The Coronavirus Transmissible Gastroenteritis Virus Evades the Type I Interferon Response through IRE1 α -Mediated Manipulation of the MicroRNA miR-30a-5p/SOCS1/3 Axis

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ABSTRACT In host innate immunity, type I interferons (IFN-I) are major antiviral molecules, and coronaviruses have evolved diverse strategies to counter the IFN-I response during infection. Transmissible gastroenteritis virus (TGEV), a member of the *Alphacoronavirus* family, induces endoplasmic reticulum (ER) stress and significant IFN-I production after infection. However, how TGEV evades the IFN-I antiviral response despite the marked induction of endogenous IFN-I has remained unclear. Inositol-requiring enzyme 1 α (IRE1 α), a highly conserved ER stress sensor with both kinase and RNase activities, is involved in the IFN response. In this study, IRE1 α facilitated TGEV replication via downmodulating the host microRNA (miR) miR-30a-5p abundance. miR-30a-5p normally enhances IFN-I antiviral activity by directly targeting the negative regulators of Janus family kinase (JAK)-signal transducer and activator of transcription (STAT), the suppressor of cytokine signaling protein 1 (SOCS1), and SOCS3. Furthermore, TGEV infection increased SOCS1 and SOCS3 expression, which dampened the IFN-I antiviral response and facilitated TGEV replication. Importantly, compared with mock infection, TGEV infection *in vivo* resulted in decreased miR-30a-5p levels and significantly elevated SOCS1 and SOCS3 expression in the piglet ileum. Taken together, our data reveal a new strategy used by TGEV to escape the IFN-I response by engaging the IRE1 α -miR-30a-5p/SOCS1/3 axis, thus improving our understanding of how TGEV escapes host innate immune defenses.

IMPORTANCE Type I interferons (IFN-I) play essential roles in restricting viral infections. Coronavirus infection induces ER stress and the interferon response, which reflects different adaptive cellular processes. An understanding of how coronavirus-elicited ER stress is actively involved in viral replication and manipulates the host IFN-I response has remained elusive. Here, TGEV inhibited host miR-30a-5p via the ER stress sensor IRE1 α , which led to the increased expression of negative regulators of JAK-STAT signaling cascades, namely, SOCS1 and SOCS3. Increased SOCS1 or SOCS3 expression impaired the IFN-I antiviral response, promoting TGEV replication. These findings enhance our understanding of the strategies used by coronaviruses to antagonize IFN-I innate immunity via IRE1 α -mediated manipulation of the miR-30a-5p/SOCS axis, highlighting the crucial role of IRE1 α in innate antiviral resistance and the potential of IRE1 α as a novel target against coronavirus infection.

KEYWORDS transmissible gastroenteritis virus (TGEV), IRE1 α , miR-30a-5p, SOCS, type I interferon

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Endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) are common consequences of coronavirus infection (1–5). Our groups and others have demonstrated that coronaviruses, including severe acute respiratory syndrome coronavirus (SARS-CoV), infectious bronchitis virus (IBV), porcine epidemic diarrhea virus (PEDV), and transmissible gastroenteritis virus (TGEV), are all capable of inducing significant ER stress following infection and simultaneously trigger multiple UPR pathways to restore ER homeostasis (1, 5–8). During ER stress, inositol-requiring enzyme 1 α (IRE1 α) is activated by oligomerization and autophosphorylation (9–11). IRE1 α activation initiates diverse downstream signaling of the UPR either through splicing of X-box binding protein 1 (XBP1) or through posttranscriptional modifications via IRE1 α -dependent mRNA degradation (RIDD) (9, 12). IRE1 α regulates genes involved in protein entry into the ER, folding, glycosylation, and ER-associated degradation (ERAD) to facilitate ER homeostasis (11, 13). Moreover, IRE1 α degrades ER-localized mRNAs via RIDD to reduce the burden of protein entry into the ER (14). In addition to degrading mRNA, activated IRE1 α has recently been demonstrated to cleave precursor microRNAs (pre-miRNAs) and to degrade microRNAs (miRNAs) during noninfection-derived ER stress (15, 16). Given the crucial roles of IRE1 α signaling in cellular fate determination, many RNA viruses, such as influenza A virus (IAV) and Japanese encephalitis virus (JEV), employ IRE1 α signaling to facilitate their replication (17, 18). However, whether and how IRE1 α specifically modulates coronavirus replication are not well established.

Type I interferons (IFN-I) (alpha/beta interferons [IFN- α/β]) play crucial roles in host antiviral responses. Upon viral infection, host cells react quickly to the invading viruses by synthesizing and secreting IFN-I. Binding of IFN-I to its receptor (interferon alpha/beta receptor [IFNAR]) results in activation of Janus family kinases (JAKs) and the subsequent activation of signal transducer and activator of transcription (STAT) signaling cascades, thereby inducing the expression of IFN-stimulated genes (ISGs) (19). However, over the course of the long evolutionary competition between viruses and host cells, coronaviruses have evolved diverse mechanisms to counteract the IFN-I response (20–23). At least 11 viral proteins of the coronaviruses SARS-CoV and PEDV have been identified as IFN-I antagonists (20, 23, 24). In contrast, TGEV induces high levels of IFN-I *in vivo* and *in vitro* after infection (25–27). Despite a wealth of knowledge regarding how TGEV triggers IFN-I production, how TGEV counters the antiviral activity of IFN-I has not been fully elucidated.

MicroRNAs (miRNAs) are a large family of short (19- to 24-nucleotide [nt]) noncoding RNAs that regulate gene expression posttranscriptionally through translational repression and/or mRNA degradation by binding their seed regions to complementary sites present in the 3' untranslated region (UTR) of target genes (28, 29). Given the critical roles of miRNAs in regulating gene expression, unsurprisingly, viruses take advantage of host miRNAs to target vital components of the IFN-I response and impair IFN-I antiviral activity for optimal infection (28, 30, 31). JEV evades IFN-I and enhances viral infection by downregulating the expression of the miRNA miR-432, which directly targets the suppressor of cytokine signaling protein 5 (SOCS5), a negative regulator of the JAK-STAT1 signaling cascade (32). Porcine reproductive and respiratory syndrome virus (PRRSV) dampens the JAK-STAT signaling of IFN-I to facilitate its replication by upregulating host miR-30c, which directly targets JAK1 (30). However, the potential role of miRNAs in coronavirus escape from the IFN-I response has remained elusive.

Aberrant miRNA expression is integrally related to the progression and pathogenesis of diseases (30, 33, 34). Although we have gained considerable insights into aberrant miRNA expression by *cis*-regulatory elements and *trans*-acting factors caused by viral infection, the contribution of the virus-induced UPR to aberrant miRNA expression has rarely been investigated. Recent studies have shown that activated IRE1 α affects the cell fate by directly degrading a subset of host miRNAs (miR-17, miR-34a, miR-96, and miR-125b) under the persistent ER stress induced by chemicals or noninfectious diseases (15, 35). However, whether and how viruses exploit IRE1 α to manipulate miRNA expression for optimal viral infection remain unknown.

In this study, TGEV infection downregulated the expression of host miR-30a-5p via

virally triggered IRE1 α -mediated UPR induction. miR-30a-5p suppressed TGEV infection by enhancing the IFN-induced antiviral signaling pathway by directly targeting the negative regulators of IFN signaling, SOCS1 and SOCS3. Moreover, TGEV infection *in vivo* suppressed miR-30a-5p expression and significantly elevated the expression of SOCS1 and SOCS3 in the ileum. Altogether, these data contribute new insights into the roles of IRE1 α in regulating the innate immune response and help to explain how TGEV escapes host IFN-I innate immunity.

RESULTS

TGEV infection downregulates miR-30a-5p expression. The host miR-30 family (five members, consisting of miR30a to miR30e) plays important roles in cancers and viral infections (30, 34, 36, 37). We recently reported that miR-30a-5p, a member of the miR-30 family, is downregulated and that its expression is inversely correlated with the levels of ER stress in renal cancer, indicating that ER stress might inhibit miR-30a-5p expression (34). To assess whether ER stress suppresses the expression of miR-30a-5p, we initially analyzed the levels of miR-30a-5p in swine testicular (ST) cells following treatment with the ER stress inducer thapsigargin (Tg). Tg treatment substantially diminished the abundance of miR-30a-5p and exhibited dose-dependent suppression (Fig. 1A), indicating that Tg-derived ER stress reduces miR-30a-5p abundance. Our labs and others have shown that, similar to other coronaviral infections, TGEV infection triggers significant ER stress and initiates all three UPR pathways (1, 8). To explore whether miR-30a-5p could be regulated by TGEV infection, we initially monitored miR-30a-5p expression in ST cells after TGEV infection at different multiplicities of infection (MOIs). Compared with mock infection, TGEV infection significantly reduced the levels of miR-30a-5p at 24 h postinfection (hpi) and displayed an MOI-dependent response (Fig. 1B). To determine the stage at which miR-30a-5p suppression by TGEV infection occurs, we analyzed miR-30a-5p expression at different time points after TGEV infection. TGEV infection at an MOI of 1 caused a typical cytopathic effect (CPE), including syncytium formation in ST cells at 24 hpi, and resulted in approximately 35% cell death at 48 hpi. The miR-30a-5p reduction occurred after 12 hpi and then gradually decreased up to 48 hpi (Fig. 1D), indicating that TGEV infection decreases miR-30a-5p abundance at the late stage of infection. TGEV infection in ST cells was confirmed by quantifying the viral genomes (Fig. 1C and E). TGEV primarily infects villous epithelial cells in the small intestine *in vivo* and causes watery diarrhea. To assess whether TGEV infection also decreases miR-30a-5p expression *in vivo*, we quantified miR-30a-5p expression in piglet ilea at 48 hpi. TGEV infection resulted in an approximately 5-fold reduction in miR-30a-5p abundance in the ileum *in vivo* (0.021 ± 0.003) compared with that in uninfected control ileum (0.096 ± 0.016) ($P < 0.01$) (Fig. 1F). TGEV infection in the ileum was confirmed by quantifying TGEV RNA (Fig. 1G). These results demonstrate that TGEV infection decreases miR-30a-5p expression.

IRE1 α -mediated UPR induction inhibits miR-30a-5p expression. We next investigated the mechanisms responsible for the suppression of miR-30a-5p by TGEV infection. In recent studies, IRE1 α , a highly conserved ER stress sensor comprising a protein kinase and RNase, has the ability to degrade miRNAs, in addition to degrading mRNA under ER stress (35, 38, 39). IRE1 α activation by TGEV infection was assessed by real-time PCR (RT-PCR) and PstI digestion, which showed a recognition site located within the 26-nt region of XBP1 cDNA removed by IRE1 α -mediated splicing, as previously described (12, 40). Consistent with previous results for the coronavirus mouse hepatitis virus (MHV) (41), TGEV infection caused substantial cytoplasmic cleavage of the XBP1u transcript into the XBP1s transcription factor starting at 24 hpi, indicating that IRE1 α is activated by TGEV infection (Fig. 2A). IRE1 α activation was further confirmed by analyzing the expression of the XBP1s downstream target gene, namely, ER-localized DnaJ homologue 4 (ERdj4) (Fig. 2B). The activity of IRE1 α (the ratio of spliced XBP1 DNA to total XBP1u DNA) was inversely correlated with the levels of miR-30a-5p expression (Fig. 2A and 1D) ($R = 0.933$, $P < 0.01$). The decrease in miR-30a-5p expression primarily occurred within 24 to 48 hpi, the period in which

FIG 1 TGEV infection suppresses miR-30a-5p expression *in vitro* and *in vivo*. (A) The ER stress inducer Tg decreased miR-30a-5p expression in ST cells. The miR-30a-5p levels in ST cells were measured by RT-qPCR after Tg treatment for 24 h. (B to E) TGEV infection downregulated miR-30a-5p expression *in vitro*. The miR-30a-5p levels (B) and TGEV infection (C) in ST cells were measured by RT-qPCR at 24 hpi at different MOIs. For time kinetics, the levels of miR-30a-5p (D) and TGEV genomes (E) in ST cells were quantified at the indicated time points after infection with TGEV at an MOI of 1. The results from three independent experiments are shown. (F, G) TGEV infection suppressed miR-30a-5p expression *in vivo*. Piglets were orally inoculated with 5 ml 1×10^5 TCID₅₀ TGEV or DMEM. Total cellular RNA from each ileum was collected at 48 hpi, and the levels of miR-30a-5p (F) and TGEV viral RNA in the ileum (G) were measured by RT-qPCR. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant versus the mock-infected control.

significant IRE1 α activation was triggered by TGEV infection (Fig. 1D and 2A). These findings suggest that TGEV-induced IRE1 α activation may involve decreased miR-30a-5p expression during TGEV infection. To demonstrate that activated IRE1 α is responsible for the downregulation of miR-30a-5p, we monitored the expression of miR-30a-5p in TGEV-infected or Tg-treated cells after inhibiting the IRE1 α function with

4 μ 8c, a highly specific and selective inhibitor of the RNase activity of IRE1 α (14). The effective blockage of the IRE1 α RNase function by 4 μ 8c was confirmed by PCR analysis of XBP1 cleavage (Fig. 2C). The inhibition of IRE1 α RNase by 4 μ 8c almost completely abolished the suppression of miR-30a-5p by TGEV or Tg (Fig. 2D and E). To further verify the contribution of IRE1 α to miR-30a-5p expression, we knocked down IRE1 α expression by specific small interfering RNAs (siRNAs), and the efficiency of IRE1 α knockdown was confirmed by Western blotting (Fig. 2F). The silencing of IRE1 α by siRNAs significantly rescued the decreased miR-30a-5p induced by TGEV infection (Fig. 2G) or Tg treatment (Fig. 2H) ($P < 0.05$). In addition, the efficiency of miR-30a-5p restored by IRE1 α siRNAs (siIRE1 α #1 to siIRE1 α #3) was correlated with the knockdown efficiency of IRE1 α siRNAs (Fig. 2F to H). Taken together, these results show that activated IRE1 α reduces miR-30a-5p expression.

miR-30a-5p suppresses TGEV propagation. In agreement with our previous results, compared with the results of control dimethyl sulfoxide (DMSO) treatment, blocking IRE1 α with 4 μ 8c significantly reduced TGEV replication, as revealed by measuring the quantities of viral genomes (Fig. 3A) and infectious virions (Fig. 3B) (8). Consistent with 4 μ 8c treatment results, knockdown of IRE1 α by siIRE1 α #3, which was the most efficient at silencing IRE1 α , significantly reduced the TGEV genome quantity and titer. Both siIRE1 α #1 and siIRE1 α #2 also exhibited a tendency to decrease TGEV propagation in ST cells but not significantly because of the silencing efficiency (Fig. 3C and D). The observed levels of TGEV reduction were correlated with the knockdown efficiency of IRE1 α siRNAs (Fig. 2F and 3 and 3D). These data demonstrate that the IRE1 α pathway promotes TGEV replication.

Since TGEV replication was reduced after blocking IRE1 α signaling cascades by 4 μ 8c or IRE1 α -specific siIRE1 α #3, which rescued the IRE1 α -mediated downregulation of miR-30a-5p expression, we reasoned that miR-30a-5p may inhibit TGEV infection. To investigate the role of miR-30a-5p in TGEV propagation, we monitored TGEV infection in ST cells after transfecting miR-30a-5p mimics at 24 h prior to infection. Compared with negative control (NC) mimics, the miR-30a-5p mimics decreased the TGEV genome quantity and progeny viral titer by up to 17-fold. The inhibition of TGEV by preexisting miR-30a-5p mimics was dose dependent, and TGEV infection (MOI = 0.01) was substantially reduced by 80 nM and 160 nM miR-30a-5p mimics ($P < 0.001$) (Fig. 3E and F). The miR-30a-5p mimics also significantly decreased TGEV infection when an MOI of 1 was used (data not shown). The suppression of TGEV infection by miR-30a-5p was further confirmed by a TGEV nucleocapsid protein immunofluorescence assay (IFA) (Fig. 3G). To determine the phase at which preexisting miR-30a-5p overexpression suppresses TGEV infection, we measured TGEV infection at different time points in the presence of miR-30a-5p overexpression. miR-30a-5p significantly suppressed TGEV replication at 24 and 36 hpi, indicating that the miR-30a-5p-mediated reduction in TGEV replication occurs at the late stage of TGEV infection (Fig. 3H). Furthermore, the specific suppression of endogenous miR-30a-5p in ST cells by the miR-30a-5p inhibitor boosted TGEV infection compared with that achieved with the NC inhibitor (NC-i) (Fig. 3I). These results demonstrate that miR-30a-5p suppresses TGEV infection. To verify

FIG 2 Activated IRE1 α reduces miR-30a-5p abundance. (A) Analysis of IRE1 α activation by XBP1 mRNA splicing. IRE1 α activation by TGEV infection was analyzed by PCR amplification of total XBP1 cDNA and further digestion with PstI, as previously described (40). The sizes of the PCR-amplified fragments from spliced and unspliced XBP1 DNA with or without PstI cleavage are also listed. The PCR fragments of total XBP1, spliced XBP1, and unspliced XBP1 DNA in ST cells that were infected with TGEV at an MOI of 1 for various time points or treated with Tg (1 μ M) for 24 h are shown. The PCR products of the housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase), used as an internal control, are shown in the top panel. The ratio of the band intensities for spliced and total XBP1 DNA in the infected cells was normalized to that in the mock-infected cells. ORF, open reading frame. (B) TGEV infection upregulated ERdj4 expression. The relative expression of ERdj4 normalized to that of GAPDH following TGEV infection was measured and is presented. (C) Analysis of IRE1 α activation in partial samples from the assay whose results are presented in panel A, D, or E. (D, E) The inhibition of IRE1 α by 4 μ 8c rescued the suppression of miR-30a-5p by TGEV (D) or Tg (E). ST cells were pretreated with 50 or 100 μ M 4 μ 8c for 2 h, followed by TGEV infection (MOI = 1) (D) or Tg (1 μ M) treatment (E). The relative expression of miR-30a-5p normalized to that of internal U6 siRNA was measured by RT-qPCR after 24 h. The results are presented as the relative expression of miR-30a-5p in ST cells normalized to that of miR-30a-5p in the mock-infected control cells. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant. (F to H) Knockdown of IRE1 α rescued miR-30a-5p suppression following TGEV infection or Tg treatment. ST cells were transfected with siIRE1 α #1, siIRE1 α #2, siIRE1 α #3, or scrambled control siRNA (NC) at 100 nM for 24 h, followed by infection with TGEV for 24 h at an MOI of 0.01 (F, G) or treatment with Tg (1 μ M) for 24 h (H). Next, the cells were harvested to determine the efficiency of IRE1 α knockdown (F) or miR-30a-5p expression (G, H). The results represent those from three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.

FIG 3 miR-30a-5p inhibits the replication of TGEV. (A, B) The inhibition of IRE1 α by 4 μ 8c suppressed TGEV infection (MOI = 1) in ST cells. ST cells were pretreated with 50 or 100 μ M 4 μ 8c for 2 h and then infected with TGEV (MOI = 1); (Continued on next page)

whether IRE1 α facilitates TGEV replication by manipulating miR-30a-5p expression, we analyzed TGEV production in ST cells transfected with a miR-30a-5p inhibitor in the presence of 4 μ 8c at a TGEV MOI of 1.0. The suppression of endogenous miR-30a-5p by the miR-30a-5p inhibitor significantly abrogated 4 μ 8c-mediated TGEV suppression compared with that achieved with the mock (DMSO)-treated control (Fig. 3I), indicating that IRE1 α facilitates TGEV infection by manipulating miR-30a-5p expression. Taken together, our data indicate that IRE1 α enhances TGEV replication by decreasing miR-30a-5p abundance.

miR-30a-5p enhances IFN-I antiviral signaling cascades rather than IFN-I induction. Next, we explored the mechanisms responsible for miR-30a-5p-mediated TGEV inhibition. TGEV efficiently replicates in cells, despite significant amounts of endogenous IFN-I production after infection (25, 26), indicating that some underlying mechanisms are exploited by TGEV to escape IFN-I-induced antiviral responses. Given that miR-30a-5p inhibited TGEV replication at the late stage of infection (Fig. 3H), we hypothesized that miR-30a-5p suppresses TGEV replication possibly by enhancing IFN-I antiviral signaling cascades rather than by promoting the production of IFN-I. To exclude the possibility that miR-30a-5p enhances the production of IFN-I, we initially analyzed IFN- β expression in ST cells following TGEV infection when overexpressing miR-30a-5p. Consistent with the findings of previous studies (8, 25), TGEV infection elicited a substantial amount of IFN- β production at 24 hpi (Fig. 4A). Overexpression of miR-30a-5p did not significantly increase IFN- β production, as measured by quantifying IFN- β protein levels following TGEV infection relative to those observed in the presence of NC mimics (Fig. 4A), indicating that miR-30a-5p does not modulate TGEV-induced IFN-I production.

The binding of IFN-I to the IFN-I receptor primarily activates the JAK-STAT1 signaling pathway and induces hundreds of ISGs to inhibit viral infection. To verify our hypothesis that miR-30a-5p hinders TGEV replication largely by enhancing IFN-I antiviral signaling cascades, we initially monitored the kinetic profiles of interferon-stimulated gene 15 (ISG15) expression after TGEV infection (Fig. 4B). The expression of ISG15 peaked at 24 hpi and then significantly diminished, although IFN- β was continuously elevated during the period from 24 to 48 hpi (Fig. 4B) ($P < 0.05$). Consistent with the kinetic profile of ISG15, STAT1 blotting results demonstrated that STAT1 phosphorylation peaked at 24 hpi and then decreased (Fig. 4C). These results suggest that IFN-I antiviral signaling cascades are undermined during the later stage of TGEV infection, in contrast to IFN- β production. To directly verify if TGEV infection impairs IFN-I antiviral signaling at the later stage of infection, we stimulated TGEV-infected ST cells at 36 hpi by IFN- β for 30 min and evaluated STAT1 activation. Compared with uninfected control cells, TGEV-infected cells exhibited a significant reduction in IFN- β -elicited STAT1 phosphorylation (p-STAT1) (Fig. 4D), indicating that TGEV infection impairs IFN-I antiviral signaling at a later stage. Furthermore, the time frame of the observed decrease in ISG15 expression matched that of the observed significant reduction in miR-30a-5p abundance during TGEV infection (Fig. 4B), suggesting that miR-30a-5p may be relevant for the suppression of the IFN-I antiviral response at the later stage of infection. To validate the role of miR-30a-5p in modulating IFN-I antiviral signaling, we initially monitored the activity of

FIG 3 Legend (Continued)

TGEV RNA (A) and titers (B) were measured at 24 hpi. *, $P < 0.05$; **, $P < 0.01$. (C, D) IRE1 α knockdown by siRNAs decreased TGEV replication. ST cells were transfected with IRE1 α siRNA or the scrambled control siRNA (siRNA NC) at 100 nM, followed by infection with TGEV (MOI = 0.01); TGEV RNA (C) and titers (D) were quantified at 24 hpi. *, $P < 0.05$; **, $P < 0.01$ versus siRNA NC-transfected control cells. (E to G) miR-30a-5p overexpression inhibited TGEV infection. ST cells were transfected with miR-30a-5p mimics at the indicated doses (20, 80, and 160 nM) for 24 h, followed by infection with TGEV for 24 h at an MOI of 0.01. TGEV infection was determined at 24 hpi by RT-qPCR (E) or titration (F). *, $P < 0.05$; ***, $P < 0.001$ versus mimic NC. (G) The suppression of TGEV infection by miR-30a-5p was confirmed by IFA. TGEV-N, N protein of TGEV. (H) miR-30a-5p suppressed TGEV replication at the late stages of infection. ST cells were transfected with 160 nM miR-30a-5p mimics for 24 h and then infected with TGEV at an MOI of 0.01. TGEV infection was analyzed at 2, 6, 12, 24, or 36 hpi. *, $P < 0.05$; ***, $P < 0.001$. (I) The miR-30a-5p inhibitor rescued TGEV suppression by 4 μ 8c. ST cells were transfected with 160 nM miR-30a-5p inhibitor or NC inhibitor. After 24 h of transfection, ST cells were treated with 100 μ M 4 μ 8c for 2 h and then infected with TGEV (MOI = 1). TGEV infection was measured at 24 hpi. *, $P < 0.05$.

FIG 4 miR-30a-5p enhances IFN-I antiviral signaling rather than IFN-I production. (A) miR-30a-5p did not manipulate IFN- β production. ST cells were transfected with 160 nM miR-30a-5p mimics or NC mimics for 24 h, followed by infection with TGEV (MOI = 0.01) for 24 h. The IFN- β levels in the supernatant were measured by ELISA. NS, not significant. (B, C) TGEV infection antagonized interferon signaling at the late stages of infection. ST cells were infected with TGEV at an MOI of 1, and then the samples were collected at different times for the quantification of ISG15, IFN- β , and miR-30a-5p expression (B) or the Western blotting of pSTAT1, STAT1, or β -actin (C). * , $P < 0.05$. (D) TGEV infection impaired IFN-I-elicited STAT1 signaling. ST cells were infected with TGEV at an MOI of 1 for 36 h, followed by stimulation with IFN- β for 30 min. Then, cells were lysed and collected for Western blotting of pSTAT1, STAT1, or β -actin. * , $P < 0.05$. (E) miR-30a-5p modulated the activity of the ISRE reporter vector following IFN- β stimulation. The ISRE reporter vector and pRL-TK were cotransfected with the indicated miR-30a-5p mimics, NC mimics, miR-30a-5p inhibitor, or NC inhibitor into ST cells for 24 h, followed by stimulation with IFN- β (100 ng/ml). Cells were harvested for luciferase (luc) assay at 12 h after IFN- β stimulation. * , $P < 0.05$; *** , $P < 0.001$. (F to H) miR-30a-5p promoted IFN- β signaling. The cells were

(Continued on next page)

the IFN-stimulated response element (ISRE) with a luciferase assay following IFN- β stimulation. Compared with NC mimics, miR-30a-5p mimics significantly enhanced the activity of ISRE stimulated by IFN- β ; in contrast, compared with the NC inhibitor (NC-i), the miR-30a-5p inhibitor suppressed IFN- β -derived ISRE activity (Fig. 4E). We next measured the expression of antiviral ISG genes (ISG15, 2'-5'-oligoadenylate synthetase-like [OASL], and myxovirus resistance protein 1 [MxA]) in the presence of miR-30a-5p overexpression during TGEV infection (Fig. 4F) or IFN- β stimulation (Fig. 4G). Compared to the NC mimic control, miR-30a-5p overexpression greatly upregulated the expression of antiviral ISG genes in response to either TGEV infection or IFN- β stimulation (Fig. 4F and G). Consistent with the results of ISRE luciferase and ISG expression assays, the overexpression of miR-30a-5p significantly promoted p-STAT1, whereas the inhibition of endogenous miR-30a-5p decreased p-STAT1 levels following IFN- β stimulation or TGEV infection (Fig. 4H). These data demonstrate that miR-30a-5p promotes IFN-I signaling. Overall, we conclude that miR-30a-5p reinforces IFN-I antiviral signaling rather than IFN-I production.

Consistent with previously published results (42, 43), we demonstrated that IFN- β substantially reduced TGEV infection and exhibited anti-TGEV activity *in vitro* (Fig. 4I). In agreement with the result that miR-30a-5p enhanced IFN-I antiviral signaling compared with NC mimics, overexpression of miR-30a-5p reduced TGEV infection and increased the efficiency of IFN- β in inhibiting TGEV infection by more than 100-fold (Fig. 4I) ($P < 0.01$). The inhibition of endogenous miR-30a-5p by the inhibitor significantly increased TGEV infection and decreased the efficiency of IFN- β in suppressing TGEV infection (Fig. 4I). Therefore, these data demonstrate that miR-30a-5p inhibits TGEV replication by enhancing IFN-I antiviral signaling rather than by modulating IFN-I production.

miR-30a-5p directly targets SOCS1 and SOCS3. To elucidate the underlying mechanisms by which miR-30a-5p enhances IFN-I signaling, we performed computational analysis by using the TargetScan prediction program to identify the potential target genes of miR-30a-5p. Since miR-30a-5p promoted IFN-I signaling rather than IFN-I induction (Fig. 4), we primarily focused on the target genes of miR-30a-5p that enhance IFN signaling pathways. Computational analysis showed that miR-30a-5p could potentially target SOCS1 and SOCS3 through a 3' UTR site that is conserved in mammals (Fig. 5A). The members of the SOCS family of proteins are endogenous potent negative regulators of JAK-STAT signaling transduction (19, 44). Next, we explored whether miR-30a-5p directly targets SOCS proteins and enhances IFN-I signaling by downregulating SOCS expression. To verify whether miR-30a-5p directly targets SOCS1 and SOCS3, we cloned the predicted target sites in the 3' UTRs of SOCS1 or SOCS3 into a firefly luciferase reporter vector. In addition, a mutant vector was constructed to eliminate possible recognition by replacing five seed nucleotides (in which the underlined nucleotides in the sequence UGUUUAC were changed, resulting in the sequence UAGGGUC) as previously described (Fig. 5A) (30). Compared with the NC mimic treatment, overexpression of miR-30a-5p in ST cells decreased the activity of the luciferase reporter containing the SOCS1 or SOCS3 wild-type target sequence but not that of the luciferase reporter containing the mutant target site of SOCS1 or SOCS3. In contrast, compared with the NC inhibitor, the miR-30a-5p inhibitor increased the activity of the luciferase reporter containing the SOCS1 or SOCS3 wild-type target sequence but not that of the luciferase reporter containing the mutant target site (Fig. 5B). These results confirm that miR-30a-5p directly targets the 3' UTRs of SOCS1 and SOCS3. To further verify that SOCS1 and SOCS3 are direct targets of miR-30a-5p, we examined the expression of

FIG 4 Legend (Continued)

transfected with 160 nM miR-30a-5p mimics, NC mimics, miR-30a-5p inhibitor, or NC inhibitor for 24 h, followed by IFN- β treatment or TGEV infection (MOI = 1). The samples were collected at 24 h after TGEV infection or IFN- β stimulation for the quantification of ISG expression (F, G) and for Western blotting of pSTAT1, STAT1, or β -actin (H). Quantifications were normalized to those for the mock-treated and uninfected NC. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. (I) miR-30a-5p enhanced the anti-TGEV activity of IFN- β . After transfection with miR-30a-5p mimics or inhibitor for 24 h, cells were pretreated with IFN- β or DMEM for 24 h and then infected with TGEV (MOI = 0.01) and harvested at 24 hpi for viral RNA quantification. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.

FIG 5 miR-30a-5p targets the 3' UTRs of SOCS1 and SOCS3. (A) Schematic diagram of the predicted target sites of miR-30a-5p in the SOCS1 and SOCS3 3' UTRs of six representative mammals. The predicted target sites and mutated target sites of miR-30a-5p are underlined and were mutated as indicated. (B) Results of the luciferase assay. ST cells were cotransfected with SOCS1 or SOCS3 wild-type or mutant luciferase vectors (500 ng) and 160 nM miR-30a-5p mimics or NC mimics, miR-30a-5p inhibitor, or NC inhibitor, and the luciferase activity was analyzed at 30 h after transfection. FL, firefly luciferase; RL, *Renilla* luciferase. (C) The suppression of SOCS1 and SOCS3 mRNA levels by miR-30a-5p under TGEV-uninfected conditions. ST cells were transfected with 160 nM NC mimics, miR-30a-5p mimics, NC inhibitor, or miR-30a-5p inhibitor. The expression levels of SOCS1 and SOCS3 were analyzed by RT-qPCR at 48 h after transfection. The relative expression of SOCS1 and SOCS3 was normalized to that of the NC control. Bars represent the means $\pm$ SEM ($n = 3$). (D) The suppression of SOCS1 and SOCS3 protein levels by miR-30a-5p under TGEV-uninfected and -infected conditions. ST cells were transfected as described in the legend to panel C for 24 h, followed by infection with TGEV (MOI = 1) or mock infection with DMEM, and the samples were collected at 24 h for Western blotting of SOCS1, SOCS3, or β -actin. Quantifications were normalized to those for the uninfected NC. (E to G) TGEV infection upregulated SOCS1 and SOCS3 expression. (E) The SOCS1 and SOCS3 expression levels in ST cells were measured by RT-qPCR at 24 hpi at different MOIs. P values represent the difference from the mock-infected control. For time kinetics, the SOCS1 and SOCS3 mRNA levels (F) or SOCS1 and SOCS3 protein levels (G) in ST cells were measured at the indicated time points after infection with TGEV at an MOI of 1. (H) Treatment with 4 μ 8c abolished the upregulation of SOCS1 and SOCS3 by TGEV infection by modulating miR-30a-5p. ST cells were transfected with 160 nM miR-30a-5p inhibitor for 24 h. Next, ST cells were pretreated with 100 μ M 4 μ 8c or DMSO for 2 h, followed by infection with TGEV (MOI = 1). Cells were collected for RT-qPCR analysis for determination of SOCS1 and SOCS3 expression at 24 hpi. (I, J) Elevated expression of SOCS1 and SOCS3 in the ilea after TGEV infection. The expression of SOCS1 (I) and SOCS3 (J) in the ilea at 48 hpi was quantified by RT-qPCR. Bars represent the means $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; NS, not significant.

SOCS1 and SOCS3 in ST cells transfected with the miR-30a-5p mimics or inhibitor. As expected, the miR-30a-5p mimics significantly decreased the transcript levels of SOCS1 and SOCS3 in ST cells (Fig. 5C). The diminished expression of SOCS1 and SOCS3 induced by miR-30a-5p overexpression was verified by Western blotting (Fig. 5D). Conversely, compared with the NC inhibitor, the miR-30a-5p inhibitor increased the expression of SOCS1 and SOCS3 in ST cells (Fig. 5C and D). The modulation of miR-30a-5p abundance exerted an effect on the expression of SOCS1 and SOCS3 in TGEV-infected ST cells similar to that in TGEV-uninfected ST cells (Fig. 5D). Altogether, these data demonstrate that miR-30a-5p downregulates the expression of SOCS1 and SOCS3 by directly targeting their 3' UTRs.

TGEV infection downregulated miR-30a-5p expression through activation of IRE1 α (Fig. 1 and 2). Reasonably, TGEV infection would upregulate the expression of SOCS1 and SOCS3. As expected, TGEV infection substantially elevated the expression of SOCS1 and SOCS3 in ST cells at 24 hpi and exhibited a dose-dependent induction of MOIs (Fig. 5E). Furthermore, the increased expression of SOCS1 and SOCS3 began at 12 hpi, and expression was substantially elevated at 24 hpi following TGEV infection, as observed by measuring both mRNA and protein levels, which were inversely correlated with the kinetic expression profiles of miR-30a-5p (Fig. 5F and G). To further validate the role of miR-30a-5p in manipulating SOCS1 and SOCS3 expression via IRE1 α , we assessed the expression of SOCS1 and SOCS3 after TGEV infection in the presence of 100 μ M 4 μ 8c pretreatment. Compared with the DMSO-pretreated mock-treated control, 4 μ 8c pretreatment strongly decreased the expression of SOCS1 and SOCS3 in ST cells following TGEV infection, and the reduction in SOCS1 and SOCS3 expression by 4 μ 8c was counteracted by the miR-30a-5p inhibitor (Fig. 5H), indicating that TGEV upregulates the expression of SOCS1 and SOCS3 through IRE1 α -mediated modulation of miR-30a-5p expression. Consistent with the *in vitro* SOCS expression results, the expression of both SOCS1 (Fig. 5I) and SOCS3 (Fig. 5J) in the TGEV-infected ileum was also upregulated more than 10-fold. Taken together, these data demonstrate that TGEV infection upregulates the expression of SOCS1 and SOCS3 via the IRE1 α -mediated modulation of miR-30a-5p abundance.

Increased expression of SOCS1 or SOCS3 disrupts the IFN-I antiviral response and facilitates TGEV replication. Because miR-30a-5p hindered TGEV infection (Fig. 3) and enhanced IFN-I signaling (Fig. 4), next, we explored whether TGEV-derived suppression of miR-30a-5p facilitates TGEV infection via modulation of IFN-I signaling by SOCS1 and SOCS3. Initially, we analyzed the activation of the JAK-STAT1 pathway in response to IFN-I stimulation or TGEV infection of ST cells transfected with pCAGGS-SOCS1, pCAGGS-SOCS3, or the empty vector. The expression of SOCS1 or SOCS3 was confirmed by an IFA (Fig. 6A). As expected, the overexpression of SOCS1 or SOCS3 reduced p-STAT1 and the ratio of p-STAT1 to total STAT1 in response to either IFN- β stimulation or TGEV infection (Fig. 6B), indicating that the elevated expression of SOCS1 or SOCS3 suppresses the p-STAT1 induced by IFN- β or TGEV infection. Consistent with the disruption of IFN-I-activated JAK-STAT1 signaling by either SOCS1 or SOCS3, transient expression of SOCS1 substantially dampened the anti-TGEV activity of IFN- β and enhanced TGEV infection, as indicated by quantification of the TGEV genomes and the viral titers, and the transient expression of SOCS3 also dampened the anti-TGEV activity of IFN- β but significantly elevated the levels of TGEV genomes only, despite the trend of an increase in TGEV titers (Fig. 6C). Importantly, the overexpression of either SOCS1 or SOCS3 enhanced TGEV infection under physiological infection conditions in the absence of IFN- β stimulation (Fig. 6D). Overexpression of SOCS1 suppressed p-STAT1, IFN- β antiviral activity, and TGEV infection more efficiently than did overexpression of SOCS3 (Fig. 6B to D). These results demonstrate that the increased expression of SOCS1 and SOCS3 hinders the JAK-STAT1 signaling elicited by IFN-I or TGEV and enhances TGEV infection. To further verify the roles of SOCS1 and SOCS3 in IFN-I anti-TGEV activity, we silenced the expression of endogenous SOCS1 and SOCS3 using siRNAs. The knockdown efficiency of SOCS1 siRNAs (siSOCS1s) and SOCS3 siRNAs was confirmed by Western blotting (Fig. 6E). In contrast, knockdown of endogenous SOCS1

FIG 6 Increased expression of SOCS1 or SOCS3 dampens the IFN-I antiviral response and promotes TGEV replication. (A) Overexpression of SOCS1 and SOCS3 in ST cells. ST cells were transfected as indicated with pCAGGS-HA, pCAGGS-SOCS1, or pCAGGS-SOCS3 for 48 h, and the transient expression of SOCS1 and SOCS3 was confirmed by IFA with anti-HA staining. (B) SOCS1 or SOCS3 overexpression suppressed the activation of STAT1 by IFN-β and TGEV infection. ST cells were transfected as indicated with pCAGGS-HA, pCAGGS-SOCS1, or pCAGGS-SOCS3 for 24 h, followed by incubation with porcine IFN-β (100 ng/ml) or infection with TGEV (MOI = 1). Cells were collected for Western blotting of pSTAT1, STAT1, or β-actin after 24 h. *P* values represent the difference from the vector control. (C, D) SOCS1 or SOCS3 overexpression enhanced TGEV infection and undermined the anti-TGEV activity of IFN-β. ST cells were transfected as described in the legend to panel B, followed by incubation with porcine IFN-β (C) or DMEM (D) for 24 h. Then, cells were infected with TGEV at an MOI of 0.01; TGEV infection was determined at 24 hpi. (E) Knockdown of SOCS1 and SOCS3 by siRNAs in ST cells. ST cells were transfected for Western blotting of SOCS1 and SOCS3 expression at 48 h after transfection with 100 nM siSOCS1s, siSOCS3s, or scrambled control siRNA. (F) Enhancement of the anti-TGEV activity of IFN-β by knockdown of SOCS1 or SOCS3 in ST cells. ST cells were transfected with siSOCS1s, siSOCS3s, or the scrambled control siRNA for 24 h, followed by incubation with IFN-β for 24 h. Then, the cells were infected with TGEV (MOI = 0.01) and harvested for quantification of TGEV infection at 24 hpi. (G) Silencing of SOCS1 or SOCS3 boosted IFN-β signaling under IFN-β-stimulated conditions. ST cells were stimulated with IFN-β at 24 h after transfection with siSOCS1#1, siSOCS3#3, or the scrambled control siRNA, and cells were collected for RT-qPCR analysis of ISG15, OASL, or MxA expression relative to that of GAPDH after 24 h of stimulation. (H) Silencing of SOCS1 or SOCS3 decreased TGEV infection under IFN-β-unstimulated conditions. ST cells were transfected with siSOCS1#1, siSOCS3#3, or the scrambled control siRNA for 24 h. Then, cells were infected TGEV (MOI = 0.01) and harvested for quantification of TGEV infection at 24 hpi. (I) Silencing of SOCS1 or SOCS3 boosted IFN-β signaling under TGEV-infected conditions. ST cells were treated as described in the legend to panel H, and cells were collected for RT-qPCR analysis of ISG15, OASL, or MxA expression relative to that of GAPDH. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ****, *P* < 0.0001; NS, not significant.

or SOCS3 by specific siRNAs in ST cells substantially enhanced the anti-TGEV activity of IFN- β (Fig. 6F) and the expression of IFN-I-induced ISGs (Fig. 6G). Overall, the silencing of endogenous SOCS1 and SOCS3 expression greatly decreased TGEV titers by up to 10-fold (Fig. 6H) and enhanced the expression of IFN-induced ISGs (Fig. 6I) in non-IFN- β -stimulated ST cells compared with those in siRNA NC-transfected cells. In agreement with SOCS1 and SOCS3 overexpression results, the silencing of SOCS1 enhanced the IFN- β -mediated antiviral response and hindered TGEV infection more efficiently than did the knockdown of SOCS3 (Fig. 6E to I), suggesting that SOCS1 has a more important role in IFN-I signaling than does SOCS3. Thus, these data collectively indicate that TGEV infection upregulates the expression of SOCS1 and SOCS3, which undermines JAK-STAT1 signaling and facilitates TGEV infection.

IRE1 α facilitates TGEV infection by modulating the miR-30a-5p/SOCS axis. We demonstrated above that TGEV-activated IRE1 α decreased the levels of miR-30a-5p (Fig. 2) and that miR-30a-5p directly targeted SOCS family proteins and downregulated their expression, which enhanced STAT1 activation and hindered TGEV infection (Fig. 3 to 6). To further verify whether TGEV escapes the IFN-I response by disrupting the JAK-STAT1 pathway through IRE1 α -mediated modulation of the miR-30a-5p/SOCS axis, we initially monitored TGEV infection after inhibiting STAT1 with fludarabine (a STAT1-specific inhibitor) in ST cells treated with 4 μ 8c. The suppression of IRE1 α by 4 μ 8c promoted STAT1 activation, resulting in TGEV inhibition (Fig. 7A). The disruption of 4 μ 8c-mediated enhancement of STAT1 signaling by fludarabine significantly rescued TGEV suppression by 4 μ 8c (Fig. 7A). In agreement with these results, the overexpression of miR-30a-5p enhanced STAT1 activation and hindered TGEV infection, and fludarabine-mediated blockade of STAT1 activation significantly rescued the suppression of TGEV infection by miR-30a-5p (Fig. 7C). The inhibition of STAT1 activation by fludarabine was confirmed by Western blotting for p-STAT1 and total STAT1 (Fig. 7B and D). The rescue of 4 μ 8c- or miR-30a-5p-mediated TGEV inhibition by fludarabine treatment was not due to cellular cytotoxicity since treatment with 4 μ 8c, fludarabine, or 4 μ 8c plus fludarabine did not cause cellular cytotoxicity at the concentrations used in the study (Fig. 7E). These results suggest that IRE1 α enhances TGEV infection through miR-30a-5p/SOCS-mediated disruption of the JAK-STAT1 pathway. Finally, to directly verify whether the effect of the miR-30a-5p/SOCS axis on TGEV infection is dependent on the IFN-I antiviral response, we assessed the effect of the suppression of miR-30a-5p on TGEV in ST cells after knockdown of the IFN-I receptor IFNAR1. The silencing efficacy of IFNAR1 siRNAs (siIFNAR1) was verified by measuring the transcripts of IFNAR1 (Fig. 7F). Among the 3 tested siRNAs, only siIFNAR1#1 substantially silenced IFNAR1 expression (Fig. 7F). The silencing of IFNAR1 by siIFNAR1#1 compared with that by the siRNA control was further confirmed by the reduction in ISG expression (ISG15, OASL, and MxA) after IFN- β stimulation (Fig. 7G). Unlike the siRNA control, no inhibitory effect of miR-30a-5p on TGEV was observed in IFNAR1-silenced cells (Fig. 7H). In line with the TGEV titer results, miR-30a-5p overexpression did not result in the enhancement of STAT1 activation in IFNAR1-silenced ST cells, whereas miR-30a-5p overexpression did in wild-type ST cells (Fig. 7I). These data demonstrate that miR-30a-5p overexpression suppresses TGEV infection via the IFN-I response. In summary, IRE1 α enhances TGEV infection through miR-30a-5p/SOCS axis-mediated escape from the IFN-I response.

DISCUSSION

IFN-I has vital roles in the host innate immunity response against viral infections, and in turn, coronaviruses have evolved diverse strategies to counter the antiviral activity of IFN-I during infection (20, 23, 45). Coronavirus replication is structurally and functionally related to the ER, and ER stress is a common outcome in coronavirus-infected cells (1, 2, 45). The UPR constitutes a vital aspect of the virus-host interaction and modulates both viral replication and pathogenesis. However, an understanding of how UPR induction by coronaviruses actively participates in viral replication and manipulates the host innate immune responses has remained elusive. In the present study, we observed that TGEV infection induced the activation of IRE1 α , which facilitated TGEV infection by

FIG 7 IRE1 α facilitates TGEV infection by modulating the miR-30a-5p/SOCS axis. (A, B) The blockage of STAT1 activation rescued the viral suppression of 4μ8c. ST cells were pretreated with 100 μM 4μ8c for 2 h or 100 μM 4μ8c for 2 h plus 10 μM fludarabine (Flud) for 24 h, followed by infection with TGEV (MOI = 1). Then, the TGEV titer (A) was measured at 24 hpi, and STAT1 and p-STAT1 were analyzed by Western blotting (B). (C, D) The blockage of STAT1 activation rescued the viral suppression of miR-30a-5p. ST cells were transfected with 160 nM NC mimics, miR-30a-5p mimics, or miR-30a-5p mimics plus stimulation with 10 μM fludarabine for 24 h, followed by infection with TGEV (MOI = 1). Next, the TGEV titer (C) was measured at 24 hpi, and STAT1 and p-STAT1 were analyzed by Western blotting (D). (E) Effect of 4μ8c and fludarabine on cell viability. ST cells were treated with 4μ8c, 4μ8c plus fludarabine, fludarabine only, or the carrier control (DMSO) as described above. Cell cytotoxicity was analyzed with a CCK-8 system as described in Materials and Methods. (F) Knockdown of IFNAR1 by siRNAs in ST cells. ST cells were harvested for RT-qPCR analysis of IFNAR1 expression at 48 h after transfection with 100 nM siRNAs or scrambled control siRNA (siCtrl). (G) Silencing of IFNAR1 dampened IFN-β signaling under IFN-β-stimulated conditions. ST cells were transfected with 100 nM siIFNAR1#1 or scrambled control siRNA for 24 h, followed by incubation with IFN-β for 24 h. Then, the cells were collected for RT-qPCR analysis of ISG15, OASL, or MxA expression relative to that of GAPDH. (H, I) Silencing of IFNAR1 abolished viral suppression (H) and enhancement of p-STAT1 (I) of miR-30a-5p. NC mimics or miR-30a-5p mimics (160 nM) were cotransfected with 100 nM siIFNAR1#1 or the scrambled control siRNA in ST cells for 24 h, followed by infection with TGEV (MOI = 1). The TGEV titer (H) and STAT1 and p-STAT1 levels (I) were measured at 24 hpi. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; NS, not significant.

modulating the miR-30a-5p/SOCS axis. Our results revealed an unappreciated mechanism employed by TGEV to escape the IFN-I antiviral response via IRE1 α -mediated modulation of the miR-30a-5p/SOCS axis (Fig. 8).

IRE1 α , the most conserved UPR transducer with both kinase and RNase activities,

FIG 8 TGEV antagonizes IFN-I-related innate immunity via IRE1 α -mediated manipulation of the miR-30a-5p/SOCS axis. During TGEV infection, TGEV activates IRE1 α , which reduces miR-30a-5p abundance. The decreased level of miR-30a-5p dampens IFN-I antiviral signaling by increasing the expression of SOCS1 and SOCS3, leading to viral escape from the IFN-I response. Pri-miR-30a, primary miR-30a.

plays a critical role in restoring ER homeostasis (38, 46). Many viruses have evolved strategies to employ IRE1 α signaling to facilitate their infection. The inhibition of IRE1 α signaling by specific IRE1 α inhibitors or siRNA decreases the replication of IAV, JEV, and hepatitis C virus (HCV) (17, 18, 47). We also showed that the inhibition of IRE1 α by the specific inhibitor 4 μ 8c or by siRNA knockdown diminished TGEV replication *in vitro*, similar to that observed for IAV, JEV, and HCV. However, the mechanisms by which IRE1 α facilitates viral infection remain elusive. IRE1 α may facilitate viral infection by conferring the resistance of the infected cells to apoptosis, as observed for HCV (47). IRE1 α facilitates JEV replication by modulating viral RNA translation through the RIDD pathway (18). In this study, we identified another mechanism of IRE1 α enhancement of viral infection, which occurs through reducing the endogenous abundance of miR-30a-5p. We also observed that IRE1 α mediated the decrease in miR-125b and miR-125a levels in Tg-treated ST cells (data not shown). Thus, the downregulation of miR-30a-5p by IRE1 α is not specific to miR-30a-5p. This finding is consistent with those of previous studies reporting that IRE1 α manipulates the expression of subsets of cellular miRNAs, including miR-125b, miR-150, and miR-17, in response to ER stress (15, 38, 47). However, the specificity and mechanisms of IRE1 α -mediated manipulation of cellular miRNAs remain elusive and merit further study.

In contrast to PEDV, which inhibits double-stranded RNA-mediated IFN-I production (20), TGEV infection results in the very rapid and massive production of IFN-I *in vitro* and *in vivo* (26, 27, 43, 48). Because TGEV infection is sensitive to IFN-I activity, TGEV has

several strategies to antagonize IFN-I signaling rather than IFN-I production. This information is consistent with our finding that TGEV impairs IFN-I JAK-STAT1 signaling at the late stage of infection (Fig. 4B to D). SOCS family proteins are negative regulators of cytokine-mediated JAK-STAT signaling. Here, TGEV infection inhibited miR-30a-5p expression, resulting in the strongly upregulated expression of SOCS1 and SOCS3 (Fig. 1 and 5), allowing efficient TGEV replication despite high IFN-I levels (Fig. 6). Knock-down of the physiological expression levels of SOCS1 or SOCS3 resulted in up to a 4-fold increase in the expression of ISG15 and a more than a 200-fold increase in the anti-TGEV activity of IFN-I (Fig. 6), indicating that SOCS3 and especially SOCS1, which suppresses IFN-I signaling more strongly than SOCS3, play vital roles in negatively regulating the IFN-I response during TGEV infection. Our data indicate that TGEV largely evades IFN-I innate immunity largely by modulating IFN-I antiviral signaling rather than by manipulating the production of IFN-I. The induction of SOCS1 and SOCS3 to counteract IFN antiviral responses is also observed in other viruses, such as SARS-CoV (49), herpes simplex virus 1 (50), respiratory syncytial virus (51), HCV (31), and IAV (52). SOCS1 and SOCS3 act on multiple STAT family members and potently suppress both the JAK-STAT1 and JAK-STAT3 signaling pathways (53, 54). Thus, our study provides more evidence that SOCS family proteins play a crucial role in antagonizing IFN-I responses during viral infections.

Our results showed that miR-30a-5p enhanced IFN-I signaling and significantly suppressed viral infection by directly targeting SOCS1 and SOCS3, whose 3' UTRs are conserved in mammals (Fig. 5). Consistent with our data, during the preparation of this article, a paper published by Xu and colleagues revealed that miR-30a-5p directly targets the 3' UTR of SOCS3 and manipulates the expression of SOCS3 in mouse B cell lymphoma (55). Importantly, we detected decreased expression of miR-30a-5p and increased expression of SOCS1 and SOCS3 in TGEV-infected ileum. Reasonably, we speculate that the miR-30a-5p/SOCS axis may have a role in TGEV pathogenesis *in vivo*. Given that activation of IRE1 α and upregulation of SOCS family proteins by many other viruses are not uncommon, the IRE1 α -miR-30a-5p/SOCS axis may also be involved in the pathogenesis of other viral infections, and further study is worthwhile.

In conclusion, here we report a previously unappreciated mechanism by which TGEV escapes IFN antiviral activity via IRE1 α -mediated miR-30a-5p suppression and subsequent SOCS1 and SOCS3 upregulation (Fig. 8). Our findings underscore the importance of IRE1 α in the regulation of IFN-I signaling and TGEV infection, as well as broaden our knowledge regarding the role of the UPR in aberrant host miRNA expression following viral infection. In addition, our study sheds light on the crucial roles of SOCS1 and SOCS3 in coronavirus infection.

MATERIALS AND METHODS

Cell culture, viruses, and virus infection. ST cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, USA), 100 U/ml penicillin, and 100 μ g/ml streptomycin in an incubator with 5% CO₂ at 37°C. TGEV strain H87, derived from the virulent strain H16 (GenBank accession number [FJ755618](#)), was propagated in ST cells. A TGEV stock was prepared and titrated as previously described (56). For TGEV infection, ST cells were infected with TGEV H87 at the desired MOI or mock infected with DMEM. After a 2-h incubation at 37°C, cells were washed and cultured in DMEM supplemented with 1% DMSO and 0.3% trypsin (0.25%; Gibco, USA) until harvested.

Synthetic miRNAs, siRNAs, and transfection. All miRNA mimics, miRNA inhibitors, and siRNAs listed in Table 1 were designed and synthesized by GenePharma (China). Cells were seeded for 16 h before transfection with plasmid DNA or synthetic oligonucleotides using the Lipofectamine 2000 reagent (Invitrogen, USA) or the Lipofectamine RNAiMAX reagent (Invitrogen) according to the manufacturer's protocol. The cells were infected with TGEV or stimulated by IFN- β at 24 h after transfection. The cells were harvested for real-time reverse transcription (RT)-quantitative PCR (qPCR) or Western blotting after a 24-h incubation.

Chemical treatments. Thapsigargin (Tg; a potent inducer of ER stress) and 4 μ 8c (a specific inhibitor of IRE1 α) (14) were purchased from Sigma-Aldrich, and fludarabine (a STAT1-specific inhibitor) was purchased from Selleck. ST cells were pretreated with different concentrations of chemicals or DMSO for 2 or 24 h, followed by inoculation with TGEV. After a 2-h incubation, the supernatant was removed and replaced with culture medium containing different doses of chemicals. The samples were harvested for RT-qPCR and viral titration at 24 hpi.

TABLE 1 Sequences of miRNA mimics, inhibitors, and siRNAs

Small RNA	Sequence (5'–3')
miR-30a-5p	UGUAAACAUCUCGACUGGAAG
miR-30a-5p inhibitor	CUUCCAGUCGAGGAUGUUUACA
siIRE1 α #1	GCACAGACCUGAAGUUCAATT
siIRE1 α #2	GGAGGUUAUCGACUGGUUTT
siIRE1 α #3	CCAUCAUCCUGAGCACCUUTT
siSOCS1#1	CCUGCACGGAGCAUUAACUTT
siSOCS1#2	UCUUCGCCCUCAGUGUGAATT
siSOCS1#3	GCCGACAAUGCAAUCUCCATT
siSOCS3#1	UCAAGCUGGUGCGUCACUATT
siSOCS3#2	CCUGGACUCCUAGAGAAATT
siSOCS3#3	UCUUCACGCUCAGCGUCAATT
siIFNAR1#1	CCAGCUUUACCCACUAAUUTT
siIFNAR1#2	CCGGGUCUAUGUUCUAAATT
siIFNAR1#3	GCCUGGAUGUCAAUUGUUTT

RNA extraction, reverse transcription, and qPCR. Total cellular RNA was extracted using the TRIzol reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. cDNA was prepared with a PrimeScript II 1st-strand cDNA synthesis kit (TaKaRa, Japan). The expression pattern of each gene was analyzed by RT-qPCR using a LightCycler 480 II system (Roche, Swiss) as previously described (56). For miRNA analyses, RNA was reverse transcribed by using an miRNA first-strand cDNA synthesis kit (Sangon Biotech, China), and miRNA expression was assessed by RT-qPCR. PCR amplification was performed in triplicate using the following conditions: 95°C for 10 min, followed by 40 cycles of two steps (95°C for 15 s and 60°C for 40 s). The relative expression levels of miRNAs were normalized to those of U6. The RNA expression results are presented as the means $\pm$ standard errors of the means (SEM) from 3 independent experiments. The sequences of the RT-qPCR primers are listed in Table 2.

TABLE 2 Sequences of primers used in the present study

Primer	Sequence (5'–3')
miR-30a-5p-qPCR-F	TGTAAACATCCTCGACTGGAAG
Uni-miR-qPCR-R	GCGAGCACAGAATTAATACGACTCAC
ISG15-qPCR-F	AGC ATG GTC CTG TTG ATG GTG
ISG15-qPCR-R	CAG AAA TGG TCA GCT TGC ACG
MxA-qPCR-F	CACGTCTTTGATACAAGGAGAGG
MxA-qPCR-R	GCACTCCATCTGCAGAACTCAT
OASL-qPCR-F	TCCCTGGGAAGAATGTGCAG
OASL-qPCR-R	CCCTGGCAAGAGCATAGTGT
SOCS1-qPCR-F	CGCCCTCAGTGTGAAGATGG
SOCS1-qPCR-R	GCTCGAAGAGGCGAGTCGAAG
SOCS3-qPCR-F	CACCTCCAGCATCTCTGTC
SOCS3-qPCR-R	TCGTAAGTGTCCAGGAATC
TGEV-qPCR-F	GCTTGATGAATTGAGTGCTGATG
TGEV-qPCR-R	CCTAACCTCGGCTTGTCTGG
GAPDH-qPCR-F	CCTCCGTGTCCCTACTGCCAAC
GAPDH-qPCR-R	GACGCCTGCTTACCACCTTCT
IFN- β -qPCR-F	AGCACTGGCTGGAATGAAAC
IFN- β -qPCR-R	TCCAGGATTGTCTCCAGGTC
ERdj4-qPCR-F	CAGAGAGATTGCAGAAGCATATGA
ERdj4-qPCR-R	GCTTCTTGGATCGAGTGTTTTG
IFNAR1-qPCR-F	ACATCACCTGCCTTACCAG
IFNAR1-qPCR-R	CATGGAGCCACTGAGCTTGA
XBP1-F	AAACAGAGTAGCAGCTCAGACTGC
XBP1-R	GAATCTCTAAGACTAGGGGCTTTGTA
SOCS1-EcoR I-F	CGGAATTCATGGTAGCACACAACAGGTG
SOCS1-KpnI-R	GGGGTACCTCATATCTGGAAGGGGAAGGAG
SOCS3-EcoR I-F	CGGAATTCATGGTACCCACAGCAAGTTC
SOCS3-KpnI-R	GGGGTACCTTAAAGTGGGGCATCGTACTGC
SOCS1-3' UTR-NheI-F	CTAGCTAGCATTATTTCTTGGAAACCATGTG
SOCS1-3' UTR-XbaI-R	GCTCTAGACACAGCAGAAAAATAAGCCAG
SOCS3-3' UTR-NheI-F	CTAGCTAGCTTCTATTTTGTGCCTCTGAC
SOCS3-3' UTR-XbaI-R	GCTCTAGAGTTTGACTTGGATTGGTATTTT
SOCS1-3' UTR-MT-F	CTTCATAGGGTCATATACCCAGTATCTTGCACAAAC
SOCS1-3' UTR-MT-R	TATATGACCCTATGAAGAGGTAGGAGGTACTGAGTTC
SOCS3-3' UTR-MT-F	ATAATAGGGTCAATCTGCCTCAATCACTCTGTCTTTTA
SOCS3-3' UTR-MT-R	TTGACCCTATTATTAATAAAACACAAACAAACCCAAAC

microRNA target prediction and plasmid construction. The TargetScan web server (version 7.1; <http://www.targetscan.org>) was used to predict SOCS1 and SOCS3 3' UTRs as potential targets of miR-30a-5p. The 3' UTR of porcine SOCS1 (GenBank accession number [GQ421919.1](#)) or SOCS3 (GenBank accession number [AY785557.1](#)) was amplified and inserted into the pmirGLO luciferase reporter vector (Promega, USA) using the NheI and XbaI restriction sites. The mutant types of SOCS1 or SOCS3 3' UTR vectors were constructed by mutating five seed nucleotides using a site-directed mutagenesis kit (Stratagene, USA) according to the manufacturer's instructions. To construct the SOCS1 and SOCS3 expression vectors, we amplified the full-length coding DNA sequence regions by specific primers, and the amplicons were cloned into the vector pCAGGS-HA (Addgene) using the EcoRI and KpnI restriction sites. The porcine ISRE reporter plasmid was kindly provided by Wenhai Feng from China Agricultural University and was previously described (30). All primers are listed in Table 2.

Dual-luciferase reporter assays. For miRNA target verification, wild-type or mutant SOCS1 and SOCS3 3' UTR luciferase reporter vectors were cotransfected with miR-30a-5p mimics, NC mimic, miR-30a-5p inhibitor, or NC inhibitor (NC-i) into ST cells for 36 h. Furthermore, the ISRE reporter plasmid and pRL-TK were cotransfected with either miR-30a-5p mimics, NC mimic, inhibitor, or NC inhibitor for 24 h, followed by incubation with porcine IFN- β (100 ng/ml) for 12 h. Next, the cells were collected for reporter activity testing with a dual-luciferase reporter assay system (Promega, USA) according to the manufacturer's protocol. All reporter assays were independently repeated at least three times.

IFAs. Immunofluorescence assay (IFAs) were performed as previously described (57). Briefly, ST cells were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100 and then blocked with blocking buffer for 2 h at 37°C. Cells were incubated with an anti-TGEV nucleocapsid monoclonal antibody (1:1,000) stocked in the State Key Laboratory of Veterinary Biotechnology or an antihemagglutinin (anti-HA) monoclonal antibody (1:5,000; Sigma-Aldrich,) at 37°C for 2 h. The cells were then labeled with an Alexa Fluor 546 goat anti-mouse immunoglobulin G (IgG) antibody (1:500; Thermo Fisher Scientific, USA) for 1 h at 37°C. Cellular nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; 1:100). Stained cells were visualized using an AMG EVOS F1 fluorescence microscope.

ELISA. Culture supernatants were collected from treated cells at the times indicated above and stored at -80°C until testing. IFN- β protein levels in the culture supernatants were determined by the use of porcine IFN- β enzyme-linked immunosorbent assay (ELISA) kits (Bio-Swamp, China) according to the manufacturer's instructions.

Protein extraction and Western blotting. To analyze the levels of host IRE1 α , phospho-STAT1, STAT1, SOCS1, and SOCS3 proteins, we analyzed the cellular lysate by Western blotting as previously described (56). Briefly, ST cells were lysed with Nonidet P-40 (NP-40) lysis buffer (Beyotime, China) supplemented with a protease inhibitor cocktail (Roche). The protein concentrations of the supernatant fraction were measured with a bicinchoninic acid (BCA) assay kit (Beyotime, China) and were equalized with the extraction reagent. Equal amounts of proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred onto a nitrocellulose membrane (GE Healthcare, USA). Membranes were blocked and incubated with the corresponding primary antibody at 4°C overnight. The primary antibodies used were as follows: β -actin (1:5,000; Sigma-Aldrich), SOCS1 (1:500; Sigma-Aldrich), SOCS3 (1:1,000; Cell Signaling Technology), phospho-STAT1 (1:1,000; Abcam), STAT1 (1:1,000; Abcam), and IRE1 α (1:500; Santa Cruz Biotechnology). After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-mouse IgG or anti-rabbit IgG for 1 h at room temperature. Then, immunolabeled proteins were visualized using an electrochemiluminescence (ECL) reagent (Thermo Fisher Scientific). The intensity of each band was analyzed using ImageJ software.

TGEV infection of piglets. Twelve 2-day-old specific-pathogen-free (SPF) piglets were randomly divided into two groups. The piglets in group 1 were orally inoculated with 5 ml 1×10^5 50% tissue culture infective doses (TCID₅₀) of the TGEV H87 strain. After viral infection, clinical signs were recorded on a daily basis. All piglets were euthanized at 48 hpi. Piglet small intestine samples were collected for RT-qPCR analyses. The TGEV infection experiment was approved by the Animal Care and Ethics Committee of the Harbin Veterinary Research Institute.

Cellular cytotoxicity assay. ST cells were cultured in 96-well plates for 24 h, followed by incubation with Tg, 4 μ 8c, fludarabine, 4 μ 8c plus fludarabine, or the same volume of DMSO for 24 h or 48 h. Next, cellular cytotoxicity assays were performed using a cell counting kit 8 (CCK-8) assay as previously described (56).

Statistical analysis. All results in the figures are presented, where appropriate, as the means $\pm$ SEM from three independent experiments and were analyzed in GraphPad Prism software (GraphPad Software, Inc.). Differences were considered significant if the *P* value was <0.05.

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REFERENCES

1. Cruz JL, Sola I, Becares M, Alberca B, Plana J, Enjuanes L, Zuniga S. 2011. Coronavirus gene 7 counteracts host defenses and modulates virus virulence. *PLoS Pathog* 7:e1002090. <https://doi.org/10.1371/journal.ppat.1002090>.

2. Fung TS, Liu DX. 2014. Coronavirus infection, ER stress, apoptosis and innate immunity. *Front Microbiol* 5:296. <https://doi.org/10.3389/fmicb.2014.00296>.
3. Favreau DJ, Desforages M, St-Jean JR, Talbot PJ. 2009. A human coronavirus OC43 variant harboring persistence-associated mutations in the S glycoprotein differentially induces the unfolded protein response in human neurons as compared to wild-type virus. *Virology* 395:255–267. <https://doi.org/10.1016/j.virol.2009.09.026>.
4. Versteeg GA, van de Nes PS, Bredenbeek PJ, Spaan WJ. 2007. The coronavirus spike protein induces endoplasmic reticulum stress and upregulation of intracellular chemokine mRNA concentrations. *J Virol* 81:10981–10990. <https://doi.org/10.1128/JVI.01033-07>.
5. DeDiego ML, Nieto-Torres JL, Jimenez-Guardeno JM, Regla-Nava JA, Alvarez E, Oliveros JC, Zhao J, Fett C, Perlman S, Enjuanes L. 2011. Severe acute respiratory syndrome coronavirus envelope protein regulates cell stress response and apoptosis. *PLoS Pathog* 7:e1002315. <https://doi.org/10.1371/journal.ppat.1002315>.
6. Liao Y, Fung TS, Huang M, Fang SG, Zhong Y, Liu DX. 2013. Upregulation of CHOP/GADD153 during coronavirus infectious bronchitis virus infection modulates apoptosis by restricting activation of the extracellular signal-regulated kinase pathway. *J Virol* 87:8124–8134. <https://doi.org/10.1128/JVI.00626-13>.
7. Wang Y, Li JR, Sun MX, Ni B, Huan C, Huang L, Li C, Fan HJ, Ren XF, Mao X. 2014. Triggering unfolded protein response by 2-deoxy-D-glucose inhibits porcine epidemic diarrhea virus propagation. *Antiviral Res* 106:33–41. <https://doi.org/10.1016/j.antiviral.2014.03.007>.
8. Xue M, Fu F, Ma Y, Zhang X, Li L, Feng L, Liu P. 2018. The PERK arm of the unfolded protein response negatively regulates transmissible gastroenteritis virus replication by suppressing protein translation and promoting type I interferon production. *J Virol* 92:e00431-18. <https://doi.org/10.1128/JVI.00431-18>.
9. Oikawa D, Tokuda M, Hosoda A, Iwakawa T. 2010. Identification of a consensus element recognized and cleaved by IRE1 alpha. *Nucleic Acids Res* 38:6265–6273. <https://doi.org/10.1093/nar/gkq452>.
10. Fung TS, Liao Y, Liu DX. 2014. The endoplasmic reticulum stress sensor IRE1alpha protects cells from apoptosis induced by the coronavirus infectious bronchitis virus. *J Virol* 88:12752–12764. <https://doi.org/10.1128/JVI.02138-14>.
11. Safra M, Ben-Hamo S, Kenyon C, Henis-Korenblit S. 2013. The ire-1 ER stress-response pathway is required for normal secretory-protein metabolism in *C. elegans*. *J Cell Sci* 126:4136–4146. <https://doi.org/10.1242/jcs.123000>.
12. Calton M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG, Ron D. 2002. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415:92–96. <https://doi.org/10.1038/415092a>.
13. Kimmig P, Diaz M, Zheng J, Williams CC, Lang A, Aragon T, Li H, Walter P. 2012. The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis. *Elife* 1:e00048. <https://doi.org/10.7554/eLife.00048>.
14. Cross BC, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, Silverman RH, Neubert TA, Baxendale IR, Ron D, Harding HP. 2012. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. *Proc Natl Acad Sci U S A* 109:E869–E878. <https://doi.org/10.1073/pnas.1115623109>.
15. Heindryckx F, Binet F, Ponticos M, Rombouts K, Lau J, Kreuger J, Gerwits P. 2016. Endoplasmic reticulum stress enhances fibrosis through IRE1alpha-mediated degradation of miR-150 and XBP-1 splicing. *EMBO Mol Med* 8:729–744. <https://doi.org/10.15252/emmm.201505925>.
16. Belmadani S, Matrougui K. 2017. The unraveling truth about IRE1 and microRNAs in diabetes. *Diabetes* 66:23–24. <https://doi.org/10.2337/dbi16-0058>.
17. Hassan IH, Zhang MS, Powers LS, Shao JQ, Baltrusaitis J, Rutkowski DT, Legge K, Monick MM. 2012. Influenza A viral replication is blocked by inhibition of the inositol-requiring enzyme 1 (IRE1) stress pathway. *J Biol Chem* 287:4679–4689. <https://doi.org/10.1074/jbc.M111.284695>.
18. Bhattacharyya S, Sen U, Vratil S. 2014. Regulated IRE1-dependent decay pathway is activated during Japanese encephalitis virus-induced unfolded protein response and benefits viral replication. *J Gen Virol* 95:71–79. <https://doi.org/10.1099/vir.0.057265-0>.
19. Porritt RA, Hertzog PJ. 2015. Dynamic control of type I IFN signalling by an integrated network of negative regulators. *Trends Immunol* 36:150–160. <https://doi.org/10.1016/j.it.2015.02.002>.
20. Zhang Q, Yoo D. 2016. Immune evasion of porcine enteric coronaviruses and viral modulation of antiviral innate signaling. *Virus Res* 226:128–141. <https://doi.org/10.1016/j.virusres.2016.05.015>.
21. Menachery VD, Einfeld AJ, Schafer A, Jossel L, Sims AC, Proll S, Fan S, Li C, Neumann G, Tilton SC, Chang J, Gralinski LE, Long C, Green R, Williams CM, Weiss J, Matzke MM, Webb-Robertson BJ, Schepmoes AA, Shukla AK, Metz TO, Smith RD, Waters KM, Katze MG, Kawaoka Y, Baric RS. 2014. Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. *mBio* 5:e01174-14. <https://doi.org/10.1128/mBio.01174-14>.
22. Kindler E, Gil-Cruz C, Spanier J, Li Y, Wilhelm J, Rabouw HH, Züst R, Hwang M, V'kovski P, Stalder H, Marti S, Habjan M, Cervantes-Barragan L, Elliot R, Karl N, Gaughan C, van Kuppeveld FJ, Silverman RH, Keller M, Ludewig B, Bergmann CC, Ziebuhr J, Weiss SR, Kalinke U, Thiel V. 2017. Early endonuclease-mediated evasion of RNA sensing ensures efficient coronavirus replication. *PLoS Pathog* 13:e1006195. <https://doi.org/10.1371/journal.ppat.1006195>.
23. Kamitani W, Narayanan K, Huang C, Lokugamage K, Ikegami T, Ito N, Kubo H, Makino S. 2006. Severe acute respiratory syndrome coronavirus nsp1 protein suppresses host gene expression by promoting host mRNA degradation. *Proc Natl Acad Sci U S A* 103:12885–12890. <https://doi.org/10.1073/pnas.0603144103>.
24. Kopecky-Bromberg SA, Martinez-Sobrido L, Frieman M, Baric RA, Palese P. 2007. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. *J Virol* 81:548–557. <https://doi.org/10.1128/JVI.01782-06>.
25. Zhou Y, Wu W, Xie L, Wang D, Ke Q, Hou Z, Wu X, Fang Y, Chen H, Xiao S, Fang L. 2017. Cellular RNA helicase DDX1 is involved in transmissible gastroenteritis virus nsp14-induced interferon-beta production. *Front Immunol* 8:940. <https://doi.org/10.3389/fimmu.2017.00940>.
26. Riffault S, Carrat C, van Reeth K, Pensaert M, Charley B. 2001. Interferon-alpha-producing cells are localized in gut-associated lymphoid tissues in transmissible gastroenteritis virus (TGEV) infected piglets. *Vet Res* 32:71–79. <https://doi.org/10.1051/vetres:2001111>.
27. Becares M, Pascual-Iglesias A, Nogales A, Sola I, Enjuanes L, Zuniga S. 2016. Mutagenesis of coronavirus nsp14 reveals its potential role in modulation of the innate immune response. *J Virol* 90:5399–5414. <https://doi.org/10.1128/JVI.03259-15>.
28. Forster SC, Tate MD, Hertzog PJ. 2015. MicroRNA as type I interferon-regulated transcripts and modulators of the innate immune response. *Front Immunol* 6:334. <https://doi.org/10.3389/fimmu.2015.00334>.
29. O'Neill LA, Sheehy FJ, McCoy CE. 2011. MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol* 11:163–175. <https://doi.org/10.1038/nri2957>.
30. Zhang Q, Huang C, Yang Q, Gao L, Liu HC, Tang J, Feng WH. 2016. MicroRNA-30c modulates type I IFN responses to facilitate porcine reproductive and respiratory syndrome virus infection by targeting JAK1. *J Immunol* 196:2272–2282. <https://doi.org/10.4049/jimmunol.1502006>.
31. Xu G, Yang F, Ding CL, Wang J, Zhao P, Wang W, Ren H. 2014. MiR-221 accentuates IFNs anti-HCV effect by downregulating SOCS1 and SOCS3. *Virology* 462:343–350. <https://doi.org/10.1016/j.virol.2014.06.024>.
32. Sharma N, Kumawat KL, Rastogi M, Basu A, Singh SK. 2016. Japanese encephalitis virus exploits the microRNA-432 to regulate the expression of suppressor of cytokine signaling (SOCS) 5. *Sci Rep* 6:27685. <https://doi.org/10.1038/srep27685>.
33. Trobaugh DW, Klimstra WB. 2017. MicroRNA regulation of RNA virus replication and pathogenesis. *Trends Mol Med* 23:80–93. <https://doi.org/10.1016/j.molmed.2016.11.003>.
34. Wang C, Cai L, Liu J, Wang G, Li H, Wang X, Xu W, Ren M, Feng L, Liu P, Zhang C. 2017. MicroRNA-30a-5p inhibits the growth of renal cell carcinoma by modulating GRP78 expression. *Cell Physiol Biochem* 43:2405–2419. <https://doi.org/10.1159/000484394>.
35. Upton JP, Wang L, Han D, Wang ES, Huskey NE, Lim L, Truitt M, McManus MT, Ruggero D, Goga A, Papa FR, Oakes SA. 2012. IRE1alpha cleaves select microRNAs during ER stress to derepress translation of proapoptotic caspase-2. *Science* 338:818–822. <https://doi.org/10.1126/science.1226191>.
36. Jia Z, Wang K, Wang G, Zhang A, Pu P. 2013. MiR-30a-5p antisense oligonucleotide suppresses glioma cell growth by targeting SEPT7. *PLoS One* 8:e55008. <https://doi.org/10.1371/journal.pone.0055008>.
37. Fu Y, Xu W, Chen D, Feng C, Zhang L, Wang X, Lv X, Zheng N, Jin Y, Wu Z. 2015. Enterovirus 71 induces autophagy by regulating has-miR-30a expression to promote viral replication. *Antiviral Res* 124:43–53. <https://doi.org/10.1016/j.antiviral.2015.09.016>.
38. Hassler J, Cao SS, Kaufman RJ. 2012. IRE1, a double-edged sword in

- pre-miRNA slicing and cell death. *Dev Cell* 23:921–923. <https://doi.org/10.1016/j.devcel.2012.10.025>.
39. Emde A, Eitan C, Liou LL, Libby RT, Rivkin N, Magen I, Reichenstein I, Oppenheim H, Eilam R, Silvestroni A, Alajajian B, Ben-Dov IZ, Aebischer J, Savidor A, Levin Y, Sons R, Hammond SM, Ravits JM, Moller T, Hornstein E. 2015. Dysregulated miRNA biogenesis downstream of cellular stress and ALS-causing mutations: a new mechanism for ALS. *EMBO J* 34:2633–2651. <https://doi.org/10.15252/embj.201490493>.
 40. Yu CY, Hsu YW, Liao CL, Lin YL. 2006. Flavivirus infection activates the XBP1 pathway of the unfolded protein response to cope with endoplasmic reticulum stress. *J Virol* 80:11868–11880. <https://doi.org/10.1128/JVI.00879-06>.
 41. Bechill J, Chen Z, Brewer JW, Baker SC. 2008. Coronavirus infection modulates the unfolded protein response and mediates sustained translational repression. *J Virol* 82:4492–4501. <https://doi.org/10.1128/JVI.00017-08>.
 42. Jordan LT, Derbyshire JB. 1995. Antiviral action of interferon-alpha against porcine transmissible gastroenteritis virus. *Vet Microbiol* 45: 59–70. [https://doi.org/10.1016/0378-1135\(94\)00118-G](https://doi.org/10.1016/0378-1135(94)00118-G).
 43. Zhu L, Yang X, Mou C, Yang Q. 2017. Transmissible gastroenteritis virus does not suppress IFN-beta induction but is sensitive to IFN in IPEC-J2 cells. *Vet Microbiol* 199:128–134. <https://doi.org/10.1016/j.vetmic.2016.12.031>.
 44. Vlotides G, Sorensen AS, Kopp F, Zitzmann K, Cengic N, Brand S, Zachoval R, Auernhammer CJ. 2004. SOCS-1 and SOCS-3 inhibit IFN-alpha-induced expression of the antiviral proteins 2,5-OAS and MxA. *Biochem Biophys Res Commun* 320:1007–1014. <https://doi.org/10.1016/j.bbrc.2004.06.051>.
 45. Minakshi R, Padhan K, Rani M, Khan N, Ahmad F, Jameel S. 2009. The SARS coronavirus 3a protein causes endoplasmic reticulum stress and induces ligand-independent downregulation of the type 1 interferon receptor. *PLoS One* 4:e8342. <https://doi.org/10.1371/journal.pone.0008342>.
 46. Credle JJ, Finer-Moore JS, Papa FR, Stroud RM, Walter P. 2005. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *Proc Natl Acad Sci U S A* 102:18773–18784. <https://doi.org/10.1073/pnas.0509487102>.
 47. Fink SL, Jayewickreme TR, Molony RD, Iwawaki T, Landis CS, Lindenbach BD, Iwasaki A. 2017. IRE1alpha promotes viral infection by conferring resistance to apoptosis. *Sci Signal* 10:eaa17814. <https://doi.org/10.1126/scisignal.aai7814>.
 48. Charley B, Laude H. 1988. Induction of alpha interferon by transmissible gastroenteritis coronavirus: role of transmembrane glycoprotein E1. *J Virol* 62:8–11.
 49. Okabayashi T, Kariwa H, Yokota S, Iki S, Indoh T, Yokosawa N, Takashima I, Tsutsumi H, Fujii N. 2006. Cytokine regulation in SARS coronavirus infection compared to other respiratory virus infections. *J Med Virol* 78:417–424. <https://doi.org/10.1002/jmv.20556>.
 50. Yokota S, Yokosawa N, Okabayashi T, Suzutani T, Miura S, Jimbow K, Fujii N. 2004. Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 contributes to inhibition of the interferon signaling pathway. *J Virol* 78:6282–6286. <https://doi.org/10.1128/JVI.78.12.6282-6286.2004>.
 51. Zheng J, Yang P, Tang Y, Pan Z, Zhao D. 2015. Respiratory syncytial virus nonstructural proteins upregulate SOCS1 and SOCS3 in the different manner from endogenous IFN signaling. *J Immunol Res* 2015:738547. <https://doi.org/10.1155/2015/738547>.
 52. Pauli EK, Schmolke M, Wolff T, Viemann D, Roth J, Bode JG, Ludwig S. 2008. Influenza A virus inhibits type I IFN signaling via NF-kappaB-dependent induction of SOCS-3 expression. *PLoS Pathog* 4:e1000196. <https://doi.org/10.1371/journal.ppat.1000196>.
 53. Babon JJ, Kershaw NJ, Murphy JM, Varghese LN, Laktyushin A, Young SN, Lucet IS, Norton RS, Nicola NA. 2012. Suppression of cytokine signaling by SOCS3: characterization of the mode of inhibition and the basis of its specificity. *Immunity* 36:239–250. <https://doi.org/10.1016/j.immuni.2011.12.015>.
 54. Yang Y, Yang L, Liang X, Zhu G. 2015. MicroRNA-155 promotes atherosclerosis inflammation via targeting SOCS1. *Cell Physiol Biochem* 36: 1371–1381. <https://doi.org/10.1159/000430303>.
 55. Xu Z, Ji J, Xu J, Li D, Shi G, Liu F, Ding L, Ren J, Dou H, Wang T, Hou Y. 2017. MiR-30a increases MDSC differentiation and immunosuppressive function by targeting SOCS3 in mice with B-cell lymphoma. *FEBS J* 284:2410–2424. <https://doi.org/10.1111/febs.14133>.
 56. Xue M, Zhao J, Ying L, Fu F, Li L, Ma Y, Shi H, Zhang J, Feng L, Liu P. 2017. IL-22 suppresses the infection of porcine enteric coronaviruses and rotavirus by activating STAT3 signal pathway. *Antiviral Res* 142:68–75. <https://doi.org/10.1016/j.antiviral.2017.03.006>.
 57. Fu F, Li L, Shan L, Yang B, Shi H, Zhang J, Wang H, Feng L, Liu P. 2017. A spike-specific whole-porcine antibody isolated from a porcine B cell that neutralizes both genogroup 1 and 2 PEDV strains. *Vet Microbiol* 205:99–105. <https://doi.org/10.1016/j.vetmic.2017.05.013>.