Identification of key genes and signaling pathways during Sendai virus infection in vitro

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Abstract

Sendai virus (SeV) has been used as a model strain to reveal molecular features of paramyxovirus biology. In this study, we comprehensively analyzed the gene profiling of murine macrophages and airway epithelial cells in response to SeV using gene expression data. The significantly differentially expressed genes (DEGs) were screened by GEO2R. Gene ontology (GO) and pathway enrichment analyses were performed by DAVID. The protein-protein interaction (PPI) map of DEGs was constructed by STRING. The modules of PPI network are produced by molecular complex detection (MCODE) plug-in of Cytoscape. In total, 241 up- and 83 downregulated DEGs were identified in airway epithelial cells while 130 up- and 148 downregulated in macrophage. Particularly, *Tmem119* and *Colla2* are significantly downregulated in airway epithelial cells and macrophages, respectively. Functional enrichment analysis showed that upregulated DEGs are clustered in innate immunity and inflammatory response in both cell types, whereas downregulated DEGs are involved in host metabolic pathway in airway epithelial cells. PI3K-AKT signaling pathway is downregulated in macrophages. PPI network analysis indicated that some high degree of nodes exist in both cell types, such as *Stat1*, *Tnf*, and *Cxcl10*. In conclusion, SeV infection can induce different host cell responses in airway epithelial cells and macrophages.

Keywords SeV · Differentially expressed genes · Immunity · Metabolism · Signaling pathway

Introduction

Sendai virus (SeV) is one member of the *Paramyxoviridae* family. It contains a negative-sense single-stranded RNA with a size of 15.3 kb. SeV has been widely used as a model strain to study the characteristics of paramyxovirus biology. SeV can cause respiratory tract infection in mice and rat. It has the potential to be used as a vaccine vector. Ishii et al. used SeV

as an antigen delivery tool to induce immune response against simian immunodeficiency virus (SIV) infection in a macaque model of acquired immune deficiency syndrome (AIDS) [1]. The recombinant SeV expressing a truncated human metapneumovirus (hMPV) can protect cotton rats against hMPV infection [2]. An intranasal SeV-based human parainfluenza virus type 1 (hPIV-1) displays good antigenicity and immunogenicity and protects 3- to 6-year-old children from hPIV-1 infection [3]. Moreover, SeV can be used as a gene therapy vector. Mizokami et al. explored the potential of SeV for treatment of laryngotracheal stenosis (LTS) in injured mucosa of LTS model and the SeV-mediated transgene expression can be detected after administration [4].

SeV infection can induce host immune responses and cause tissue injury. SeV 53 strain can cause robust respiratory tract infection and pathogenesis [5]. Wetzel et al. found that interferon regulatory transcription factor 3 (IRF-3) plays an important role in host defense against SeV partially by inducing IFN and interferon-stimulated genes (ISGs) [6]. They also found that Ifit2 can prevent the pathogenesis resulting from SeV infection while interferon can exacerbate the pathogenesis [7]. Peter et al. found that IRF-3 determines the fate of SeV-infected cells by

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promoting cellular apoptosis [8]. Persistent infection of SeV can be established in these cells without the expression of IRF-3 or with a low level of IRF-3 expression [9]. The persistent infection of SeV can stimulate the expression of many innate immune genes, but interferon signaling was suppressed [9].

Furthermore, SeV infection can activate IFN signaling protein STAT1 in airway epithelial cells, which is essential for the control of viral replication [10]. The innate immunity response leads to clearance of SeV in the early stage of infection [11]. SeV C protein can impair both IFN-stimulated tyrosine phosphorylation and dephosphorylation process of STAT1 [12]. The anti-IFN capacity of SeV C protein is required for the viral pathogenicity in vivo [11]. Besides, the type 1 interferon can regulate SeV replication and promote the maturation and migration of dendritic cells (DCs) [5]. The number of dendritic cells is increased and the expression of major histocompatibility complex class II (MHC II) is upregulated in rat DCs after SeV infection [13]. A recent study showed that 742 proteins are differentially regulated in SeV-infected 293 T cells and type 1 interferon signaling pathway and defense response may be involved in the SeV infection [14].

By now, the detailed analysis on the similarities and differences of host responses between epithelial cells and immune cells during SeV infection is still rare. This study gives a comprehensive analysis of host responses against SeV infection at the transcription level in airway epithelial cells and macrophages. The results showed that airway epithelial cells and macrophages showed different cellular responses to SeV infection in some aspects. Studying the host defense response against SeV infection will provide clues for the treatment of other respiratory virus and the control of immune-mediated respiratory tract injury as well as the optimization of SeV in vaccine design and gene therapy.

Materials and methods

Microarray data

The gene expression data (GSE2935, GSE10211) [10, 15] of SeV-infected cells were downloaded from the GEO database [16]. The GSE2935 dataset contains four samples, including two controls treated by UV-inactivated SeV, and two SeV-infected samples, while the GSE10211 dataset contains ten samples, including six controls treated by UV-inactivated SeV, and four SeV-infected samples (Table 1).

Identification of DEGs

GEO2R online tool (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>) was used to screen the significantly differentially expressed genes (DEGs) ($p < 0.05$) in SeV-infected cells, using Benjamini-Hochberg multiple testing correction. DEGs with fold change ≥ 2 . 0 were selected for the gene ontology (GO) and pathway analyses.

Gene ontology and pathway enrichment

Gene ontology analysis and Kyoto encyclopedia of Genes and Genomes (KEGG) pathway were conducted by DAVID online program [17]. Results with $p < 0.05$ were considered statistically significant.

Protein-protein interaction network and module analysis

STRING [18] was used to build the protein-protein interaction (PPI) network of highly DEGs. Only the interactions with a combined score > 0.4 were selected to build the PPI network. The high-degree genes were chosen and the network was visualized using the Cytoscape software [19]. The modules of the PPI network were produced by molecular complex detection (MCODE) plug-in of Cytoscape (MCODE > 3 ; node number > 4). The pathway and function analyses of DEGs in each module were also carried out using DAVID. Results with $p < 0.05$ were considered statistically significant.

Results

DEG analysis

The GEO2R analysis showed that, 241 genes are upregulated in airway epithelial cells while 130 genes are upregulated in macrophages after SeV infection. In particular, ten genes from top 20 upregulated genes, including *Cc15*, *Ifit2*, *Gbp2*, *Cmpk2*, *Mx1*, *Ifit1*, *Rsad2*, *Ifih1*, *Tgtp2/1*, and *Cxcl2*, are strongly induced in both macrophages and airway epithelial cells, suggesting that these genes may play important roles during SeV infection (Table 2). Besides, 83 genes are downregulated in airway epithelial cells while 148 genes are downregulated in macrophages. The top 20 downregulated genes in both cell types are shown in Table 3.

Gene ontology analysis

GO analysis showed that the upregulated genes in both of macrophages and airway epithelial cells were significantly clustered in immunity, innate immunity, antiviral defense, inflammatory response and cytokine, phosphoprotein, glycoprotein, ubl conjugation, and disulfide bond (Figs. 1 and 2).

The downregulated genes in airway epithelial cells are significantly enriched in oxidoreductase, phosphoprotein, cytoplasm, acetylation, and metal-binding (Additional file 1) while the downregulated genes in macrophages are significantly clustered in signal, phosphoprotein, disulfide bond, glycoprotein, and secreted proteins (Additional file 2). The results of GO analysis demonstrated that antiviral immune responses are activated in

Table 1 Introduction on the gene expression data of SeV-infected cells

Accession	Samples	Virus features	Cell types	Organism
GSE2935	GSM64057	UV-inactivated SeV	Macrophage	<i>Mus musculus</i>
	GSM64058	UV-inactivated SeV	Macrophage	<i>Mus musculus</i>
	GSM64059	Wild-type SeV	Macrophage	<i>Mus musculus</i>
	GSM64060	Wild-type SeV	Macrophage	<i>Mus musculus</i>
GSE10211	GSM257861	UV-inactivated SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257863	UV-inactivated SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257864	UV-inactivated SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257867	UV-inactivated SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257869	UV-inactivated SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257870	UV-inactivated SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257862	Wild-type SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257865	Wild-type SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257866	Wild-type SeV	Airway epithelial cell	<i>Mus musculus</i>
	GSM257868	Wild-type SeV	Airway epithelial cell	<i>Mus musculus</i>

viral infected cells, and diverse protein modifications are also regulated by SeV.

KEGG pathway enrichment

The KEGG pathway enrichment analysis indicated that TNF signaling is significantly enriched based on the high-regulated

genes in SeV-infected airway epithelial cells and macrophages (Figs. 3 and 4). In addition, several pathways involving in innate immune response are enriched in both kinds of cells, including Toll-like receptor signaling, chemokine signaling, NF-kappaB signaling, RIG-1-like receptor signaling, cytosolic DNA-sensing signaling, cytokine-cytokine receptor interaction, NOD-like receptor signaling, and antigen processing and presentation.

Table 2 The top 20 upregulated differentially expressed genes (DEGs) in airway epithelial cells and macrophages

Airway epithelial cells			Macrophages		
Gene symbol	<i>p</i> value	Fold change	Gene symbol	<i>p</i> value	Fold change
<i>Ccl5</i> *	2.47E-06	7.12	<i>Ccl5</i>	4.59E-06	8.98
<i>Gbp3</i>	3.64E-07	6.44	<i>Acod1</i>	1.60E-05	5.76
<i>Ifit2</i>	1.11E-07	6.06	<i>Omn2/1</i>	6.22E-05	5.44
<i>Gbp2</i>	5.67E-07	6.05	<i>Hp</i>	1.18E-04	5.08
<i>Cxcl1</i>	1.62E-07	5.54	<i>Slfn4</i>	3.69E-04	4.76
<i>Cmpk2</i>	3.14E-09	5.54	<i>Ifit1</i>	2.28E-06	4.59
AW112010	1.49E-08	5.52	<i>Ifih1</i>	3.90E-04	4.43
<i>Ifit3</i>	3.72E-10	5.52	<i>Tgtp2/1</i>	1.92E-04	4.28
<i>Mx1</i>	1.91E-08	5.04	<i>Rsad2</i>	4.22E-03	4.17
<i>Ifit1</i>	4.35E-07	5.04	<i>Slpi</i>	3.78E-04	4.07
<i>Igtp</i>	1.46E-08	4.99	<i>Ifit2</i>	4.43E-03	3.74
<i>Rsad2</i>	6.94E-07	4.91	<i>Slpi</i>	3.26E-05	3.71
<i>Mndal</i>	6.96E-09	4.85	<i>Fpr1</i>	2.59E-05	3.70
<i>Iigp1</i>	1.79E-07	4.82	<i>Klf4</i>	3.33E-04	3.62
<i>Mpeg1</i>	7.29E-06	4.77	<i>C78513</i>	1.38E-04	3.44
<i>Ifi204</i>	7.97E-08	4.64	<i>Gbp2</i>	4.50E-05	3.43
<i>Atf3</i>	1.29E-05	4.62	<i>Mx1</i>	1.51E-04	3.34
<i>Ifih1</i>	3.33E-05	4.57	<i>Marco</i>	8.65E-04	3.32
<i>Tgtp2/1</i>	2.39E-06	4.43	<i>Cmpk2</i>	1.31E-04	3.30
<i>Cxcl2</i>	4.70E-10	4.40	<i>Cxcl2</i>	7.16E-04	3.27

* Represents the top upregulated genes in both macrophages and airway epithelial cells

Table 3 The top 20 downregulated differentially expressed genes (DEGs) in airway epithelial cells and macrophages

Airway epithelial cells			Macrophages		
Gene symbol	<i>p</i> value	Fold change	Gene symbol	<i>p</i> value	Fold change
Tmem119	6.24E-06	5.54	Col1a2	1.74E-03	27.08
Bag2	2.74E-02	4.92	Fbln2	1.27E-02	17.98
Slc16a7	4.82E-04	4.77	Mmp13	1.27E-02	14.44
Cmb1	5.27E-03	4.05	Acta2	1.63E-04	11.34
Cadm1	1.64E-03	3.72	Spp1	1.74E-03	11.34
Casp6	1.67E-02	3.54	Mmp12	9.47E-04	10.95
Adi1	7.11E-03	3.36	Cd28	3.77E-04	9.86
Map1lc3b	2.79E-02	3.34	Antxr2	9.82E-04	9.55
Pdcd4	1.16E-03	3.15	Cyp1b1	1.33E-04	9.03
Slc4a5	1.81E-06	3.00	Pea15a	6.62E-03	9.03
Adarb1	4.59E-03	2.99	Col4a1	1.89E-04	8.86
Cbr1	7.89E-05	2.95	Itgax	4.18E-04	7.90
Hes6	2.06E-03	2.94	Plbd1	5.15E-05	7.29
Gpam	1.92E-02	2.94	Rgs2	1.79E-03	7.04
Calml4	4.27E-04	2.91	Ccr5	2.78E-03	6.97
Ssx2ip	1.85E-03	2.90	Ptgis	6.98E-04	6.82
Antxr2	8.70E-03	2.89	Entpd1	7.20E-04	6.74
Fgfr2	2.40E-02	2.84	Mfge8	2.53E-03	6.65
Cd59a	5.82E-03	2.82	Lpl	4.37E-03	5.96
Bpifb1	2.54E-02	2.80	Lgals1	7.99E-03	5.91

For airway epithelial cells, MAPK signaling, JAK-STAT signaling, and p53 signaling are also enriched (Fig. 3).

Downregulated genes are mainly clustered in metabolic pathway in SeV-infected airway epithelial cells, including *Acaa2*, *Ldhd*, *Cyp2s1*, *Adh5*, *Echs1*, *Cyp2b10*, *Acss1*, *Dhrs3*, *Galm*, *Aox1*, *Idh2*, *Cat*, *Scd1*, *Cpt1a*, *Pdxk*, *Akl*, *Chpt1*, *Lpin1*, *Cmb1*, *Adi1*, *Cbr1*, *Csad*, and *Gpam*. In particular, antibiotic biosynthesis, retinol metabolism, fatty acid metabolism, carbon metabolism, propanoate metabolism, and valine, leucine, and isoleucine degradation are significantly enriched (Additional file 3). However, for macrophages, PI3K-AKT signaling pathway is significantly enriched (Additional file 4). Taken together, SeV infection activates the immune response in two kinds of cells, but it inhibits cell metabolism in airway epithelial cells and PI3K-AKT pathway in macrophages.

Protein-protein interaction network and module analysis

All the DEGs found in macrophages and airway epithelial cells were submitted into STRING tool and the PPI network was produced. The PPI network of DEGs in SeV-infected macrophages contains 245 nodes and 874 edges, whereas there are 223 nodes and 1414 edges in SeV-infected airway epithelial cells. For macrophages, the top 20 high-degree nodes include *Tnf*, *Ccl5*, *Stat1*, *Cxcl10*, *Icam1*, *Itgax*, *Irf7*, *Cd40*, *Ccr5*, *Cc68*, *Ifit1*, *Hck*, *C1qa*, *Apoe*, *Acta2*, *Spp1*, *Ifit3*, *Ifih1*, *Fcgr1*, and

Cxcl2. For airway epithelial cells, the top 20 high-degree nodes are *Stat1*, *Tnf*, *Il6*, *Irf1*, *Cxcl10*, *Jun*, *Irf7*, *Ifit1*, *Isg15*, *Ifit3*, *Cc15*, *Ifih1*, *Ifit2*, *Psm8*, *Uba7*, *Igtp*, *Ccl2*, *Gbp2*, *Irf9*, and *Usp18*.

Based on the PPI network information, the top 3 modules were produced using MCODE plug-in of Cytoscape (Fig. 5). In airway epithelial cells, the top 3 modules were generated (Fig. 5A): module 1 consisting of 28 nodes and 349 edges (Fig. 5A-a), module 2 of 14 nodes and 64 edges (Fig. 5A-b), module 3 of 6 nodes and 15 edges (Fig. 5A-c). The highest degree nodes in modules 1 and 2 are *Stat1* and *Il6*, respectively. The functional annotation indicated that the DEGs in modules 1, 2, and 3 are mainly involved in Toll-like receptor signaling, RIG-1-like receptor signaling, NF-kappaB signaling, TNF signaling, and protein processing in the endoplasmic reticulum.

For macrophages, module 1 contains 18 nodes and 142 edges (Fig. 5B-a), module 2 contains 15 nodes and 64 edges (Fig. 5B-b), module 3 contains 11 nodes and 19 edges (Fig. 5B-c). The highest degree nodes in modules 1 and 2 are *Stat1* and *Tnf*, respectively. The functional annotation indicated that the DEGs in modules 1, 2, and 3 are mainly involved in Toll-like receptor signaling, cytosolic DNA-sensing signaling, RIG-1-like receptor signaling, TNF signaling, cytokine-cytokine receptor interaction, and ECM-receptor interaction. Totally, PPI network analyses suggest that high-degree nodes are clustered in host innate immune and inflammatory responses.

Fig. 1 Functional annotation of upregulated differentially expressed genes (DEGs) with fold change ≥ 2.0 in SeV-infected airway epithelial cells. The x-axis represents the number of DEGs, and the y-axis represents the GO categories

Discussion

SeV is widely used as a model virus to study the interaction between paramyxovirus and host cells. In this study, we analyzed the potential functions of DEGs in SeV-infected airway epithelial cells and macrophages using gene expression profiles downloaded from GEO. The results showed 324 genes (241 up- and 83 downregulated) are significantly regulated in airway epithelial cells while 278 genes (130 up- and 148 downregulated) are significantly regulated in macrophages. The most significantly upregulated gene in both kinds of cells is *Ccl5* while the most significantly downregulated gene is *Tmem119* in airway epithelial cells, and *Colla2* in macrophages. *Ccl5* has been known to inhibit apoptosis of virus-

infected mouse macrophages by activation of the *Ccr5* receptor and G(alphai)-PI3K-Akt and G(alphai)-MEK-ERK signaling pathways [15]. The functions of *Tmem119* and *Colla2* in SeV infection are still unknown.

The GO and pathway enrichment analyses showed that the upregulated genes are involved in TNF signaling and innate immune responses in both kinds of cell types. A previous study found that respiratory syncytial virus (RSV) infection can enhance airway hyperresponsiveness and promote macrophage and neutrophil lung infiltration, accompanied with increased levels of inflammatory cytokines, such as TNF-alpha, MCP-1, and keratinocyte-derived protein chemokine. These host immune responses may contribute to the exacerbation of asthma [20]. Influenza H5N1 can induce the production of cytokines,

Fig. 2 Functional annotation of upregulated differentially expressed genes (DEGs) with fold change ≥ 2.0 in SeV-infected macrophages. The x-axis represents the number of DEGs, and the y-axis represents the GO categories

Fig. 3 Kyoto encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of upregulated DEGs in SeV-infected airway epithelial cells. The *x*-axis represents the amounts of DEGs, and the *y*-axis represents enriched KEGG pathways

such as TNF-alpha, IFN-beta, and Ccl2/MCP-1 in primary macrophages and respiratory epithelial cells, which may contribute to severity of H5N1 disease [21]. Therefore, TNF signaling may play a role in SeV-mediated pathogenesis in mice.

Interestingly, several canonical signaling pathways are also upregulated in SeV-infected airway epithelial cells. It can be speculated that these pathways may play important roles in regulating SeV replication in airway epithelial cells.

First, MAPK signaling is activated by SeV. Some viruses also upregulate MAPK signaling to enhance virus infectivity,

such as *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) [22]. A previous study showed that type 1 IFN can activate p38 MAPK [23]. As SeV infection can induce the production of type I IFN, it is possible that SeV activate MAPK signaling through the induction of type I IFN.

Besides, SeV infection can enhance p53 signaling in airway epithelial cells. This is consistent with the previous study, which found that SeV can induce cell apoptosis by IRF-3 mediated activation of Bax in HT1080-derived cell line [6,

Fig. 4 Kyoto encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of upregulated DEGs in SeV-infected macrophages. The *x*-axis represents the amounts of DEGs, and the *y*-axis represents enriched KEGG pathways

Fig. 5 Top three modules in the protein-protein interaction (PPI) network of differentially expressed genes (DEGs) in SeV-infected airway epithelial cells (A) and macrophages (B). Nodes indicate the DEGs and edges indicate the interactions of two gene-encoded proteins

8]. p53 signaling and its involvement in cell apoptosis have been well established. The induction of cell apoptosis can inhibit viral persistent infection [8]. However, some viruses can express the viral proteins to inhibit cell apoptosis. For example, AcMNPV P35 can inhibit insect cell and mammalian cell apoptosis [24]. A recent study showed that p53 stimulates the expression of Epstein-Barr virus latent membrane protein 1, which may inhibit p53-induced cell apoptosis [25]. It is unknown whether certain viral proteins of SeV can inhibit p53 signaling.

Thirdly, SeV infection can activate JAK-STAT signaling in airway epithelial cells. JAK-STAT signaling is not only involved in the cell proliferation, differentiation, but also responsible for the production of a range of cytokines and growth factors [26]. A recent study showed that the varicella-zoster virus (VZV) can activate the JNK pathway and its activation is crucial for viral lytic activation and reactivation [27]. SeV can activate JAK/STAT signaling through IFNAR in the U937 cells at the lower virus concentration [28]. In particular, SeV infection can activate STAT1, which is required for controlling viral replication in airway epithelial cells [10]. However, the activation of JAK/STAT signaling and production of ISGs cannot inhibit the cytopathic effect (CPE) induced by SeV infection [29]. The authors proposed that the activation of canonical IFN signaling and ISGs induction may not be informative about their protective role against SeV infection [29]. The detailed mechanisms that cause CPE

in SeV-infected cells appear to be elusive. Besides, different cell types may display diverse antiviral responses. For SeV, there would be more specific profiles of antiviral genes rather than canonical markers in certain cell types. Furthermore, the activation of type I IFN signaling and ISG production in SeV-infected cells may have other non-antiviral functions [29]. It should be noted that the inability of IFN signaling to inhibit SeV infection is not due to viral proteins suppressing innate immune response [29]. Although SeV C protein can inhibit the JAK/STAT signaling, it has no effect on the induction of type I IFNs and ISGs [29].

The downregulated genes are involved in host metabolism in airway epithelial cells, such as retinol metabolism, fatty acid metabolism, carbon metabolism, and amino acid metabolism. A number of viruses can regulate the host metabolism for viral infectivity. For example, human cytomegalovirus (HCMV) can increase the expression of elongase enzyme 7 (ELOVL7) in an mTOR-dependent way. ELOVL7 is required for the synthesis of saturated very long-chain fatty acids and virus replication [30, 31]. Dengue virus (DENV) can activate the glycolytic pathway to promote virus replication. DENV infection causes increased glucose consumption through increasing the expression of glucose transporter 1 and hexokinase 2 [32]. Hepatitis B virus (HBV) can influence the host metabolic pathways, including glucose, lipid, nucleic acid, bile acid, and vitamin, contributing to the pathological process of HBV and metabolic disorders [33]. Meanwhile, some host

metabolic products can inhibit viral infection. For example, interferon-induced cholesterol-25-hydroxylase can inhibit viral entry due to the production of 25-hydroxycholesterol [34]. The mechanisms that SeV downregulates the cellular metabolism and the functions of these downregulated genes in SeV infection remain elusive. It is possible that these metabolism-associated genes are involved in antiviral responses and SeV can downregulate them to promote viral replication. Moreover, SeV may induce cell apoptosis by inhibiting cellular metabolism.

KEGG pathway analysis indicated that PI3K-AKT signaling is downregulated in macrophages. PI3K-AKT signaling plays a role in the proliferation, motility, and survival of immune cells, such as macrophage and monocyte [35]. PI3 kinase and AKT can regulate IFN responses and are involved in the subsequent initiation of mRNA translation of ISGs, which complements the roles of IFN-activated JAK-STAT pathway [36, 37]. It has been known that both DNA and RNA viruses can regulate the PI3K-AKT signaling pathway [38, 39]. For example, influenza virus, porcine circovirus type 2 (PCV2), and AcMNPV can activate the PI3K-AKT pathway, which is required for virus propagation [38–40]. However, Sindbis virus can inhibit the AKT/mTOR pathway in HEK cells, and it is not sensitive to rapamycin and torin1 [41]. The previous study showed that SeV infection can activate the PI3 kinase pathway in HT1080 cells. Moreover, blocking the PI3 kinase pathway can activate caspase 8-mediated apoptosis [8]. Unexpectedly, our analysis indicated that the genes involving the PI3K-AKT signaling were downregulated in SeV-infected macrophages. It can be speculated that SeV may suppress host immune response by inhibiting PI3K-AKT signaling. Further studies need to be done to determine whether inhibition of PI3K/AKT signaling is related to SeV-induced CPE and the underlying mechanisms that SeV regulates PI3K-AKT signaling.

The highest degree of nodes were found in PPI networks of both airway epithelial cells and macrophages, including *Stat1*, *Tnf*, *Cxcl10*, *Irf7*, *Ifit1*, *Ifit3*, *Ccl15*, *Ifih1*, and *Ccl2*. The antiviral function of STAT1 against SeV infection has been uncovered in the previous study [10]. It was found that *Stat1*^{−/−} mice showed enhancement of virus replication and overexpression of TNF- α and CXCL2 [10]. This phenomenon is also observed in *Stat1*^{−/−} airway epithelial cells, suggesting an important role of epithelial cells as a barrier against virus replication [10].

In addition, interferon-induced proteins with tetratricopeptide repeats (IFITs) play an essential antiviral role downstream of type I IFN signaling [42]. *Ifit1* was found to be involved in the antiviral response mediated by type I IFN through inhibiting viral mRNA translation [43]. Strikingly, *Rubulavirus* members of *Paramyxoviridae*, such as parainfluenza virus type 5, are sensitive to the antiviral action of IFIT1, but other non-*Rubulavirus* members of

Paramyxoviridae, such as SeV, are resistant [43]. It is proposed that the methylation of 5' guanosine nucleoside and other structural elements of viral mRNAs may influence the sensitivity of IFIT1 [43]. Further study showed that IFIT1 is not essential in inhibiting negative-sense RNA viruses [44]. Sensitivity difference of paramyxovirus to interferon may lead to the outcome difference of paramyxovirus infection [44]. Another antiviral protein IFIT3 can promote the production of IFN- β and inhibit the replication of porcine reproductive and respiratory syndrome virus (PRRSV) [45]. Interestingly, IFIT3 also has a protective role against dengue virus infection following IFN- α production in human lung epithelial cells [46].

Moreover, a variety of chemokines are implicated in antiviral immune response. For example, *Cxcl10* and *Ccl2* are significantly upregulated during African swine virus infection [47]. They may play an important role in the activation of lymphocytes and recruitment of monocytes from bone marrow [47]. The existence of these DEGs in the PPI network suggests their involvement in the host defense against SeV. The functions of the abovementioned DEGs in PPI network during SeV infection is worth studying in the future.

In conclusion, SeV can regulate the expression of a number of host genes related to immune response and metabolism of host cells. SeV infection may regulate host cellular signaling pathways to enhance the viral infectivity. More studies are needed to screen the corresponding proteins of SeV that involve the regulation of these cellular pathways to better understand the molecular mechanism of SeV infection.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

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