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Identification of human guanylate-binding protein 1 gene (hGBP1) as a direct transcriptional target gene of p53

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ABSTRACT

Human guanylate-binding protein 1 (hGBP1) plays an important role in antitumor and antiviral immune responses. Here, we show that tumor suppressor p53 positively regulated *hGBP1* transcription via binding to the p53 response element (p53RE) present in the *hGBP1* promoter region. p53 activation by 5-fluorouracil significantly increased hGBP1 expression in wild-type p53 cells, but not in p53-null cells. Knockdown of p53 expression remarkably impaired hGBP1 expression induced by 5-fluorouracil, type I interferon treatment, or influenza A virus infection. Among three deductive p53REs present in the *hGBP1* promoter region, two p53REs were found to be transactivated by p53.

1. Introduction

Human guanylate-binding protein 1 (hGBP1) is an interferon (IFN)-inducible protein, which is the most well-characterized member of the guanylate-binding proteins and involved in various biological processes, such as antitumor and antiviral immune responses [1,2]. hGBP1 has been shown to inhibit cell proliferation, invasion, migration, mammary tumor growth, and mediate the antiviral response against influenza A virus (IAV), encephalomyocarditis virus, vesicular stomatitis virus, coxsackie virus, hepatitis B virus, and hepatitis C virus [2,3].

As an IFN-inducible gene, *hGBP-1* expression is induced by IFN-α/β and -γ [4]. In addition, interleukin (IL)-1α, IL-1β, tumor necrosis factor-α, and lipopolysaccharides are also capable of inducing hGBP1 expression [5]. A nuclear factor-kappaB (NF-κB) binding site and IFN-α-stimulated response element have been identified in the *hGBP1* promoter region, which co-operate in the activation of hGBP1 expression via inflammatory cytokines in endothelial cells [5].

Tumor suppressor p53 plays an important role in the regulation of various biological processes, such as cell cycle arrest, apoptosis, as well as antitumor and antiviral immune responses [6]. p53 primarily functions as a transcription factor for regulation of target genes, which have important roles in biological processes [7]. For instance, p53 transactivates the expression of its target gene p21 to arrest the cell cycle [8] and its target gene retinoic acid-inducible gene I (RIG-I) to regulate cell survival and growth [9].

In response to genotoxic stimuli, such as 5-fluorouracil (5-FU) or ultraviolet (UV) irradiation, p53 is activated and translocated to the nucleus where it binds to a DNA sequence motif, the so-called p53 response element (p53RE), present in the promoter region of its target gene to transactivate target gene expression [10,11]. The consensus p53RE is quite complicated and consists of two decameric half-sites (5′-RRRCWWGYYY-3′, where W can be A or T, and R and Y indicate purine and pyrimidine bases, respectively), which are usually directly adjacent but can be separated by up to 13 bp [12]. DNA binding is critical for p53 biological functions [13,14].

We previously observed that GBP1 expression was significantly reduced in p53-deficient mice compared with wild-type p53 mice during IAV infection (unpublished data), suggesting a potential role of p53 in the regulation of GBP1 expression. Here, we present evidence to demonstrate that hGBP1 is a direct transcriptional target gene of p53 and that p53 plays an essential role in hGBP1 gene activation in cellular responses to DNA damage, cytokines, and viral infection.

2. Materials and methods

2.1. Cells, antibodies and cell treatment

Human hepatic carcinoma HepG2 (wild-type p53), Hep3B cells (p53-null), human colon carcinoma HCT116 (wild-type p53),

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human lung epithelial A549 (wild-type p53) cells were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). A549 cells were maintained in F-12K Nutrient Mixture, Kaighn's Modification (Invitrogen, Carlsbad, CA, USA), other cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen). All cells were cultured in their medium supplemented with 10% fetal bovine serum (Invitrogen) at 37 °C under 5% CO_2. Commercial antibodies were an anti-hGBP1 monoclonal antibody (1B1, Santa Cruz Biotechnology, Santa Cruz, CA, USA), an anti-p21 monoclonal antibody (F-5, Santa Cruz), an anti-p53 monoclonal antibody (DO-1, Santa Cruz) and anti-β-actin monoclonal antibody (AC-15, Sigma, St. Louis, MO, USA). For 5-FU treatment, 5-FU (Sigma) was added at 10 μg/ml or the indicated concentrations to the medium and incubated for the indicated times at 37 °C. For UV radiation, cells were treated with UVB (100 mJ/cm^2). For IFN-α treatment, the cells were incubated with 1000 U/ml IFN-α for 12 h. For viral infection, A549 cells were infected with influenza virus A/Puerto-Rico/8/34 at a multiplicity of infection of five as described previously [15] and incubated for the indicated times.

2.2. Plasmid construction and transfection

DNA fragments containing the deductive hGBP1-p53REs were cloned by polymerase chain reaction (PCR) and inserted into pGL3 Luciferase Reporter Vector (Promega, Madison, WI, USA) to generate the luciferase reporter plasmids including p-hGBP1-p53RE1, p-hGBP1-p53RE2&3, p-hGBP1-p53RE2 and p-hGBP1-p53RE3. The deletion mutants of p-hGBP1-p53RE2 (p-hGBP1-p53RE2-mutant) and p-hGBP1-p53RE3 (p-hGBP1-p53RE3-mutant), in which the DNA sequence motifs of the deductive hGBP1-p53REs were deleted, were generated by modified PCR-based site-directed mutagenesis [16]. All plasmids described above were verified by DNA sequence analysis. For transfection, cells were cultured with appropriate medium and transfected using Lipofectamine™ 2000 (Invitrogen), according to the manufacturer's protocol.

2.3. Luciferase assays

Hep3B cells were transfected with a combination of the luciferase reporter plasmids, control plasmid Renilla luciferase pRL-TK (Promega) and plasmid expressing wild-type p53 (pCMV-p53, Clontech, Laboratories Inc., Mountain View, CA, USA) and incubated for 48 h. For 5-FU stimulation, Hep3B or HepG2 cells were transfected with a combination of the luciferase reporter plasmids and control plasmid Renilla luciferase pRL-TK. 5-FU was added at the indicated concentrations to the medium 6 h post-transfection. After 24 h 5-FU treatment, the cells were collected for luciferase assays. The firefly luciferase activity was measured using the Dual Luciferase Reporter Assay System (Promega), according to the manufacturer's protocol, and normalized to Renilla luciferase activity.

2.4. Chromatin immunoprecipitation assay (ChIP)

HepG2 cells were cultured on 150 mm dishes at a seeding density of 2×10^7 cells/dish for 12 h. The cells were then incubated with 5-FU (10 μg/ml) for 24 h. To fix the cells, 550 μl of 37% formaldehyde were added into 20 ml of growth media and incubated at room temperature for 10 min. Glycine (125 mM) was added and incubated at room temperature for 5 min to quench unreacted formaldehyde. The fixed cells were rinsed with phosphate-buffered saline containing protease inhibitor cocktail. The cell pellet was collected and re-suspended in 1 ml of lysis buffer (50 mM Tris (pH 8.0), 1% SDS, 10 mM EDTA, and 5 μg/ml sonicated salmon sperm DNA). The cell lysates were sonicated on ice to shear cross-linked DNA to ~200–1000 bp in length. Immunoprecipitation was conducted with anti-p53 antibodies for overnight at 4 °C, followed by pre-blocked protein G Sepharose beads. Normal mouse IgG was used for control purpose. Following elution and proteinase K digestion, DNA was recovered by phenol/chloroform extraction and ethanol precipitation. The bound target DNA fractions were detected by PCR with appropriate primers (Supplementary Table 1). The PCR products were analyzed by 4% agarose gel electrophoresis.

2.5. RNA interference

RNAi-mediated knockdown of endogenous p53 was performed as described [17]. The target sequence for human p53 was 5′-AAGACTCCAGTGGTAATCTAC-3′.

2.6. Western blot and quantitative real-time RT-PCR (qRT-PCR)

Western blot and qRT-PCR were performed as described previously [15]. Primer sequences are listed in Supplementary Table 1.

2.7. Statistical analysis

All measured values are expressed as the means with standard error. Significance was analyzed using the Student's *t*-test. Value of $p < 0.05$ was considered significant.

3. Results

3.1. p53 Positively regulates hGBP1 expression

We previously observed that GBP1 expression in p53-deficient mice was impaired (unpublished data). To analyze whether p53 regulates hGBP1 expression, wild-type p53 HepG2 cells were treated with p53-inducing stimuli, such as 5-FU or UV irradiation, and hGBP1 expression was subjected to western blot analysis. In response to stimulation, hGBP1 showed a remarkable increase in protein levels in HepG2 cells, but not in p53-null Hep3B cells, and displayed an up-regulated expression pattern similar to that of p53 and p21, which is a direct transcriptional target of p53 [8] (Fig. 1A). This up-regulated expression of hGBP1 was further confirmed at the transcriptional level. In response to 5-FU stimulation, hGBP1 mRNA levels were significantly increased by up to 10–13-fold in a time- and dose-dependent manner in HepG2 cells (Fig. 1B). The p53 transcriptional target gene RIG-I was used as a positive control. The up-regulated expression of hGBP1 mRNA in response to 5-FU stimulation was also observed in other wild-type p53 cells, such as HCT116 and A549 cells, but not in p53-null Hep3B cells (Fig. 1C).

To further clarify the regulatory ability of p53 on hGBP1 expression, we evaluated hGBP1 expression in p53-knockdown HepG2 cells silenced by RNA interference (p53 siRNA) (Fig. 1D). The p53 siRNA cells were treated with 5-FU, and the expression of hGBP1 and RIG-I (positive control) was analyzed. The expression of hGBP1 and RIG-I was significantly impaired in the p53 siRNA cells compared with that in cells transfected with non-targeting siRNA (NC siRNA) (Fig. 1E). Taken together, these results demonstrated that p53 positively regulated hGBP1 expression.

3.2. p53 Transactivates the hGBP1 promoter fragment containing the deductive p53REs

Because p53 positively regulated hGBP1 expression and knockdown of p53 resulted in substantial down-regulation in hGBP1 expression (Fig. 1), we predicted that p53, as a transcription factor,

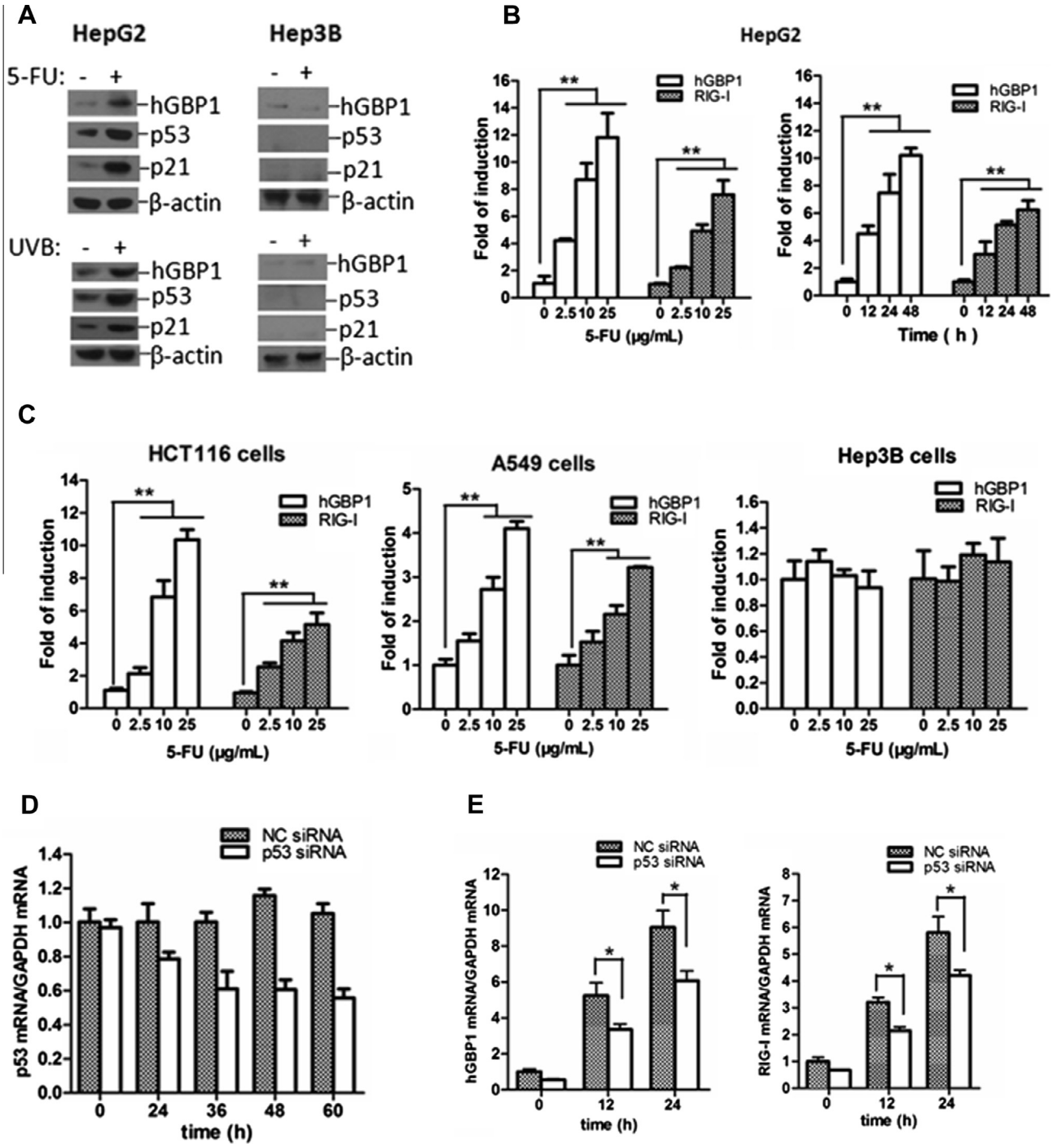

Fig. 1. p53 Induced hGBP1 expression. (A) HepG2 and Hep3B cells were treated with 5-FU or UV radiation (UVB). The expression levels of hGBP1, p53, and p21 were determined by western blot analysis. (B) HepG2 cells were treated with the indicated 5-FU concentrations for 48 h (left panel) or 10 μg/mL of 5-FU for the indicated times (right panel). hGBP1 and RIG-I expression was analyzed by qRT-PCR. (C) HCT116, A549 and Hep3B cells were treated with the indicated 5-FU concentrations for 48 h and then hGBP1 and RIG-I expression was analyzed by qRT-PCR. (D) HepG2 cells were transfected with synthesized small interfering RNA (p53 siRNA) to silence p53 expression or non-targeting small RNA (NC siRNA). p53 Expression was analyzed by qRT-PCR. (E) HepG2 cells were transfected with p53 siRNA or NC siRNA for 36 h and subsequently treated with 10 μg/mL of 5-FU for the indicated times. hGBP1 and RIG-I expression was analyzed by qRT-PCR. Results are presented as the mean ± standard error from three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$.

directly regulates *hGBP1* transcription. Indeed, alignment of the p53REs motif 5′-RRRCWWGYYY-3′ with the *hGBP1* gene revealed three deductive p53REs, designated *hGBP1*-p53RE1, -p53RE2 and -p53RE3, respectively, at −6924/−6901, −4141/−4112 and −2440/−2416 within 8 kb of the *hGBP1* promoter region (Fig. 2A). To investigate whether p53 activates the *hGBP1* promoter, two luciferase reporter plasmids (p-hGBP1-p53RE1 and p-hGBP1-p53RE2&3) were generated, which contained the luciferase reporter gene driven by the *hGBP1* promoter containing the deductive p53REs (Fig. 2B). The luciferase reporter plasmids were co-transfected with increasing concentrations of plasmid pCMV-p53, which expressed the wild-type p53 in Hep3B cells. The empty pGL3-basic vector was parallelly transfected as a control. The p-hGBP1-p53RE1-transfected cells showed constant and basal

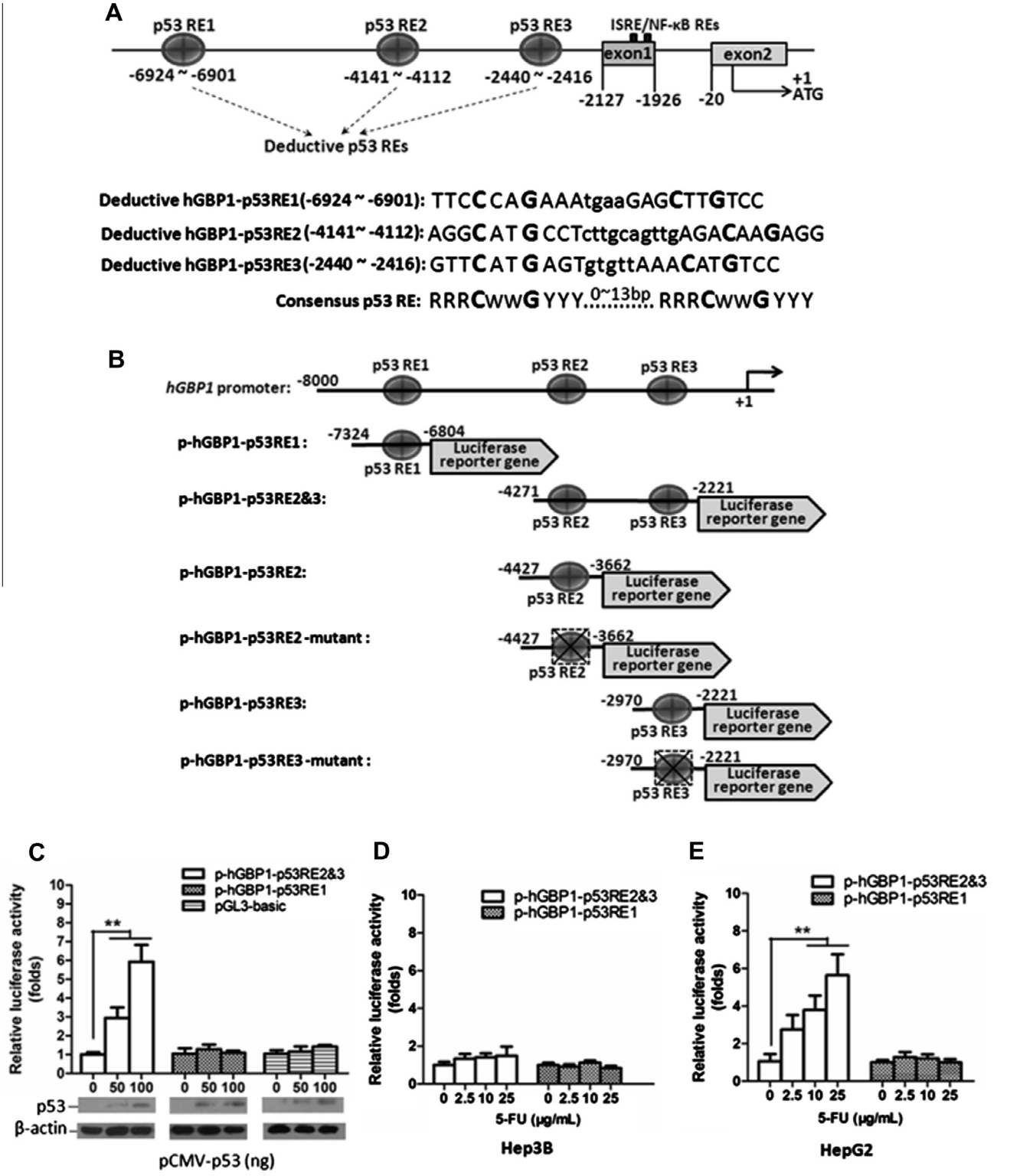

Fig. 2. p53 Transactivated *hGBP1* promoter fragments. (A) Locations and sequences of the deductive *hGBP1*-p53REs in the *hGBP1* promoter region. The location of each site in the promoter is labeled relative to the translational starting site. ISRE/NF-κB REs, NF-κB binding site and IFN-α-stimulated response element. (B) Schematic representation of the deductive *hGBP1*-p53REs in the *hGBP1* promoter region and the constructs of luciferase reporter plasmids. (C) Hep3B cells were co-transfected with the luciferase reporter plasmid p-hGBP1-p53RE1 or p-hGBP1-p53RE2&3 or the pGL3-basic vector with the indicated amounts of pCMV-p53 plasmids and incubated for 48 h. The luciferase activity of the transfectants was analyzed. p53 Expression was evaluated by Western blot analysis. (D and E) Hep3B or HepG2 cells were transfected p-hGBP1-p53RE1 or p-hGBP1-p53RE2&3 plasmids, respectively. The transfectants were incubated for 6 h and subsequently treated with increasing concentration of 5-FU for 24 h. The luciferase activity of the transfectants was analyzed. Results are presented as the means ± standard error from three independent experiments. $^{**}p < 0.01$.

luciferase activities regardless of the presence or absence of p53, which were similar to those of cells transfected with the pGL3-basic vector (Fig. 2C), suggesting that p53 did not activate the *hGBP1* promoter fragment containing the deductive *hGBP1*-p53RE1 regions. In contrast, p-hGBP1-p53RE2&3-transfected cells displayed a significant increase in luciferase activity in the presence of p53

compared with that in the absence of p53 (Fig. 2C), suggesting that p53 activated the *hGBP1* promoter fragment containing the deductive *hGBP1*-p53RE2 and -p53RE3 regions. Similar results were also observed in HepG2 and A549 cells transfected with p-hGBP1-p53RE1 or p-hGBP1-p53RE2&3 (data not shown). To determine whether endogenous p53 activates the *hGBP1* promoter fragment containing the deductive p53REs, HepG2 and Hep3B cells were transfected with p-hGBP1-p53RE1 or p-hGBP1-p53RE2&3 in the presence of increasing 5-FU concentrations. As expected, p53-null Hep3B cells transfected with p-hGBP1-p53RE1 or p-hGBP1-p53RE2&3 showed no significant difference in luciferase activity between 5-FU-treated and mock-treated cells (Fig. 2D). However, in response to 5-FU stimulation, the luciferase activity increased significantly in the wild-type p53 HepG2 cells transfected with p-hGBP1-p53RE2&3, but not in the cells transfected with p-hGBP1-p53RE1 (Fig. 2E). Taken together, these results demonstrated that p53 positively activated the *hGBP1* promoter fragment containing the deductive *hGBP1*-p53RE2 and -p53RE3 regions.

3.3. p53 Transactivates and binds to both the deductive hGBP1-p53RE2 and -p53RE3

To further identify which of the deductive *hGBP1*-p53REs was activated by p53, we generated the luciferase reporter plasmids p-hGBP1-p53RE2 and p-hGBP1-p53RE3 driven by the *hGBP1* promoter fragment containing the deductive *hGBP1*-p53RE2 or -p53RE3 regions (Fig. 2B). These luciferase reporter plasmids were co-transfected with increasing plasmid pCMV-p53 concentrations into Hep3B cells and the luciferase activity of the transfectants were measured. The plasmids p-hGBP1-p53RE2&3 and empty pGL3-basic vector were parallelly transfected as controls. The luciferase activity of p-hGBP1-p53RE2&3-transfected cells was significantly increased in the presence of p53 compared with that in the absence of p53 (Fig. 3A), which was consistent with the observation shown in Fig. 2C. The cells transfected with plasmids p-hGBP1-p53RE2 or p-hGBP1-p53RE3 also showed a significant increase in the luciferase activity in the presence of p53; however, the increase was relatively lower than that of cells transfected with plasmid p-hGBP1-p53RE2&3 (Fig. 3A). We also analyzed luciferase activity in cells transfected with a deletion mutant of p-hGBP1-p53RE2 (p-hGBP1-p53RE2-mutant) or p-hGBP1-p53RE3 (p-hGBP1-p53RE3-mutant), in which the DNA sequence motifs of the deductive *hGBP1*-p53REs were deleted (Fig. 2B). As expected, no significant increase in luciferase activity in cells transfected with p-hGBP1-p53RE2-mutant or p-hGBP1-p53RE3-mutant was observed regardless of the presence or absence of p53 (Fig. 3A). These results suggested that both deductive *hGBP1*-p53RE2 and -p53RE3 were activated by p53.

As a transcription factor, binding of p53 to p53REs is indispensable for its transcriptional activity [14]; therefore, we determined whether p53 binds to the deductive *hGBP1*-p53REs. HepG2 cells were treated with 5-FU and subjected to a chromatin immunoprecipitation (ChIP) assay. A ChIP assay for p21 was parallelly performed as a control. The anti-p53 antibodies immunoprecipitated a DNA fragment that contained *hGBP1*-p53RE2 or -p53RE3, but not *hGBP1*-p53RE1 (Fig. 3B). The DNA sequence analysis of the

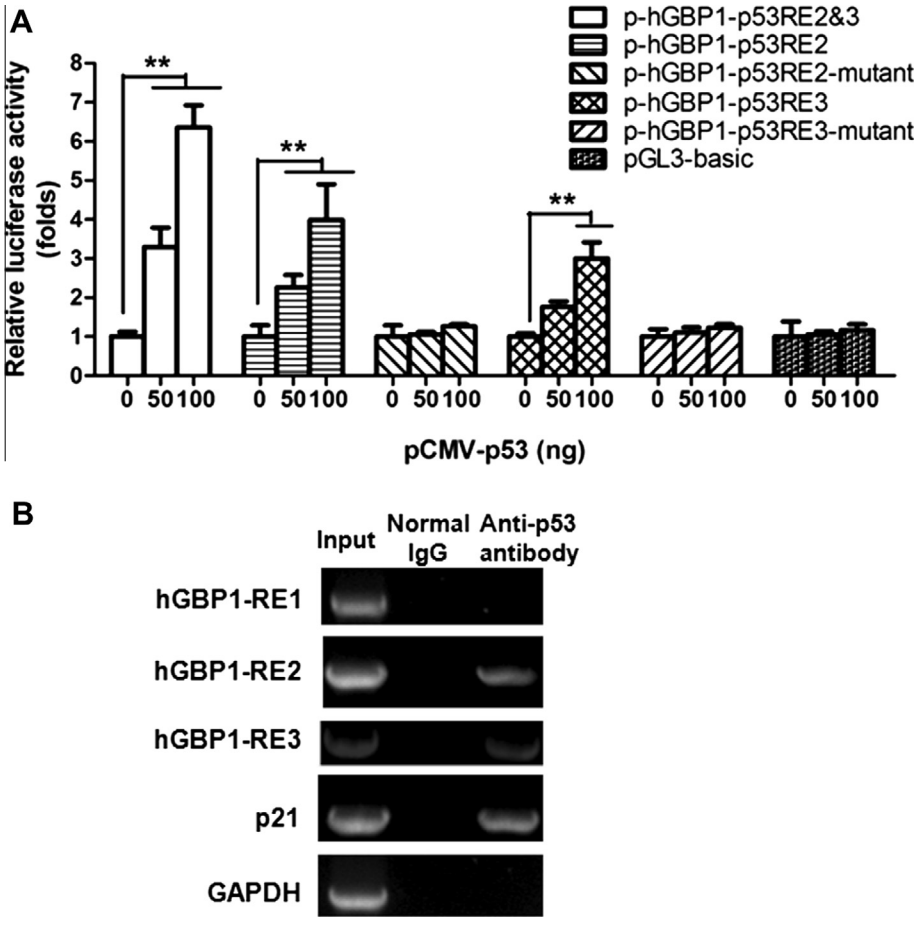

Fig. 3. p53 transactivated and bound to the *hGBP1*-p53RE2 and -p53RE3 regions. (A) Hep3B cells were co-transfected with the indicated plasmids with increasing amounts of pCMV-p53 plasmids and incubated for 48 h. The luciferase activity of the transfectants was analyzed. Results are presented as the means ± standard error from three independent experiments. $^{**}p < 0.01$. (B) ChIP analysis of p53 binding to the *hGBP1*-p53REs. HepG2 cells were treated with 5-FU for 24 h and subjected to ChIP analysis.

PCR products amplified from the immunoprecipitated DNA fragments revealed that the DNA fragments corresponded to the *hGBP1*-p53RE2 or -p53RE3 regions (data not shown). These data suggested that p53 bound to both *hGBP1*-p53RE2 and -p53RE3. Taken together, these results demonstrated that p53 activated and bound to both of the deductive *hGBP1*-p53RE2 and -p53RE3 regions.

3.4. IAV infection and IFN-α treatment induce hGBP1 expression in a p53-dependent manner

Reportedly, hGBP1 plays a role in anti-IAV infection and p53 is activated in IAV-infected cells [3,18]. In response to IAV infection, hGBP1 was up-regulated similar to p53 (Fig. 4A). Because *hGBP1* is a direct transcriptional target gene of p53 (Figs. 2 and 3), we determined whether p53 is responsible for up-regulated *hGBP1* expression in IAV-infected cells. p53 Expression in A549 cells was silenced by siRNA (Fig. 4B). In response to IAV infection, hGBP1 expression was up-regulated in the NC siRNA cells, but was notably impaired, especially at 24 and 48 h post-infection, in the p53 siRNA cells (Fig. 4C). These results indicated that p53 was, at least partially, responsible for up-regulated hGBP1 expression during IAV infection.

As an IFN-stimulated gene, *hGBP1* expression can be induced by IFN-α [5]. To determine whether p53 is involved in IFN-induced *hGBP1* expression, we analyzed the effect of IFN-α on hGBP1 expression in p53 siRNA cells. It is known that IFN-α transcriptionally induces p53 expression [19]. However, in response to IFN-α

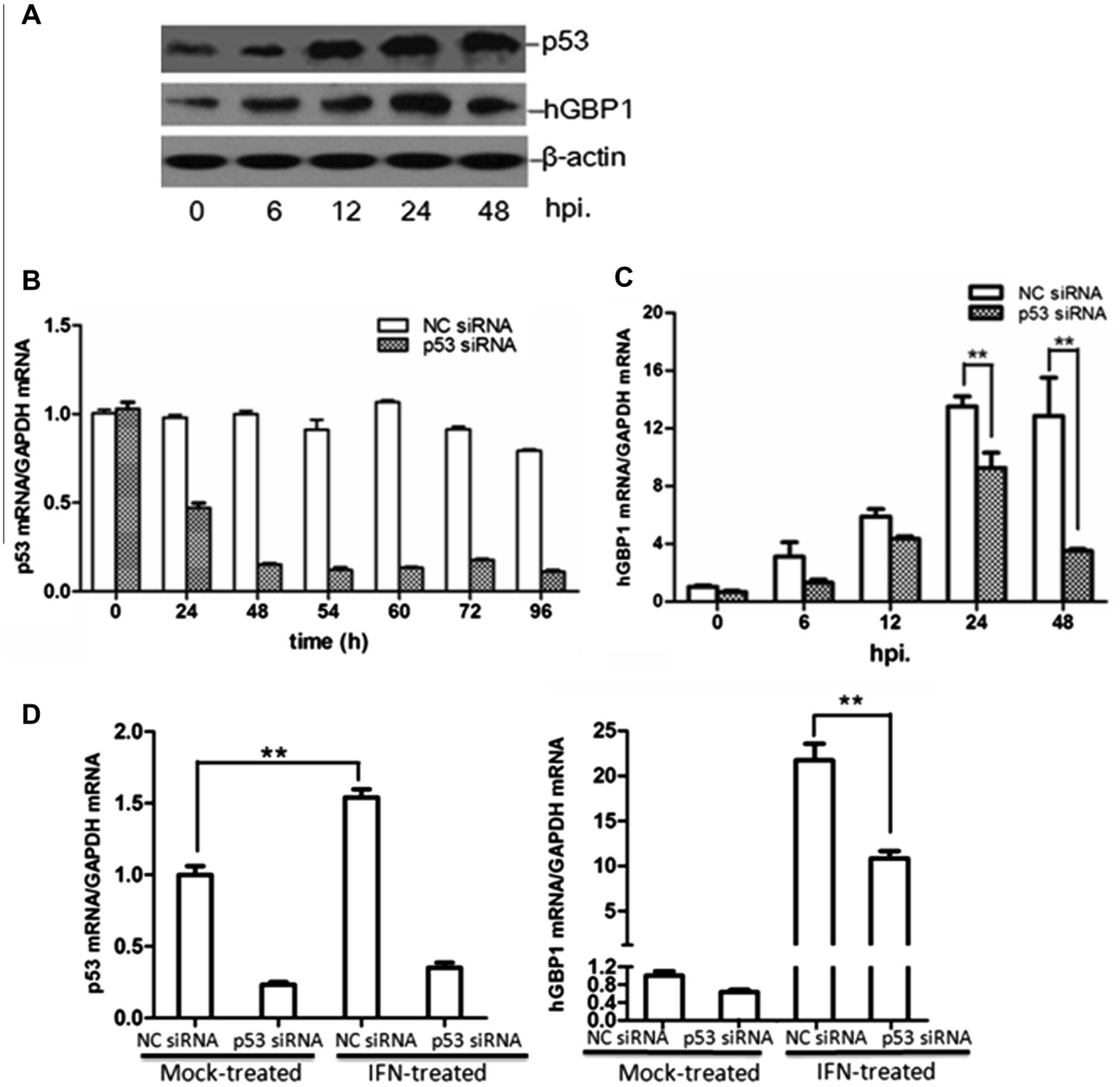

Fig. 4. IAV infection and IFN-α treatment induced hGBP1 expression in a p53-dependent manner. (A) Western blot analysis of the hGBP1 and p53 expression in IAV-infected A549 cells. (B) A549 cells were transfected with synthesized small interfering RNA (p53 siRNA) for silencing p53 expression or non-targeting small RNA (NC siRNA). p53 Expression was analyzed by qRT-PCR at the indicated times. (C and D) A549 cells were transfected with p53 siRNA or NC siRNA and incubated for 48 h. The transfectants were subsequently infected with IAV for the indicated times (C). The transfectants were subsequently treated with IFN-α for 12 h (D). The expression of p53 and hGBP1 were analyzed by qRT-PCR. Results are presented as the mean ± standard error from three independent experiments. $^{**}p < 0.01$. hpi, hours post-infection.

treatment, p53 expression was only slightly increased in the p53 siRNA cells, but was remarkably lower than that in the NC siRNA cells (Fig. 4D, left panel), suggesting that p53 expression was effectively silenced by siRNA. Analysis of hGBP1 expression in response to IFN-α treatment revealed that IFN-α induced a significant up-regulation in hGBP1 expression in the NC siRNA cells, but this response was remarkably impaired in the p53 siRNA cells (Fig. 4D, right panel). These results indicated that p53 was essential for up-regulation of hGBP1 expression in response to IFN-α treatment.

4. Discussion

Our previous observation that GBP1 expression was significantly decreased in p53-deficient mice compared with wild-type p53 mice in response to IAV infection (unpublished data) suggested a potential role of p53 in regulation of GBP1 expression. In this study, we found that p53 positively regulated hGBP1 expression. In response to p53-inducing stimuli, hGBP1 expression was significantly induced at both of the transcriptional and protein levels in wild-type p53 cells, but not in p53-null cells or p53-knockdown cells (Fig. 1). Among three deductive *hGBP1*-p53REs (Fig. 2A), both *hGBP1*-p53RE2 and -p53RE3 were transactivated by p53 (Figs. 2 and 3 A). ChIP analysis indicated that p53 was able to bind to the *hGBP1*-p53RE2 and -p53RE3 regions (Fig. 3B). These results demonstrated that *hGBP1* is a direct transcriptional target gene of p53.

Binding to the p53RE region, which comprises a motif of two 5′-RRRCWWGYYY-3′ decamers, is critical for p53 to perform its biological functions [12,14]. High-affinity binding of p53 to p53RE generally requires the conserved CWWG motif, but nucleotide changes can exist throughout the rest of the p53RE sequence [20,21]. Although three possible p53REs in the *hGBP1* promoter region were deduced from alignment of the p53RE and *hGBP1* sequences (Fig. 2A), only *hGBP1*-p53RE2 and -p53RE3 were activated and bound by p53 (Figs. 2 and 3). A comparison of the motifs of the deductive *hGBP1*-p53REs with the conserved p53RE revealed that the *hGBP1*-p53RE2 and -p53RE3 sequences were more similar to the conserved p53RE sequences than the *hGBP1*-p53RE1 (Fig. 2A), which may explain why p53 did not activate and bind to the *hGBP1*-p53RE1 region despite some noncanonical functional p53RE apart from that of the conserved p53RE region has been previously identified [13,22].

Exogenous p53 expression in p53-null Hep3B cells significantly up-regulated *hGBP1*-p53RE2 and -p53RE3 activation (Fig. 3A). It is known that p53 enhances IFN signaling [23] and that *hGBP1* is an IFN-stimulated gene [4]; therefore, it was a possibility that p53 activates *hGBP1*-p53RE2 and -p53RE3 via enhancement of IFN signaling rather than via binding to the *hGBP1*-p53REs. Analysis of the locations of the NF-κB binding site and IFN-α-stimulated response element (ISRE/NF-κB RE) in the *hGBP1* promoter region revealed that the ISRE/NF-κB RE was absent in the DNA fragments used for construction of the luciferase reporter plasmids (Fig. 2A and B), excluding this possibility. Our failure to detect significant increase in luciferase activity in cells transfected with the p-hGBP1-p53RE2-mutant or p-hGBP1-p53RE3-mutant further supported this conclusion (Fig. 3A).

Our observations that endogenous hGBP1 expression was induced at the mRNA and protein levels by 5-FU-induced p53 stimulation (Fig. 1) and that IFN-α- and IAV-induced hGBP1 expression was significantly impaired in the absence of p53 (Fig. 4) indicated an essential role of p53 in *hGBP1* gene activation in cellular responses to DNA damage, cytokines, and viral infection. Both p53 and hGBP1 are involved in the regulation of antitumor and antiviral immune responses. By establishing a direct link between p53 and transcriptional *hGBP1* activation, the functional implications of p53-dependent *hGBP1* expression are worth noting. As a transcription factor, p53 activation leads to the up-regulation of various target genes responsible for antitumor and antiviral immune responses, which implies that induction of hGBP1 expression by p53 might be a part of a p53-mediated cascade to initiate antitumor and antiviral activities. On the other hand, although hGBP1 expression is induced by several cytokines, the mechanism of how cytokines induce GBP1 expression is largely unknown [2]. The ISRE/NF-κB RE has been identified in the *hGBP1* promoter region [24]. In this study, we identified two p53REs in the *hGBP1* promoter region, suggesting an alternative mechanism to regulate hGBP1 activity.

In addition to the induction of hGBP1 expression, type I IFN induces p53 transcription [19]. Interestingly, p53 also directly transactivates the expression of several genes involved in type I IFN signaling, suggesting a positive feedback loop between p53 and type I IFN [6]. In this study, we observed that IFN-α-induced hGBP1 expression was significantly impaired in the absence of p53 (Fig. 4D), suggesting a potential role of this positive feedback loop in the regulation of hGBP1 expression as well as the subsequent antitumor and antiviral immune responses.

In conclusion, we demonstrated that *hGBP1* is a direct transcriptional target gene of p53. The hGBP1 expression induced by 5-FU or IFN-α treatment or IAV infection was significantly impaired in the absence of p53, suggesting an essential role of p53 in hGBP1 activation in cellular responses to DNA damage, cytokines, and viral infection.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.05.074.

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