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BAF53 Interacts with p53 and Functions in p53-mediated p21-gene Transcription

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BAF53, a component of chromatin remodelling and histone acetyltransferase complexes, has been shown to be essential for cell survival in human cells and plays roles in p53-mediated gene transcription. However, the mechanism concerned in the process needs to be further explored. In this study, we show that BAF53 is involved in the repression of p53-dependent p21-gene transcription by interacting with p53 both *in vivo* and *in vitro*. Through electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation analyses, we demonstrate that BAF53 can reduce the p53-binding ability to p21 promoter. By western-blot experiments, we find that BAF53 can decrease p53-Lys382 acetylation, which may be partially responsible for the repression of p53-binding ability. Furthermore, BAF53 represses p21-promoter activity in a BRG1-independent manner. These data contribute to elucidating the molecular mechanisms of BAF53 in regulating p53-mediated gene transcription.

Key words: BAF53, p53, p21-gene transcription, p53 acetylation.

Abbreviations: Arps, actin-related proteins; BAFs, BRG1-associated factors; PREs, p53-response elements; ChIP, chromatin immunoprecipitation; EMSA, electrophoretic mobility shift assay.

INTRODUCTION

Actin-related proteins (Arps), a group of protein families with moderate sequence similarity among themselves and to actin, have been found in a variety of eukaryotic organisms (*1*, *2*). Arps are components of two distinct families of chromatin remodelling enzyme complexes, histone acetyltransferases (HATs) and SWI/SNF-related ATPases (*3*). These two enzymes play pivotal roles in chromatin remodelling via either acetylating the lysine residues of histones or altering the mobility and spacing of the nucleosome arrays in an ATP-dependent manner, respectively (*4*–*6*). BAF53, a 53-kDa human Arp, is originally identified as a component of the BRG1-associated factors (BAFs). It has been reported that BAF53 can directly interact with BRG1 and is required for its maximum ATPase activity (*7*). BAF53 is also found to be a component of the SWI2/SNF2-related p400 and Tip60 histone acetyltransferase complexes, and each of these complexes acetylates the lysine residues of histones and induces conformational alterations of chromatin, and plays important roles in transcription, apoptosis, DNA repair and oncogenic transformation by stimulating transcription accessibility (*8*, *9*). BAF53 homologues, from yeast to humans, have a highly conserved N-terminal motif, and overexpression of an N-terminal deletion mutant or the mutants in which serine 2 or tyrosine 6 on the N-terminal has been replaced with alanine both can greatly reduce cell viability and stimulate p53-mediated transcription, indicating that BAF53 is also essential for cell survival (*10*).

p53 is a tumor suppressor which can induce cell-cycle arrest or apoptosis in response to a variety of stress signals, such as DNA damage, oncogenic stimuli or hypoxia (*11*). The activity of p53 can be regulated by several approaches including protein stabilization and modification by phosphorylation and acetylation. As a transcriptional factor, p53 can recognize specific binding sites within numerous target genes, including mdm2, cyclin G, bax and p21 (*12*, *13*). While multiple downstream targets are involved in the mediation of apoptosis, the main target for p53-induced cell-cycle arrest seems to be the p21 (*14*). Although it has been known that BAF53 functions on p53-mediated transcription (*15*), the mechanism of BAF53-involved transcription regulation and the relations between BAF53 and p53 need to be further explored. In this report, we showed that BAF53 was associated with p53 *in vivo* and *in vitro* and could repress p53-mediated p21-gene transcription. The interaction of BAF53 and p53 led to the decrease of p53-binding ability to p53-response elements (PREs) on p21 promoter, which might be partially due to the decrease of p53-Lys382 acetylation regulated by BAF53. In addition, BAF53 could repress p21-promoter activity in a BRG1-independent manner.

MATERIALS AND METHODS

Plasmids and Antibodies—Luciferase reporter plasmid with p21 promoter was kindly provided by Dr Masataka Nakamura (Human Gene Sciences Center) (*16*). HA-BAF53 construct was obtained from Michael. D. Cole (*17*). pcDNA3-p53 (wild-type) was a kind gift of

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Dr Bert. Vogelstein (Johns Hopkins Medical University, Maryland). pcDNA3-p53ΔBD, pcDNA3-p53ΔAD1ΔBD, GST-p53ΔBD and GST-p53ΔAD1ΔBD plasmids were reconstructed by inserting the PCR-amplified fragments into pcDNA3 or pGEX-6p-1 plasmids. GST-p53 and GST-p53CTD were obtained from Prof. Alan Weiner (University of Washington). pREP7-Rluc vector was kindly provided by Dr Keji Zhao (NIH, Maryland). Anti-β-actin and anti-BAF53A antibodies were both obtained from Sigma Corporation and Gene Tex, Inc., respectively. Anti-acetylated-p53 (Lys382) and anti-p53 (1801) antibodies were purchased from Cell Signaling-technology and Biosciences Pharminger, respectively. Anti-GST monoclonal antibody was obtained from Sigma Corporation. Horseradish peroxidase (HRP)-conjugated secondary antibody against mouse or rabbit IgG was purchased from Jackson Laboratory (Bar Harbor, ME).

Cell Culture and Transfection—U2OS (the human osteosarcoma cell line, which expresses wild-type p53) and SW13 (adrenal adenocarcinoma cell line, which does not express BRG1) cells purchased from the Institute of Cell Biology (Shanghai, China) were cultured at 37°C with 5% CO_2 in IMDM and L15 medium (Invitrogen), respectively, supplemented with 10% FBS and appropriate amount of penicillin/streptomycin. Transient transfections of U2OS and SW13 cells were performed by the calcium–phosphate method and by an electroporator with the Gene Pulser X cell TM electroporation system (Bio-Rad), respectively. The transfected cells were cultured for 48 h and harvested for luciferase activity assay and western blot analyses.

Dual-Luciferase Assay—U2OS or SW13 cells were transfected with 3 μg of expression plasmids. All the aliquots of cells were co-transfected with 1 μg of the reporter plasmid encoding firefly luciferase gene driven by the p21 promoter and 200 ng of pREP7-Rluc vector. The equal quantity of transfected DNA was adjusted with the pcDNA3 plasmid. Luciferase activity was quantified by using the dual-luciferase assay system (Promega), and was normalized with data generated from pREP7-Rluc. Results shown are the mean ± SD of three independent experiments performed in triplicate.

RNA Extraction and Quantitative PCR Analyses—Total RNA isolation and reverse transcription reaction were performed by using the RNA extraction and RT Systems supplied by Promega from U2OS cells transfected with indicated plasmids for 48 h. Quantitative real-time PCR was performed on an ABI PRISM 7000 Sequence Detection System following the manufacturer's protocol, and SYBR Green (TaKaRa, Japan) was used as a double-stranded DNA-specific fluorescent dye. GAPDH was used as a control for standardizing p21 mRNA expression. Expression of p21 gene was measured in triplicate and normalized to GAPDH expression. GAPDH primers: 5-agggggggagccaaaaggg-3, 5-gaggagtgggtgtcgctgt tg-3. p21 primers: 5-gagcctccctccatccctatg-3, 5-ctcccagcac acactcacac-3. Data were analysed by calculating the $2^{-\Delta\Delta Ct}$. All the results represent means ± SD of three independent experiments.

Western Blotting and Co-Immunoprecipitation (Co-IP)—Transfected U2OS cells were harvested and resuspended in buffer A (10 mM Hepes, pH 7.9; 10 mM KCl; 0.1 mM EDTA; 0.1 mM EGTA; 1 mM DTT; 0.5 mM PMSF), then NP-40 was added. After centrifugation, the pellets were resuspended in buffer B (20 mM Hepes, pH 7.9; 0.4 M NaCl; 1 mM EDTA; 1 mM EGTA; 1 mM DTT; 1 mM PMSF), and then were centrifuged and the supernatant was nuclear extract. Proteins were separated by SDS–PAGE, analysed by immunoblotting, and detected with the ECL method (Amersham Life Science). For Far-Western blotting, the nuclear extract or no protein were separated by SDS–PAGE and subjected to electrotransfer onto a nitrocellulose membrane. Binding reaction was then performed in TBS-Tween (20 mM Tris base, 1.37 M NaCl, 0.1% Tween 20, pH 7.6) containing an equal amount of the GST-fusion protein (GST or GST-p53) (10 μg/ml) for 10 h at room temperature. The membranes were then washed three times with TBS-Tween. To detect the GST-tagged protein, the membrane was probed with anti-GST monoclonal antibody and then visualized with an ECL immunoblotting detection kit (Amersham-Pharmacia) according to the manufacturer's instructions. For co-immunoprecipitation assay, the nuclear extracts of transfected or untransfected U2OS cells were precleared with protein A- or G Sepharose bead suspension (Upstate) and then incubated with 2 μg of the respective antibodies at 4°C for 3 h. After addition of 20 μl of protein A- or G Sepharose bead suspension, the mixture was further incubated under gentle shaking at 4°C for 12 h. After three washes with extraction buffer, the beads were resuspended in 50 μl of extraction buffer and 30 μl of aliquots were examined for protein expression by western blotting.

GST Pull-Down Assay—The GST fusion proteins were expressed in *E. coli* strain BL21. The bacteria were grown overnight and stimulated with 250 μM IPTG at 37°C for 4 h, and then were harvested, re-suspended in phosphate-buffered saline (PBS), sonicated for 1 min in PBS on ice, lysed by the addition of Triton X-100 to a final concentration of 1%, and cleared by centrifugation at 8000 rpm, at 4°C for 10 min. Glutathione–Sepharose 4B beads were equilibrated in PBS and mixed with appropriate amount of bacterially expressed GST fusion proteins on a rotary shaker at 4°C for 2 h. The beads were washed once with an equal volume of interaction buffer (40 mM Hepes, pH 7.9, 10 mM $MgCl_2$, 0.4% NP-40, 15% glycerol, 0.4% bovine serum albumin, 2 mM PMSF, 10 mg/ml aprotinin). Coupling efficiency was monitored by SDS–PAGE and Coomassie-Blue-staining. For the binding reaction, 20 μg of 1:1 bead slurry in 100 μl of interaction buffer was combined with 500 μg of nuclear extract of U2OS cells in a final volume of 600 μl on a rotary shaker at 4°C for 90 min. The beads were then washed three times with washing buffer (20 mM Hepes, pH 7.9, 100 mM KCl, 5 mM $MgCl_2$, 0.2% NP-40, 2 mM PMSF, 10 mg/ml aprotinin), and the bound proteins were eluted by boiling in SDS–PAGE loading buffer and subjected to SDS–PAGE. The bound proteins were visualized by western blotting.

Chromatin Immunoprecipitation (ChIP)—The treated U2OS cells were cross-linked with 1% formaldehyde and then re-suspended in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.1) with protease inhibitors,

followed by sonication. Antibodies were added to each of the samples, which were then rotated at 4°C overnight. After interaction with protein A or G beads and incubation at 65°C for 4 h, the DNA was dissolved and analysed by polymerase chain reaction (PCR). The following primers were used for detecting p21 promoter sequences: 5-gagcctccctccatccctatg-3 and 5-ctcc cagcacacactcacac-3(distal p53: dis.p53); 5-tgcgttagaggaag aagact-3 and 5-ctgtgcctgaaacatttgc-3(proximal p53: prox.p53).

Electrophoretic Mobility Shift Assay (EMSA)—Biotin end-labelled or unlabelled sense and antisense oligonucleotides were synthesized as described in (*18*). The oligonucleotides were annealed to generate the double-stranded probe. EMSA was performed by pre-incubating 3 μg of nuclear extract in the mixture containing 2 μg of poly dI:dC, 2 μl of binding buffer on ice for 10 min. Twenty-five femtomoles biotin-labelled double-stranded probe was added to the mixture and incubated on ice for another 15 min. DNA/protein complexes were resolved in a 5% polyacrylamide gel and analysed according to the EMSA kit instructions (Pierce). Competition EMSA experiments were performed by incorporating excess concentrations (5 pmol) of unlabelled probe in the pre-incubation step of the assay, and supershift assays were performed by adding 2 μg of anti-BAF53 antibody before DNA/protein incubation.

RESULTS

BAF53 is Associated with p53 in vivo and in vitro—It has been reported that BAF53 plays a role in p53-mediated gene transcription. We proposed that BAF53 and p53 might physically interact in cells. To this aim, the association of BAF53 and p53 was analysed by immunoprecipitation experiments in U2OS cells. As shown in Fig. 1A, the endogenous BAF53 was co-immunoprecipitated with p53, whereas no BAF53 could be detected in the control. Correspondingly, p53 was also found in the BAF53 immunoprecipitated complexes. To further analyse the association of BAF53 and p53, GST pull-down assay was conducted with a series of GST-p53 fusion proteins or GST alone (Fig. 1B, up and middle panels). As shown in Fig. 1B bottom panel, BAF53 interacted with GST-wild-type p53 (wt) and GST-p53ΔBD, but the binding ability of BAF53 to GST-p53ΔBD was much weaker than that to GST-p53wt. BAF53 did not bind to GST, GST-p53ΔAD1ΔBD and GST-p53CTD proteins. In order to test whether BAF53 can directly bind to p53, we conducted Far-Western blotting assays and found that there was a positive signal specifically in the BAF53 position incubated with GST-p53, but not in no-protein position or that incubated with GST protein (Fig. 1C). These results indicate that BAF53 is associated with p53 *in vivo* and i*n vitro*.

BAF53 Represses p53-Mediated p21-Gene Transcription—Based on the earlier results, we further investigated the effect of BAF53 on p53-mediated p21 promoter. First, U2OS cells were transfected with the plasmids of pcDNA3-BAF53, pcDNA3-p53 and the combination of pcDNA3-p53 and pcDNA3-BAF53, respectively. As a result, the luciferase activity of the p21

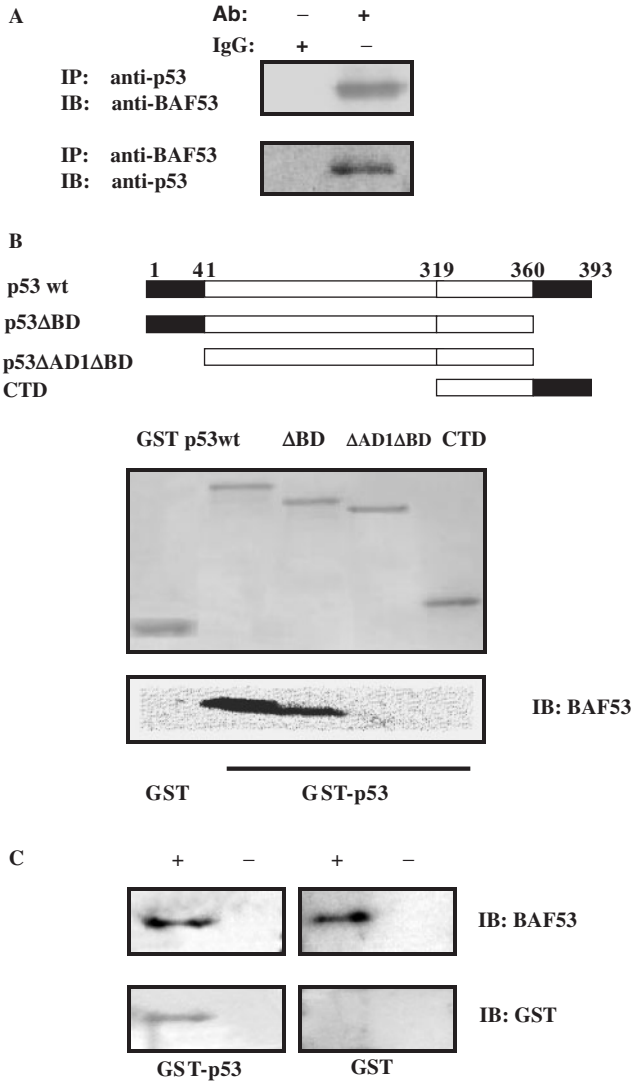

Fig. 1. **BAF53 interacts with p53 *in vivo* and *in vitro*.** (A) Nuclear lysates of U2OS cells were immunoprecipitated (IP) with anti-p53 or anti-BAF53 antibody. The precipitates were detected with anti-BAF53 or anti-p53 antibody, respectively. Rabbit IgG was used as a negative control for BAF53 antibody and mouse IgG was used as negative for p53 antibody, respectively. (B) Schematic representation of p53 used in the GST pull-down assays (upper). Coomassie-Blue-stained GST-p53 fusion proteins used for the pull-down assays (middle). GST-fusion proteins were incubated with nuclear extracts of U2OS cells and detected by immunoblotting (IB) with anti-BAF53 antibody (bottom). (C) Nuclear lysates of U2OS cells or no protein were detected with anti-BAF53 antibody, and then the membranes were stripped and incubated with GST-p53 or GST protein, respectively, and then detected with GST antibody.

promoter in the cells transfected with BAF53 plasmid alone decreased about 2-folds, and the transfection of p53 plasmid alone increased the luciferase activity 4–5-folds compared with that transfected with empty vector. The luciferase activity of the cells co-transfected with BAF53 and p53 expression plasmids decreased 3–4-folds compared with that transfected with p53 plasmid alone (Fig. 2A, upper). As BAF53 overexpression

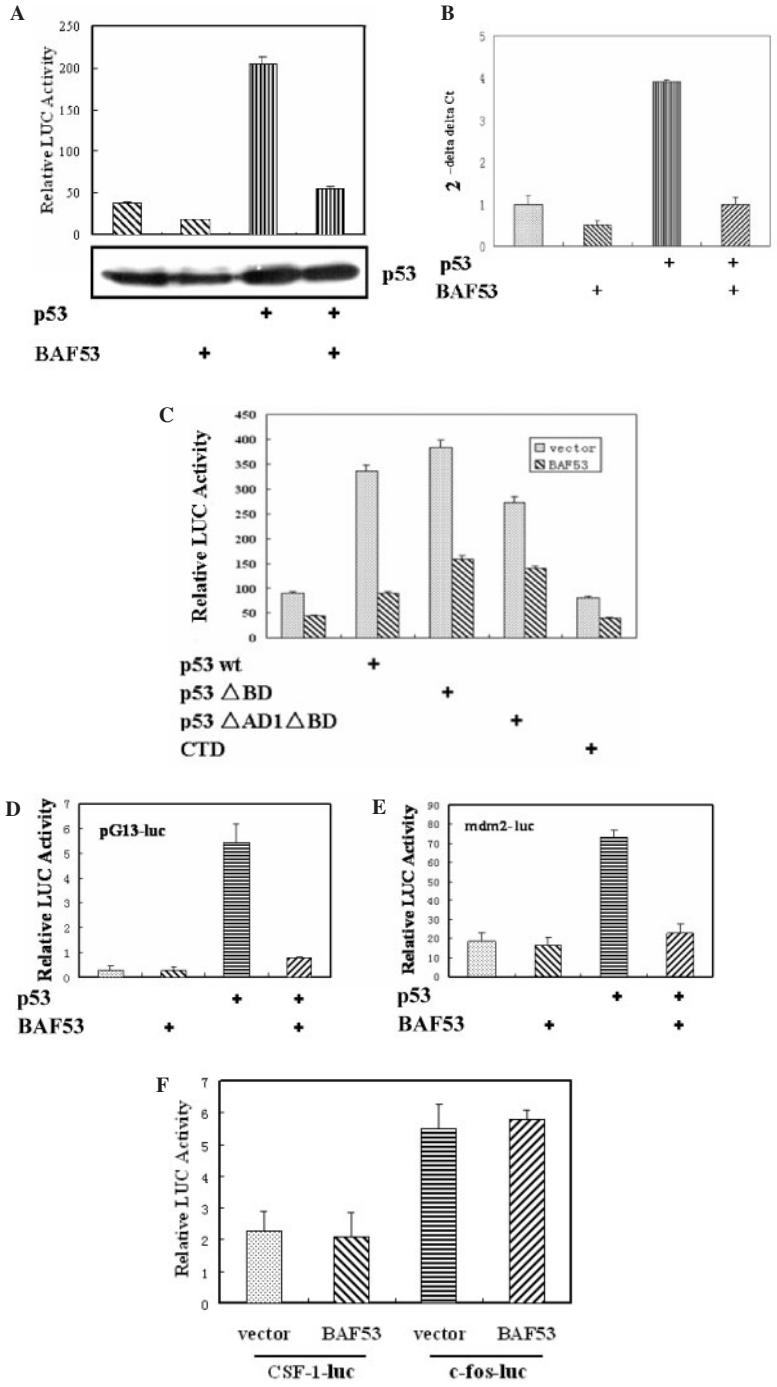

Fig. 2. **BAF53 represses p53-mediated p21-promoter activity by interacting with p53.** (A) U2OS cells were transiently transfected with p21 promoter reporter plasmid alone or in combination with the expression plasmids of pcDNA3-p53 and pcDNA3-BAF53. A portion of nuclear extracts as described earlier was analysed for p53 expression by western blotting (bottom). (B) Quantitative real-time PCR analysis of the endogenous p21-gene transcription repressed by BAF53. (C) U2OS cells were co-transfected with pcDNA3-BAF53 in combination with the indicated plasmids: pcDNA3-p53, pcDNA3-p53ΔBD, pcDNA3-p53ΔAD1ΔBD, along with p21 promoter reporter plasmid. Luciferase activity obtained from each pcDNA3-BAF53 transfectant was indicated as fold change compared with that transfected with empty vector. (D) U2OS cells were transiently transfected with pG13-luc in combination with the plasmids of pcDNA3-BAF53 and pcDNA3-p53. (E) U2OS cells were transiently transfected with mdm2-luc in combination with the plasmids of pcDNA3-BAF53 and pcDNA3-p53 as indicated. F, U2OS cells were transiently transfected with CSF-luc or c-fos-luc in combination with the expression plasmid of pcDNA3-BAF53.

did not change the expression of p53 (Fig. 2A, bottom), the reason for the decrease of p21 promoter activity should be due to the interaction between BAF53 and p53. To confirm the role of BAF53 in regulating p53-mediated p21-gene transcription, we transiently transfected pcDNA3-BAF53 or co-transfected it with pcDNA3-p53 into U2OS cells and analysed the endogenous p21-gene transcription. The real-time PCR results showed that BAF53 overexpression alone decreased the endogenous p21-gene expression about 2-folds, compared with that transfected with empty vector alone, and co-transfection of BAF53 and p53 plasmids decreased the endogenous p21-gene expression 3–4-folds, compared with that transfected with p53 plasmid alone (Fig. 2B). This is consistent with the luciferase assay results.

To further confirm the interaction of BAF53 and p53 functions in p53-mediated p21-promoter activity, we conducted dual-luciferase assays by co-transfecting U2OS cells with p21 promoter reporter plasmid and the combination of expression plasmids of pcDNA3-BAF53 and pcDNA3-wild-type p53 (p53 wt) or its deletion mutants. As shown in Fig. 2C, co-transfection of BAF53 and p53 wt plasmids repressed the luciferase activity of p21 promoter 3–4-folds, compared with that transfected with p53 wt plasmid alone, but the repression folds was 2.4, 1.9 and 2.0, respectively, when pcDNA3-BAF53 was co-transfected with pcDNA3-p53ΔBD, pcDNA3-p53ΔAD1ΔBD or pcDNA3-p53CTD. These results further indicate that the function of BAF53 on repressing p21-promoter activity is dependent on the interaction of BAF53 with p53.

Based on the earlier indication, we then asked whether the regulation of BAF53 in p53-mediated gene activation was specific. To this aim, we transfected U2OS cells with BAF53 expression vector to conduct dual luciferase assays and found that BAF53 could repress the transcription of pG13-luc and mdm2 promoter, which are both regulated by p53 (*13*) (Fig. 2D and E), while BAF53 could not repress the p53-unrelated genes transcription, such as CSF-1 and c-fos (Fig. 2F), which can be stimulated by some components of BAF complex (*7*, *19*, *20*). These results indicate that BAF53 can specifically interact with p53 and function in p53-mediated gene transcription.

BAF53 Inhibits p53 Binding to the p53-Response Elements (PREs)—As a multidomain transcription factor, the tumour suppressor p53 binds to specific DNA response elements (PREs) and is integrated in various signalling networks by a multitude of protein–protein interactions (*21*, *22*). In this study, we want to know whether BAF53 can influence the binding of p53 to its PREs on p21 promoter. EMSA was carried out with nuclear extracts from U2OS cells, and the binding reactions were performed by using wild-type oligonucleotides corresponding to one p53-binding site in the p21 promoter as described in (*18*). As a result, the large DNA–protein complex specific to the probe was observed (Fig. 3A, lanes 2–3 and 6–8), however, excess amount of unlabelled probe (specific competitor) diminished the DNA band (lanes 4 and 9). To determine whether the DNA–protein complex contain BAF53, we incubated the nuclear extracts with anti-BAF53 antibody and found

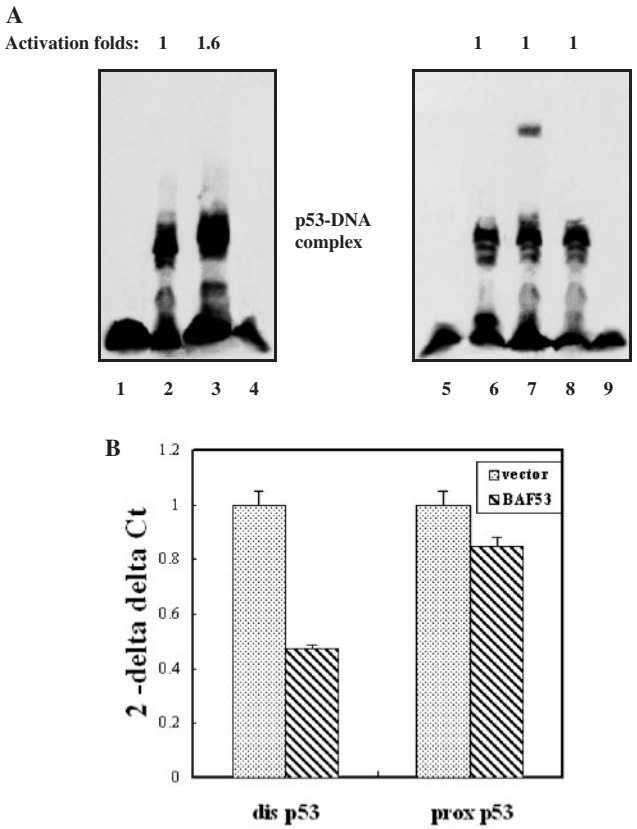

Fig. 3. **BAF53 represses p53 binding to the PREs.** (A) Nuclear extracts from U2OS cells were incubated with a biotin-labelled oligonucleotide known as p21-dec1 + 2. The free probe without nuclear extracts was used as control (lanes 1 and 5). Lanes 2–4 and 6–9 were the free probes with nuclear extracts. Anti-BAF53 antibody was added before the addition of free probes (lane 3). Anti-p53 (lane 7) or anti-GAPDH (lane 8) antibody was added as a positive or negative control, respectively. Excess amounts of unlabelled probe were used for competition experiments (lanes 4 and 9). B, U2OS cells were transfected with pcDNA3-BAF53 or pcDNA3 vector, ChIP assays with anti-p53 antibody were performed, and the immunoprecipitated DNA was analysed by quantitative real-time PCR using primers specific to the indicated regions of the p21 promoter. The DNA intensity from the cells transfected with pcDNA3 vector was defined as 1. Error bars denote the SD. Mouse IgG was used as a negative control and its signals were subtracted from the corresponding immunoprecipitated samples.

that the addition of BAF53 antibody led to an increase of the bound DNA quantity by incubating with the probe (lane 3). In addition, the addition of p53 antibody supershifted the complex (lane 7, 2 μg) and negative control antibody had no effect (lane 8, 2 μg GAPDH antibody), showing that p53 protein is on the gel and the experiment is specific. These results indicate that BAF53 is present in the DNA–protein complex and can repress p53 binding to PREs.

To confirm this result, we transfected U2OS cells with either pcDNA3-BAF53 or pcDNA3 and performed ChIP assays with anti-p53 antibody to examine the effect of BAF53 on the p53-binding ability to the PREs regions on p21 promoter. As outlined in Fig. 3B, when cells were

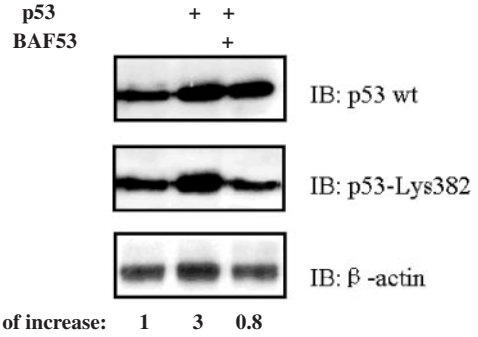

Fig. 4. **Overexpressing BAF53 reduces p53 Lys382 acetylation.** A portion of nuclear extracts as described earlier in Fig. 1A was analysed for expression of the wild-type p53 and p53-Lys382 acetylation by western blotting. β-actin as a loading control was shown under every panel. The protein quantity obtained from each transfectant was indicated as fold change compared with that transfected with empty vector.

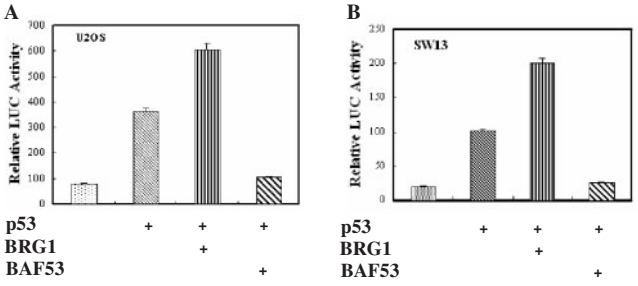

Fig. 5. **BAF53 represses p53-mediated p21-promoter activity in a BRG1-independent manner.** (A) U2OS cells were transiently transfected with p21 promoter reporter plasmid alone or in combination with pcDNA3-p53, pcDNA3-BRG1 and pcDNA3-BAF53. (B) SW13 cells were transiently transfected with p21 promoter reporter plasmid or in combination with pcDNA3-p53, pcDNA3-BAF53 and pcDNA3-BRG1 as indicated.

transfected with pcDNA3-BAF53 plasmid, decreased p53 accumulations were observed in the PRE regions on p21 promoter, and the decrease was nearly 50% when the primers to the distal PRE (dis.p53) were used, and only 20% decrease was observed when the primers to proximal PRE (prox.p53) were used, compared with that transfected with pcDNA3. These results demonstrate that BAF53 functions in repressing p53 binding to PREs on p21 promoter.

Overexpression of BAF53 Results in the Decrease of p53-Lys382 Acetylation—Acetylation is one kind of p53 post-translational modifications observed in response to DNA damage (*23*, *24*). It has been reported that p53 deacetylation can decrease p53-binding ability to p53-response elements (PREs) and the ability of p53 to activate its target genes (*25*). To investigate whether the repression of BAF53 on p53 binding to p21-gene promoter is related to p53 post-translational modifications, western blot assays were performed with antibody recognizing acetylated p53-Lys382 in U2OS cells. The results showed that when the cells were transfected with p53 plasmid, the total p53 (p53wt) and the p53-Lys382 acetylation both increased about 3-folds, compared with the controls. When BAF53 and p53 plasmids were co-transfected, the p53-Lys382 acetylation decreased 3–4-folds, while the total p53 had no change, compared with that transfected with p53 plasmid alone (Fig. 4), indicating that BAF53 overexpression inhibits p53-Lys382 acetylation.

BAF53 Represses p53-Mediated p21-Promoter Activity in a BRG1-Independent Manner—It has been reported that BAF53 as a subunit of ATP-dependent chromatin remodelling complex can enhance the ATPase activity of BRG1 (*7*), and BRG1 can interact with p53 and activate p53-mediated gene transcription (*26*). We want to know whether BRG1 was involved in the transcription repression of BAF53 on p21 promoter. First, we co-transfected U2OS cells with p21 promoter reporter plasmid in combination with the expression plasmids of pcDNA3-BAF53, pcDNA3-p53 and pcDNA3-BRG1, and detected the luciferase activity of the transfected cells. As a result, BRG1 could activate p53-mediated p21-promoter activity about 2 folds, while BAF53 could repress the p21-promoter activity about 4-folds, compared with that transfected with p53 alone (Fig. 5A). Furthermore we co-transfected the BRG1-negative SW-13 cells with the combinations of p21 promoter reporter plasmid and the expression plasmids of pcDNA3-p53, pcDNA3-BAF53 and pcDNA3-BRG1. As shown in Fig. 5B, BAF53, without BRG1, could still reduce the p53-mediated p21-promoter activity 4–5-folds, compared with that transfected with pcDNA3-p53 alone. These data indicate that BAF53 functions in the p53-mediated p21-gene transcription in a BRG1-independent manner.

DISCUSSION

Though BAF53 has been identified as a component of several kinds of chromatin remodelling enzyme complexes, little is known about the roles of BAF53 in these complexes. A recent work reported that serine 2 or tyrosine 6 mutation of BAF53 on the N-terminal stimulates p53-mediated transcription (*15*), but the mechanism involved in the process has not been clearly clarified. Here, we showed that BAF53 is associated with p53 *in vivo* and *in vitro* by co-immunoprecipitation, GST pull-down and far-Western blot assays (Fig. 1), implicating that the interaction might function in the regulation of a subset of p53-targeted genes. p21 gene is a transcriptional target of p53, and increased expression of p21 may play a crucial role in cell growth arrest (*27*). Based on the results of relative luciferase and real-time PCR analyses, we found that BAF53 can repress the p53-mediated p21-promoter activity and endogenous p21-gene transcription, and BAF53 overexpression does not change the expression of p53, suggesting that the effect observed in this study that BAF53 represses p21-gene transcription is not due to the p53 protein expression (Fig. 2). Besides p21 gene, we also found that BAF53 can inhibit other p53-mediated gene activation, such as pG13 and mdm-2, but cannot inhibit the transcription of p53-unregulated genes, such as CSF and c-fos, which can be activated by some subunits of BAF complex (*7*, *13*, *19*, *20*). We believe that the repression of BAF53 in

p53-mediated gene transcription by interacting with p53 is specific.

Since the transcription activation of p53 is related to its binding ability to PREs, we tested whether the interaction between BAF53 and p53 affects p53 binding to PREs. The results from our ChIP experiments showed that BAF53 overexpression could repress p53 binding to PREs on the p21 promoter (Fig. 3). Previous study reported that p53 can be acetylated by p300/CBP at multiple lysine residues at the C-terminal DNA binding regulatory domain, and the acetylation can activate its sequence-specific DNA-binding activity and increase the activation of its target genes (*28*). Our western blot assays showed that BAF53 overexpression results in the reduction of p53-Lys382 acetylation (Fig. 4), implying that BAF53 is responsible for p53 deacetylation, which may result in the decrease of p53-binding ability. Since no evidence has shown that BAF53 is a deacetylase, we guess that BAF53 might be a cofactor of some deacetylases or associated proteins. In our EMSA, we found that the addition of BAF53 antibody can activate p53 binding to PREs. Since BAF53 in the reaction of EMSA could not have function on protein post-transcription modification, we presumed that the addition of BAF53 antibody might affect the interaction of BAF53 and p53 or other p53-associated proteins, then enhance the p53-binding ability.

Previous studies have reported that BAF53 and BRG1 are both the subunits of BAF complex (SWI/SNF) which is necessary for p53-driven transcription activation and plays a role in p53-mediated cell-cycle control (*26*). Here we further explored the relationship between BAF53 and BRG1 in regulating p53-mediated p21-gene transcription. We found that BAF53 represses p21-promoter activity, while BRG1 stimulates p21-promoter activity, and BAF53 represses p53-mediated p21-promoter activity in a BRG1-independent manner (Fig. 5). We speculate that the possible reason for the functional disagreement between BAF53 and BRG1 in this process might be that BAF53 also exists in other chromatin modification complexes in which BAF53 may function differently from BRG1 (*8*, *9*). Recent studies reported that p400, a histone acetyltransferase complex containing BAF53, can repress p21 expression (*29*, *30*), implying that BAF53 might participate in the process as a co-repressor.

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