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Circular RNA GATAD2A promotes H1N1 replication through inhibiting autophagy

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ABSTRACT

Circular RNAs (circRNAs) play critical roles in various diseases. However, whether and how circular RNA regulates influenza A virus (IAV) infection is unknown. Here, we studied the role of circular RNA GATA Zinc Finger Domain Containing 2A (circ-GATAD2A) in the replication of IAV H1N1 in A549 cells. Circ-GATAD2A was formed upon H1N1 infection. Knockdown of circ-GATAD2A in A549 cells enhanced autophagy and inhibited H1N1 replication. By contrast, overexpression of circ-GATAD2A impaired autophagy and promoted H1N1 replication. Similarly, knockout of vacuolar protein sorting 34 (VPS34) blocked autophagy and increased H1N1 replication. However, the expression of circ-GATAD2A could not further enhance H1N1 replication in VPS34 knockout cells. Collectively, these data indicated that circ-GATAD2A promotes the replication of H1N1 by inhibiting autophagy.

1. Introduction

Influenza virus, belonging to the Orthomyxoviridae family, is a negative single-stranded RNA virus (Peiris et al., 2007). Its genome contains 8 negative-sense segments, which encode 17-18 viral proteins (Zhou et al., 2009). Among these proteins, different matrix (M) proteins and antigenicities of nucleic proteins (NP) divide the influenza virus into three serotypes (A, B and C) (Bouvier and Palese, 2008). Influenza A virus (IAV) is the most well-known dominant pathogen in causing annual seasonal epidemics and results in great economic loss in the world every year. Currently, according to the antigenicity of these membrane-associated glycoproteins, IAVs have been divided into 16 hemagglutinin (HA) subtypes (H1 to H16) and 9 neuraminidase (NA) subtypes (N1 to N9) (Stohr, 2005). The H1N1 subtype is generally known to have caused two pandemics (1918 and 2009) and therefore is a threat to human health.

Autophagy is a digestion process for removing intracellular toxic protein, protein aggregates, damaged organelles, and pathogens (Kudchodkar and Levine, 2009). IBDV, HSV-1 and Sindbis virus have been shown to be engulfed in host cell autophagosomes by transmission electron microscope (TEM) analysis (Hu et al., 2015; Orvedahl et al., 2010; Yordy et al., 2012; Yoshimori, 2010). Overexpression of the autophagy gene Beclin-1 improves survival of mice infected with Sindbis virus. Furthermore, Anthony Orvedah proved that autophagy protects neurons from Sindbis virus by promoting clearance of viral protein (Orvedahl et al., 2010). However, several studies suggested that incomplete autophagy induced by IAV infection promotes its replication (Gannage et al., 2009; Jung et al., 2018; Liu et al., 2016a; Yeganeh et al., 2018; Zhou et al., 2009). In contrast, Beclin-1 and TUFM-regulated autophagy inhibit IAV titers (Feizi et al., 2017; Kuo et al., 2017). Thus, the precise role of complete autophagy on IAV infection is still controversial.

Circular RNAs (circRNAs) have been identified as a class of non-coding RNAs and are spread largely in a variety of eukaryotic organisms (Enuka et al., 2016; Jeck and Sharpless, 2014; Liu et al., 2016b). These RNAs are generated from back-spliced exons and form a covalently closed continuous loop. Because they do not have accessible ends for exonucleases, circRNAs are more stable than their linear counterparts (Memczak et al., 2013). The extraordinary stability, large abundance and evolutionary conservation of circRNAs indicate their important

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regulatory role in cells. Although circRNAs are being studied largely in human disease, such as cardiovascular disease, Alzheimer's disease, diabetes and cancer, the mechanism remains to be explored (Greene et al., 2017; Lei et al., 2018; Zhang et al., 2018). Moreover, whether and how circular RNA regulates IAV infection is largely unknown.

GATA zinc finger domain containing 2A (GATAD2A) plays an important role in replication of virus (Komarova et al., 2011; Konig et al., 2008). The circRNA database showed that GATAD2A is able to produce circular RNA (ID: hsa_circ_30753) (http://202.195.183.4:8000/circrnadb/detail_info.php?circ_id=hsa_circ_30753). The circ-GATAD2A amplified by RT-PCR was determined to be derived from exon 3 and exon 4. We showed that IAV infection greatly promoted the formation of circ-GATAD2A. Correspondingly, circ-GATAD2A strongly inhibited autophagy and promoted IAV replication. Thus, circ-GATAD2A might be a potential therapeutic target for the inhibition of influenza virus infection.

2. Materials and methods

2.1. Vectors and virus

The circ-GATAD2A overexpression vector was generated using the lentiviral overexpression vector pCDH-ciR-EGFP, and a nonrelated sequence served as a control. The primers are listed in Table 1. Two sgRNAs of circ-GATAD2A were designed on the website (http://crispr.mit.edu) and inserted into the pLenti-Crispr-V2-HMID.v1 vector. The sgRNA sequences and knockdown cell detection primers of circ-GATAD2A are listed in Table 1. Laboratory Influenza virus H1N1 (A/Puerto Rico/8/34) (refered to H1N1 in this study), wild-type variants of H1N1 (Pandemic 2009) and H9N2 (A/Quail/Hangzhou/1/2013(H9N2)) were stored by our laboratory.

2.2. Cell culture and transfection

A549 cells were purchased from the Chinese Academy of Sciences (Shanghai, China). It was cultured in DMEM (Thermo Scientific Gibco™, 12100038) supplemented with 10% fetal bovine serum (Thermo Scientific Gibco™, 10099141) at 37 °C in a humidified atmosphere containing 5% CO_2.

The circ-GATAD2A-overexpressing A549 cell line was constructed by a lentiviral system. The lenti-circ-GATAD2A and lenti-control A549 cell lines were both cultured in DMEM (Thermo Scientific Gibco™, 12100038) supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2.

The circ-GATAD2A knockdown A549 cell line was constructed by the Crispr-Cas9 system. The circ-GATAD2A knockdown A549 cell line and control cell line were both cultured in DMEM (Thermo Scientific Gibco™, 12100038) supplemented with 10% fetal bovine serum and puromycin (2 μg/ml) (InvivoGen, ant-pr) at 37 °C in a humidified atmosphere containing 5% CO_2.

The VPS34-knockout A549 cell line was constructed by the Crispr-Cas9 system, which was described in our recent report (Liu et al., 2018). Briefly, sgRNA (5'-AACTAGTCCTTTTGTTTCTCATTGG-3') was cloned into PX459 and was transfected into A549. The VPS34 KO cell line was then screened, verified and generated.

2.3. RNA extraction and quantitative real-time PCR

Total RNA was isolated by the TRIzol reagent (Vazyme, R401-01) following the instructions from the manufacturer. The concentration of RNA was measured, and RNA was reverse transcribed into cDNA using the reverse transcription kit (Vazyme, R211-01). Quantitative real-time PCR was performed on a Roche LC96 real-time PCR system using AceQ qPCR SYBR Green Master Mix (Vazyme, Q111-02) with specific primers. The primers were listed in Table 1. GAPDH was used as an internal control for normalization. The results were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.4. Western blot assay

Total protein was extracted from cells with lysis buffer (2% sodium dodecyl sulfate [SDS], 1% Triton X-100, 50 mM Tris-HCl, 150 mM NaCl, pH 7.5). The lysate concentration was measured using a Detergent Compatible Bradford Protein Assay Kit (Beyotime, P0006C). Equal amounts of total protein from different samples were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and the protein bands were transferred onto a nitrocellulose (NC) blotting membrane (GE Healthcare Life Science, 10600001). After blocking with 5% nonfat dry milk containing 0.1% Tween 20 for 30 min at 37 °C, the membranes were incubated with specific primary antibodies at 4 °C overnight, followed by incubation with HRP-conjugated secondary antibodies (KPL, 074-1806/074-1506) at room temperature for 1 h. GAPDH (GoodHere Technology, AB-P-R001) was used as an internal control. Finally, the bands were detected using the ECL detection system (Vazyme, E411-05).

2.5. Viral infection

Circ-GATAD2A-overexpression, circ-GATAD2A-knockdown and VPS34-knockout A549 cell lines were infected with H1N1 (MOI = 0.5). After 1 h, the cells were washed twice with sterile PBS buffer, and DMEM containing 2% fetal bovine serum was then added. Then, cells were statically cultured in an incubator for 12, 24, and 36 h. Viral titers in culture supernatants were measured by determining log10 TCID50/ml values in MDCK cells. The lysates from the above cells at 24 hpi were analyzed by Western blot.

Table 1
The primers for verification and sgRNA sequences of knockdown circ-GATAD2A.

Primer name	Sequence (5'-3')
Circ-F	TTCTGAAATATGCTATCTTACAGTGACATGAAGTCCGAGAGGA
Circ-R	ATCCTCAAGAAAAAATATATTCACCTTCTGGGCGGTGGCTTCCT
P1	GTGGCCTTGAAGGAGACTAGC
P2	CGTGGTCAGCCCATTCACTCT
circ-GATAD2A-F	CGGCAGAGTCAAATACAAAAGG
circ-GATAD2A-R	GGTCTGGAGTGATGGCTTG
GAPDH-F	GTCAGCCGCATCTTCTTTTG
GAPDH-R	GCGCCCAATACGACCAAATC
Detection-F	TCCTGAGTCCAGCATCTGGCGAGTC
Detection-R	GTCACAAACGTCTAAGAGTGTGGCT
CAS-1	TCTAGTTGATAATTAGACCC
CAS-2	GCCGCAGCTCAAAGTTGTCC

CAS-1 and CAS-2 are two different sgRNAs of circ-GATAD2A.

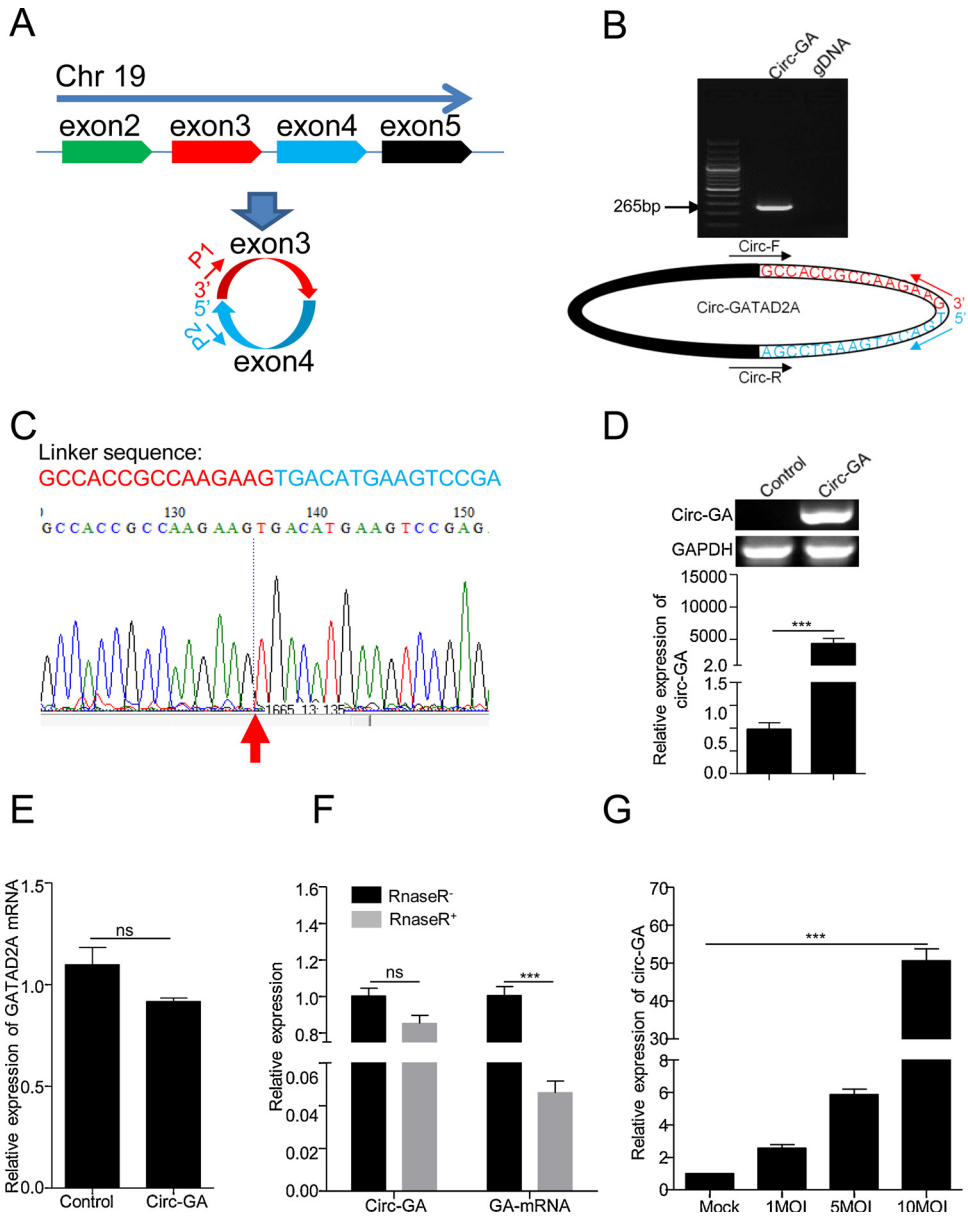

Fig. 1. H1N1 infection upregulates circGATAD2A in A549 cells. (A) Structure of circ-GATAD2A. (B) The expected size of the PCR product was detected by PCR (upper panel). The model of circ-GATAD2A formation (lower panel). (C) The exact junction sequence of the circ-GATAD2A PCR product was sequenced. (D) Verification of the circ-GATAD2A vector by nucleic acid electrophoresis and real-time PCR. (E) GATAD2A linear mRNA level was detected in circ-GATAD2A-transfected (Circ-GA) or not transfected cells. (F) Detection of circ-GATAD2A and linear GATAD2A mRNA expression in RNAse R-treated and untreated cells. (G) Detection of circ-GATAD2A by real-time PCR in H1N1-infected A549 cells at different doses.

2.6. Immunofluorescence staining

Confocal microscopy was used to observe the formation of LC3 puncta in both circ-GATAD2A overexpression and knockdown cell lines. Cells were planted in the confocal plate (In Vitro Scientific) overnight. Then, the mCherry-LC3B vector was transfected into both circ-GATAD2A overexpression and knockdown cells with jetPRIME™ Transfection Reagent (Polyplus Transfection, PT-114-07) for 24 h. Finally, the cells were fixed with 4% paraformaldehyde for 20 min at room temperature and then permeabilized with 0.1% Triton X-100 for 10 min at 4 °C. After that, the cells were scanned with a Nikon A1R/A1 laser scanning confocal microscope.

2.7. Immunoprecipitation assay

Circ-GATAD2A-overexpression cell line was separately transfected with Flag-VPS34 and Flag-N empty vector for 48 h and then lysed using NP40 lysis buffer. Cellular lysates were incubated with anti-Flag antibody and Protein A/G beads for 4 h at 4 °C. After centrifugation, the supernatants were removed and the pellets were resuspended in washing buffer. Centrifugation and resuspension of the pellets in fresh washing buffer were performed five times. Finally, the pellets were divided into two parts. One part was lysed in lysis buffer for western blot analysis, and the other part was lysed in TRIzol reagent for RNA extraction for further detection of PCR analysis.

2.8. Statistical analysis

All data were analyzed by GraphPad Prism 6 software. The comparisons between groups were performed by one-way ANOVA and Student's *t*-test. The means of $2^{-\Delta\Delta ct}$ in groups for each gene were compared. $p < 0.05$ was considered to indicate statistically significant differences. Each experiment was repeated at least three times.

3. Results

3.1. Circ-GATAD2A was formed upon H1N1 infection

A search of GATAD2A in the circRNA database showed that this gene is able to produce circular RNA derived from exon 3 and exon 4. We extracted RNA from H1N1-infected A549 cells and performed RT-PCR using a pair of inverse PCR primers, which localized at the 5' end of

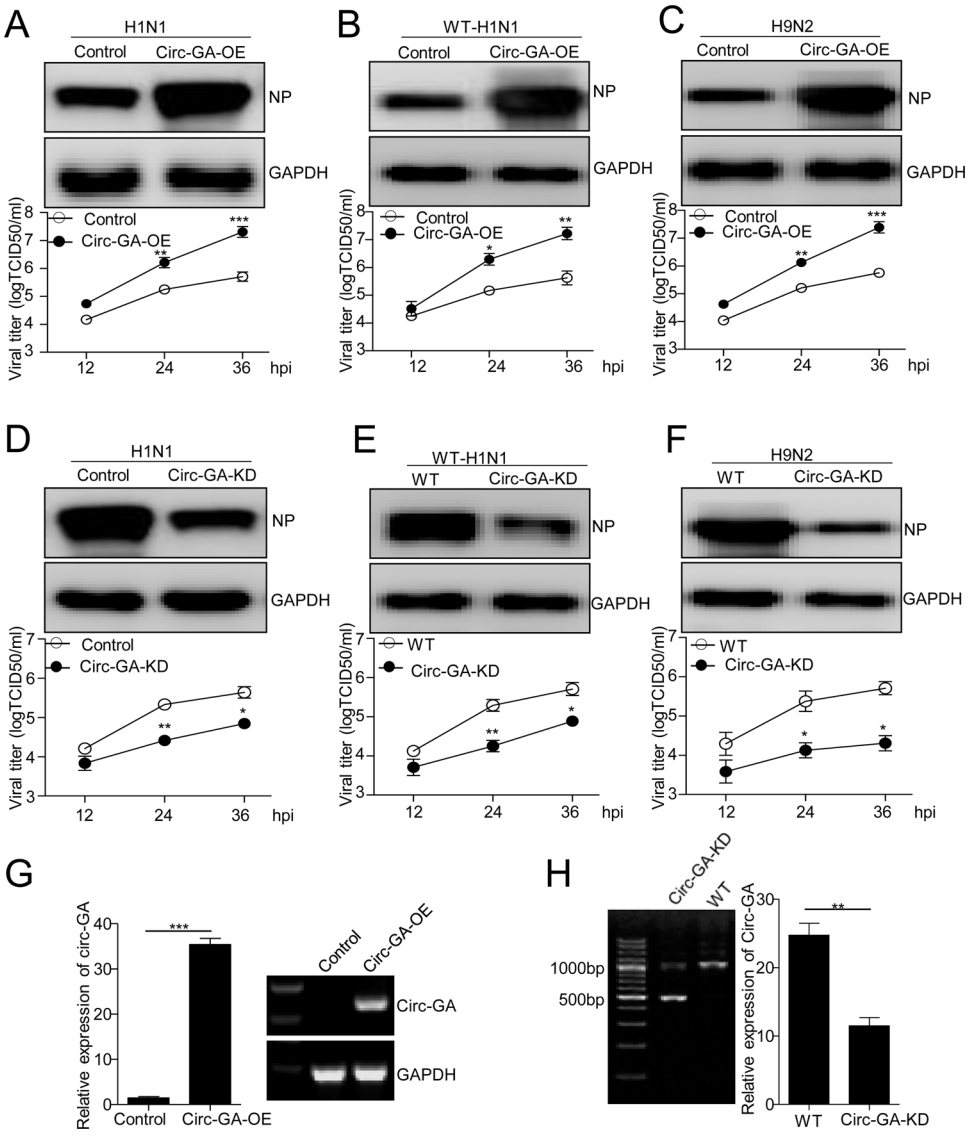

Fig. 2. Circ-GATAD2A promotes the replication of H1N1. (A–C) The expression levels of viral protein NP and titers of H1N1, WT-H1N1 or H9N2 were separately examined in circ-GATAD2A-overexpressing cells by Western blot and virus titer tests, respectively. (D–F) The expression level of the viral protein NP and titers of H1N1 were detected separately in circ-GATAD2A-knockdown cells by Western blot and virus titer tests, respectively. (G) The verification of the lenti-circ-GATAD2A cell line in the observation of the GATAD2A product by nucleic acid electrophoresis and of the circRNA expression level by real-time PCR. (H) The verification of the circ-GATAD2A-knockdown cell line in the observation of the GATAD2A genome by nucleic acid electrophoresis and of the circRNA expression level by real-time PCR.

exon 3 and the 3' end of exon 4 (Table 1, Fig. 1A). A fragment with a size of 265 bp was observed by nucleic acid electrophoresis (Fig. 1B). Then, the PCR product was cloned and sequenced (Fig. 1C). The sequencing results revealed the exact same junction (arrow) sequence of circ-GATAD2A with deposit sequence (ID: hsa_circ_30753) in circBase. To further explore the function of circ-GATAD2A, the fragment was cloned into a lentiviral overexpression vector (pCDH-ciR-EGFP). The expression of circ-GATAD2A was confirmed by real-time PCR, and the products were visually identified by nucleic acid electrophoresis (Fig. 1D, Table 1). To further understand whether circ-GATAD2A had an effect on GATAD2A linear mRNA levels, we detected linear mRNA in circ-GATAD2A-expressing cells. As shown in Fig. 1E, circ-GATAD2A expression had no influence on its mRNA level. To confirm the circularization of the expression vector, we treated RNAs extracted from vector-transfected and circ-GATAD2A-transfected A549 cells with or without Rnase R, followed by real-time PCR. Treatment with Rnase R decreased the level of GATAD2A linear mRNA but not of circ-GATAD2A (Fig. 1F).

We then tested whether H1N1 dose affects the expression level of circ-GATAD2A in A549 cells. A549 cells were seeded in 12-well plates and then infected with H1N1 virus at 1 MOI, 5 MOI and 10 MOI for 24 h. The circ-GATAD2A level was detected by real-time PCR. As shown in Fig. 1G, the relative expression level of circ-GATAD2A was indeed upregulated in a virus dose-dependent manner. Collectively, circ-GATAD2A was largely formed in response to H1N1.

3.2. Circ-GATAD2A promotes the replication of H1N1

To further explore the function of circ-GATAD2A, we generated the overexpression A549 cell line by transfecting lenti-circ-GATAD2A vector. The green fluorescence of the cell line was observed by Nikon inverted fluorescence microscopy, and the RNA level was examined by real-time PCR. Fig. 2G shows that lenti-circ-GATAD2A and lenti-control A549 cell lines both had green fluorescence, while the relative expression level of circ-GATAD2A in overexpression cells was much higher than in control cells. We found that the level of H1N1 encoding viral protein NP and virus titer were both higher in lenti-circ-GATAD2A cells than in control cells, suggesting that the expression of circ-GATAD2A promotes the replication of H1N1, as well as wild-type variants of H1N1 and H9N2 variantsin A549 cells (Fig. 2A and C).

In addition, we generated the circ-GATAD2A-knockdown cell line. Two sgRNAs against circ-GATAD2A were seperately inserted into the pLenti-Crispr-V2-HMID.v1 vector, which were then cotransfected into A549 cells. Then, the modification of the target sequence from the above cell line was verified by PCR using a pair of primers shown in Table 1. As shown in Fig. 2C, the size of the PCR product from the

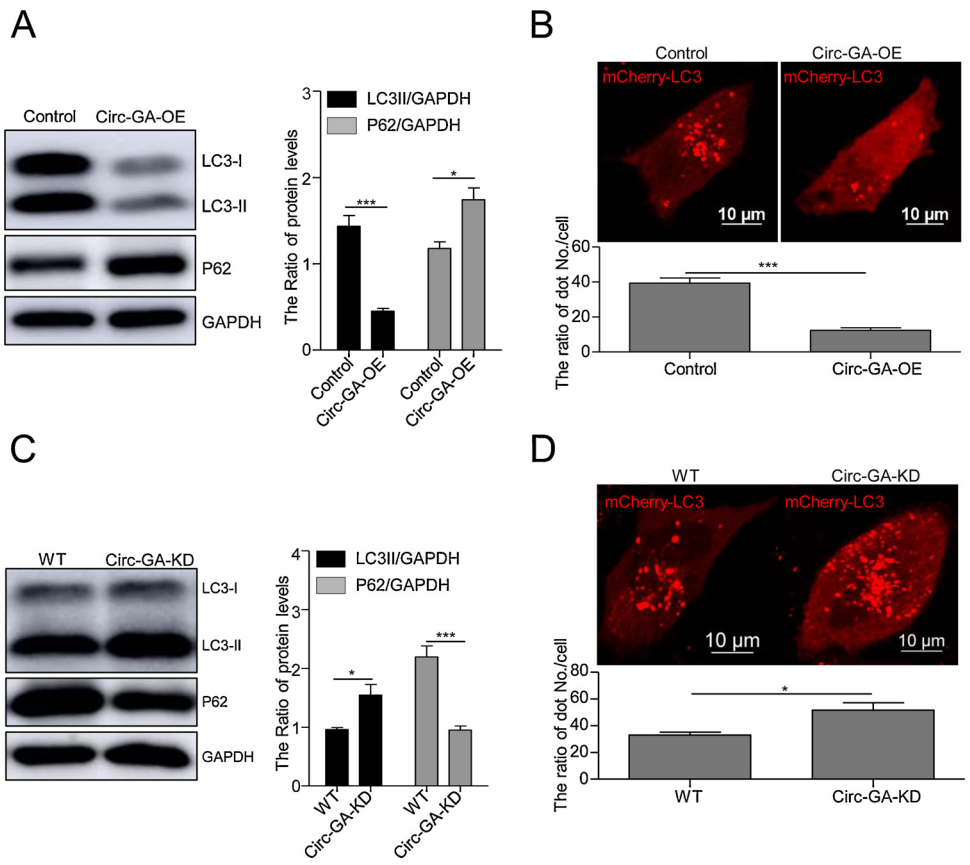

Fig. 3. Circ-GATAD2A inhibits the activity of cell autophagy. (A) The levels of LC3-II and P62 were detected by Western blot in circ-GATAD2A-overexpression cells. (B) The puncta of mCherry-LC3B were observed by laser scanning confocal microscopy in circ-GATAD2A-overexpressing cells. (C) The levels of LC3-II and P62 were detected by Western blot in circ-GATAD2A-knockdown cells. (D) The puncta of mCherry-LC3B were observed by laser scanning confocal microscopy in circ-GATAD2A-knockdown cells.

control cell line was approximately 1000 bps, while the size of the product from the circ-GATAD2A-KD cell line decreased to 500 bp, suggesting that the target sequence was indeed modified following the expression of sgRNAs in cells (Fig. 2H). The single cell line for circ-GATAD2A knockdown was then screened and generated. The circ-RNA level was examined by real-time PCR. As shown in Fig. 2C, the level of circ-GATAD2A in circ-GATAD2A-KD cells was lower than that in control cells. The effect of circ-GATAD2A on the replication of H1N1 was measured by Western blot and virus titer tests. The results showed that the level of viral protein NP in circ-GATAD2A-knockdown cells was less than that in control cells. Similarly, the titer of H1N1, as well as wild-type variants of H1N1 (WT-H1N1) and H9N2 were lower in circ-GATAD2A-knockdown cells than that in the control cells (Fig. 2D–F). Collectively, circ-GATAD2A promotes H1N1 replication.

3.3. circ-GATAD2A inhibited autophagy

Autophagy plays a critical role in the removal of intracellular viruses, so we then tested whether circ-GATAD2A affected autophagy. P62 and LC3-II are two well-known markers for measuring autophagy. Therefore, the levels of P62 and LC3-II were tested in circ-GATAD2A-overexpressing and circ-GATAD2A-knockdown A549 cell lines. The results showed that the P62 level increased and the LC3-II level decreased significantly in circ-GATAD2A expression cell line in comparison to the control cell line. In contrast, the P62 level decreased dramatically and the LC3-II level increased greatly in circ-GATAD2A knockdown A549 cells compared to control cells (Fig. 3A and C).

After that, we also performed statistical analysis of LC3-II puncta in both cell lines by using laser confocal microscopy to further verify the effect of circ-GATAD2A on autophagy. We transfected the mCherry-LC3B vector into both cell lines for 24 h. Finally, we observed that puncta of LC3-II in lenti-circ-GATAD2A cells were less than in lenti-control cells, while puncta of LC3-II in circ-GATAD2A-knockdown cells increased greatly in comparison to control cells (Fig. 3B and D). The above results strongly suggest that circ-GATAD2A negatively regulates cell autophagy.

3.4. Circ-GATAD2A promotes H1N1 replication by inhibiting autophagy

Since circ-GATAD2A could promote the replication of H1N1 and inhibit autophagy, we further explored whether autophagy affects H1N1 replication. We generated an autophagy-deficient cell line by knocking out VPS34, which is necessary for autophagy in mammalian cell lines. VPS34 KO cell lines and control cell lines were infected with H1N1 for the indicated times, and then virus titer tests and WB blot analyses were performed. The results showed that the level of viral protein NP and the titer of H1N1 increased in VPS34 KO cells (Fig. 4A), suggesting that H1N1 replication was enhanced in autophagy-deficient cells. Correspondingly, the level of viral protein NP and H1N1 titer decreased when VPS34 was expressed in VPS34 KO cells (Fig. 4B), suggesting that rescue of autophagy impaired replication of H1N1 in VPS34 KO cells. We then tested whether circ-GATAD2A further promotes H1N1 replication in VPS34 KO cell lines. As shown in Fig. 4C, the levels of viral protein NP or virus titer are similar between circ-GATAD2A transfected and nontransfected VPS34-KO cell lines (Fig. 4C), suggesting that circ-GATAD2A promotes the replication of H1N1 through autophagy.

Since KO of VPS34 compromised effects of circ-GATAD2A on increasing H1N1 titers. We therefore tested weather circ-GATAD2A interacted with VPS34. Flag-VPS34 or Flag-Vector was overexpressed in circ-GATAD2A overexpressed cell lines. RT-PCR analysis of Flag IP samples showed that circ-GATAD2A could be detected in VPS34 overexpressed samples (Fig. 4D), suggesting VPS34 interacted with circ-GATAD2A.

Collectively, *Circ-GATAD2A promotes H1N1 replication by inhibiting autophagy*.

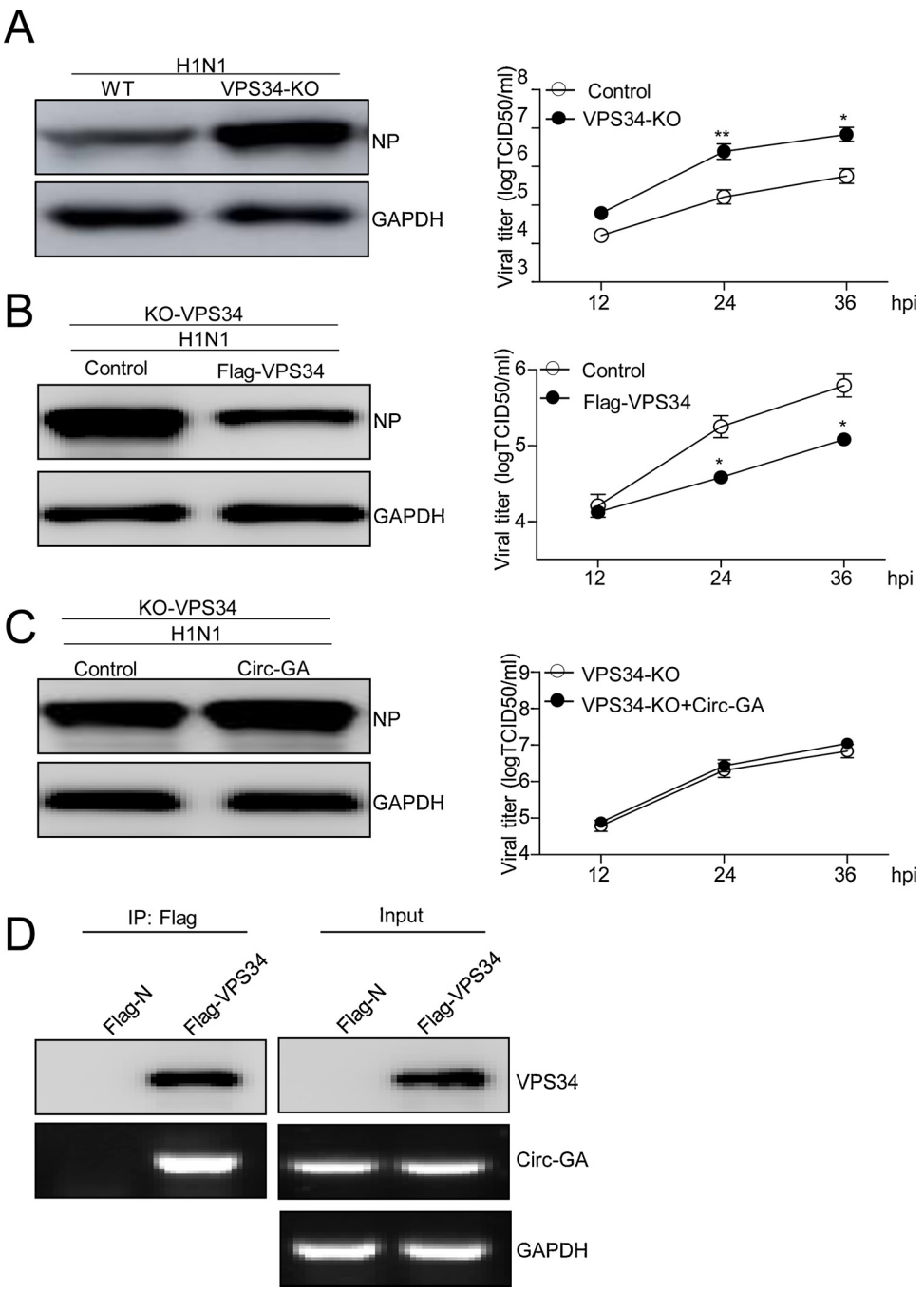

Fig. 4. Circ-GATAD2A promotes H1N1 virus replication by inhibiting autophagy. (A) The expression level of the viral protein NP and titers of H1N1 were detected in VPS34-KO cells by Western blot and virus titer tests, respectively. (B) The expression level of the viral protein NP and titers of H1N1 were detected separately in VPS34-overexpressing cells by Western blot and virus titer tests, respectively. (C) The expression level of viral protein NP and titers of H1N1 were detected by Western blot and virus titer tests, respectively, in VPS34-KO cells, which were transfected with circ-GATAD2A or with the control. (D) VPS34 interacts with Circ-GATAD2A.

3.5. Inhibition of LC3-II expression by circ-GATAD2A is compromised by M2

We measured the autophagy in wild-type, lenti-circ-GATAD2A and circ-GATAD2A knockdown A549 cells after infection by H1N1. The results showed that H1N1 infection promoted the formation of LC3-II, and also increased the level of P62, suggesting that H1N1 infection inhibits the maturation of autophagosome (Fig. 5A). Interestingly, H1N1 infection also increased the LC3-II level in circ-GATAD2A over-express cell lines (Fig. 5A), suggesting that H1N1 infection compromised the inhibition of LC3-II formation by circ-GATAD2A. It's well known that M2 encoded by IAV is involved in induction of autophagy (Zhirnov and Klenk, 2013) and inhibits the maturation of autophagosome (Gannage et al., 2007). Although circ-GATAD2A inhibits the formation of LC3-II and increase the P62 level, M2 compromised the role of circ-GATAD2A on inhibition of LC3-II formation (Fig. 5B), but it still inhibited the maturation of autophagosome, suggesting that circ-GATAD2A might plays role in inhibiting autophagosome maturation in parallel.

4. Discussion

Our study showed that circ-GATAD2A is highly expressed in response to IAV infection. Circ-GATAD2A promotes IAV infection by inhibiting autophagy.

A previous study showed that IAV infection inhibits fusion between autophagosomes and lysosomes by binding M2 to Beclin-1 (Zhang et al., 2014). However, whether autophagy protects hosts from IAV infection is still largely unknown. TUFM-regulated autophagy has been shown to inhibit influenza virus replication (Kuo et al., 2017). Other studies

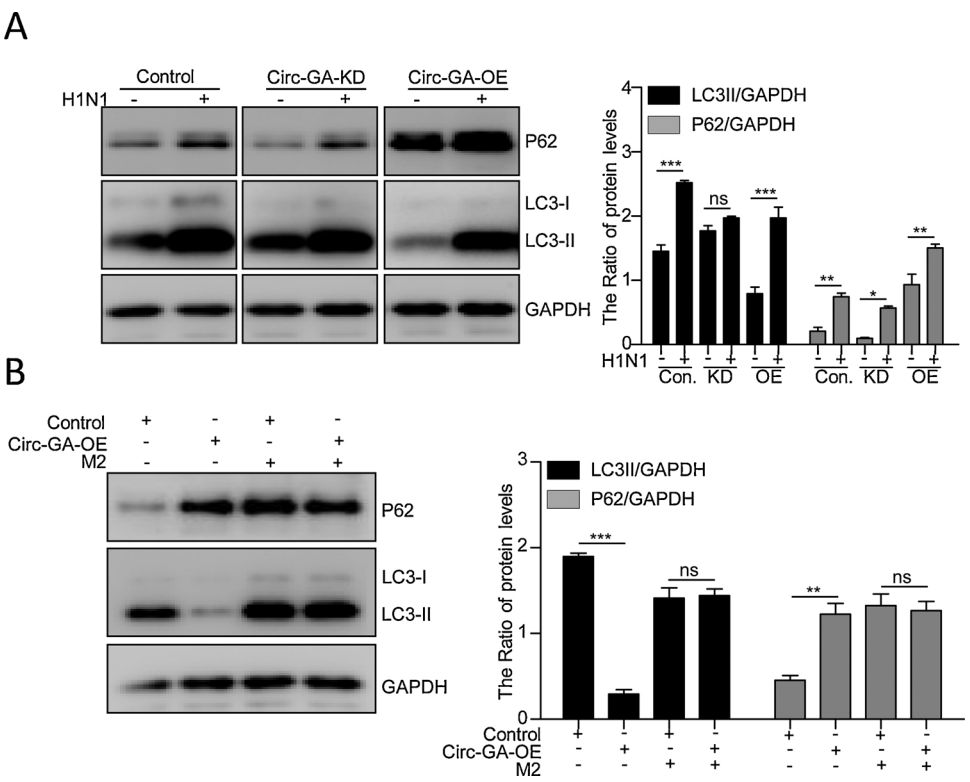

Fig. 5. H1N1 infection or M2 expression rescue the expression of LC-II in circ-GATAD2A overexpression cell lines. (A) Impacts of H1N1 infection on autophagy in WT A549, circ-GATAD2A-overexpression cell lines, or circ-GATAD2A-KD cell lines. (B) Impacts of H1N1 M2 expression on autophagy in WT or circ-GATAD2A-overexpression A549 cell lines.

suggested that induction of autophagy in a therapeutic approach inhibited IAV replication (Feizi et al., 2017). Therefore, induction of autophagy in a specific manner is able to inhibit IAV replication. In our study, VPS34 KO resulted in blocking autophagy and increasing the IAV titer, indicating that VPS34 might be a very important modulator in controlling autophagy specific to IAV inhibition. Similarly, circ-GATAD2A inhibited autophagy and promoted replication of H1N1. Interestingly, circ-GATAD2A did not increase virus replication more in VPS34 KO cell lines than in control cell lines, suggesting that it regulates virus replication through VPS34-dependent autophagy. Interestingly, circ-GATAD2A interacted with VPS34 (Supplementary Fig. 4D), suggesting that circ-GATAD2A works on upstream of VPS34). But whether circ-GATAD2A impacts on activity of VPS34 requires further study. Therefore, circ-GATAD2A is emerging as a modulator utilized by viruses to block autophagy and promote virus replication.

Circular RNA regulates autophagy and subsequently affects many downstream functions. Circular RNA ACR inhibits autophagy and therefore impairs myocardial ischemia and reperfusion injury (Zhou et al., 2018a). Knockdown of circRNA2837 inhibits autophagy and subsequently protects sciatic Nerve from injury (Zhou et al., 2018b). By contrast, circular RNA circ-DNMT1 increases autophagy and promotes breast cancer progression (Du et al., 2018). In our study, circ-GATAD2A inhibited autophagy and therefore promoted IAV infection. Thus, we could expect that circ-GATAD2A might play multiple roles in various cellular functions due to its effect on autophagy.

CircRNA plays a complicated role in inhibiting virus infection in different ways. Foreign circular RNA can be recognized by RIGI and subsequently induced expression of 84 innate immunity-related genes (Chen et al., 2017). In comparison to linear RNA-expressing cells, the Venezuelan equine encephalitis virus infection rate decreases by 10-fold in circRNA-expressing cells. By contrast, overexpression of circ-POLR2A inversely facilitates VSV infection by competing with viral mRNA for binding to immune factor NF90 and NF110 (Li et al., 2017). In our study, circ-GATAD2A facilitates the replication of influenza virus in A549 cell lines by blocking VPS34-dependent autophagy, which inhibits virus replication. Therefore, circ-GATAD2A could be a potential drug target for inhibiting viral infection.

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Conflicts of interest

The authors declare that there is no conflicts of interest associated with this manuscript.

Contribution statement

All authors made substantial contributions to the concept and design of this study, acquisition of data or analyses and interpretation of data and drafting of the article. All authors gave final approval of the version to be published.

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