2B and 3C Proteins of Senecavirus A Antagonize the Antiviral Activity of DDX21 via the Caspase-Dependent Degradation of DDX21

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INTRODUCTION

Senecavirus A (SVA), also known as Seneca Valley virus, is a recently discovered picornavirus that can cause swine vesicular disease, posing a great threat to the global swine industry. It can replicate efficiently in cells, but the molecular mechanism remains poorly understood. This study determined the host’s differentially expressed proteins (DEPs) during SVA infection using dimethyl labeling based on quantitative proteomics. Among the DE proteins, DDX21, a member of the DEAD (Asp-Glu-Ala-Asp)-box RNA helicase (DDX) family, was downregulated and demonstrated inhibiting SVA replication by overexpression and knockdown experiment. To antagonize this antiviral effect of DDX21, SVA infection induces the degradation of DDX21 by 2B and 3C proteins. The Co-IP results showed that 2B and 3C did not interact with DDX21, suggesting that the degradation of DDX21 did not depend on their interaction. Moreover, the 3C protein protease activity was necessary for the degradation of DDX21. Furthermore, our study revealed that the degradation of DDX21 by 2B and 3C proteins of SVA was achieved through the caspase pathway. These findings suggest that DDX21 was an effective antiviral factor for suppressing SVA infection and that SVA antagonized its antiviral effect by degrading DDX21, which will be useful to guide further studies into the mechanism of mutual regulation between SVA and the host.

Keywords: senecavirus A (SVA), DDX21, 2B protein, 3C protein, caspase pathway
Our study has determined changes in the host cell protein expression during SVA infection using dimethyl labeling-based quantitative proteomics. Among these proteins, the expression level of DDX21 was significantly downregulated during SVA infection, and the antiviral effect of DDX21 was also verified by overexpressing and knockdown experiments. Moreover, SVA 2B and 3C proteins induce the degradation of DDX21 via caspase to antagonize the antiviral activity of DDX21. The results helped study the interaction between host and virus.

MATERIALS AND METHODS

Cells, Viruses, and Drug
Porcine kidney-15 (PK-15), human embryonic kidney (HEK293T) cells, and baby hamster kidney cells (BHK-21) cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Cells were maintained at 37°C with 5% CO2. The SVA virus (GenBank accession number: MZ375462) was used for all the experiments. The proteasome inhibitor MG-132, the lysosomal inhibitor chloroquine (CQ), and the caspase inhibitor carboxbenzoxo-valyl-alanyl-aspartyl-[O-methyl]-fluoro-methyl ketone (Z-VAD-FMK) was purchased from Beijing Solarbio Science & Technology Co. Ltd.

Construction of Plasmids
The porcine DDX21 gene (GenBank accession number: XM_005657387.3) was amplified and cloned into pCAGEN (modified from pCAGGS) to generate pCAGEN-DDX21-HA. The other proteins of SVA were cloned into pCAGGS to generate pCAGGS-V1-Flag, pCAGGS-V2-Flag, pCAGGS-V3-Flag, pCAGGS-2B-Flag, pCAGGS-2C-Flag, pCAGGS-3A-Flag, pCAGGS-3C-Flag, and pCAGGS-3D-Flag. All plasmids were constructed by homologous recombination with the NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs, Ipswich, MA, USA) according to the manufacturer’s instructions. Mutagenesis of SVA 3C protein was generated using overlap PCR and cloned into vector pCAGGS-3C-Flag (H48A), pCAGGS-3C-Flag (C160A, USA), and pCAGGS-3C-Flag (double-site mutation (DM) H48A-C160A). The primers used for gene amplification are listed in Table 1.

Dynamics of SVA in PK-15 Cells
PK-15 cells were infected with SVA at a multiplicity of infection (MOI) of 0.5, and normal cells were set as the control. Cytopathic effects (CPEs) were observed at 12, 24, 36, 48, 60, and 72 h postinfection (hpi) under a microscope. Subsequently, the cell culture supernatant at different time points was collected to determine 50% tissue culture infective doses (TCID50). The viral titers were determined by the Reed–Muench method and expressed as TCID50/ml. Mean values and standard deviations were calculated from the results of three independent experiments. According to the flow chart, the cells infected or uninfected with SVA at 48 hpi were chosen for the following quantitative proteomics.

Sample Preparation, Digestion, and TMT Labeling
Mock- or SVA-infected PK-15 cells were cultured in 6-well plates for 48 hpi. The cells were then washed with cold phosphate-buffered...
saline (PBS), lysed by sonication in STD lysate buffer [4% (w/v) SDS, 100 mM Tris/HCl pH 7.6, 0.1 M DTT] (12), and quantified using a BCA assay. 200 ug of protein per sample for trypsin digestion using filter-aided sample preparation (FASP) (12), after trypsin digestion, the peptides were desalted on a C18 column and 40 ml of dissolution buffer was added after the lyophilization of peptides. The peptides were quantified by OD280. According to the manufacturer’s instructions, peptides were labeled using a TMT Reagent kit.

**LC-MS/MS Analysis**

Labeled samples were analyzed using an Easy-nLC 1200 nano flow HPLC system (Thermo Fisher Scientific, Waltham, MA, USA). Buffer: solution A is 0.1% formic acid in the water, and solution B is 0.1% formic acid in acetonitrile. The sample was loaded by the autosampler onto the loading column (Thermo Scientific EASY column, 100 μm * 2 cm, 5 μm, C18) and then separated by an analytical column (Thermo Scientific EASY column, 75 μm * 10 cm, 3 μm, C18) with a flow rate of 250 nl/min. After chromatographic separation, the samples were analyzed by a Q Exactive mass spectrometer (13).

**Quantitative Real-Time PCR**

Total RNA was extracted with Trizol (Thermo Fisher Scientific, Waltham, MA, USA). The PrimeScript™ 1st-Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) was used for reverse transcription. The SYBR Premix Ex Taq™ (TaKaRa, Dalian, China) was used to quantify the mRNA levels of ZC3H11A, DDX21, IL-18, ZCCHC9, STAT1, OAS1, MX2, ZAP, and IFIT1.

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**TABLE 1 |** The sequence of primers and siRNAs used in the study.

| Primers and siRNAs | Sequence (5′-3′) |
|--------------------|------------------|
| pCAGGS-VP1-F       | CATCATTTTGGCAAAGATGTCCACCGACAACGCCGAGAC |
| pCAGGS-VP1-FLAG-R  | CGAGAGATCTGAATTTCACTTATCGTCGTCATCTTGTTAATCTTGCATCAGCATCTTCTGCT |
Relative expression levels were analyzed using the 2^{-ΔΔCt} method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as a control. Primers are listed in Table 1.

**Plasmid Transfection and Virus Challenge**

To investigate the effect of DDX21 on SVA replication, BHK-21 cells cultured in 6-well plates were transfected with 2 μg of pCAGEN-DDX21-HA using X-tremeGENE HP DNA reagent (Roche Applied Science, Penzberg, Germany). Next, 36 h posttransfection (hpt), the cells were infected with SVA at a MOI of 0.1. After inoculation for 1.5 h at 37°C, the supernatants were discarded, and the cells were washed three times with PBS. The supernatant was harvested at 12, 24, 36, and 48 hpi, and the cells were lysed using RIPA lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA). Viral titers in the supernatants were determined using a microtitration assay. The amount of the lytic supernatant was used as an input control, and the remaining lysates were incubated with anti-Flag agarose beads for 8 h at 4°C. The beads were washed with the lysis buffer five times and then analyzed by WB with the indicated antibodies.

**Western Blotting**

Cell lysates were prepared by harvesting virus-infected or plasmid-transfected cells in RIPA or IP-lysate buffer at 4°C for 15 min containing 1 mM of phenylmethylsulfonyl fluoride and 1 mg/ml of protease inhibitor cocktail (Roche). After centrifuging at 12,000 rpm/min for 10 min, the supernatants of cell lysates were mixed with 5x sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample loading buffer (Beyotime) and placed in boiling water for 5 min. The proteins were then separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was blocked in 5% skim milk for 2 h at room temperature and then incubated with the indicated primary antibody for 1 h at room temperature. After washing three times with Tris-buffered saline with 0.1% Tween 20, the membrane was incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (H+L) and/or goat anti-rabbit IgG (H+L) secondary antibody (1:5,000) for 1 h at room temperature. The target proteins were visualized by treating the membrane with Pierce ECL WB substrate (Thermo Fisher Scientific, Waltham, MA, USA). To quantify target proteins, their levels were normalized to the levels of β-actin.

**RNA Interference**

Three small interfering RNAs (siRNAs) against hamster DDX21 (GenBank No. XM_013113937.3) were synthesized by GenePharma (Shanghai, China). The sequence of siRNA is listed in Table 1. BHK-21 cells were seeded in 6-well plates and transfected with 100 pM of indicated siRNA or NC using Lipofectamine™ RNAiMAX transfection reagent (cat. No. 13778075; Thermo Fisher Scientific, Waltham, MA, USA). The cells were lysed in RIPA lysis buffer after 36 h of transfection, and the effects of siRNAs were analyzed by WB using an anti-DDX21 polyclonal antibody (cat. No. 10528-1-AP; Proteintech; 1:1,000). Next, efficient siRNA and NC were selected for transfection. At 36 hpt, the cells were infected with SVA at a MOI of 0.1. The supernatant and cells were harvested at 12, 24, 36, and 48 hpi and analyzed based on virus titers and WB.

**Cell Viability Assay**

Cells at 10^4 were seeded into 96-well plates and treated with the proteasome inhibitor MG132 concentrations (0–50 μM), the caspase inhibitor Z-VAD-FMK (0–50 μM), or the lysosomal inhibitor CQ (0–100 μM) for 24 h, at five replicate wells per dilution. Subsequently, the 3-(4,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide (MTT) assay (14) was used to detect cytotoxicity.

**Statistical Analysis**

All the experiments mentioned above were performed with three independent experiments. The GraphPad Prism software was used for statistical analysis by a two-tailed Student’s t-test. The data shown are three independent experiments’ means ± standard variations (SD).

**RESULTS**

**Virus Infection and Determination of Optimal Collection Time**

PK-15 cells were infected with SVA at a MOI of 0.5, and the CPE began to appear at 36 hpi. Subsequently, the cell showed typical CPE, characterized as rounding, shrinkage, and detachment (Figure 1A). The virus titers of SVA almost reached the highest value at 48 hpi (Figure 1B). At 48 hpi, the CPE of the cells was typical, and the virus titers reached the peak, so the cells were collected and subjected to proteomics analyses by LC-MS/MS (Figure 1C).

**Proteomics Analysis of Host Proteins Upon SVA Infection**

SVA infection affects various host cellular pathways. Knowledge of changes in host protein expression caused by SVA infection helps to understand the interaction between the virus and the host. Our study employed a dimethyl labeling-based quantitative proteomics strategy to characterize the host cell proteome changes upon SVA infection in PK-15 cells at 48 hpi. The results showed that 333 proteins were downregulated and 137 were upregulated in the cells infected with SVA. In contrast, the expression level of 5,122 proteins was not affected by SVA infection (Figure 2A). These results were also visualized by clustering the samples according to differential treatment (Figure 2B). To further verify the accuracy of the proteomics and the relationships of the differentially expressed proteins (DEPs) at the translational and transcriptional levels, the
transcriptional level of some DEPs (STAT1, IFIT1, OAS1, ZAP, MX2, DDX21, IL-18, ZCCHC9, and ZC3H11A) were verified by qPCR. The results showed that the transcriptional and translational levels are almost identical to DEPs except for DDX21 during SVA infection (Figures 2C, D). The translational level of DDX21 was downregulated during SVA infection (Figure 2B). In contrast, the transcription level of DDX21 was upregulated in the SVA-infected cells (Figure 2C). Thus, the SVA infection inevitably affects the protein function of DDX21.

**DDX21 Restricts the Replication of SVA**

To further examine the effects of DDX21 on SVA replication, DDX21 was overexpressed in the BHK-21 cells by transfecting them with pCAGEN-DDX21-HA. As shown in Figure 3A, when DDX21 was overexpressed, the VP2 protein levels of SVA were lower than those in the control cells, especially at 12–36 hpi. Furthermore, there was a significant difference in virus titers between cells transfected with pCAGEN-DDX21-HA or pCAGEN, with an approximate 0.5–1.0 log decrease in virus titers from 12 to 36 hpi (p < 0.05; Figure 3B). These observations suggest that DDX21 is a cellular antiviral factor that represses SVA infection.

**DDX21 and SVA 2B and 3C Degrade DDX21**

As shown in Figure 3A, the expression level of DDX21-HA decreased gradually upon SVA infection, which was consistent with the proteomics results. Furthermore, the transcription level of DDX21 was upregulated during SVA infection. Therefore, we speculated that SVA infection could degrade DDX21. To verify this hypothesis, BHK-21 cells infected with SVA were lysed at 12, 24, 36, and 48 hpi, and the expression level of DDX21 was analyzed by WB. The results revealed that the expression of DDX21 decreased in SVA-infected cells with the prolongation of infection time (Figure 4A). Furthermore, to confirm which protein of SVA induces the degradation of DDX21, DDX21 was cotransfected with the protein of SVA. The results revealed that proteins 2B and 3C of SVA induced the degradation of DDX21 (Figure 4B). Furthermore, the 2B and 3C of SVA degraded DDX21 in a dose-dependent manner (Figures 4C, D).

**3C Protein of SVA Degrades DDX21 Depending on Its 3C Protein Protease Activity**

The SVA 3C protein contained a conserved catalytic box with histidine (His) and cysteine (Cys) residues, which was essential for its protease activity (15). Single-site mutation H48A (3C-H48A) or C160A (3C-C160A) and double-site mutation H48A-C160A (3C-DM) were catalytically inactive (16). To detect whether 3C protease...
activity was involved in DDX21 degradation, HEK293T cells were cotransfected with plasmids encoding wild-type 3C protein (3C protein-WT) or its mutants, and HA-DDX21 for 30 h. The results showed that all 3C protein mutants losing protease activity failed to induce DDX21 to degrade (Figures 5A, C).

DDX21 Does Not Interact With SVA 2B and 3C Proteins

DDX21 was degraded by SVA 2B and 3C proteins, so we speculate that the 2B and 3C may interact with DDX21. To verify this hypothesis, we performed coimmunoprecipitation (Co-IP) assays to analyze the interaction between nonstructural proteins (2B and 3C) and DDX21. HEK293T cells were cotransfected with plasmids expressing HA-tagged DDX21 and Flag-tagged 2B or 3C proteins. Co-IP assays were performed with anti-Flag monoclonal antibody at 48 hpt. Interestingly, coimmunoprecipitation assays showed that DDX21 did not precipitate 2B and 3C proteins (Figures 6A, B).

Effect of Inhibitors on the Viability of Cells

We examined the effects of different inhibitors on the growth of BHK-21 cells at 24 h by an MTT assay. As shown in Figures 7A, C, compared with the DMSO-treated group, MG132 (0–20 µM) and Z-VAD-FMK (0–20 µM) did not affect the cell viability. However, a concentration of 50 µM reduced the cell viability. In contrast, CQ (0–100 µM) did not affect cell viability. So, we choose the proteasome inhibitor MG-132 (10–20 µM), the caspase inhibitor Z-VAD-FMK (10–20 µM), and the lysosomal inhibitor CQ (50–100 µM) for subsequent experiments.

2B and 3C Protein of SVA Degrade the DDX21 via the Caspase Pathway

To further confirm whether DDX21 was degraded upon virus infection, cells were treated with the proteasome inhibitor MG-132, the caspase inhibitor Z-VAD-FMK, and the lysosomal inhibitor CQ, followed by SVA for 36 h. As shown in Figures 7D–F, DDX21 degradation was inhibited after treatment with Z-VAD-FMK. Notably, CQ also seemed to inhibit the degradation of DDX21 (Figure 7F). However, viral protein expression was also inhibited after treatment with CQ, indicating that the inhibition of DDX21 degradation may be due to its severe inhibition of virus replication. Next, we investigated which pathways were involved in the degradation of DDX21 by 2B and 3C proteins. The results showed that 2B and 3C degraded DDX21 through the caspase pathway and not via the lysosomal and proteasome pathways (Figures 7G–L). These results suggest that DDX21 was degraded through the caspase pathways.

DISCUSSIONS

As an emerging infectious disease pathogen of swine, SVA has demonstrated its capacity to cause vesicular diseases, neonatal mortality, and persistent infection in pigs. Furthermore, it
FIGURE 3 | DDX21 affects the replication of SVA. (A) BHK-21 cells were transfected with pCAGEN-DDX21-HA or pCAGEN. At 36 hpt, the cells were inoculated with SVA at an MOI of 0.1. The cells and supernatant were harvested at the indicated times, and the expression level of the VP2 protein of SVA was analyzed by WB with an anti-VP2 pAb. DDX21-HA was detected with an anti-HA mAb. β-Actin served as the internal reference. (B) Virus titers were calculated and expressed as TCID₅₀/ml at the indicated times. (C) BHK-21 cells seeded in 6-well plates were transfected with siRNA-1651, siRNA-673, siRNA-1712, or negative control (NC) (50 pM/well). At 36 hpt, the cells were lysed and analyzed by Western blotting (WB) with an anti-DDX21 polyclonal antibody (mAb), and β-actin was probed as the internal reference. (D) Knocking down the expression of DDX21 promoted the replication of SVA in BHK-21 cells. BHK-21 cells were transfected with NC or siRNA-1651 at 50 pM. At 36 hpt, the cells were infected with SVA at a MOI of 0.1. The cells and the supernatant were harvested at indicated times. The cells were lysed and analyzed by WB using an anti-VP2 pAb. (E) The infectious virus titers in the supernatant were determined by a TCID₅₀/ml assay. The statistical significance of differences was determined using a Student’s t-test (NS, not significant; *p < 0.05).

FIGURE 4 | SVA degrades endogenous DDX21 during viral infection, and DDX21 is degraded by SVA 2B and 3C. (A) BHK-21 cells in 6-well plates were infected with SVA at a MOI of 0.1, cells were harvested at indicated times, and the expression level of endogenous DDX21 protein was analyzed by WB. (B) HEK293T cells in six-well plates were cotransfected with HA-DDX21 and Flag-VP1, VP2, VP3, 2B, 2C, 3A, 3C, or 3D, cells were harvested at 30 h, and the samples were analyzed by WB. (C, D) HEK293T cells were cotransfected with HA-DDX21 and different concentrations of Flag-2B or Flag-3C for 30 h. The cell lysates were analyzed by WB.
induces immunodepression in suckling piglets and effectively replicates in piglets’ tonsils, mesenteric lymph nodes, and spleen (17, 18). With SVA disease appearing in new countries each year, the virus has become a virus with a global prevalence (19). There must be a molecular mechanism by which SVA antagonizes host antiviral immunity. Although much work has been done in SVA research, there is currently no effective vaccine to control the disease. The interaction of SVA with the host immune response and the pathogenesis remains largely unknown, resulting in the continuous spreading of the disease.

Cell lines have been used as important tools to study virus–host interactions during viral infection. It allows the investigation of the host proteins and pathways involved in viral replication. SVA can infect a variety of cells, such as swine testis (ST), human embryonic kidney-293T, swine kidney (IBRS-2 and PK-15), and human lung cancer cells (NCI-H1299) and BHK-21 (20, 21). To explore the mechanism of SVA infection, the PK-15 cell line can be used as a model for evaluating SVA-induced innate immune signaling and the involved antagonistic effects caused by viral proteins (22). In this study, we screened several candidate cellular proteins in SVA-infected PK-15 by proteomic analysis. In total, 470 DEPs were verified, of which 333 were downregulated and 137 were upregulated. Among the DE proteins, four host proteins with antiviral effects on other viruses were used for screening (10, 23–25). Only DDX21 and IL-18 can suppress the expression of VP2 of SVA (date not shown). Interestingly, unlike IL-18, DDX21 decreased during SVA infection (Figure 2B), while the transcription level of DDX21 surprisingly increased (Figure 2C). Similar phenomena were previously reported for DDX21, DDX23, and DDX1, whose mRNA levels are higher in FMDV-infected cells than in mock-infected cells (11, 26, 27). The transcription level of DDX21 was inconsistent with the translation level, implying that SVA infection affects the function of DDX21. DDX21 is involved in a variety of cell functions, including transcription, protein translation, processing, and modification of pre-rRNA, mediating and sensing transcription during nucleotide stress, as well as regulating various innate immunity (28–31). Many studies have confirmed that DDX21 may act as an antiviral protein. It has been reported that DDX21 restricts infection and replication of several viruses, including the influenza virus, Borna disease virus (BDV), dengue virus (DENV), FMDV, and SARS-CoV-2 (10, 11, 28, 32, 33). To further verify the effect of DDX21 on the virus replication, overexpression and knockdown experiments of DDX21 were conducted. The results demonstrated that DDX21 could effectively inhibit SVA replication in BHK-21 cells (Figures 3A, E). However, many viruses have evolved general strategies to evade host defenses, such as influencing mRNA translation, protein localization, and cleaving or degrading host proteins (15, 34). In a natural infection, SVA can effectively replicate; therefore, we assumed that SVA could evade the antiviral effect by some means. During the overexpression experiments, we found that as the virus replicated, the expression of DDX21 dropped, which was consistent with the proteomics results. All of these suggest that SVA may escape the antiviral effect of DDX21 by degrading DDX21.

To investigate the mechanism of SVA degrading the DDX21, SVA and the viral proteins were used for verification. With the prolongation of virus infection, the more severe the degradation of DDX21 (Figure 4A). In addition, only the 2B and 3C proteins of SVA can induce the degradation of DDX21 (Figure 4B). With the
increase of 2B and 3C proteins, the degradation of DDX21 was more significant (Figures 4C, D). The 2B and 3C proteins are important nonstructural proteins of SVA with multiple roles, especially in antagonizing host innate immunity. 2B protein can suppress type I interferon production by inducing the degradation of MAVS, indicating that 2B protein has the function of degrading and antagonizing innate immunity. It likely enhances membrane permeability, acting like a viroporin (4).

Furthermore, SVA 2AB protein promoted MARCHF8 and MAVS degradation to inhibit IFN-I signaling during SVA infection (35). In contrast, the 3C protein of SVA cleaves PABPC1 to promote viral replication through 3C protease activity (36) and 3C protein-induced hnRNP A1 degradation through its protease activity to promote virus replication (37). SVA suppressed the innate immune response and mediated host cell autophagy and apoptosis to improve viral replication through 2C and 3C proteins (38). Similar to other picornaviruses, the protease activity of the SVA 3C protein depends on a conserved catalytic box with Cys and His residues (16). When the Cys and His residues of 3C protein were mutated (H48A, C160A, or H48A/C160A), it failed to induce the degradation of DDX21, indicating that the degradation of DDX21 by 3C was dependent on protease activity. Since 2B and 3C proteins did not interact with DDX21, the degradation of DDX21 did not depend on their interaction (Figures 6A, B).

Subsequently, we investigated the involvement of the lysosome, proteasome, and caspase pathways in SVA, 2B, and 3C protein-dependent degradation of DDX21. The results revealed that SVA, 2B, and 3C proteins use the caspase pathway to degrade DDX21 (Figure 7). Although infection with SVA after treatment of cells with CQ can alleviate the degradation of DDX21 (Figure 7F), it may be related to autophagy. As shown by Hou et al. (39), SVA infection induces autophagy in BHK-21 cells, and SVA utilizes autophagy machinery to promote its proliferation in BHK-21 cells. After treatment with CQ (a specific inhibitor of autophagosome-lysosome fusion), the VP1 protein expression and viral yields were appreciably reduced. Similarly, we obtained the same results as above after CQ treatment. Therefore, we speculated that the inhibition of DDX21 degradation might be due to inhibition of autophagy and decreased VP2 expression in SVA-infected BHK-21 cells. CQ did not alleviate the degradation effects of 2B and 3C proteins.
proteins on DDX21, so we believe that the lysosomal pathway is not the main way for SVA to degrade DDX21 (Figures 7G–I).

In summary, we confirmed that DDX21 inhibits SVA replication. Furthermore, SVA can evade the antiviral activity of DDX21 by decreasing its expression through the 2B and 3C proteins of SVA. The degradation of DDX21 by 2B and 3C proteins depends on the caspase pathway. Moreover, SVA 3C protein can degrade DDX21 through 3C protease activity. Our findings provide new insight into the development of antivirals against SVA infection.

DATA AVAILABILITY STATEMENT
The data presented in the study are deposited in the ProteomeXchange Consortium (http://proteomcentral.proteomeexchange.org) repository, accession number PXD034197.

AUTHOR CONTRIBUTIONS
KZ designed the experiments. KZ, XRG, SFL, XNL, YH, LLW, BSL, WKC, and LML performed the experiments. KZ and XRG designed the experiments. KZ, XRG, SFL, XNL, YH, LLW, WKC, and LML contributed to the article and approved the submitted version.

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