A novel IFNbeta-induced long non-coding RNA ZAP-IT1 interrupts Zika virus replication in A549 cells

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ABSTRACT

Zika virus (ZIKV) infection can cause severe neurological diseases including neonatal microcephaly and Guillain-Barre syndrome. Long noncoding RNAs (lncRNAs) are the by-products of the transcription process, which are considered to affect viral infection. However, it remains largely unexplored whether host lncRNAs play a role in ZIKV infection. Here, we identified a group of human lncRNAs that were up-regulated upon ZIKV infection and were dependent on the type I interferon (IFN) signaling. Overexpression of lncRNA ZAP-IT1 leads to an impairment of ZIKV infection. Correspondently, deficiency of ZAP-IT1 led to an enhancement of ZIKV infection. We further confirmed that ZAP-IT1, an intronic lncRNA with total 551 nt in length, is mainly located in the nuclear upon ZIKV infection. Knockout of ZAP-IT1 also led to the increase of dengue virus (DENV), Japanese encephalitis virus (JEV), or vesicular stomatitis virus (VSV) infection. Mechanically, we found that the antiviral effect of ZAP-IT1 was independent of the type I IFN signaling pathway. Therefore, our data unveiled that host lncRNA ZAP-IT1 induced by the type I IFN signaling, showed robust restriction on ZIKV infection, and even on DENV, JEV, and VSV infection, which may benefit the development of antiviral therapeutics.

1. Introduction

Zika virus (ZIKV) is an emerging arthropod-borne virus that belongs to the Flavivirus genus of the Flaviviridae family (Pierson and Diamond, 2018), which includes dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), and Japanese encephalitis virus (JEV) (Pierson and Diamond, 2020). ZIKV has been reported that can lead to a devastating congenital syndrome in the fetuses of pregnant mothers and cause the Guillain-Barre syndrome in adults (Zhang et al., 2021). Nonetheless, there are no approved vaccines or therapeutic countermeasures.

Long non-coding RNAs (lncRNAs) are a class of RNA transcripts exceeding 200 nucleotides (nt) in length as well known that they do not code for proteins (Batista and Chang, 2013; Zhang et al., 2021). LncRNAs play an important role in modulating the expression of the gene at the epigenetic, transcription, and post-transcriptional processes (Qiu et al., 2018; Liu et al., 2019; Wang et al., 2019a). Some lncRNAs participate in the response after virus infection, such as lincRNA-Cox2 (Carpenter et al., 2013; Xue et al., 2019), NRAV (Ouyang et al., 2014; Li et al., 2020), and NEAT1 (Zhang et al., 2013; Inamura et al., 2014; Ma et al., 2017), which can inhibit virus replication. Other lncRNAs, such as lncRNA-ACOD1 (Wang et al., 2017), NeST (Gomez et al., 2013), and lncRNA-CMPK2 (Kambara et al., 2014), are hijacked by viruses to promote virus replication. Lnc-ITPRIP-1 (Xie et al., 2018), NEAT1 (Zhang et al., 2013; Ma et al., 2017), lnc-Lsm3b (Jiang et al., 2018), and lncRHOXF1 (Penkala et al., 2016) have been reported to be involved in the regulation of innate immunity.

RNA-seq analysis in human neural progenitor cells (hNPCs) by Hu et al. has shown that 149 lncRNAs are differentially expressed in response to ZIKV infection (Hu et al., 2017), although the function of the differentially expressed lncRNAs has not been determined. Recently, our group has demonstrated that 79 lncRNAs and 140 mRNAs screened by lncRNA-microarray assay were differentially expressed in ZIKV-infected human lung epithelial A549 cell line (Wang et al., 2021). Three lncRNAs RPL27-OT1, Rec8-OT3, and OASL-IT1 exhibited a potent...
restriction effect on ZIKV infection. Importantly, OASL-IT1 exerted a significant antiviral activity, and OASL-IT1 and IFNs regulated each other in a positive feedback loop. However, only a few hundred lncRNAs have been annotated and reported to participate in the response of viral infection, and the functions of lncRNAs in ZIKV infection still remained to be known.

In order to explore whether more lncRNAs might be involved in ZIKV replication, we analyzed the microarray assay (GSE124094) in NCBI’s Gene Expression Omnibus (GEO) database. We found that lncRNAs including LAP1-AS1, LIN2C1762, VAMP1-AS1, ZAP-IT1 were differentially expressed after ZIKV infection. In this study, we demonstrated that ZIKV enhanced the expression of these lncRNAs through the type I IFN pathway. And we found that IncRNA ZAP-IT1 could inhibit the replication of ZIKV, as well as DENV, JEV and vesicular stomatitis virus (VSV). In addition, we found that the antiviral effect of ZAP-IT1 was independent of the type I IFN signaling. Taken together, our work identified that IncRNA ZAP-IT1 induced by IFN-β might interrupt viral replication, which could be served as a potential molecular target for anti-ZIKV agents.

2. Materials and methods

2.1. Cell culture

Human lung epithelial cell A549 (ATCC CCL-185), human glioblastoma cells LN229 (ATCC CRL-2611), human embryonic kidney cells 293T (ATCC CRL-3216), and African green monkey kidney cells Vero (ATCC CCL-81) were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% or 10% (v/v) fetal bovine serum (FBS) (Gibco, New York, USA). The mosquito cell line C6/36 (ATCC, CRL-1660) was maintained in RPMI-1640 medium (Invitrogen, Carlsbad, USA) supplemented with 10% FBS, 1% sodium pyruvate. All cells were supplemented with 100 units/mL of streptomycin and penicillin (G). In the detection of viral titers, the primary substrates were Vero cells and C6/36 cells. VSV carrying ZAP-IT1 sgRNA together with pSPAX2 and pMD2.G using FuGENE® HD Transfection Reagent (Promega). And lentiCRISPR vector was transfected into 293T cells to select the Survifé vector. The primer sequences used in cloning were listed in Supplementary Table S3. 293T cells were transfected with lentiCRISPR plasmids were transfected into A549 cells by Lipofectamine 2000 (Invitrogen). The IncRNA-expressing cells were selected by G418 (Invivogen) for 7–10 days.

2.6. Plasmid construction

5'- and 3'-rapid amplification of cDNA ends (RACE) PCR assays were performed to amplify IncRNAs using the SMARTer RACE cDNA amplification kit (TaKaRa, 6106, Osaka, Japan) and cloned into the pcDNA3.1(+) vector. The primer sequences used in cloning were listed in Supplementary Table S2. The sequences of plasmids were verified by sequencing.

2.7. Generation of IncRNA expressing cells

The pcDNA3.1(+-) lncRNA plasmids were transfected into A549 cells by Lipofectamine 2000 (Invitrogen). The IncRNA-expressing cells were selected by G418 (Invivogen) for 7–10 days.

2.8. Generation of knockout cells by CRISPR/Cas9 gene editing

CRISPR/Cas9 system was utilized to generate knockout cell clones as previously described (Ma et al., 2019). The single-guide RNAs (sgRNAs) targeting ZAP-IT1 were inserted into the lentivirus vector. The single-guide RNAs (sgRNAs) targeting ZAP-IT1 were inserted into the lentivirus vector. The single-guide RNAs (sgRNAs) targeting ZAP-IT1 were inserted into the lentivirus vector. The single-guide RNAs (sgRNAs) targeting ZAP-IT1 were inserted into the lentivirus vector. The single-guide RNAs (sgRNAs) targeting ZAP-IT1 were inserted into the lentivirus vector. The single-guide RNAs (sgRNAs) targeting ZAP-IT1 were inserted into the lentivirus vector. The single-guide RNAs (sgRNAs) targeting ZAP-IT1 were inserted into the lentivirus vector.

2.9. IFN-β treatment

A549 cells were treated with 500 units/mL of IFN-β (Sigma-Aldrich, 19032, Germany) for 24 h to harvest the total cellular RNA. Cells that had not received IFN-β treatment were served as control, and total RNA was extracted.

2.10. Cytoplasm and nuclear RNA fraction assay

Cells were washed, trypsinized and collected as a cell pellets prior to lysis. RNase-free TRI reagent (Promega, Madison, USA) was used to degrade the genomic DNA. Then the reverse was transcribed using M-MLV reverse transcriptase (Promega) according to the manufacturer’s instructions. qRT-PCR analysis was performed using the LightCycler 480 SYBR Green I Master (Roche, Basle, Switzerland) on a CFX96 Real-Time System (Bio-Rad, Basle, Switzerland). Differences of gene expression from qRT-PCR data were analyzed with 2−ΔΔCT values as described previously (Huang et al., 2020). Human U6 or β-actin level was measured as an internal control. The primer sequences used in qRT-PCR were listed in Supplementary Table S1.

2.5. Western blotting

Cells were harvested at indicated time and treated with RIPA lysis buffer (pH 7.4) (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.5% NP-40, 1% Triton X-100, 1 mmol/L EDTA, 1 mmol/L PMSF, 1% protease inhibitor cocktails, 1 mmol/L Na3VO4, and 1 mmol/L NaF). Western blotting was then performed as previously described (Wang et al., 2019b).

The primary antibodies used in this study were shown as follows: rabbit polyclonal antibodies against ZIKV E (GeneTex, GTX133314), rabbit polyclonal antibodies against ZAP (Proteintech, 16820-1-AP), and rabbit polyclonal antibodies against GAPDH (Proteintech, 10494-1-AP). Secondary antibodies included IRDye 800 CW-conjugated goat-anti-rabbit IgG (LI-COR, 926-32211, Lincoln, USA). Immuno-reactive bands were analyzed with the Odyssey infrared imaging system (LI-COR). Quantity One program (Bio-rad) was used to quantify the Western blotting results.

2.2. Virus, virus infection, and titration

ZIKV strain H/PF/2013 (GenBank accession number KJ776791), Dengue-2 virus New Guinea C (DENV2 NGC, GenBank accession number AF038403), and JEV (14-14-2 vaccine strain) were provided by the Guangzhou Centres for Disease Control. The Vesicular Stomatitis Virus (VSV, Indiana Strain) and the herpes simplex virus 1 (HSV-1, Kos strain) were kindly provided by Prof. Dongyan Jin (University of Hong Kong).

2.3. lncRNA microarray analysis

The IncRNA microarray data are available on the NCBI GEO database, (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124094). The GEO accession number is GSE124094 (GPL25961).

2.4. Quantitative real-time PCR (qRT-PCR)

Total RNAs were extracted using Trizol reagent (Invitrogen). Before reverse transcribing, the RNase-Free DNase (Promega, Madison, USA) was used to degrade the genome DNA. Then the reverse was transcribed using M-MLV reverse transcriptase (Promega) according to the manufacturer’s instructions. qRT-PCR analysis was performed using the LightCycler 480 SYBR Green I Master (Roche, Basle, Switzerland) on a CFX96 Real-Time System (Bio-Rad, Basle, Switzerland). Differences of
centrifuge tube. Cytoplasmic RNA was extracted by RNeasy Mini Kit (Qiagen, Germany). The pellet contained nuclei washed with RLT (a guanidine-thiocyanate-containing lysis buffer supplied by the RNeasy Mini Kit) buffer and was applied to extract nucleic RNA using TRIzol reagent (Invitrogen). qRT-PCR was performed to measure the levels of β-actin, U6, and ZAP-IT1 (primer sequences were listed in Supplementary Table S1).

2.11. RNA interference

The sequences of siRNAs targeting human ZAP mRNA are GCAGTCAGAGGACCTTT. A control siRNA with the scrambled sequence was used as a negative control (siNC). Transfection was carried out with 16 nmol of siRNAs by using Lipofectamine 2000 Reagent (Invitrogen) according to the manufacturer's instructions. At 48 h.p.t., cells were harvested for further analysis.

2.12. Statistical analysis

All statistical analyses of viral RNA levels or viral titers were performed with an unpaired, two-tailed Student's t-test. Data were presented as mean ± standard deviation (SD).

3. Results

3.1. Differential expression of lncRNAs is induced by ZIKV

In order to explore whether more lncRNAs may be involved in ZIKV replication, we analyzed the microarray assay (GSE124094) in NCBI's GEO database precisely. We found that the abundance of lncRNAs including NONHSAT095710, ENST00000621762, ENST00000619166, and lnc-ZC3HAV1L-1:1 was increased more than two folds in the microarray assay (Table 1 and Supplementary Table S4). To confirm the differential expression of lncRNAs from the microarray assay, A549 cells were infected with ZIKV, and the total RNA was collected at 24 h.p.i. to detect the expression of lncRNA by qRT-PCR. These lncRNAs were increased 10 folds or more than 20 folds upon ZIKV infection compared to uninfected cells (control) (Fig. 1A). According to the HUGO Gene Nomenclature Committee (HGNC, www.genenames.org) decision tree for naming lncRNA genes with unknown functions (Seal et al., 2020), NONHSAT095710, ENST00000621762, ENST00000619166, and lnc-ZC3HAV1L-1:1 were named into LAP3-AS1, LINC21762, VAMP1-AS1, and ZAP-IT1, respectively.

To find out whether the differential expression of these four lncRNAs is cell type-specific, monocyte-derived macrophages cells isolated from...
peripheral blood mononuclear cell (PBMC) were infected with ZIKV and the expression of these four lncRNAs was detected by qRT-PCR. As expectedly, LAP3-AS1, LINC21762, VAMP1-AS1, and ZAP-IT1 were induced to increase about two to eight folds after ZIKV infection (Fig. 1B). These results demonstrated that the differential expression of the selected lncRNAs was not cell type-specific. We examined the expression of these four lncRNAs in human glioma cells (LN229) as well. Up-regulation of lncRNAs was also shown in LN229 after ZIKV infection (Fig. 1C). This suggested that lncRNAs might play a role in ZIKV infected nervous system.

The kinetic assays were also performed in A549 cells. And the results demonstrated that the differential expression of these lncRNAs triggered by ZIKV was in a time- and dose-dependent manner (Fig. 1D and E).

3.2. Differential expression of lncRNAs induced by different viruses

To find out whether other viruses in Flavivirus genus like DENV and JEV have impact on the expression of the selected lncRNAs, A549 cells were infected with DENV2 or JEV, respectively. And the total RNAs were extracted for qRT-PCR. Increased expression level of all four lncRNAs was observed after DENV2 or JEV infection (Fig. 2A and B). To test whether negative single-stranded RNA virus or DNA virus could affect the expression of these lncRNAs, A549 cells were infected with VSV or HSV-1 for 12 h.p.i. Surprisingly, LAP3-AS1 could not be induced by VSV, while LINC21762, VAMP1-AS1, and ZAP-IT1 were significantly up-regulated after VSV infection (Fig. 2C). As for HSV-1, the expression level of LAP3-AS1 and LINC21762 increased more than 10 folds after HSV-1 infection, while only 5 folds of increase was observed in that of VAMP1-AS1 and ZAP-IT1. This was different from the RNA virus (Fig. 2D). The above observations indicated that the selected lncRNAs were responsive to other viral infections as well.

3.3. The expression of lncRNAs is dependent on the production of IFN and the activation of IFN signaling pathway

To elucidate the mechanism of the up-regulated expression of lncRNAs induced by ZIKV infection, we carried out bioinformatics analysis to predict potential binding sites of transcription factors on the promoter region by analyzing ENCODE ChIP-seq data. As shown in Fig. 3A, putative transcription factor binding sites on the promoter region of LAP3-AS1, LINC21762, VAMP1-AS1, and ZAP-IT1, respectively.

A 0 kb 1.0 kb 2.0 kb
B 0 kb 1.0 kb 2.0 kb
C 0 kb 1.0 kb 2.0 kb
D 0 kb 1.0 kb 2.0 kb
E 0 kb 1.0 kb 2.0 kb

Fig. 2. Differential expression of lncRNAs induced by different viruses. A549 cells were infected with DENV2 NGC (MOI = 5) (A) or JEV (MOI = 5) (B), and total RNAs were harvested at 24 h.p.i. to detect the lncRNA level by qRT-PCR. A549 cells were infected with VSV (MOI = 1) (C) or HSV-1 (MOI = 1) (D), and total RNAs were harvested at 12 h.p.i. to detect the lncRNA level by qRT-PCR. Human U6 level was measured as an internal control and normalized to uninfected cell (mock). All experiments were independently repeated for three times. Data were shown as means ± standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001. NS, not significant; h.p.i., hours post-infection.

Fig. 3. IFN pathway is involved in the induction of lncRNAs. A Putative transcription factor binding sites on the promoter region of LAP3-AS1, LINC21762, VAMP1-AS1, and ZAP-IT1, respectively. B A549 cells were treated with 500 units/mL of IFN-β for 24 h and total RNAs were harvested to detect the lncRNA level by qRT-PCR. Human U6 level was measured as an internal control and normalized to IFN-β (−) cell. C–E Control cells and MAVS K0 cells (C), IFNAR1 K0 cells (D), or STAT1 K0 cells (E) were infected with ZIKV (MOI = 3). Total RNAs were harvested at 24 h.p.i. to detect the lncRNA level by qRT-PCR. Human U6 level was measured as an internal control and normalized to uninfected cell (mock, 0 h or MOI 0). All experiments were independently repeated for three times. Data were shown as means ± standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001. K0, knockout.
binding sites of NF-xB and STAT1 were identified, implying a potential association between IncRNAs and NF-xB or STAT1. We hypothesized that ZIKV might induce the expression of these IncRNAs through activating the IFN signaling pathway. To verify this hypothesis, A549 cells were treated with IFN-β. The expression of four IncRNAs was significantly enhanced after the IFN-β treatment (Fig. 3B). To further confirm this result, we employed the MAVS<sup>−/−</sup>, IFNAR1<sup>−/−</sup>, or STAT1<sup>−/−</sup> A549 cells, which blocked the production of IFN and the activation of IFN signaling pathway. We found that the enhanced expression of IncRNAs was blocked after knockout of MAVS, IFNAR1, or STAT1 compared to the control cells (Fig. 3C and E). Taken together, our results indicated that LAP3-AS1, LINC21762, VAMP1-AS1, and ZAP-IT1 probably belonged to interferon-stimulated genes (ISGs) and were specifically induced by IFN and the activation of IFN pathway.

### 3.4. Overexpression of ZAP-IT1 restricts the replication of ZIKV

Next, we investigated the role of LAP3-AS1, LINC21762, VAMP1-AS1, and ZAP-IT1 in ZIKV infection. Firstly, the natural abundance of IncRNAs in uninfected cells was detected by qRT-PCR (Supplementary Fig. S1 and Supplementary Table S5). Next, A549 cells stably overexpressing LAP3-AS1, LINC21762, VAMP1-AS1 or ZAP-IT1 were successfully established, with 5–8 folds of expression level of that in control cells (empty vector) (Fig. 4A), and were then infected with ZIKV. No significant change of ZIKV RNA was detected after overexpressing LAP3-AS1, LINC21762, and VAMP1-AS1, while overexpression of ZAP-IT1 led to a 70% decrease of ZIKV RNA (Fig. 4B). The expression of ZIKV E protein was validated by Western blotting. And the result showed that overexpression of these four IncRNAs, especially ZAP-IT1, led to a decreased ZIKV E protein level (Fig. 4C). Consistently, overexpression of ZAP-IT1 significantly decreased the infectious level of progeny ZIKV (Fig. 4D). These results suggested that ZAP-IT1 conferred the most robust antiviral activity. In view of this and the above results that the highest level of ZAP-IT1 was observed after ZIKV infection among these four IncRNAs (Fig. 1), ZAP-IT1 was chosen for further analysis.

### 3.5. Identification of ZAP-IT1 as a nuclear non-coding RNA

5′ and 3′ rapid amplification of cDNA ends (RACE) of total RNA from A549 cells was carried out, and one transcript from Zap-it loci was identified (Fig. 5A). Human ZAP-IT1 is located on the reverse strand of chromosome 7, at position 139,049,456–139,050,006 (551 nt) and is in the intron 12 of ZAP. The nucleotide sequence of human ZAP-IT1 shared 92.3% identity with that of rhesus, but didn’t show any homology with mouse, dog, and elephant (USCS genome browser, hg19, Supplementary Fig. S2A), suggesting that ZAP-IT1 was highly species-specific.

To define if ZAP-IT1 had any protein-coding potential, we used Coding Potential Assessment Tool (CPAT) to explore the coding possibility of ZAP-IT1. The CPAT analysis predicted that the ZAP-IT1 possessed an extremely low coding potential to encode protein as other IncRNAs, NEAT1 and OASL-IT1, compared to the protein-coding genes ACTB and GAPDH (Fig. 5B). To further validate these predictions, the sequence of ZAP-IT1 fused with HA tag was inserted into pcDNA3.1 vector (pcDNA3.1-ZAP-IT1-HA). The pcDNA3.1 (empty vector), pcDNA3.1-ZAP-IT1-HA, and the positive control pcDNA3.1-GFP-HA were transfected into 293T cells. A specific HA band was shown in the pcDNA3.1-GFP-HA-transfected cells, while no band was detectable in the cells transfected with pcDNA3.1-ZAP-IT1-HA (Fig. 5C). These data demonstrated that ZAP-IT1 was indeed a non-protein-coding RNA.

To examine the subcellular localization of ZAP-IT1, a cytoplasm and nuclear RNA fraction assay were performed. β-actin and U6 mainly presented in the cytoplasm or nuclei as the control, respectively (Fig. 5D and E). ZAP-IT1 was rarely detectable in uninfected A549 cells but mainly presented in the nuclei fraction upon ZIKV infection (Fig. 5F). These results demonstrated that ZAP-IT1 was a nuclear non-coding transcript upon ZIKV infection.

### 3.6. Depletion of ZAP-IT1 promotes ZIKV replication

To find out whether deficiency of ZAP-IT1 may affect the replication of ZIKV, CRISPR/Cas9 gene editing-based knockout technology was used to construct ZAP-IT1 knockout (ZAP-IT1<sup>−/−</sup>) A549 cells. To generate large fragment deletions of ZAP-IT1, two sgRNAs were designed that targeted both the N and C terminal of ZAP-IT1 (Fig. 6A). About 372 nt was removed out of ZAP-IT1 in the knockout cells (Fig. 6B and Supplementary Fig. S2B). And the expression of ZAP-IT1 in the ZAP-IT1<sup>−/−</sup> cells would not be induced by ZIKV infection compared to control cells (Fig. 6C).

A significant enhancement in the level of ZIKV genomic RNA and ZIKV E protein was observed in ZAP-IT1<sup>−/−</sup> cells compared to control cells after ZIKV infection (Fig. 6D and E). Furthermore, the increased viral yield of ZIKV was also shown in ZAP-IT1<sup>−/−</sup> cells (Fig. 6F). Since ZAP-IT1 could be induced by DENV, JEV, VSV, and HSV-1 at different levels, we further examined whether ZAP-IT1 affected the replication of these viruses. Intriguingly, the viral yields of DENV, JEV and VSV were significantly increased in ZAP-IT1<sup>−/−</sup> cells compared to the control cells after infection (Supplementary Figs. S3A–C), while the titer of HSV-1 did not show remarkable change (Supplementary Fig. S3D). Together, these results suggested that ZAP-IT1 might play a restriction role in ZIKV, DENV, JEV and VSV infection, but not in HSV-1.
3.7. The antiviral effect of ZAP-IT1 is independent of the type I IFN signaling pathway and ZAP

To determine if the impact of ZAP-IT1 deficiency on ZIKV infection was mediated by the production of IFN or the IFN response directly, we evaluated the transcriptional level of IFN-β, and Mx1, known as a protein-coding ISG with antiviral activity against ZIKV. The results showed that the mRNA level of IFN-β and Mx1 didn’t be affected by knockout of ZAP-IT1 compared to control after ZIKV infection (Fig. 7A and B).

To test whether the knockout of ZAP-IT1 affects the expression of ZAP, the protein-coding gene sharing the same sequence of its intron 12 with ZAP-IT1, the mRNA and protein level of ZAP were detected. Intriguingly, the mRNA and protein level of ZAP-IT1 did not show a remarkable change in ZAP-IT1Ko cells compared to control (Fig. 7C and D), indicating that the knockout of ZAP-IT1 had no effects on the expression of ZAP. To find out whether ZAP plays a role in ZIKV infection, siRNA targeting ZAP (siZAP) or siNC was transfected into A549 cells. Neither the level of ZIKV E protein nor the viral titer showed the obvious change in siZAP cells compared to siNC cells (Fig. 7E and F).

These results were consistent with the findings in Chiu’s study (Chiu et al., 2018). Taken together, these results suggested that the antiviral effect of ZAP-IT1 was independent of the type I IFN signaling pathway and ZAP.
Current study revealed that a group of IncRNAs differentially expressed after virus infection and confirmed that a novel IncRNA, ZAP-IT1, played an antiviral role in ZIKV, DENV, JEV, and VSV replication in a type I IFN signaling independent way.

IncRNAs are found to be up- or down-regulated following TNF-α, LPS, IL-1β, IFN-α/β/γ treatment or after viral infection (Carrero et al., 2016; Covarrubias et al., 2017; Liu et al., 2019; Barriocanal et al., 2022; Chen et al., 2022; Ghafouri-Fard et al., 2022). In our study, we predicted multiple putative NF-κB and STAT1 binding sites on the promoter region of the selected IncRNAs (LAPI-AS1, LINC21762, VAMP1-AS1, and ZAP-IT1) and experimentally demonstrated that both NF-κB and STAT1 were important for the up-regulation of the selected IncRNAs induced by IFN-β and ZIKV. Therefore, the differential expression of host IncRNAs is closely related to viral infection or cytokines treatment.

4. Discussion

ZIKV is an emerging arbovirus and can cause severe congenital abnormalities in the fetuses and Guillain-Barre Syndrome in adults (Pierson and Diamond, 2018, 2020; Zhang et al., 2021). Long non-coding RNAs (IncRNAs) constitute for approximate 95% of the human genome transcripts and possess low coding possibility (Djebali et al., 2012; Qiu et al., 2017, 2018; Li et al., 2019; Li et al., 2020; Wang et al., 2020). IncRNAs are considered to be dysregulated during ZIKV infection. ZIKV infection can cause differential expression of IncRNAs profile in Aedes aegypti Mosquitoes cells and human neural progenitor cells (hNPCs) (Etebari et al., 2017; Hu et al., 2017). Our previous study also found that ZIKV infection could cause differential expression of 149 IncRNAs in AS549 cells and confirmed the IncRNA OASL-IT1 was a defense against viral replication via regulating innate immune response (Wang et al., 2021).

5. Conclusions

Overall, our study investigated the role of IncRNAs in ZIKV infection, and unveiled that ZAP-IT1 induced by IFN-β interrupted ZIKV replication.
independent of IFN signaling pathway, which might serve as a novel molecular target for anti-ZIKV therapeutics.

Data availability

The datasets generated for this study can be found in the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124094). The accession number is GSE124094.

Ethics statement

Experiments involving human monocyte-differentiated macrophages were reviewed and approved by the local Ethics Committee of the Sun Yat-sen University, and were conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All donors provided written informed consent. All samples were anonymized before use.

Author contributions

Yanxia Huang: conceptualization, formal analysis, investigation, methodology, software, writing-original draft, writing-review & editing. Yu Su: conceptualization, data curation, investigation, methodology, writing-original draft, writing-review & editing. Xin Li: supervision, writing-original draft, writing-review & editing. Tian Xiu: investigation, software. Ning Li: investigation. Yang Chao: funding acquisition, project administration, supervision, writing-review & editing.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2022.08.003.

References

Batista, P.J., Chang, H.Y., 2013. Long noncoding RNA: cellular address codes in development and disease. Cell 152, 1298–1307.

Carrero, E., Barriocanal, M., Prior, C., Udvardy, P., Varela, I., Garcia-Leon, R., 2018. The role of long noncoding RNA B2ST2-2 in innate immune response to viral infection. J. Virol. 96, e02076-18.

Chen, S., Huang, X., Yeo, E., Liu, B., Zhu, H., 2018. Inhibition of Japanese encephalitis virus infection by the host zinc-finger antiviral protein. PLoS Pathog. 14, e1007166.

Gribnau, S., Robinson, E.K., Shapleigh, B., Vollmers, A., Katsman, S., Hanley, N., Fong, N., Mcmanus, M.T., Carpenter, S., 2017. CRISPR/Cas-based screening of long non-coding RNAs (lncRNAs) in macrophages with an NF-kappaB reporter. J. Biol. Chem. 292, 20911–20920.

Djebali, S., Davis, C.A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., Tanzer, A., Lagarde, J., Lin, W., Schlesinger, F., Xue, C., Marinov, G.K., Khatun, J., Williams, B.A., Zalecki, C., Rozovskiy, J., Rodier, M., Kokocinski, F., Abad-Rodriguez, R.F., Alioto, T., Antoschek, I., Bae, M.T., Bax, B., Bell, K., Bell, I., Chakraborty, S., Chen, X., Christ, J., Ciocchi, L., Debernardi, J., Descello, J., Derrieux, E., Eiben, J., Ewing, A.J., Flicek, P., Funke, B., Guccione, J., Huntington, A.B., Iovino, N., Issaeva, T., Jacobs, L., Janes, S., Jones, R., Khodr, R., Khosravi, S., Kistenmacher, T., Kool, M., Kriz, A., Kung, P., Kutyakov, V., Kwon, H., Land, M., Loh, Y., Lu, F., Mccarthy, D.J., Mcvicker, H., Nightingale, C., Nisewander, J., Pancenko, S., Patrick, R., Pavesi, G., Pietsch, A., Poustka, A., Ramalho-Santos, M., Righi, L., Saha, S., Sastry, S., Seppala, H., Schones, D.E., Sinha, S., Skibber, J., Song, D., Stadler, D., Stadler, P.K., Stoeckloed, F., Tanaka, Y., Thomas, Y., Thompson, A., Tse, M., van Wijk, D., Velculescu, V., Vezina, L., Xie, G., Xu, Y., Yang, J., Young, R.A., Zaleski, C., Zehir, A., Zinn, K., Zschaechner, J.D., Zuzak, S., Zwiller, J., Zweier, L., Zyla, J., Zhong, W., Zheng, Y., Zuccato, C., Zuccato, C., Zuccato, C.

The role of long noncoding RNA BST2—repression of immune response genes. Science 341, 789–792.

Zhang, L., Cao, X., 2018. Self-recognition of an inducible host lncRNA by RIG-I promotes innate immune response. Cell 173, 906–919.

Zhang, L., Cao, X., 2018. The emergence of Zika virus and its new clinical syndromes. Nature 560, 573–581.

Zhang, L., Cao, X., 2018. The role of non-coding RNAs in the regulation of virus replication and resultant cellular pathologies. Int. J. Mol. Sci. 23, 815.

Zhang, L., Cao, X., 2018. Long non-coding RNAs: cellular address codes in development and disease. Nature 549, 101–108.

Zhang, L., Cao, X., 2018. The continued threat of emerging flaviviruses. Nat. Rev. Microbiol. 16, 616–626.

Zhang, L., Cao, X., 2018. Inhibition of Japanese encephalitis virus infection by the host zinc-finger antiviral protein. PLoS Pathog. 14, e1007166.

Zhang, L., Cao, X., 2018. Inhibition of Japanese encephalitis virus infection by the host zinc-finger antiviral protein. PLoS Pathog. 14, e1007166.
Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., Zhang, F., 2013. Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. 8, 2281–2308.

Rapicavoli, N.A., Qu, K., Zhang, J., Mikhail, M., Laberge, R.M., Chang, H.Y., 2013. A mammalian pseudogene IncRNA at the interface of inflammation and anti-inflammatory therapeutics. Elife 2, e00762.

Schneider, W.M., Chevillotte, M.D., Rice, C.M., 2014. Interferon-stimulated genes: a complex web of host defenses. Annu. Rev. Immunol. 32, 513–545.

Seal, R.L., Chen, L.L., Griffiths-Jones, S., Lowe, T.M., Mathews, M.B., O’reilly, D., Pierce, A.J., Stadler, P.F., Ulitsky, I., Wolin, S.L., Bruford, E.A., 2020. A guide to naming human non-coding RNA genes. EMBO J. 39, e103777.

Wang, P., Luo, M.L., Song, E., Zhou, Z., Ma, F., Wang, J., Jia, N., Wang, G., Nie, S., Liu, Y., Hou, F., 2018. Long noncoding RNA Inc-TSI inhibits renal fibrogenesis by negatively regulating the TGF-beta/Smad3 pathway. Sci. Transl. Med. 10, eaat2039.

Wang, P., Xu, J., Wang, Y., Cao, X., 2017. An interferon-independent IncRNA promotes viral replication by modulating cellular metabolism. Science 358, 1051–1055.

Wang, J., Zhang, Y., Li, Q., Zhao, J., Yi, D., Ding, J., Zhao, F., Hu, S., Zhou, J., Deng, T., Li, X., Guo, F., Liang, C., Cen, S., 2019a. Influenza virus exploits an interferon-independent IncRNA to preserve viral RNA synthesis through stabilizing viral RNA polymerase PB1. Cell Rep. 27, 3295–3304 e3294.

Wang, Y., Chen, X., Xie, J., Zhou, S., Huang, Y., Li, Y.P., Li, X., Liu, C., He, J., Zhang, P., 2019b. RNA helicase A is an important host factor involved in dengue virus replication. J. Virol. 93, e01306–e01318.

Wang, Y., Hoo, Q., Lin, Q., Liu, Y., Chen, C., Huang, Y., Huang, C., Zhang, J., He, J., Liu, C., Zhang, P., 2021. Positive feedback loop of long noncoding RNA OASL-IT1 and innate immune response restricts the replication of zika virus in epithelial A549 cells. J. Innate Immun. 13, 179–193.

Xie, Q., Chen, S., Tian, R., Huang, X., Deng, R., Xue, B., Qin, Y., Xu, Y., Wang, J., Guo, M., Chen, J., Tang, S., Li, G., Zhu, H., 2018. Long noncoding RNA ITFPR1-1 positively regulates the innate immune response through promotion of oligomerization and activation of MDAS. J. Virol. 92, e00507–e00518.

Xue, Z., Zhang, X., Liu, H., Li, W., Guo, X., Zhang, X., Liu, Y., Jin, L., Li, Y., Ren, Y., Yang, H., Zhang, L., Zhang, Q., Da, Y., Hao, J., Yao, Z., Zhang, R., 2019. lincRNA-Coxl2 regulates NLRP3 inflammasome and autophagy mediated neuroinflammation. Cell Death Differ. 26, 130–145.

Yang, W., Wu, Y.H., Liu, S.Q., Sheng, Z.Y., Zhen, Z.D., Gao, R.Q., Cui, X.Y., Fan, D.Y., Qin, Z.H., Zheng, A.H., Wang, P.G., An, J., 2020. S100A4+ macrophages facilitate zika virus invasion and persistence in the seminiferous tubules via interferon-gamma mediation. PLoS Pathog. 16, e1009019.

Yin, X., Gao, J., Liu, Z., Han, M., Ji, X., Wang, Z., Li, Y., He, D., Zhang, F., Liu, Q., Xin, T., 2022. Mechanisms of long non-coding RNAs in biological phenotypes and ferroptosis of glioma. Front. Oncol. 12, 941327.

Zhang, Q., Chen, C.Y., Yedavalli, V.S., Jeang, K.T., 2013. NEAT1 long noncoding RNA and paraspeckle bodies modulate HIV-1 posttranscriptional expression. mBio 4, e00596–512.

Zhang, X., Li, G., Chen, G., Zhu, N., Wu, D., Wu, Y., James, T.D., 2021. Recent progresses and remaining challenges for the detection of Zika virus. Med. Res. Rev. 41, 2039–2108.

Zheng, H., Chen, C., Luo, Y., Yu, M., He, W., An, M., Gao, B., Kong, Y., Ya, Y., Lin, Y., Li, Y., Xie, K., Huang, J., Lin, T., 2021. Tumor-derived exosomal BCYRN1 activates WNT5A-VEGF-C/VEGFR3 feedforward loop to drive lymphatic metastasis of bladder cancer. Clin. Transl. Med. 11, e497.

Zhou, S., Yang, C., Zhao, F., Huang, Y., Lin, Y., Huang, C., Ma, X., Du, J., Wang, Y., Long, G., He, J., Liu, C., Zhang, P., 2019. Double-stranded RNA deaminase ADAR1 promotes the Zika virus replication by inhibiting the activation of protein kinase PKR. J. Biol. Chem. 294, 18168–18180.