antiviral strategies. *Front Cell Infect Microbiol,* 14:1488527, 2024. DOI: 10.3389/fcimb.2024.1488527

20. Collins AR: *In vitro* detection of apoptosis in monocytes/macrophages infected with human coronavirus. *Clin Diagn Lab Immunol,* 9 (6): 1392-1395, 2002. DOI: 10.1128/cdli.9.6.1392-1395.2002

21. Lim YX, Ng YL, Tam JP, Liu DX: Human coronaviruses: A review of

virus-host interactions. *Diseases,* 4 (3):26, 2016. DOI: 10.3390/diseases4030026

22. Fu Y, Ishii KK, Munakata Y, Saitoh T, Kaku M, Sasaki T: Regulation of tumor necrosis factor alpha promoter by human parvovirus B19 NS1 through activation of AP-1 and AP-2. *J Virol,* 76 (11): 5395-5403, 2002. DOI: 10.1128/jvi.76.11.5395-5403.2002

23. Duechting A, Tschöpe C, Kaiser H, Lamkemeyer T, Tanaka N, Aberle S, Lang F, Torresi J, Kandolf R, Bock CT: Human parvovirus B19 NS1 protein modulates inflammatory signaling by activation of STAT3/PIAS3 in human endothelial cells. *J Virol,* 82 (16): 7942-7952, 2008. DOI: 10.1128/JVI.00891-08

24. Morita E, Nakashima A, Asao H, Sato H, Sugamura K: Human parvovirus B19 nonstructural protein (NS1) induces cell cycle arrest at G(1) phase. *J Virol,* 77 (5): 2915-2921, 2003. DOI: 10.1128/jvi.77.5.2915-2921.2003

25. Kang H, Liu D, Tian J, Hu X, Zhang X, Yin H, Wu H, Liu C, Guo D, Li Z, Jiang Q, Liu J, Qu L: Feline panleucopenia virus NS2 suppresses the host IFN-β induction by disrupting the interaction between TBK1 and STING. *Viruses,* 9 (1):23, 2017. DOI: 10.3390/v9010023

26. Radford AD, Chapman D, Dixon L, Chantrey J, Darby AC, Hall N: Application of next-generation sequencing technologies in virology. *J Gen Virol,* 93 (Pt 9): 1853-1868, 2012. DOI: 10.1099/vir.0.043182-0

27. Zhang H, Zhang W, Pan Y, Li H, He T, Dong Q, Song W, Zhang W, Zhang L, Kareem K, Jiang S, Sheng J: Evolutionary dynamics and pathogenicity analysis of feline panleukopenia virus in Xinjiang, China. *Microorganisms,* 12 (11):2205, 2024. DOI: 10.3390/microorganisms12112205

28. Zhang L, Liang R, Raheem A, Liang L, Zhang X, Cui S: Transcriptomics analysis reveals key lncRNAs and genes related to the infection of feline kidney cell line by panleukopenia virus. *Res Vet Sci,* 158, 203-214, 2023. DOI: 10.1016/j.rvsc.2023.03.027

29. Drechsler Y, Vasconcelos EJR, Griggs LM, Diniz PPPV: CRFK and primary macrophages transcriptomes in response to feline coronavirus infection differ significantly. *Front Genet,* 11:584744, 2020. DOI: 10.3389/fgene.2020.584744

30. Wang Y, Hassan HM, Nisar A, Zahara SS, Akbar A, Al-Emam A: Cardioprotective potential of tectochrysin against vanadium induced heart damage via regulating NLRP3, JAK1/STAT3 and NF-κB pathway. *J Trace Elem Med Biol,* 87:127588, 2025. DOI: 10.1016/j.jtemb.2025.127588

31. Misiukiewicz-Stępień P, Zajusz-Zubek E, Górska K, Krenke R, Paplińska-Goryca M: The different response of PM2.5 stimulated nasal epithelial spheroids in control, asthma and COPD groups. *Respir Res,* 26 (1):8, 2025. DOI: 10.1186/s12931-025-03097-w

32. Lai Y, Qiu R, Zhou J, Ren L, Qu Y, Zhang G: Fecal microbiota transplantation alleviates airway inflammation in asthmatic rats by increasing the level of short-chain fatty acids in the intestine. *Inflammation,* 2025:2025. DOI: 10.1007/s10753-024-02233-w

33. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B: An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A,* 72 (9): 3666-3670, 1975. DOI: 10.1073/pnas.72.9.3666

34. Nukuzuma S, Nakamichi K, Kameoka M, Sugiura S, Nukuzuma C, Tasaki T, Takegami T: TNF-α stimulates efficient JC virus replication in neuroblastoma cells. *J Med Virol,* 86 (12): 2026-2032, 2014. DOI: 10.1002/jmv.23886

35. Wu H, Jiang X, Gao Y, Liu W, Wang F, Gong M, Chen R, Yu X, Zhang W, Gao B, Song C, Han D: Mumps virus infection disrupts blood-testis barrier through the induction of TNF-α in Sertoli cells. *FASEB J,* 33 (11): 12528-12540, 2019. DOI: 10.1096/fj.201901089R

36. Chen K, Eddens T, Trevejo-Nunez G, Way EE, Elsegeiny W, Ricks DM, Garg AV, Erb CJ, Bo M, Wang T, Chen W, Lee JS, Gaffen SL, Kolls JK: IL-17 receptor signaling in the lung epithelium is required for mucosal chemokine gradients and pulmonary host defense against *K. pneumoniae. Cell Host Microbe,* 20 (5): 596-605, 2016. DOI: 10.1016/j.chom.2016.10.003

37. Wang X, Chan CC, Yang M, Deng J, Poon VK, Leung VH, Ko KH, Zhou J, Yuen KY, Zheng BJ, Lu L: A critical role of IL-17 in modulating the B-cell response during H5N1 influenza virus infection. *Cell Mol Immunol,* 8 (6): 462-468, 2011. DOI: 10.1038/cmi.2011.38https://www.nature.com/articles/cmi201138

38. Jain A, Pandey N, Garg RK, Kumar R: IL-17 level in patients with Dengue virus infection & its association with severity of illness. *J Clin Immunol,* 33 (3): 613-618, 2013. DOI: 10.1007/s10875-012-9855-0

39. Mebratu YA, Tesfaigzi Y: IL-17 plays a role in respiratory syncytial virus-induced lung inflammation and emphysema in elastase and LPS-injured mice. *Am J Respir Cell Mol Biol,* 58 (6): 717-726, 2018. DOI: 10.1165/rcmb.2017-0265OC

40. Li C, Yang P, Sun Y, Li T, Wang C, Wang Z, Zou Z, Yan Y, Wang W, Wang C, Chen Z, Xing L, Tang C, Ju X, Guo F, Deng J, Zhao Y, Yang P, Tang J, Wang H, Zhao Z, Yin Z, Cao B, Wang X, Jiang C: IL-17 response mediates acute lung injury induced by the 2009 pandemic influenza A (H1N1) virus. *Cell Res,* 22 (3): 528-538, 2012. DOI: 10.1038/cr.2011.165

41. Zheng S, Gu H, Han G, Xu H, Liu Z, Lu Y, He F: Porcine reproductive and respiratory syndrome virus nsp11 antagonizes broad antiviral effects of MCPIP1 by inducing interleukin-17 expression. *J Virol,* 95 (22):e0111921, 2021. DOI: 10.1128/JVI.01119-21

42. Ronkina N, Gaestel M: MAPK-activated protein kinases: Servant or partner? *Annu Rev Biochem,* 91, 505-540, 2022. DOI: 10.1146/annurev-biochem-081720-114505

43. Wang L, Xia Z, Tang W, Sun Y, Wu Y, Kwok HF, Sun F, Cao Z: p38 activation and viral infection. *Expert Rev Mol Med,* 24:e4, 2022. DOI: 10.1017/erm.2021.29

44. Subaramaniyam U, Allimuthu RS, Vappu S, Ramalingam D, Balan R, Paital B, Panda N, Rath PK, Ramalingam N, Sahoo DK: Effects of microplastics, pesticides and nano-materials on fish health, oxidative stress and antioxidant defense mechanism. *Front Physiol,* 14:1217666, 2023. DOI: 10.3389/fphys.2023.1217666