INTRODUCTION

Sea perch, *Lateolabrax japonicus*, an economically important species, is widely cultured in Asia, especially in China. It has been increasingly threatened by various diseases caused by bacteria and viruses due to its extensive farming (Jia, Jia, & Yi, 2015; Zhao, Fu, Wang, Jiao, & Qiu, 2016). Of which, viral nervous necrosis (VNN), caused by nervous necrosis virus (NNV), has been widely reported in more than 40 cultured fish, including sea perch and grouper, and resulted in significant economic losses (Nakai et al., 2009). However, there is still no effective method to prevent or control VNN. In teleost fish, the innate immune system plays a critical role in host against virus infection, but studies on the immune system of sea perch are still limited. In recent years, researches on immunity of sea perch against NNV infection were mainly focused on single genes, and only a few immune-related genes, including LGP2 (Jia, Zhang et al., 2015), MDA5 (Jia, Jia et al., 2016), MAVS (Jia, Jin et al., 2016), TRAF3 (Zhang et al., 2018),
HSP27 (Le, Jia et al., 2017) and RAVER1 (Jia et al., 2017), have been identified and characterized in sea perch. However, knowledge about the immunity system of sea perch, especially the immune response–related signalling pathways implicated in NNV infection, still remains incomplete. Systematic study of sea perch immune-related signalling pathway is necessary to provide the fundamental understanding of the interaction between sea perch and NNV.

Transcriptome analysis is a fast and cost-effective way to understand the underlying pathways and mechanisms of host against pathogen infection by evaluating immune responses of gene expression (Liu, Wang, Kwang, Yue, & Wong, 2016; Zhong et al., 2017). Many aquatic species have been sequenced to study pathogenic processes during virus infection, including mandarin (Hu et al., 2015), orange-spotted grouper (Huang et al., 2011), Pacific white shrimp (Zeng et al., 2013) and rainbow trout (Aquino, Castro, Fischer, & Tafalla, 2014).

It was well known that the brain and retina were the main target organs of NNV (Poisa-Beiro et al., 2008). Therefore, cells originated from sea perch brain should be a good material for studying sea perch–NNV interaction. In our previous study, a continuous cell line Lateolabrax japonicus brain (LJB), derived from the brain of sea perch, was established and exhibited susceptibility to red-spotted grouper nervous necrosis virus (RGNNV) (Le, Li et al., 2017). In this study, transcriptome sequencing libraries were constructed with RGNNV-infected or mock-infected LJB cells. Based on the analysis of differentially expressed genes (DEGs), we found that p53 signalling pathway might be involved in the immune response against RGNNV, and experimentally revealed the role of L. japonicus p53 (Ljp53) in the regulation of the type I IFN response and cellular apoptosis during RGNNV infection. This study provides insight into the immune response of sea perch against RGNNV infection and the important role of Ljp53 in inhibiting RGNNV infection.

2 | MATERIALS AND METHODS

2.1 | Cells, virus stock and plasmid

Lateolabrax japonicus brain cells were grown and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 15% foetal bovine serum (FBS) (Gibco) at 28°C (Le, Li et al., 2017). HEK293T (human embryonic kidney 293) cells were maintained in DMEM supplemented with 10% FBS at 37°C in a 5% CO2 incubator (Russell, Graham, Smiley, & Nairn, 1977).

Red-spotted grouper nervous necrosis virus was isolated from diseased sea perch in Guangdong Province of China and kept in our laboratory (Jia, Jia et al., 2015). RGNNV was propagated in LJB cells at 28°C, and virus titres were detected by 50% tissue culture infective dose (TCID50) (Jia, Zhang et al., 2015).

DrIFN1-pro-luc plasmid was kindly provided by Professor Yibing Zhang at the Institute of Hydrobiology, Chinese Academy of Sciences (Zhang & Gui, 2012).

2.2 | RNA preparation, library construction and transcriptome sequencing

Lateolabrax japonicus brain cells were infected with RGNNV (multiplicity of infection [MOI] = 5) at 28°C for 4 hr. Then, the medium containing RGNNV was discarded and the same volume of growth medium with 15% FBS was added. The control (mock-infected LJB cells) was treated with the same volume of medium. RGNNV-infected or mock-infected LJB cells were harvested for RNA isolation at 48 hr post-infection (hpi), respectively. Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The concentration of total RNA was determined using NanoDrop 2000 UV-Vis Spectrophotometer.

For cDNA library construction and sequencing, mRNA was first isolated from total RNA treated with DNase I using Magnetic Oligo (dT) Beads (Illumina) and was fragmented. Then, the double-stranded cDNA was synthesized with random hexamer primers and was further subjected to end-repair and adapter ligation using T4 DNA ligase. The products of ligation reaction were purified on 2% agarose gel, and cDNA fragments (about 200 bp) were recovered. PCR was carried out to enrich the purified cDNA template. Finally, the cDNA library was constructed. After validating on an Agilent Technologies 2100 Bioanalyzer, the library was sequenced using Illumina HiSeq 4000 according to the manufacturer’s instruction. All data sets have been submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database PRJNA497762.

2.3 | De novo assembly and functional annotation

After sequencing, we carried out a stringent filtering process of raw sequencing reads as described previously (Gui et al., 2013). The raw reads were cleaned by removing adapter sequences, non-coding RNA, low-quality sequences (reads with ambiguous bases “N” and the ratio of “N” > 10%) and reads with average length <20 bases. De novo transcriptome assembly was performed by Trinity program (v2.0.6) as described elsewhere (full-length transcriptome assembly from RNA-seq data without a reference genome). The function of unigenes was annotated depending upon following databases: NR (NCBI non-redundant protein sequences), NT (NCBI nucleotide sequences), GO (Gene Ontology), COG (Clusters of Orthologous Groups of proteins), KEGG (Kyoto Encyclopedia of Genes and Genomes) and Swiss-Prot (a manually annotated and reviewed protein sequence database).

2.4 | Gene expression level analysis, DEG identification and classification

For differential gene expression analysis, reads per kilobase of exon model per million mapped reads value was used to normalize the gene expression levels (Poisa-Beiro et al., 2008). Statistical comparison between two different groups was conducted using a web tool DESeq (http://www.huber.embl.de/users/anders/DESeq) (Lu et al., 2017). False discovery rate (FDR) <0.05 was used as the threshold
of p-value in multiple tests to judge the significance of gene expression difference. Genes were considered differentially expressed in a given library when the p-value < 0.05, and a greater than twofold change (absolute value of log2 ratio >1) in expression across libraries was observed. GO and KEGG pathway enrichment of differential unigenes were analysed. KO analysis of differential expression unigenes in metabolite pathways was conducted on website (http://www.genome.jp/kegg/tool/map_pathway2.html).

2.5 | Expression analysis of DEGs involved in p53 signalling pathway post-RGNNV challenge

Red-spotted grouper nervous necrosis virus infection and sample collection were performed as described above. Total RNA was extracted using TRIzol reagent (Invitrogen) and reverse-transcribed into cDNA by PrimeScript™ First Strand cDNA Synthesis Kit (Takara) according to the manufacturer's instructions. The relative expression levels of Mdm2, Mdm4, p53, Bax, Casp8 and Casp9 were analysed by quantitative real-time PCR (qRT-PCR). qRT-PCR was performed as described previously (Le, Li et al., 2017). Primers for qRT-PCR are listed in Supporting Information Table S1. Expression levels of target genes were normalized with β-actin of sea perch by the 2-ΔΔCT method. Data from each sample were shown as mean ± SD from three independent experiments in triplicates.

2.6 | Effects of Ljp53 on RGNNV replication

To investigate the effects of Ljp53 overexpression on RGNNV replication, encoding region of Ljp53 was amplified by PCR using gene-specific primers and sub-cloned into pcDNA 3.1 (+) vectors (Invitrogen) to generate pcDNA-Ljp53, which was confirmed through DNA sequencing analysis (Supporting Information Table S1). LJB cells in six-well plates at 70%–80% confluence were transfected with pcDNA-Ljp53 or pcDNA 3.1(+) using Lipofectamine 3000 (Invitrogen) to generate pEGFP-Ljp53, which was confirmed through DNA sequencing analysis (Supporting Information Table S1). LJB cells were transfected with pEGFP-Ljp53 or pEGFP-N3 or treated with pifithrin-α as above. Cells were harvested for RNA isolation at 48 hpi. The relative expression levels of Casp3, Casp9 and CytC were analysed by qRT-PCR. Primers for qRT-PCR are listed in Supporting Information Table S1.

2.7 | Luciferase activity assay

Luciferase activity assay was carried out as described previously (Jia et al., 2017). In brief, the plasmids pRL-TK (Promega), pcDNA-Ljp53 or pcDNA 3.1(+), and DrIFN1-pro-luc were co-transfected into HEK293T cells using Lipofectamine 3000. At 48 hr post-transfection, cells were harvested with passive lysis buffer and subjected to luciferase activity measurement using GloMax 20/20 Luminometer (Promega). Data were expressed as mean ± SD from three independent experiments performed in triplicates.

2.8 | Expression analysis of apoptosis-related genes

To evaluate the effect of Ljp53 on apoptosis-related genes during RGNNV infection, the encoding region of Ljp53 was sub-cloned into pEGFP-N3 vectors (Invitrogen) to generate pEGFP-Ljp53, which was confirmed through DNA sequencing analysis (Supporting Information Table S1). LJB cells were transfected with pEGFP-Ljp53 or pEGFP-N3 or treated with pifithrin-α as above. Finally, cells were harvested for RNA isolation at 48 hpi. The relative expression levels of Casp3, Casp9 and CytC were analysed by qRT-PCR. Primers for qRT-PCR are listed in Supporting Information Table S1.

2.9 | Statistics analyses

All statistics were calculated using SPSS version 19. Differences between control and treatment groups were assessed by one-way ANOVA. p < 0.05 (*) and p < 0.01 (**) were, respectively, considered to be statistically significant and very significant.

3 | RESULTS

3.1 | De novo assembly and function annotation

To understand the gene expression patterns of LJB cells in response to RGNNV infection, RNA-seq libraries were generated from RGNNV-infected and mock-infected LJB cells. A total of 4.751 × 10⁷ and 4.525 × 10⁷ raw reads were produced in mock-infected and RGNNV-infected samples, respectively. After filtration, 4.51 × 10⁷ clean reads obtained from the mock-infected sample (control group) and RGNNV-infected group were used for de novo assembly (Table 1). In control group, transcriptome assembly

| Sample     | Total raw reads (Mb) | Total clean reads (Mb) | Total clean bases (Gb) | Clean reads Q20 (%) | Clean reads Q30 (%) | Clean reads ratio (%) |
|------------|----------------------|------------------------|------------------------|---------------------|---------------------|-----------------------|
| Ljb_control | 47.51                | 45.1                   | 4.51                   | 94.25               | 87.07               | 94.93                 |
| Ljb_nnv     | 45.25                | 44.85                  | 4.48                   | 94.57               | 87.54               | 99.12                 |
yielded 38,354 unigenes with a mean length of 1,067 and N50 length of 1,979, and in RGNNV-infected group, 30,209 unigenes with a mean length of 697 and N50 length of 1,110 were produced. Moreover, combined transcriptome assembly of the two groups generated 39,954 unigenes with a mean length of 1,038 and N50 length of 1,931 (Table 2). The length distribution of these unigenes is shown in Figure 1.

After assembly, 39,954 unigenes were annotated based on six databases including NR, NT, GO, COG, KEGG and Swiss-Prot. A total of 32,685 unigenes were matched in at least one database. Of all the unigenes, 24,738 (61.92%), 21,854 (54.68%), 18,029 (45.12%) and 9,001 (22.53%) unigenes showed significant matches (E-value ≤ 1E-5) against the databases of NR, Swiss-Prot, KEGG and COG, respectively (Table 3). GO analysis assigned 1,569 unigenes to biological process (BP), 1,078 to cellular component (CC) and 649 to molecular function (MF). For BP, the most assigned genes were cellular process with 294 unigenes. For CC, the most assigned genes were cell and cell part with 234 unigenes. For MF, binding with 292 unigenes was the most highly represented group (Figure 2). COG classification indicated that 9,001 unigenes were assigned to 25 different COG categories. The cluster for "General function prediction only" represented the largest group (3,237 unigenes), followed by "replication, recombination and repair" (1,426 unigenes) and "transcription" (1,375 unigenes) (Figure 3). A total of 18,029 unigenes were assigned to 301 KEGG pathways, covering six main categories, include cellular processes, environmental information processing, genetic information processing, human diseases, metabolism and organismal systems. Metabolic pathway (1,883 unigenes) was the most abundant KEGG pathway, followed by "pathway in cancer" (844 unigenes) and "focal adhesion" (678 unigenes) (Supporting Information Table S2).

### 3.2 Differentially expressed genes

To identify DEGs, the unigenes between LJB-Control and LJB-NNV groups were compared. A large number of genes were found to be differentially expressed in LJB cells post-RGNNV infection, including 1,969 up-regulated genes (DUGs) and 9,858 down-regulated genes (DDGs) (FDR ≤ 0.001 and log2 ratio ≥ 1) (Supporting Information Table S3). Of these genes, many well-known immune-related genes were strongly inhibited after RGNNV infection, including NOD1, TLR2, IRF3, STAT1, IFN-stimulated gene 15 (ISG15). Among all the DDGs, heat-shock protein beta-1 (HSPB1), also named HSP27, was mostly down-regulated (decreased by 18.60 times).

Gene Ontology function and KEGG pathway enrichment analysis were performed to identify the biological function of DEGs. Based on GO classification, DEGs were mainly divided into three categories: BP (129 DEGs), CC (105 DEGs) and MF (158 DEGs). Furthermore, KEGG mapping annotated DEGs to 311 signalling pathways. Lots of DEGs were related to immune-related signalling pathways, such as MAPK signalling pathway (228 DEGs), Epstein-Barr virus infection (199 DEGs), p53 signalling pathway (79 DEGs), NF-kB signalling pathway (74 DEGs), NOD-like receptor signalling pathway (59 DEGs) and RIG-I-like receptor signalling pathway (43 DEGs) (Supporting Information Table S4).

### 3.3 qRT-PCR verification for DEGs related to p53 signalling pathway

Comparative transcriptome analysis showed that 79 DEGs involved in p53 signalling pathway were regulated in RGNNV-infected LJB cells compared to control cells (Supporting Information Table S5). A majority of these DEGs were down-regulated, such as p53, Fos, CytC and Casp8, but only six DEGs, including Mdm2, Cdc2, Igf, P21, Gadd45 and Igf-bp3, were up-regulated. To verify the expression pattern of DEGs involved in p53 signalling pathway, the transcriptional levels of six randomly selected DEGs relating to p53 signalling pathway (Mdm2, Mdm4, p53, Bax, Casp8 and CytC) were detected by qRT-PCR. As shown in Figure 4, the expression levels of these genes showed similar fold change to the transcriptome data. Meanwhile, in accordance with the high-throughput sequencing data, the expressions of all these DEGs were significantly reduced post-RGNNV infection, except for

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**TABLE 2** Summary of de novo assembly of transcriptomic profiles

| Sample    | Total number | Total length (nt) | Mean length (nt) | N50 (nt) | N70 (nt) | N90 (nt) | GC (%) |
|-----------|--------------|-------------------|------------------|---------|---------|---------|--------|
| Ljb_control | 38,354       | 40,935,337        | 1,067            | 1,979   | 1,119   | 404     | 48.37  |
| Ljb_nnv    | 30,209       | 21,081,274        | 697              | 1,110   | 575     | 279     | 48.4   |
| All-Unigenes | 39,954       | 41,498,627        | 1,038            | 1,931   | 1,085   | 391     | 48.18  |

**FIGURE 1** Length distribution of All-unigenes
the expression of Mdm2 was up-regulated by 5.2 times at 48 hpi (Supporting Information Figure S1). These results suggested that the expression profiling of DEGs determined by RNA-seq was reliable. These results indicated that p53 signalling pathway was not only involved in RGNNV infection but also inhibited by RGNNV at 48 hpi.

| Values          | Total | Nr    | Nt    | Swiss-Prot | KEGG   | COG  | GO   | Overall |
|-----------------|-------|-------|-------|------------|--------|------|------|---------|
| Number          | 39,954| 24,738| 31,651| 21,845     | 18,029 | 9,001| 518  | 32,685  |
| Percentage      | 100   | 61.92 | 79.22 | 54.68      | 45.12  | 22.53| 1.30 | 81.81   |

Note. COG: Clusters of Orthologous Groups; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.
Given the important role of p53 in innate antiviral immunity, in the present work, Ljp53 was further cloned and analysed. Fortunately, the partial sequence of Ljp53 obtained from high-throughput sequencing data contained the complete coding sequence of Ljp53 (GenBank accession number MH940221). To elucidate the role of Ljp53 during RGNNV infection in vitro, the transcription level of RDRP was detected in Ljp53 overexpressing LJB cells. As shown in Figure 5a,b, a significantly attenuated expression of RDRP was observed in Ljp53 expressing LJB cells, whereas pifithrin-α caused down-regulation of Ljp53 led to opposite results, suggesting the antiviral role of Ljp53 during RGNNV infection. Many works have demonstrated that p53 contributed to the innate antiviral response by enhancing type I IFN-dependent antiviral activity (Ding et al., 2018; Muñoz-Fontela et al., 2008). To further reveal the potential antiviral mechanism of Ljp53, the luciferase reporter assay was performed. Our results indicated that zebrafish IFN1 promoter was activated by Ljp53 overexpression (Figure 5c), indicating that Ljp53 might exert its anti-RGNNV activity partially by activating the IFN signalling pathway.
Ljp53 promoted the expression of apoptotic genes

To validate the antiviral role of Ljp53 was associated with its pro-apoptotic activity in RGNNV infection, the mRNA levels of Casp3, Casp9 and CytC were measured in Ljp53 overexpressing LJB cells. Our results showed that overexpression of Ljp53 up-regulated the transcription of pro-apoptotic Casp3, Casp9 and CytC at 48 hpi, whereas the suppression of Ljp53 caused by pifithrin-α led to the opposite effect (Figure 6). Thus, our results suggested that the
apoptosis induced by RGNNV infection was dependent to some extent on Ljp53, and Ljp53 might be involved in promoting apoptosis in LJB cells.

4 | DISCUSSION

Sea perch is a commercially important marine fish widely cultured in Asia. However, the high mortality in a wide range of larvae and juveniles caused by NNV is an intensive threat. Nevertheless, there is limited transcriptomic information available in relation to the molecular immune mechanism of sea perch against NNV infection. In this study, transcriptome sequencing libraries were generated from RGNNV-infected or mock-infected LJB cells. In total, $4.51 \times 10^7$ and $39.954$ unigenes were annotated by six databases. And the number of unigenes was slightly different from previous reports in which the fish were infected with different pathogens (Lu et al., 2017; Zhao et al., 2016).

Gene comparative screening identified a lot of DEGs, including several known immune-related genes, such as IFR3, STAT1 and ISG15. Previous reports indicated that ISG15 could be induced by RGNNV infection in European sea bass and Senegalese sole (Álvarez-Torres et al., 2017; Moreno, García-Rosado, Borrego, & Alonso, 2016). However, in this study, the expression of ISG15 was reduced at 48 hpi. ISG15, as an antiviral effector, was demonstrated to play important roles in defence against RGNNV infection in teleost (Liu et al., 2018; Moreno, Álvarez-Torres, García-Rosado, Borrego, & Alonso, 2018). Many viruses have developed different mechanisms to escape the antiviral response, including counteracting the activity of ISG15 (Guerra, Cáceres, Knobeloch, Horak, & Esteban, 2008; Yuan & Krug, 2001). Several studies have addressed NNV persistent infections in many fish, indicating NNV has developed some mechanisms to antagonize host innate antiviral responses (Lu et al., 2016; Zhang et al., 2017). We speculated that RGNNV might counteract the antiviral activity of ISG15 to escape the host antiviral response in LJB cells. Zinc finger protein 395 (ZNF395) is one of the most downregulated genes (decreased by 18.11 times) in this study. ZNF395, as a novel hypoxia-inducible transcription factor, contributes to the maximal stimulation of a subset of ISGs, such as ISG15, IFIT1/ISG56, IFI44, CXCL10 and CXCL11 (Schroeder, Herwartz, Jordanovski, & Steger, 2017). In this study, in line with the down-regulation of ISG15, the expression of ZNF395 was also decreased. Given these results, it is possible that the declining level of ZNF395 may impairs host antiviral responses by reducing the expression of ISGs involved in the innate immune response during RGNNV infection. It was known that ZNF395-mediated activation of ISGs was depended on IKK signalling (Jordanovski et al., 2013). In present work, the expression levels of IKKα and IKKβ, the catalytic subunits of IKK, were also repressed post-RGNNV infection. Further experiments would be necessary to determine the association of ISG15, ZNF395 and IKK signalling in sea perch. In addition, among these DEGs, HSPB1, also named HSP27, was down-regulated, similar to our previous study (Le, Jia et al., 2017). HSPB1 has been reported to be widely involved in pathophysiology of oxidative stress and apoptosis. Overexpression of HSPB1 protected cells from oxidative damage and apoptosis triggered by Cd exposure (Muñoz-Fontela, 2005). In our previous study, overexpression of LjHSP27 inhibited RGNNV-induced apoptosis, supporting the vital role of HSP27 as an anti-apoptosis protein (Le, Jia et al., 2017). It has been known that many DNA and RNA viruses can trigger oxidative stress and induce host cell death in infected cells (Casavant et al., 2006; Yuan et al., 2015). The role of LjHSP27 against oxidative stress still needs further research.

Published literature about the immune-related signalling pathways involved in RGNNV infection was mainly focused either on the apoptosis pathway or on the innate immune pathways (Chen et al., 2017; Wang, Rajanbabu, & Chen, 2015). Recently, numbers of studies have shown that p53 signalling pathway is involved in the process of different virus infection and replication. For example, porcine circovirus type 2 infection induced 5 phase accumulation to promote viral replication in host cells via activation of the p53 signalling pathway (Xu et al., 2016). Classical swine fever virus Shimen regulated p53 signalling pathway to subvert host innate immune response (Ning et al., 2017). Given the important role of p53 signalling pathway during virus infection, in this study, we focused our analysis on the DEGs associated with p53 signalling pathway. qRT-PCR results indicated that p53 signalling pathway was involved in RGNNV infection and inhibited by RGNNV. In p53 signalling pathway, Mdm2 and Mdm4 are major negative regulators of p53 (Chai et al., 2018; Raja, Ronsard, Lata, Trivedi, & Banerjea, 2017). A significant negative correlation was also found between Mdm2 and Ljp53 in this study. Thus, we speculated that RGNNV might repress the expression of Ljp53 by activating Mdm2 during RGNNV infection. As Mdm2 has been initially identified as a required host factor for viral replication (Raja et al., 2017), we cannot exclude an ambivalent role of Mdm2 during infection, as a result of its multiple functions and interactions with other host factors. In contrast, there was a positive correlation between Mdm4 and Ljp53. Previous study reported that Mdm2 targeted Mdm4 for proteasomal degradation in response to DNA damage (Kawai et al., 2003). Thus, we speculated that sea perch Mdm2 might also bind to Mdm4 for degradation during RGNNV infection. Nevertheless, to clarify the relationship of sea perch Mdm2 and Mdm4, more experiments will be required.

Tumour suppressor p53, primarily famous for its vital role in protecting against cancer development, has been proven to regulate various biological process, such as cell cycle arrest, cellular metabolism, cellular apoptosis and innate antiviral immunity (Liu, Zhang, Hu, & Feng, 2015). Several studies indicated that p53 functioned as a key player in innate antiviral immunity by both enforcing the type I IFN response and inducing apoptosis in virus-infected cells (Rivas, Aaronson, & Munoz-Fontela, 2010). Here, the expression of RDRP was significant reduced in Ljp53 overexpressing cells post-RGNNV infection, whereas pifithrin-α caused opposite results. Furthermore, the results of luciferase reporter assay showed that zebrafish IFN1...
promoter was activated by Ljp53 overexpression. Thus, we speculated that Ljp53 inhibited RGNNV replication by activating the IFN signalling pathway.

It was known that the role of p53 in the control of virus infection was also associated with its ability to activate apoptosis during virus infection, which inhibited virus replication (Rivas et al., 2010). Several studies have reported that p53 can induce apoptosis through activating death pathway receptor or mitochondrial pathway (Benchimol, 2001; Moll & Zaika, 2001). Our previous study demonstrated that RGNNV infection caused apoptosis at 6 and 24 hpi in LJB cells (Le, Jia et al., 2017). Meanwhile, our results indicated that three apoptosis-related genes, including Casp3, Casp9 and CytC, were notably enhanced as the ectopic expression of Ljp53 in vitro, whereas pifithrin-α caused the opposite effect. In addition, significantly higher level of caspase 3 activities was observed in Ljp53 overexpressing cells post-RGNNV infection. All these results indicated the pro-apoptotic function of Ljp53 (Supporting Information Figure S2). It has been known that p53-dependent apoptosis could be used by host as a useful mechanism to repress virus infection (Muñoz-Fontela et al., 2005; Turpin et al., 2005). On the other hand, many viruses have also developed strategies to manipulate host p53 signalling pathways to increase virus replication (Casavant et al., 2006; Roys et al., 2006). For example, coronavirus inhibited p53-mediated host apoptosis to ensure viral growth in infected cells (Yuan et al., 2015). In our study, RGNNV infection reduced the expression of Ljp53 at 48 hpi, which at least partially inhibited the host cell apoptosis. We speculated that RGNNV might repress the p53-dependent apoptosis to promote virus replication in RGNNV-infected LJB cells and facilitate transmission of newly formed viral particles to other cells.

In this study, transcriptome sequencing libraries were constructed with LJB cells mock-infected or infected with RGNNV at 48 hpi. A large number of DEGs in response to RGNNV infection were identified. The DEGs involved in p53 signalling pathway were further investigated. Our results demonstrated that Ljp53 played an essential role in inhibiting RGNNV replication. Mechanistically, we confirmed that the antiviral role of Ljp53 was involved in its activity in mediating the IFN response and its pro-apoptotic activity.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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