Original Paper

Yin Yang 1 Dynamically Regulates Antiviral Innate Immune Responses During Viral Infection

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Key Words

Yin Yang 1 • Type I Interferon • Signal Transducer and Activator of Transcription • Interferon-Stimulated Gene • Antiviral Innate Immune Response

Abstract

Background/Aims: Type I interferon (IFN-1) production and IFN-1 signaling play critical roles in the host antiviral innate immune responses. Although transcription factor Yin Yang 1 (YY1) has been reported to have a dual activator/repressor role during the regulation of interferon beta (IFN- β) promoter activity, the roles of YY1 in the regulation of upstream signaling pathways leading to IFN-1 induction and IFN-1 signaling during viral infection remain to be elucidated.

Methods: The roles of YY1 in IFN-1 production and IFN-1 signaling were investigated using immunoblotting, real-time PCR, small interfering RNA (siRNA)-mediated YY1 knockdown, YY1 overexpression by transient transfection, and co-immunoprecipitation, using mouse cells.

Results: YY1 was shown to interact with STAT1 in the absence of viral infection. Following viral infection, YY1 protein expression levels were decreased. YY1 knockdown led to a considerable downregulation of phosphorylated (p) TBK1 and pIRF3 expressions, while YY1 overexpression significantly upregulated pTBK1 and pIRF3 expression levels and promoted virus-induced IFN- β production. Additionally, YY1 knockdown led to a significant upregulation of pSTAT1, pSTAT2 and antiviral interferon-stimulated genes, and inhibited viral replication. **Conclusion:** We demonstrated here that YY1 interacts with STAT1 and dynamically regulates the induction of IFN-1 production and activation of IFN-1 signaling in different stages during viral infection.

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Published by S. Karger AG, Basel

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Introduction

Type I Interferons (IFNs), primarily including IFN- α and IFN- β , play essential roles during the activation of antiviral innate immune responses [1]. In non-infected cells, IFN genes are maintained in a constitutive transcriptionally repressed state and are tightly regulated by latent transcription factors. Upon the recognition of invading viruses by cellular pattern-recognition receptors (PRRs), such as retinoic acid-inducible gene I (RIG-I), which senses viral double-stranded RNA, or Toll-like receptors, which recognize exogenous ligands, IFN transcription factors are activated to promote the transcription of the IFN genes, which are turned on 4 to 6 h after virus infection, and turned off 10 to 12 h after the infection [2-4]. The critical factor for the initiation of IFN response is interferon regulatory factor 3 (IRF-3), which is phosphorylated by TANK-binding kinase 1 (TBK-1) during viral infection and then forms dimers that translocate into nucleus to promote IFN expression [5].

The secreted early IFNs, predominantly IFN- β , have antiviral effects through the binding to the IFN- α/β receptor (IFNAR) on the cellular membrane surface to activate Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway [6]. IFN- α/β interacting with IFNAR induces the activation of the JAK protein tyrosine kinases that phosphorylate STAT1 and STAT2 [7]. Phosphorylated STAT1 (pSTAT1) and STAT2 (pSTAT2) interact with IFN regulatory factor 9 (IRF9) to form a heteromeric complex (IFN-stimulated gene factor 3 [ISGF3]), which translocates into the nucleus and binds to an IFN-stimulated response element to induce the expression of IFN-stimulated genes (ISGs), such as Mx1, interferon-inducible protein 204 (IFI204), interferon induced transmembrane protein 3 (IFITM3), and ISG20 [8-11]. All IFN-induced biological responses are believed to be mediated by ISGs, leading to the induction of intrinsic antiviral processes [12].

In addition to the effects of IFN- β and ISGs during antiviral response, IFN- β and ISGs are also involved in the development of several inflammatory and autoimmune diseases [13, 14]. Abnormal expression of IFN- β and ISGs is deleterious for the organism, and thus their expression need to be tightly regulated to avoid the inappropriate expression in the absence of viral infection. The molecular mechanisms underlying the induction of IFN- β expression have been investigated and it was shown that promoter transcription factors recruitment coupled with chromatin remodeling events are required for this [15, 16]. One of key transcription factors binding to *IFN- β* promoter is Yin Yang 1 (YY1), which binds to the murine *IFN- β* promoter and regulates its transcriptional capacity [17, 18]. YY1, a ubiquitous, highly conserved zinc finger transcription factor, functions to either activate or repress expression through directly binding to a consensus element in the promoters of a high number of genes, including *c-Myc*, *c-Fos*, β -casein, human immunodeficiency virus type I, and several other cellular or viral genes [19-22]. Furthermore, YY1 interacts with co-activators and co-repressors, which modulate its regulatory behavior [23, 24]. Although YY1 has been reported to suppress the IFN- β promoter by binding to the -90 site at the promoter, and to activate the IFN- β promoter by simultaneous occupancy of both sites at -90 and -122 of the promoter [17, 18], it remains to be elucidated how YY1 regulates the IFN- β upstream signaling pathways during viral infection and how YY1 modulates IFN- β -mediated transcriptional induction of antiviral ISGs.

Here, we analyzed YY1 as an antiviral innate immune response regulator in various stages of viral infection, and its effects on IFN-1 production and signaling. Additionally, we aimed to identify YY1 interaction partners in IFN-1 signaling pathways.

Materials and Methods

Animals, cells and viruses

C57BL/6 mice (5-6 weeks old) were obtained from Medical Laboratory Animal Center of Guangdong (Guangzhou, China). All animal experiments and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Guangdong Provincial Hospital of Chinese Medicine (Guangzhou, China).

Mouse embryonic fibroblast cells (MEFs) (SCRC-1040, purchased from American Type Culture Collection) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco) at 37°C with 5% CO₂. Thioglycolate-elicited peritoneal macrophages were collected from the mice by infusing their cavity with 5 mL ice-cold sterile phosphate-buffered saline solution, as previously reported [25, 26]. Collected cells were centrifuged and the cell pellets were resuspended in DMEM supplemented with 10% FBS and then incubated at 37°C with 5% CO₂. After 1 h, non-adherent cells were removed. Cells were infected with vesicular stomatitis virus (VSV), or herpes simplex virus type I (HSV-1) (provided by professor Jin-Yang Zhang, Kunming University of Science and Technology, Kunming, China) at the indicated multiplicity of infection (MOI) for the indicated hours, as described previously [27].

Plasmids, siRNA and cell transfection

Mouse *YY1* and *STAT1* genes were amplified using following primers: 5'-CCCAAGCTTATGGCCTCGGGC-GACACCCTCTA-3' and 5'-CCGCTCGAGTCACTGGTTGTTTGGCTTTA-3'; and 5'-ACGCGTCGAC CATGTCA-CAGTGGTTCGAG-3' and 5'-CGGGGTACCTTATACTGTGCTCATCAT-3', respectively, and then cloned into pC-MV-flag-N-vector or pCMV-Myc-N-vector, respectively, as described previously [27]. The small interfering RNAs (siRNAs) against *YY1* (siYY1) consisted the following sequences: 5'-GAACUCACCUCCUGAUUAUTT-3' and 5'-AUAACAGGAGGUGAGUUCTT-3'. siRNA against *STAT1* (siSTAT1) consisted the following sequences: 5'-CACAGUUUUUAUCCUGAUGA-3' and 5'-UCAUCAGGAUAAAACUGUG-3'. Scrambled siRNA (siNC) oligonucleotide duplex with the following sequences: 5'-UUCUCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGUUCG-GAGAATT-3' was used as control. siRNAs were synthesized by Shanghai GenePharma Co., Ltd. Plasmids and siRNA were transfected using Exfect Transfection Reagent (Vazyme Biotech, Nanjing, China) in the indicated cells, as described previously [27]. After 24 hours post-transfection (hpt), cells were processed for further analysis.

Western blotting and co-immunoprecipitation

Cellular samples were collected and lysed with NP40 lysis buffer (P0013F, Beyotime, China). Whole cell lysates were subjected to SDS-PAGE and immunoblotting as previously described [28]. Primary antibodies against YY1 (ab109237, Abcam), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ab8245, Abcam), TBK1 (ab40676, Abcam), phosphorylated TBK1 (pTBK1) (ab109272, Abcam), IRF3 (ab68481, Abcam), phosphorylated IRF3 (pIRF3) (ab76493, Abcam), Lamin A (ab8980, Abcam), IRF9 (ab51639, Abcam), STAT1 (14994, Cell Signal Technology), phosphorylated STAT1 (pSTAT1) (9167, Cell Signal Technology), STAT2 (72604, Cell Signal Technology), phosphorylated STAT2 (pSTAT2) (ab53132, Abcam), flag (ab18230, Abcam) and Myc (ab18185, Abcam) were used. Co-immunoprecipitation was performed as previously described [29].

IFN-β Treatment

Mouse macrophages were serum-starved for 12 h and then stimulated with 150 U/ml IFN-β (ab84998, Abcam) dissolved in 0.1% bovine serum albumin for 15, or 30 min at 37°C. Macrophage cells were then processed for further analysis.

ELISA

IFN-β in the cultural supernatants was measured by a IFN beta Mouse ProcartaPlex Simplex Kit (EPX01A-26944-901, ThermoFisher Scientific), according to the manufacturer's instructions.

Nuclear and cytoplasmic fractionation

Nuclear and cytosolic fractions were extracted from the analyzed cells using a nuclear and cytoplasmic extraction kit (Beyotime), according to the manufacturer's instructions.

Viral plaque assay

Viral titers were quantified using plaque assays. Briefly, mouse macrophages were transfected with siNC or siYY1 for 24 h and then infected with VSV at an MOI of 1 for 24 h. The supernatants of cells culture were used to infect monolayers of Vero cells. One hour later, the supernatants were removed and the infected Vero cells were washed with PBS twice followed by incubation with DMEM containing 2% methylcellulose for 48 h. The cells were fixed with 4% paraformaldehyde for 15 min and stained with 1% crystal violet for 30 min before counting the plaques.

RNA preparation and real-time PCR (RT-PCR)

Total RNA was extracted from cells using RNA Isolater Total RNA Extraction Reagent (R401-01, Vazyme). RNA (500 ng) from each sample was reverse-transcribed into cDNA using the PrimeScript RT reagent kit (Takara). RT-PCR was performed using the 7500 real-time PCR system (Applied Biosystems), with AceQ qPCR SYBR Green Master Mix (Q111-02, Vazyme). RT-PCR primers were as follows: *MX1*, 5'-CTTTC-CAGTCCAGCTCGGCA-3' and 5'-AGCTGCTGGCCGTACGTCTG-3'; *IFI204*, 5'-GACAACCAAGAGCAATACACCA-3' and 5'-ATCAGTTTGCCCAATCCAGAAT-3'; *IFITM3*, 5'-CCCCAACTACGAAAGAATCA-3' and 5'-ACCATCTTC-CGATCCCTAGAC-3'; *RSAD2*, 5'-AGCATTAGGGTGGCTAGATCC-3' and 5'-CTGAGTGCTGTCCCATCTTC-3'; *CXCL10*, 5'-CCAAGTGCTGCCGTCATTTTC-3' and 5'-GGCTCGCAGGGATGATTTC-3'; *ISG20*, 5'-TGGGCCT-CAAAGGGTGAGT-3' and 5'-CGGGTCGGATGTACTTGTCTCATA-3'; *YY1*, 5'-GTGGTTGAAGAGCAGATCATTGG-3' and 5'-TTGCTTAGGGTCTGAGAGGTC-3'; VSV (Indiana serotype), 5'-ACGGCGTACTTCCAGATGG-3' and 5'-CTCG-GTTCAAGATCCAGGT-3'; *STAT1*, 5'-TGATGGCCCTAAAGGAAGT-3' and 5'-CAGAGCCCACTATCCGAGAC-3'; *GAPDH*, 5'-TCAACAGCAACTCCCACTCTTCCA-3' and 5'-ACCCTGTTGCTGTAGCCGTATTCA-3'. The obtained data were normalized to *GAPDH* expression levels in each sample.

Statistical analysis

Data were statistically analyzed and graphed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). All results were presented as mean values $\pm$ standard deviations. Statistically significant differences between groups were determined by the Student's *t*-test. **P* < 0.05 and ***P* < 0.01 were considered statistically significant.

Results

Viral infection induces the downregulation of YY1 expression

YY1 has been reported to be an important regulator of IFN- β production [17, 18], suggesting that it may be closely involved in the antiviral innate immune responses during viral infections. We analyzed RNA virus VSV-infected MEFs at 0, 4, 6, 8, 10 and 12 hours-post infection (hpi) and showed that despite a certain degree of reduction, YY1 protein levels were relatively stable from 0 to 8 hpi (Fig. 1A and B). However, YY1 levels were significantly downregulated at 10 and 12 hpi, compared with those at 0 hpi. Furthermore, we determined YY1 protein and mRNA expression levels in MEFs or macrophages infected by VSV or DNA virus HSV with different MOIs at 12 hpi. The results showed that YY1 protein (Fig. 1C, D, E and F) and mRNA (Fig. 1G and H) levels were all significantly downregulated in VSV- or HSV-infected MEFs or macrophages.

YY1 promotes virus-induced IFN- β production

IFN- β production is usually initiated at 4 to 6 hours after virus infection [2-4], and therefore, we speculate that YY1 may play a role in IFN- β production during viral infection. To test this hypothesis, we detected the IFN- β mRNA expression levels in YY1-overexpressed MEFs infected with VSV, which was shown to induce the production of IFN- β and is widely used in IFN antiviral studies [30], at 6 hpi by RT-PCR. The results in Fig. 2A and B showed that IFN- β mRNA expression levels in YY1-overexpressed cells infected with VSV were critically higher than those in the empty vector-transfected cells infected with VSV, and that YY1 overexpression alone did not significantly affect IFN- β mRNA expression, which were consistent with the results of ELISA (Fig. 2B). Furthermore, we investigated the effects of YY1 overexpression on IFN- β transcription factors by immunoblotting. The results in Fig. 2C and D showed that YY1 overexpression led to a significant upregulation of pTBK1 and pIRF3 expression, but not that of the total TBK1 and IRF3. Consistently, YY1 knockdown, which was confirmed by Western blotting and RT-PCR assays (Fig. 2E and F), significantly inhibited IFN- β production (Fig. 2G) and led to a considerable downregulation of pTBK1 and pIRF3 expression in both the cytosolic and nuclear fractions from VSV-infected macrophages at 6 hpi (Fig. 2H and I).

Fig. 1. Viral infection induces the downregulation of YY1 expression. (A and B) MEF cells were infected with VSV at an MOI of 1 and cultured for 0, 4, 6, 8, 10 and 12 h. At different time points, cells were lysed and subjected to immunoblotting to determine the expressions of YY1 and GAPDH. Relative expression levels of YY1 were calculated by normalizing to that of GAPDH, respectively. (C and D) MEF or macrophage cells (MC) were infected with VSV at different MOIs of 0.1, 1, 5, or 10. At 12 hpi, cells were lysed and subjected to immunoblotting to determine the expressions of YY1 and GAPDH. Relative expression levels of YY1 were calculated by normalizing to that of GAPDH, respectively. (E and F) MEF or MC cells were infected with HSV at different MOIs of 0.1, 1, 5, or 10. At 12 hpi, cells were lysed and subjected to Western blotting to determine the expressions of YY1 and GAPDH. Relative expression levels of YY1 were calculated by normalizing to that of GAPDH, respectively. (G and H) MEF or MC cells were infected with VSV (G) or HSV (H) at an MOI of 1 for 12 h. The cells were then collected for RNA extraction. RT-PCR was performed to detect YY1. GAPDH gene was used as control. Data are represented as means $\pm$ SD (n=3; *represents P<0.05, **represents P<0.01).

the expressions of YY1 and GAPDH. Relative expression levels of YY1 were calculated by normalizing to that of GAPDH, respectively. (G and H) MEF or MC cells were infected with VSV (G) or HSV (H) at an MOI of 1 for 12 h. The cells were then collected for RNA extraction. RT-PCR was performed to detect YY1. GAPDH gene was used as control. Data are represented as means $\pm$ SD (n=3; *represents P<0.05, **represents P<0.01).

YY1 interferes with IFN- β signaling by interacting with STAT1

IFN- β expression leads to the phosphorylation of STAT1 and STAT2, which then combined with IRF9 to form a tripartite transcription factor ISGF3 complex, and this complex translocates into the nucleus, driving the expressions of numerous ISGs, such as MX1 and IFI204, to play antiviral effects [6, 31]. To further investigate the biological significance of the observed downregulation of YY1 expression 8 hours after viral infection, when IFN- β is already being expressed, we further analyzed the effects of YY1 knockdown on the IFN- β signaling by immunoblotting. The obtained results in Fig. 3 demonstrated that pSTAT1 and pSTAT2 expression levels were significantly upregulated in the cytosolic and nuclear fractions of IFN- β -treated macrophages at 15 and 30 minutes post-treatment, compared with those detected in the absence of IFN- β -treatment at 0 minute, which is consistent with previous study [32]. Surprisingly, upregulation of pSTAT1 and pSTAT2 expression in siYY1-transfected cells with IFN- β -treatment was markedly higher than that in siNC-transfected cells (Fig. 3). Additionally, STAT1 expression level, but not that of STAT2, significantly increased in siYY1-transfected cells, compared to that in siNC-transfected cells (Fig. 3).

Fig. 2. YY1 promotes virus-induced IFN- β production. (A, B, C and D) MEF cells were transfected with pCMV-flag or pCMV-flag-YY1 for 24 h and then infected with VSV at an MOI of 1 for 6 h. The cells were collected for cellular RNA extraction or for protein lysis. RT-PCR was performed to detect YY1 (A) and IFN- β (B). GAPDH gene was used as control. (B) IFN- β in the supernatants was measured by ELISA. (C) Western blotting was performed to determine the expressions of pTBK1, TBK1, pIRF3, IRF3 and GAPDH. (D) Relative expression levels of pTBK1, TBK1, pIRF3, and IRF3 were calculated by normalizing to that of GAPDH, respectively. (E and F) Macrophage cells were transfected with siNC or siYY1 for 24 h and then YY1 expression was detected by Western blotting (E) and RT-PCR (F). (G, H and I) siNC or siYY1-transfected macrophages were infected with VSV at an MOI of 1 for 6 h. (G) IFN- β in the supernatants was measured by ELISA. (H) Western blotting was performed to determine the expressions of pTBK1, TBK1, pIRF3, IRF3 in the cytosolic fraction (Cyto Fraction) and nuclear fraction (Nuc Fraction). GAPDH as a cytosolic protein maker, and Lamin A as a nuclear protein maker. (I) Relative expression levels of pTBK1 and pIRF3 in the cytosolic and nuclear fractions were calculated by normalizing to that of GAPDH or Lamin A, respectively. Data are represented as means $\pm$ SD (n=3; *represents P<0.05, **represents P<0.01).

Furthermore, we investigated the mechanism underlying the effects of YY1 on IFN- β signaling activation. We hypothesized that YY1 may interact with ISGF3, interfering with IFN- β signaling. Co-immunoprecipitation analyses demonstrated that flag-YY1 interacts with endogenous STAT1, but not STAT2 or IRF9 (Fig. 4A), which was further validated by reciprocal co-immunoprecipitation using Myc-STAT1 to pull down endogenous YY1 from the precipitated lysates (Fig. 4B). Moreover, co-immunoprecipitation analyses further demonstrated that endogenous YY1 and STAT1 interact with each other (Fig. 4C). Subsequently, to investigate whether the inhibitory effect of YY1 on IFN- β signaling was related to STAT1, we further analyzed the effects of STAT1 knockdown on the inhibitory effect of YY1 on IFN- β signaling by RT-PCR. The results in Fig. 4E showed that IFN- β treatment increased the expressions of *MX1* and *IFI204*, and YY1 overexpression significantly blocked IFN- β -

Fig. 3. Effects of YY1 knockdown on IFN- β signaling. Macrophages were transfected with siNC or siYY1 for 24 h and then treated with recombinant mouse IFN- β for 15 or 30 min. The cells were lysed and isolated for the cytosolic and nuclear fractions. Western blotting was performed to determine the expressions of YY1, pSTAT1, STAT1, pSTAT2, STAT2 in the Cyto fraction (A) and the Nuc fraction (C), GAPDH as a cytosolic protein maker, and Lamin A as a nuclear protein maker. Relative expression levels of pSTAT1, STAT1, pSTAT2, STAT2 in the Cyto fraction (B) and the Nuc fraction (D) were calculated by normalizing to that of GAPDH or Lamin A, respectively. Data are represented as means $\pm$ SD (n=3; *represents P<0.05, **represents P<0.01).

induced upregulation of *MX1* and *IFI204*. Besides, as expected, STAT1 knockdown, which was confirmed by RT-PCR (Fig. 4D), significantly inhibited IFN- β -induced upregulated expressions of *MX1* and *IFI204*, and the inhibitory effect was more evident than that of YY1 overexpression. Moreover, the expressions of *MX1* and *IFI204* in YY1-overexpressed and STAT1-knockdown cells were comparable to those in STAT1-knockdown cells, but were much lower than those in YY1-overexpressed, or YY1 and siNC-cotransfected cells, suggesting that YY1 suppresses IFNs signaling depends on STAT1. Taken together, these results indicated that YY1 interacts with STAT1 to suppress IFNs signaling.

YY1 knockdown promotes antiviral ISGs expression and inhibits viral replication

IFN- β signaling-induced antiviral immune responses are considered to be mediated by ISGs, such as *Mx1*, *IFI204*, *IFITM3*, *CXCL10*, and *ISG20* [8, 10, 11]. To investigate the effects of YY1 knockdown on the expressions of antiviral ISGs and viral replication, we detected the mRNA expression levels of *Mx1*, *IFI204*, *IFITM3*, *RSAD2*, *CXCL10*, and *ISG20* in siYY1-transfected macrophages infected with VSV at 24 hpi by RT-PCR. The results in Fig. 5A showed that the expression of these genes was significantly upregulated in siYY1-transfected cells, compared with those in control. Moreover, YY1 knockdown considerably inhibited VSV replication (Fig. 5B and C).

Discussion

As a repressor and an activator, YY1 plays important roles during development, tumorigenesis, and IFN production, by regulating gene expression and protein modifications [17, 18, 33]. Additionally, YY1 was reported to suppress the replication of some viruses, such as human immunodeficiency virus type I, hepatitis B virus, and Rift Valley fever virus [19, 34, 35]. The roles of YY1 in the induction of IFN-1 production and signaling during viral infection remain to be elucidated. We report here that YY1 plays crucial roles in the antiviral innate immune responses by dynamically inducing IFN-1 production and signaling during viral infection. For the first time, we demonstrated that YY1 interacts with STAT1 in the absence

Fig. 4. YY1 interacts with STAT1. (A) MEF cells were transfected with pCMV-flag or pCMV-flag-YY1 for 24 h. Whole cell lysates were blotted directly after immunoprecipitation for flag-YY1 using an anti-flag antibody. Blotting was performed using anti-STAT1, anti-STAT2, anti-IRF9 or GAPDH antibodies as indicated. (B) MEF cells were transfected with pCMV-myc or pCMV-myc-STAT1 for 24 h. Whole cell lysates were blotted directly after immunoprecipitation for myc-STAT1 using an anti-myc antibody. Blotting was performed using anti-YY1 or GAPDH antibodies as indicated. (C) Whole MEF cell lysates were blotted directly after immunoprecipitation for YY1 or STAT1 using a specific anti-YY1 or anti-STAT1 antibody. Blotting was performed using anti-YY1, anti-STAT1, or GAPDH antibodies as indicated. (D) MEF cells were transfected with siNC or siSTAT1 for 24 h and then STAT1 expression was detected by RT-PCR. (E) MEF cells were transfected with siNC, siSTAT1 and/or pCMV-flag-YY1, or not (NT) for 24 h. Then, the cells were treated with IFN- β (150 U/ml) or not for 24 h. The cells were collected for RNA extraction. RT-PCR was performed to detect MX1 and IFI204. GAPDH gene was used as control. Data are represented as means $\pm$ SD (n=3; *represents P<0.05, **represents P<0.01).

indicated. (B) MEF cells were transfected with pCMV-myc or pCMV-myc-STAT1 for 24 h. Whole cell lysates were blotted directly after immunoprecipitation for myc-STAT1 using an anti-myc antibody. Blotting was performed using anti-YY1 or GAPDH antibodies as indicated. (C) Whole MEF cell lysates were blotted directly after immunoprecipitation for YY1 or STAT1 using a specific anti-YY1 or anti-STAT1 antibody. Blotting was performed using anti-YY1, anti-STAT1, or GAPDH antibodies as indicated. (D) MEF cells were transfected with siNC or siSTAT1 for 24 h and then STAT1 expression was detected by RT-PCR. (E) MEF cells were transfected with siNC, siSTAT1 and/or pCMV-flag-YY1, or not (NT) for 24 h. Then, the cells were treated with IFN- β (150 U/ml) or not for 24 h. The cells were collected for RNA extraction. RT-PCR was performed to detect MX1 and IFI204. GAPDH gene was used as control. Data are represented as means $\pm$ SD (n=3; *represents P<0.05, **represents P<0.01).

of viral infection, most likely to avoid an exacerbated and uncontrolled IFN- β signaling. Following viral infection, YY1 protein levels were shown to progressively decrease, which may result in the attenuation of STAT1 inhibition by YY1, thus promoting the antiviral IFN- β signaling. In YY1-knockdown cells, the expression levels of STAT1, pSTAT1, and pSTAT2 were considerably upregulated following immune stimulation, while the levels of antiviral ISGs were significantly increased following the viral infection. Taken together, these data indicate that YY1 interacts with STAT1, suppressing IFN- β signaling, and identify a novel regulatory role of YY1.

Whether YY1 acts as a repressor or an activator of transcription is closely associated with its post-translational modifications, promoter sequence environment, or intracellular concentration [36, 37]. Here, we determined that in spite of a certain degree of reduction, YY1 protein levels remained relatively stable immediately after viral infection, but they were significantly decreased 8 hours after infection, suggesting a gradual, time-dependent decrease in YY1 levels. These changes were shown to be consistent with the activation of the transcriptional capacity of the IFN- β promoter, which is turned on 4 to 6 hours after the

Fig. 5. YY1 knockdown promotes antiviral ISGs expression and inhibits viral replication. Macrophages were transfected with siNC or siYY1 for 24 h and then infected with VSV at an MOI of 1 for 24 h. The cells were collected for RNA extraction. RT-PCR was performed to detect MX1, IFI204, IFITM3, RSAD2, CXCL10, ISG20 (A), VSV RNA replicates (B). GAPDH gene was used as control. (C) Virus titers in the cultural supernatants were determined by plaque assays (plaque-forming units, PFU). Data are represented as means $\pm$ SD (n=3; *represents $P < 0.05$, **represents $P < 0.01$).

initiation of viral infection, and turned off 10 to 12 h [2-4]. Moreover, YY1 overexpression was shown to significantly upregulate pTBK1 and pIRF3, and IFN- β levels at 6 hpi, while YY1 knockdown inhibited the phosphorylation and nuclear translocation of TBK1 and IRF3, indicating that a certain level of YY1 expression is essential for the activation of IFN- β production after viral infection, however, it remains unclear why. It is possible that a certain amount of YY1 molecules is required to bind to the -90 and -120 sites of IFN- β promoter, allowing the recruitment of virus-induced histone acetyltransferases (HATs) and the acetylation of lysine residues of histone H4 and H3 at the IFN- β promoter region, in order to activate IFN- β transcription [17, 18]. In addition to the interaction with HATs, YY1 was reported to interact with histone deacetylases [38], which play crucial roles in the regulation of RIG-I activation during antiviral immune response [39]. Therefore, the required levels of YY1 may facilitate the activation of TBK1 and IRF3 during viral infection. Future studies should focus on the mechanisms underlying YY1-mediated induction of IFN- β expression during viral infection. Nonetheless, our study has clearly demonstrated that certain concentration of YY1 is essential for the activation of TBK1 and IRF3 during virus infection.

Activation of STAT1 and STAT2 is essential for the induction of antiviral ISG expression through IFN-1 signaling [6, 7, 11]. Although IFN-1 signaling can help inhibit viral infections, uncontrolled IFN-1 signaling may lead to the development of autoimmune diseases [13, 14], making it the regulation of IFN-1 signaling activation in the absence of pathogen stimulation very important. We for the first time demonstrated that YY1 interacts with STAT1, suppressing IFN- β signaling in the absence of viral infection. YY1 knockdown significantly induced STAT1, pSTAT1, and pSTAT2 expression after the immune stimulation, and induced the expression of antiviral ISGs after viral infection. Our results helped identify a novel regulatory mechanism of IFN- β signaling, in which YY1 interacts with STAT1, suppressing IFN- β signaling in non-infected cells, while, during the viral infection, YY1 expression is gradually downregulated, facilitating IFN- β signaling that helps inhibit viral infection.

In conclusion, we showed that YY1 regulates antiviral innate immune responses through a dynamic induction of IFN- β expression and signaling in different stages of viral infection. Although the mechanisms underlying these processes should be elucidated in further studies, our results provide a better understanding of the YY1 regulatory role during the antiviral innate immune responses.

Acknowledgements

This study was supported by grants from the 2017 PhD Start-up Fund of Guangdong Natural Science Foundation of China (2017A030310404), National Natural Science Foundation of China (No.81301013), Guangdong Natural Science Foundation of China (No. S2013040013591), Administration of Traditional Chinese Medicine of Guangdong Province Foundation (No. 20162007) and Scientific Research Fund of Yunnan Provincial Education Department (2015Y062).

Disclosure Statement

There are no conflicts of interest to disclose for all authors.

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