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INI1/hSNF5/BAF47 represses *c-fos* transcription via a histone deacetylase-dependent manner ☆

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Abstract

INI1/hSNF5/BAF47 is a core component of the hSWI/SNF ATP-dependent chromatin-remodeling complex. It has been suggested that INI1/hSNF5/BAF47 contributes to the regulation of many genes. In this report, we showed that the overexpression of INI1/hSNF5/BAF47 repressed *c-fos* promoter activity and endogenous *c-fos* transcription in 293T cells, and the siRNA targeting INI1/hSNF5/BAF47 (siINI1) reversed the inhibitory effect. Histone deacetylation by histone deacetylases (HDACs) was necessary for the repression of *c-fos* transcription by INI1/hSNF5/BAF47. HDAC and INI1/hSNF5/BAF47 functioned together to suppress *c-fos* transcription. ChIP experiments demonstrated that INI1/hSNF5/BAF47 could be recruited to the region of *c-fos* promoter to reduce histone acetylation. Altogether, these data show that INI1/hSNF5/BAF47 represses *c-fos* transcription via a histone deacetylase (HDAC)-dependent manner.
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Keywords: INI1/hSNF5/BAF47; Repression; *c-fos*; Transcription; Histone deacetylases

INI1 gene, also known as hSNF5 or BAF47, was initially identified as encoding a human homologue of the yeast SNF5 protein and a HIV integrase interactor [1,2]. INI1/hSNF5/BAF47 is a core component of the hSWI/SNF ATP-dependent chromatin-remodeling complex that plays important roles in cell proliferation and differentiation, in cellular antiviral activities, and inhibition of tumor formation [3–9]. INI1/hSNF5/BAF47 has also been defined as a tumor suppressor gene that was mutated in a malignant rhabdoid tumors [10]. Several studies have shown that INI1/hSNF5/BAF47 activated p16*INK4a* transcription and inhibited cyclin D1, CD44, and E2F target gene, and was suggested to be involved both in activation and repression of transcription of target genes [11–13]. Histone acetylation by histone acetyltransferases is often required for transcriptional activation, while histone deacetylation by histone deacetylases (HDACs) reverses the acetylation of histones and is associated with transcriptional repression [6]. It had been reported that components of the hSWI/SNF complex mediating transcriptional repression are involved by associating with the HDAC complex, and INI1/hSNF5 repressed the transcription of cyclin D1 gene in MON in a histone deacetylase (HDAC)-dependent manner [13].

The *c-fos* proto-oncogene belongs to the class of 'immediate early genes', which are involved in converting extracellular and intracellular signals into changes in gene expression, and *c-fos* is regulated at the transcriptional level during mitogenesis and other cellular processes [14,15]. In this report, we showed that the overexpression of INI1/hSNF5/BAF47 repressed *c-fos* transcription in 293T cells, and siRNA targeting INI1/hSNF5/BAF47 (siINI1) reversed the inhibitory effect. The repression of *c-fos* transcription by INI1/hSNF5/BAF47 requires

☆ *Abbreviations:* ChIP, chromatin immunoprecipitation; RT-PCR, reverse transcription polymerase chain reaction; HDAC, histone deacetylase; BuA, sodium butyrate; Brg1, *brahma*-related gene 1.

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histone deacetylation by histone deacetylases (HDACs). In addition, the association and interaction of HDAC4 with INI1/hSNF5/BAF47 is necessary for the repression of *c-fos* transcription. INI1/hSNF5/BAF47 could be recruited to the region of *c-fos* promoter and the association of INI1/hSNF5/BAF47 with *c-fos* promoter resulted in the deacetylation of histone.

Materials and methods

Plasmids. The luciferase reporter plasmid with a *c-fos* promoter (pXP2-*c-fos*-luc) was provided by Dr. J.M. Redondo (Centro de Biologia Molecular Madrid, Spain). The pREP7-Rluc vector and the RNA interfering construct (siINI1) were generous gifts from Dr. Keji Zhao (NIH, Maryland). INI1/hSNF5/BAF47 construct in pcDNA3.0 (HA-INI1) was kindly provided by Olivier Delattre (Institute Curie, France). HDAC4 expression vector was a gift from Dr. Greene WC (University of California, San Francisco).

Cell culture and transient transfection. 293T cells purchased from the Institute of Cell Biology (Shanghai, China) were maintained at 37 °C with 5% CO_2 in IMDM supplemented with 10% FCS and 1% penicillin/streptomycin mixture. Transient transfection of 293T cells (1×10^6 cells) was conducted with the Gene Pulser X cell electroporation system (Bio-Rad). Cells in logarithmic growth were trypsinized, harvested, and resuspended in IMDM without FCS at a concentration of 1×10^6 cells/ml. 0.2 ml of the cell suspension was mixed with 3 μg DNA and pulsed at 15 μF and 130 V. 293T cells were also transfected by using a standard calcium phosphate method with 2.5×10^5 cells and 3 μg DNA.

Dual-luciferase assay. 293T cells (1×10^6 cells) were cotransfected with either 3 μg of empty vector or 3 μg HA-INI1/hSNF5/BAF47 construct in pcDNA3.0 or other expression plasmids. Simultaneously, 3 μg of the luciferase reporter vector pXP2-*c-fos*-Luc and 150 ng of pREP7-RL were transfected into the cells. Relative luciferase activity was analyzed after 24–30 h by using a Turner Designs TD20/20 Luminometer (USA) with the Dual-luciferase assay system, according to the manufacturer's instructions (Promega). Firefly luciferase activity was normalized to *Renilla* luciferase activity.

RT-PCR. 293T cells (1×10^6 cells) were transiently transfected with either 3 μg of empty vector or 3 μg siINI1 or INI1 constructs. The transfected cells were cultured at 37 °C for 24 h and then total RNA was isolated by using TRIZOL reagent (Invitrogen). Total RNA (2 μg) was reverse transcribed with an M-MLV reverse transcriptase kit (Promega). RT-PCR products were amplified within the linear range. Equal aliquots of cDNA were amplified with actin primers. Amplification primers for *c-fos* were 5′ tgc ttc aca gcg ccc acc 3′ and 5′ cct cct ccg cga ccc caa 3′, internal control actin primers were 5′ aca ctg tgc cca tct acg 3′ and 5′ ctc gtc ata ctc ctg ctt g 3′. PCR conditions were: 94 °C 30 s; 55 °C 30 s; 72 °C 30 s; repeated 20 times for the actin primers and 30 times for the *c-fos* primers. Aliquots of the PCRs were separated on 1% agarose gels and visualized with UV light after ethidium bromide staining. Semi-quantitative estimation of the RT-PCR products was accomplished by photo-densitometric analysis of the bands in the agarose gels after electrophoresis, and the results are presented as ratios of the intensities of the *c-fos* and actin PCR bands.

Real-time quantitative PCR analysis. 293T cells (1×10^6 cells) were transiently transfected with either 3 μg of empty vector or 3 μg of siINI1 construct. Total RNA was isolated and reverse transcribed with an M-MLV reverse transcriptase kit (Promega). mRNA was quantified with an ABI PRISM 7000 sequence Detection System (PE Applied Biosystems, Weiterstadt), and SYBR Green (PE Applied Biosystems) as a double-stranded DNA-specific fluorescent dye. Amplification mixtures (25 μl) contained 1 μl cDNA, 10× SYBR Green buffer, 0.24 mM $MgCl_2$, dNTP (0.2 mM dATP, dCTP, dGTP, and 0.4 mM dUTP), 1.25 U AmpliTaq Gold DNA polymerase, 0.5 U Amp Erase UNG, and 5 μM DNA primers. The amplification primers were as described for RT-PCR. Samples were incubated at 50 °C for 2 min to inhibit the carry over of contamination. Thermal cycling conditions consisted of an initial denaturation step at 95 °C for 10 s, followed by 40 cycles of 5 s at 95 °C, and 20 s at 60 °C. All PCRs were run in duplicate. The C_t (threshold cycle) was defined as the number of cycles required for the fluorescence signal to exceed the detection threshold. Data were analyzed by the $2^{-\Delta\Delta C_t}$ method, a convenient way to analyze relative changes in gene expression [16].

Western blotting. 293T cell extracts were prepared 24 or 48 h after transfection. The cells were trypsinized and washed twice with phosphate-buffered saline (PBS). The pellets were resuspended in lysis buffer (containing 1× PBS, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 5 mM NaF, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail) at 4 °C for 30 min. The resulting lysates (50 μg of protein) were mixed with sample buffer, boiled, and subjected to sodium dodecyl sulfate (SDS)–10% polyacrylamide gel electrophoresis. The separated proteins were transferred to Immobilon-P membranes (Millipore) and incubated with primary antibodies obtained from Sigma [anti-hSNF5, anti-β-actin, and anti-Flag tagged antibodies]. Horseradish peroxidase (HRP)-conjugated secondary antibodies against rabbit or mouse IgG [Biotech Company of Zhongshan (China)] were used for antibody detection, employing enhanced chemiluminescence (Amersham) according to the manufacturer's instructions.

Coimmunoprecipitation analysis. 293T cells were transiently cotransfected with 5 μg INI1/hSNF5/BAF47 and 5 μg of Flag-HADC4 expression plasmids. After 48 h, cells were harvested and washed with PBS, and then sonicated in 1 ml buffer G (20 mM Tris, pH 7.5, 100 mM NaCl, 0.5% NP-40, 0.5 mM EDTA, 0.5 mM PMSF, and 0.5% protease inhibitor cocktail (Sigma)). Lysates were precleared by incubating with 50 μl of protein A–Sepharose (50% slurry at 4 °C for 3 h). INI1/hSNF5/BAF47 antibody (Sigma) or IgG as control antibody (Biotech Company of Zhongshan (China)) was added to the precleared lysate, and the mixture was incubated at 4 °C for 1 h, which was followed by the addition of protein A–agarose beads (50 μl of a 50% slurry) and then further incubated at 4 °C overnight. Bound proteins were washed four times with buffer G without bovine serum albumin, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and immunoblotted with monoclonal Flag antibody (Santa Cruz).

ChIP. The chromatin immunoprecipitation (ChIP) assays were carried out according to the Upstate protocol. Briefly, 293T cells at 50% confluence in 10-cm-diameter dishes were transfected with 3 μg pREP4 empty vector or the RNA interference construct (siINI1). After 24 h, the cells were cross-linked with 1% HCHO at 37 °C for 15 min, resuspended in 200 μl SDS lysis buffer supplemented with 1 mM PMSF, and sonicated three times with an Ultrasonic Processor XL (HEAT System) for 30 s. Soluble chromatin was collected by centrifugation at 13,000*g* for 10 min and transferred to ChIP dilution buffer (containing 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris, pH 8.1, 167 mM NaCl, and 1 mM PMSF). Immunoprecipitation reactions (containing 800 μl of the chromatin, 60 μl of protein A–Sepharose beads, and 5 μg hSNF5 antibody or the antibody specific to acetylatated histone H4 (acetyl-H4) (Sigma)) were incubated with rotation at 4 °C overnight. The immunoprecipitates were washed sequentially with low salt immune complex wash buffer (once), high salt immune complex wash buffer (once), LiCl immune complex wash buffer (once), and TE buffer (twice). Following reverse crosslinking at 65 °C for 6 h, the DNA was purified by proteinase K digestion, phenol–chloroform extraction, and ethanol precipitation, and then resuspended in 20 μl of 1× TE. One-fifth of the immunoprecipitated DNA and 1% of the input DNA were analyzed by PCR. The following primers were used to detect *c-fos* promoter sequences: −74F, 5′ ggt tga gcc cgt gac gtt ta 3′; +185R, 5′ gta gtc tgc gtt gaa gcc cg 3′. Primers −5234R, 5′ ctcttcctcctgatagctcc atga 3′; −5436F, 5′ cactatgttagccagga tggtctc 3, were used for the upstream CSF1 control sequence. Twenty percent of the ChIP DNA was subjected to PCR amplification by *Taq* polymerase in the following conditions: 94 °C, 30 s; 55 °C, 30 s; 72 °C, 30 s; repeated 32 times for the *c-fos* primers and 35 times for the CSF1 control primers.

Results

INI1/hSNF5/BAF47 represses c-fos transcription

It has been reported that INI1/hSNF5/BAF47 contributes to the regulation of many genes. In order to investigate whether INI1/hSNF5/BAF47 functions in regulating *c-fos* transcription, the reporter plasmid pXP2-*c-fos*-Luc, containing nucleotides −711 to + 45 of the human *c-fos* promoter, was transfected into 293T cells with INI1/hSNF5/BAF47 expression vector (pcDNA3.0-INI1) or empty vector (pcDNA3.0). The luciferase activity was analyzed by dual luciferase assay. As shown in Fig. 1A, the overexpression of INI1/hSNF5/BAF47 caused about 2-fold repression of luciferase activity, indicating that INI1/hSNF5/BAF47 represses *c-fos* promoter activity.

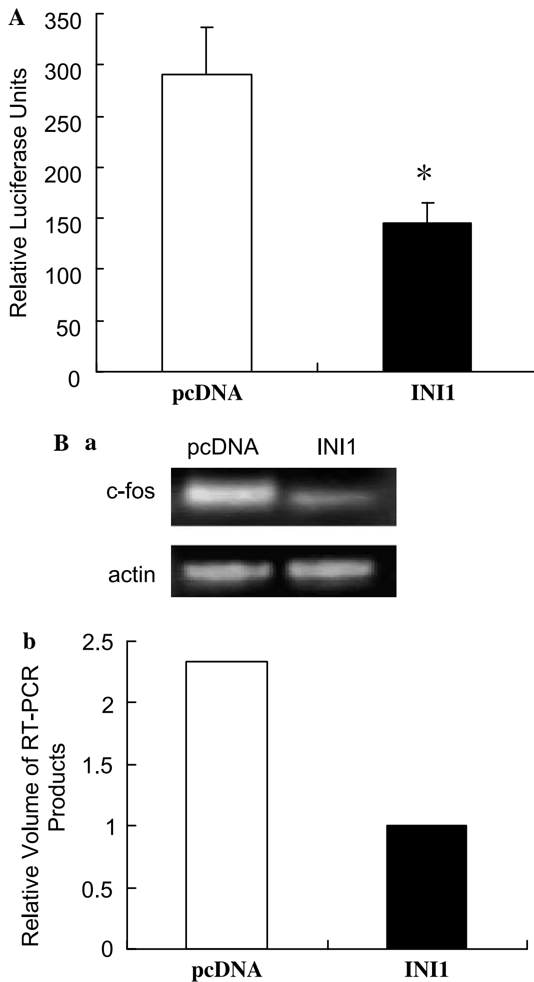

Fig. 1. INI1/hSNF5/BAF47 represses *c-fos* transcription. (A) INI1/hSNF5/BAF47 represses *c-fos* promoter activity. 293T cells were transiently transfected with 3 μg INI1/hSNF5/BAF47 expression vector (pcDNA3.0-INI1) and pXP2-*c-fos*-luc. For the negative control, 293T cells were transiently transfected with empty vector (pcDNA3.0). The luciferase activity was analyzed by the dual luciferase system after 24 h by using the pREP7-RL reporter as an internal control. The result shown is the averages of three independent experiments. *Statistically significant ($p < 0.05$) compared with the negative control. (B) INI1/hSNF5/BAF47 repressed the endogenous *c-fos* transcription. 293T cells was transfected with 3 μg of INI1/hSNF5/BAF47 expression vector (pcDNA3.0-INI1) or empty vector (pcDNA3.0). Total RNA was reverse transcribed with oligo(dT) primer, followed by PCR amplification with either *c-fos* or actin primers. Products were analyzed by agarose gel electrophoresis followed by ethidium bromide staining and photodensitometry. (a) Levels of *c-fos* mRNA in the cells transfected with pcDNA3.0 empty vector and INI1/hSNF5/BAF47 vector. (b) Photodensitometric analysis of RT-PCR products in (a).

To further confirm this result, we tested if the endogenous *c-fos* transcription could also be repressed by INI1/hSNF5/BAF47. Consistent with the results of dual luciferase assay, the RT-PCR analyses showed that INI1/hSNF5/BAF47 repressed the endogenous *c-fos* transcription by 2.3-fold (Fig. 1B). These results demonstrated that the overexpression of INI1/hSNF5/BAF47 suppressed the *c-fos* transcription.

Small interfering RNAs targeting INI1/hSNF5/BAF47 (siINI1) reversed the inhibitory effect

In order to further evaluate the repression of *c-fos* transcription by INI1/hSNF5/BAF47, a small interfering RNA target sequence was selected for INI1/hSNF5/BAF47 and cloned into the pREP4 episomal vector under the control of U6 promoter (kindly gift from Keji Zhao). The siINI1 was shown to be efficient in inhibiting INI1/hSNF5/BAF47 expression by Western bolt analysis (Fig. 2A). We next transfected control vector (pREP4) or siINI1 into 293T cells. The results of luciferase activity assay showed that siINI1 reversed the inhibitory effect and the inhibition of INI1/hSNF5/BAF47 expression up-regulated the activity of *c-fos* promoter about 1.8-fold (Fig. 2B). Consistent with the results of dual luciferase assay, the RT-PCR results showed that the transfection of siINI1 augmented endogenous *c-fos* transcription about 2.1-fold (Fig. 2C). We further quantified the *c-fos* mRNA products by real-time RT-PCR method. The results showed that the knockdown of INI1/hSNF5/BAF47 by siINI1 transfection up-regulated the *c-fos* transcription about 1.75-fold compared with the control (Fig. 2D). These data suggested that INI1/hSNF5/BAF47 plays a role in the repression of *c-fos* transcription.

Repression of c-fos transcription by INI1/hSNF5/BAF47 is dependent on HDAC activity

Histone deacetylation by histone deacetylase ($HDAC_S$) is often associated with transcription repression. To determine if histone deacetylation is required for the repression of *c-fos* transcription by INI1/hSNF5/BAF47, we transfected INI1/hSNF5/BAF47 expression vector (pcDNA3.0-INI1) with the *c-fos* promoter reporter plasmid (pXP2-*c-fos*-Luc) into 293T cells. The histone deacetylase inhibitor BUA (1 mM) was added into 293T cells. After 24 h, cells were lysed, and the luciferase activity was analyzed as described previously. As shown in Fig. 3, the addition of BUA completely reversed the repression of *c-fos* transcription by INI1/hSNF5/BAF47. The result

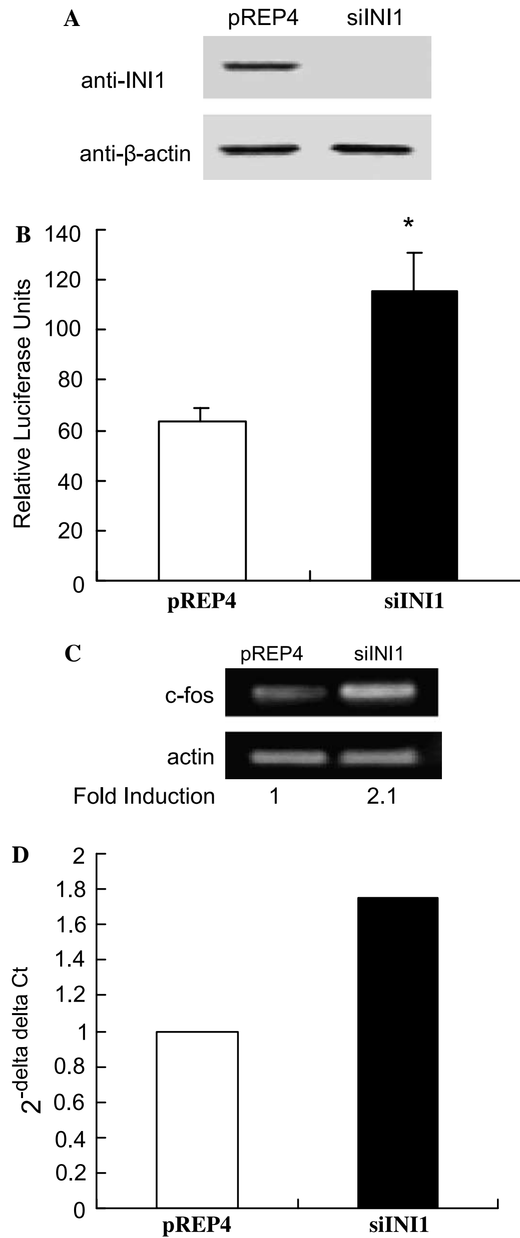

Fig. 2. Small interfering RNAs targeting INI1/hSNF5/BAF47 (siINI1) reverse the inhibitory effect. (A) INI1/hSNF5/BAF47 expression was efficiently knocked down by RNA interference. 293T cells were transfected with control vector (pREP4) or the siRNA construct targeting INI1/hSNF5/BAF47 (siINI1) and selected in 1 μg/ml of puromycin for 48 h. The remaining cells were lysed and analyzed by Western blotting with antibodies against INI1 or β-actin. (B) The pXP2-*c-fos*-Luc was transfected into 293T cells with either control vector (pREP4) or siINI1. The luciferase activity was analyzed by using a dual luciferase system as described previously. The error bars represent the range of three independent experiments. *Statistically significant ($p < 0.05$) compared with the negative control. (C) 293T cells were transfected with 3 μg of control vector or siINI1. After 24 h, total RNA was reverse transcribed with oligo(dT) primer, followed by PCR amplification with either *c-fos* or β-actin primers. Products were analyzed by agarose gel electrophoresis followed by ethidium bromide staining. (D) Real-time quantitative PCR analysis. 293T cells were transfected with 3 μg of control vector (pREP4) or siINI1. Total RNA was isolated and reverse transcribed with oligo(dT) primer, and the cDNA was used for PCR with either *c-fos* or β-actin primers. Data were analyzed by the $2^{-\Delta\Delta C_t}$ method [16].

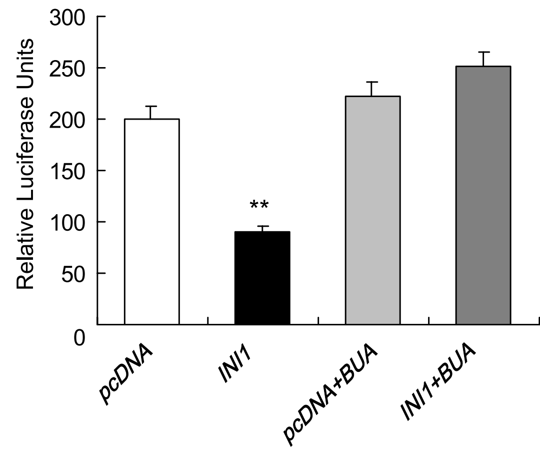

Fig. 3. Repression of *c-fos* transcription by INI1/hSNF5/BAF47 is dependent on the HADC activity. 293T cells were transfected with the INI1/hSNF5/BAF47 expression vector (pcDNA3.0-INI1) and pXP2-*c-fos*-luc. The histone deacetylase inhibitor BUA (1mM) was added into 293T cells. After 24 h, cells were lysed, and the luciferase activity was analyzed as described previously. The error bars represent the range of three independent experiments. **Statistically significant ($p < 0.01$) compared with the negative control.

suggested that histone deacetylation by histone deacetylases (HDACs) is necessary for the repression of *c-fos* transcription by INI1/hSNF5/BAF47.

HDAC and INI1/hSNF5/BAF47 function together to suppress the c-fos transcription

To further investigate the relation of HDAC activity and the repression of *c-fos* transcription, we cotransfected INI1/hSNF5/BAF47 and Flag-HDAC4 expression vector into 293T cells and performed coimmunoprecipitation analysis. Immunoblot analyses showed that the proteins of INI1/hSNF5/BAF47 and Flag-HDAC4 are expressed (Fig. 4A). The lysis of 293T cells cotransfected was assayed by immunoprecipitation with anti-INI1/hSNF5/BAF47 antibody, and the bound fractions were detected by immunoblot with Flag-tagged antibody. As shown in Fig. 4B, the overexpressed Flag-HDAC4 was recognized from the complex immunoprecipitated by anti-INI1/hSNF5/BAF47 antibody, indicating that HDAC4 is associated with INI1/hSNF5/BAF47 in 293T cells.

To determine if the association of HDAC4 and INI1/hSNF5/BAF47 is necessary for the repression of *c-fos* transcription, we transfected 293T cells with INI1/hSNF5/BAF47 or Flag-HDAC4 expression vector. The results of luciferase activity analyses showed that the overexpression of INI1/hSNF5/BAF47 or HDAC4 suppressed the *c-fos* promoter-driven luciferase by 1.8- and 1.65-fold, respectively (Fig. 4C). When 293T cells were cotransfected with INI1/hSNF5/BAF47 and HDAC4 expression vectors, the activity of *c-fos* reporter was significantly inhibited by 3-fold (Fig. 4C). The results implicate that HDAC4 and INI1/hSNF5/BAF47 function together to suppress *c-fos* transcription.

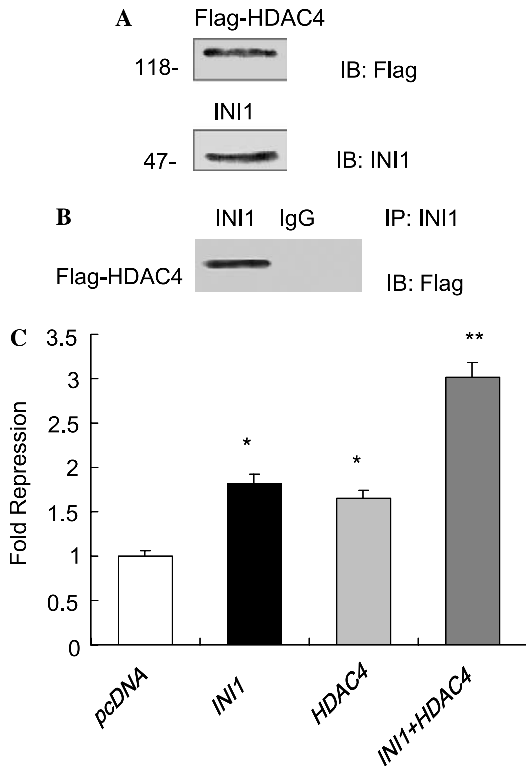

Fig. 4. HDAC and INI1/hSNF5/BAF47 function together to suppress the *c-fos* transcription. (A) Western blot analysis of the expression of HA-INI1 or Flag-HADC4 constructs in 293T cells was conducted as described under Materials and methods. (B) 293T cells were cotransfected with INI1/hSNF5/BAF47 and Flag-HADC4 expression vectors. After 24 h, lysates from the transfected cells were immunoprecipitated with anti-INI1/hSNF5/BAF47 or IgG control antibodies, and the immunoprecipitated complexes were fractioned by SDS–PAGE and detected by anti-Flag antibody. (C) 293T cells were transfected with INI1/hSNF5/BAF47 or HDAC4 expression vector, or the two expressions vectors together. The luciferase activity was analyzed as described previously. The error bars represent the range of three independent experiments. *Statistically significant ($p < 0.05$) and **statistically significant ($p < 0.01$) compared with the negative control.

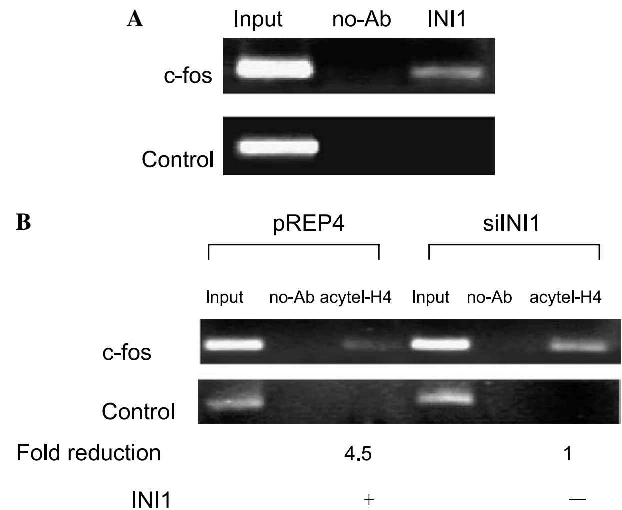

Fig. 5. INI1/hSNF5/BAF47 can be recruited to the region of *c-fos* promoter to reduce histone acetylation. (A) After cross-linking with formaldehyde, chromatin fractions of 293T cells were prepared by sonication, and the DNA purified from immunoprecipitates with antibody specific to INI1/hSNF5/BAF47 was analyzed with the primers covering *c-fos* promoter (−74 to +185) and the primers of negative control (−5436 to −5234 of the CSF1 upstream sequence) in multiplex PCR. The *c-fos* promoter sequence was amplified for 32 cycles (upper panel), and CSF1 control sequence was amplified for 35 cycles in order to detect the products (lower panel). The PCR products were analyzed by agarose gel electrophoresis, and the ethidium bromide staining images were inverted. (B) 293T cells were transfected with 3 μg of control vector (pREP4) or siINI1. ChIP assay was performed as described above, by using antibody specific to acetylated histone H4 (acetyl-H4).

INI1/hSNF5/BAF47 can be recruited to the region of c-fos promoter to reduce histone acetylation

To further understand the molecular mechanisms for the repression of *c-fos* transcription by INI1/hSNF5/BAF47, we tested if INI1/hSNF5/BAF47 functions by being recruited to the *c-fos* promoter in vivo. A ChIP assay was performed by using antibody specific to INI1, and the *c-fos* promoter sequence (−74 to +185) and a control sequence (−5436 to −5234 of the CSF1 upstream sequence) in the immunoprecipitated chromatin were analyzed by multiplex PCR, and input DNA as well as DNA immunoprecipitated in the absence of antibody was also used for PCRs. The results showed that the antibody against INI1 was able to immunoprecipitate *c-fos* promoter region, compared to those of no antibody and the control fragment (Fig. 5A), indicating that INI1/hSNF5/BAF47 is interacted with the *c-fos* promoter in living cells.

Next we further asked if the knockdown of INI1/hSNF5/BAF47 could change the levels of histone acetylation at the *c-fos* promoter. For this purpose, a ChIP experiment with antibody against tetra-acetylated histone H4 tail (acetyl-H4) was performed. The results showed that histone H4 acetylation was reduced by about 4.5-fold in the presence of INI1/hSNF5/BAF47, compared to the result in that INI1/hSNF5/BAF47 was knocked down by siINI1 (Fig. 5B), suggesting that the presence of INI1/hSNF5/BAF47 results in the deacetylation of histone H4 on specific region of the *c-fos* promoter. Therefore, we infer that HDAC4 could be recruited by INI1/hSNF5/BAF47 to the region of the *c-fos* promoter and is responsible for the observed repressive effect.

Discussion

INI1/hSNF5/BAF47 gene, which encodes a member of the hSWI/SNF ATP-dependent chromatin-remodeling complex, has been identified as a tumor suppressor gene that was mutated in sporadic and hereditary malignant rhabdoid tumors (MRT) [10]. A number of studies have shown that hSNF5 re-expression blocks MRT cell proliferation, leads to an accumulation in G0/G1, and induces cellular senescence and increased apoptosis [11–13]. Previous studies have suggested that INI1/hSNF5/BAF47 inhibits the expression of cyclin D1, CD44, and E2F

target gene [11–13]. Thus, INI1/hSNF5/BAF47 has been demonstrated to contribute to the regulation of many genes that was involved in the control of cell cycle. In this article, we showed that the overexpression of INI1/hSNF5/BAF47 represses *c-fos* promoter activity and endogenous *c-fos* transcription (Fig. 1). Moreover, based on the relative luciferase activity assay and RT-PCR analysis, we found that the siRNA targeting INI1/hSNF5/BAF47 (siINI1) significantly reversed the inhibitory effect (Fig. 2). Our results indicated that INI1/hSNF5/BAF47 plays a role in the repression of *c-fos* transcription.

INI1/hSNF5/BAF47 is a 385-amino-acid protein with three highly conserved regions including two direct imperfect repeats (repeat1 (Rpt1) and repeat 2 (Rpt2)), a C-terminal coiled-coiled domain, and a homology region 3 (HR3) [17]. Zhang et al. [13] reported that full-length INI1/hSNF5, but not the deletion fragment lacking Rpt1, is able to form a complex with HDAC1 for repression of cyclin D1. So, it is possible that HDAC is involved in the repression of *c-fos* transcription by INI1/hSNF5/BAF47. To confirm this hypothesis, we tested if histone deacetylase inhibitor BUA could overcome INI1/hSNF5/BAF47-mediated repression of *c-fos* transcription in 293T cells. We found that the addition of BUA completely reversed the repression of *c-fos* transcription by INI1/hSNF5/BAF47, indicating that INI1/hSNF5/BAF47-mediated repression of *c-fos* transcription is dependent on HDAC activity (Fig. 3). In addition, as illustrated in Fig. 4, the cotransfection of HDAC4 and INI1/hSNF5/BAF47 expression vectors significantly potentiated the inhibitory effect. Therefore, the association and interaction of HDAC4 with INI1/hSNF5/BAF47 is necessary for the repression of *c-fos* transcription.

The −77 to +1 region of human *c-fos* promoter contains binding sites for transcription factors CREB/ATF, YY1, TFII, and also contains TATA box. The human *c-fos* gene transcription is induced by the cAMP/PKA pathway through a major cAMP element (CRE) centered at −60. This element is recognized by the protein CREB/ATF [14]. It has been reported that −67 CREB/ATF binding site in the mouse *c-fos* promoter is required for the transcriptional repression of *c-fos* transcription by BRG1 and YY1 [15,18]. Our ChIP assays showed that INI1/hSNF5/BAF47 could be recruited to the region (−74 to +185, which includes the −60 ATF/CREB binding site) of human *c-fos* promoter (Fig. 5A), implying that the function of INI1/hSNF5/BAF47 in repression of *c-fos* promoter may first depend on the recruitment. Furthermore, we showed that the recruitment of INI1/hSNF5/BAF47 reduced the acetylation of histone (Fig. 5B). We infer that the association of INI1/hSNF5/BAF47 with *c-fos* promoter is likely correlated to the recruitment of HDAC4, which results in histone deacetylation in the specific region of *c-fos* promoter. Therefore, INI1/hSNF5/BAF47 represses *c-fos* transcription via a histone deacetylase (HDAC)-dependent manner.

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