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The Transcriptional Regulation Role of BRD7 by Binding to Acetylated Histone Through Bromodomain

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Abstract Studies showed that the bromodomain binds to acetyl-lysines on histone tails, which is involved in deciphering the histone codes. BRD7, a novel bromodomain gene, is the first described bromodomain gene involved in nasopharyngeal carcinoma (NPC). Previous studies showed that ectopic expression of BRD7 inhibited cell growth and cell cycle progression from G1 to S phase in HNE1 cells(a NPC cell line) by transcriptionally regulating some cell cycle related genes including E2F3 gene. In the present study, we revealed the co-localization between acetylated H3 and BRD7 and found that the bromodomain of BRD7 is required for this co-localization. More importantly, wild-type BRD7 interacted with H3 peptide acetylated at Lys14, while the bromodomain deleted mutant lost this ability. We also found that the mutant BRD7 failed to regulate E2F3 promoter activity and inhibit cell cycle progression. These results indicated that the transcriptional regulation role of BRD7 was achieved by binding to acetylated histone H3 and that the bromodomain was essential for this role. In addition, no obvious changes were observed in the acetylated level of histone H3 after transfection with BRD7, indicating that chromatin remodeling, not chromatin modification, is the major mechanism of BRD7 mediated gene transcription. Taken together, the present work shed light on the fact that a novel bromodomain gene, BRD7, is of importance in transcriptional regulation and cellular events including cell cycle. J. Cell. Biochem. 97: 882–892, 2006.

Key words: BRD7; bromodomain; nasopharyngeal carcinoma (NPC); acetylated H3

Bromodomain, an evolutionally conserved domain, is present in many chromatin associated transcription factors and some nuclear histone acetyltransferases(HATs) [Haynes et al., 1992; Tamkun et al., 1992; Jeanmougin et al., 1997; Isaacs et al., 1998]. Nuclear magnetic resonance showed that the bromodomain forms a bundle of four α helices that may serve as a chromatin-targeting module [Marcus et al., 1994; Brownell et al., 1996; Filetici et al., 1998; Winston and Allis, 1999]. Although the bromodomain function still remains to be elucidated, it is commonly recognized that bromodomain can specifically bind to acetylated chromatin and further cooperate with other transcription factors to facilitate the completion of transcriptional regulation by chromatin remodeling or chromatin modification [Dhalluin et al., 1999; Hudson et al., 2000; Jacobson et al., 2000; Owen et al., 2000]. Studies on known bromodomain proteins showed that bromodomain genes can participate in important cellular events by transcriptionally regulating some important signaling molecules [Winston and Allis, 1999; Strahl and Allis, 2000; Dyson et al., 2001], and genetic alterations of bromodomain genes contribute to diseases, including carcinogenesis. Therefore, studies on bromodomain genes have a broader significance both in understanding transcriptional regulation and its relationship with diseases.

Nasopharyngeal carcinoma (NPC) is a common cancer in the South of China and Southeast Asia, while it occurs with rare incidence in other

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parts of the world. It is now well known that both genetic alterations and environment factors are of importance in the development of NPC [Lo et al., 1994; Hu et al., 1996; Mutirangura et al., 1998; Chen et al., 1999; Hui et al., 1999; Shao et al., 2000; Feng et al., 2002; Xiong et al., 2004]. In order to find genes contributing to NPC, cDNA representational difference analysis (RDA) was performed. Some differential expressed genes between NPC tissues and their normal tissues were identified. BRD7 is one of those genes down-regulated in NPC tissue and its derived cell lines compared with normal ones [Yu et al., 2000]. Functional studies indicated that ectopic expression of BRD7 blocked cell cycle progression and cell growth in NPC cells HNE1 [Yu et al., 2001]. It was also found that BRD7 protein could interact with some nuclear transcription factors such as BRD2 and IRF2 [Staal et al., 2000]. Another research group observed that BRD7 could form a triple complex with adenovirus nuclear protein E1B-AP5 and affected the transcription activity of E1B-AP5 [Hyshkowska et al., 2003]. Our previous results showed that BRD7 could transcriptionally regulate important cell cycle related genes in Ras/MEK/ERK and Rb/E2F pathways including E2F3 molecular by cell cycle specific cDNA array analysis [Zhou et al., 2004]. It is well known that many E2F transcriptional regulation factors including retinoblastoma protein RB were mainly correlated to post-modification of H3 [Morris et al., 2000; Nielsen et al., 2001; Estelle et al., 2003; Narita et al., 2003], which offer important clue for us to study BRD7 function.

BRD7 contains a bromodomain domain, which suggest that the recognition of chromatin modifications is an important aspect of BRD7 function. So, several experiments including confocal microscopy, CHIP, peptide binding assay and differential salt extraction assay were combined to confirm the specific binding of BRD7 to acetylated histone H3. Further studies showed that bromodomain-deleted BRD7 mutant lose the affinity with acetylated histone H3. It was also found that bromodomain-deleted mutant lose the ability to inhibit E2F3 promoter activity and could not inhibit cell cycle G1–S progression. These findings indicated that binding of BRD7 to acetylated histone H3 is the underlying mechanism of BRD7's transcriptional regulation role on its target genes and cell cycle inhibition role in NPC cells.

MATERIALS AND METHODS

Plasmids and Antibodies

Plasmids used in these experiments include a full-length coding region of BRD7 and a bromodomain-deleted BRD7 mutant construct fused to green fluorescent protein (GFP) and PCMV-myc, respectively. E2F3 promoter plasmid was previously described [Zhou et al., 2004]. Antibodies against MYC was purchased from Santa Cruz. Anti-acetylated histone H3 Chip Kit and acetylated histone H3 peptide were purchased from UPSTATE.

Cell Culture and Transfection

COS-7 (African green monkey kidney) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). HNE1 (Human Nasopharyngeal Carcinoma cell line) were cultured in RPMI 1640 plus 10% FBS. Cells were plated onto sterilized glass cover slips till they were 50 to 80% confluent on the day of transfection. Cells were transiently transfected with the appropriate plasmids by using Lipofectamine reagent (Invitrogen) according to the manufacturer's protocol.

Chromatin Immunoprecipation Assay (CHIP)

Chip procedure was performed according to the directions of the manufacture(UPSTATE). Cells were cross-linked by adding formaldehyde into cell culture at the final concentration of 1% and incubated at 37°C for 10 min, then medium was aspirated. After being washed with PBS twice, cells were scraped, centrifuged at 2,000 rpm at 4°C, and then lysed with SDS lysis buffer. Extracts were incubated with antibody overnight at 4°C, and followed by adding ssDNA/Protein beads −50% slurry to collect the complex. The beads were washed in different solutions in order. The eluates were subjected to SDS–PAGE and detected by immunoblot analysis by using respectively antibodies.

Indirect Immunofluorescence

Transfected cells (COS-7) grown on coverslips were fixed with 4% paraformaldehyde for 10 min at room temperature and then were permeabilized in 4% paraformaldehyde 0.1% Triton X-100 for 10 min at room temperature. Following incubation with blocking buffer (PBS

containing 5% skim milk) for 30 min, Acetylated histone H3 was detected with the rabbit anti-acetylated H3 antibody and the myc fused BRD7 either wild type or mutant was detected by the mouse anti-myc monoclonal antibody, which were incubated for 1 h at 37°C. Secondary antibodies including anti-mouse conjugated with Cy3, anti-rabbit antibodies conjugated with FITC were used at 1:200 for 30 min at 37°C. After three washes in PBS, the cells were counterstained with DAPI stain and examined under a fluorescence microscope or confocal microscopy.

Confocal microscopy was employed to detect the immunofluorescence. Briefly, 12-bit images were collected using a Zeiss 510 laser-scanning microscope equipped with a 40× (1.3 NA) lens. For each antibody trial, the detector gain was first optimized by sampling various regions of the cover-slip and then fixed for each channel. Once set, the detector gain value was kept constant throughout the image acquisition process. As a result, qualitative measurements for signal intensities from identical channels of different images could be compiled to permit quantitative measurements and statistical comparisons. For each assay, a minimum of 50 transiently expressing interphase cells were imaged for each antibody treatment. Individual channels were separated with Zeiss LSM 510, version 3.0 (Carl Zeiss, Inc.), and subjected to quantitative analysis.

Peptide Binding Assay

Exponentially growing COS-7 cells were transiently transfected with pCMV-BRD7-myc and bromodomain-deleted BRD7. After incubation for 30 h, cells were harvested and lysed with lysis buffer (20% glycerol, 3 mM $MgCl_2$, 50 mM HEPES (pH:7.9), 500 mM KCl, 0.1% NP40, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF)) on ice for 30 min, then centrifuged at 12,000 rpm for 10 min, the supernatant was collected and incubated with 2 μg of biotin-labeled synthetic peptides corresponding to acetylated histone H3 (UPSTATE) overnight at 4°C, and followed by incubated with streptavidin beads in incubation buffer (20% glycerol, 3 mM $MgCl_2$, 50 mM HEPES (pH: 7.9), 500 mM KCl, 0.1% NP40, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF)) at room temperature for 2 h, then the beads were washed in incubation buffer and detected by Western-blot with antibody against MYC.

Differential Salt Extraction

Exponentially growing COS-7 cells were transfected with GFP-BRD7 and bromodomain-deleted GFP-BRD7. After transfection for 30 h, cells were lysed with bufferA (10 mM Tris.Cl at pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP40, 2.5 mM DTT, and protease inhibitor mixture) and incubated at 4°C for 30 min. Cell lysates were centrifuged and the extract was resuspended in buffer A and aliquots were then incubated with increasing concentration of NaCl. Eluted materials were detected by Western-blot with antibody against GFP.

Reporter Assay

COS-7 cells cultured in 6-well plates were transiently transfected (Lipofectamine) with 1.5 μg β-galactosidase expression vector containing E2F3 promoter fragment and increasing amounts of wildly-type BRD7 or BRD7-mut (0.5, 1.0, and 1.5 μg). The luciferase activity was assayed on the luminometer. β-galactosidase assays were performed as described previously [Sear et al., 2002].

Flow Cytometry

Cells were prepared and stained with PI as described recently [Schafer et al., 1999], and analyzed by fluorescence flow cytometry. Cell cycle analysis was carried out using the FLOMAX software (Dako).

RESULTS

Structure-Based Sequence Alignment of BRD7

Alignment of the amino acid sequence of BRD7 protein with known structure of proteins in PDB (protein database), we obtained three known proteins (hsP/CAF, ScGCN5, hsTAF2d2) which possess the same structure as BRD7 protein in the bromodomain region (Fig. 1). The bromodomain in these three proteins contain a four-helix-bundles (αZ, αA, αB, and αC), and the four bundles forms ZA loop and BC loop. The ZA loop varies in length between different bromodomains, but always contains conserved residues (shaded residue Phe748, Pro751, Pro758,Tyr760, and Pro767 in hsp/CAF). The ZA loop and BC loop can form a hydrophobic pocket that is important for the specific binding of bromodomain to acetylated chromatin. Based on the similar structure, we proposed that BRD7, like other known bromodomain proteins,

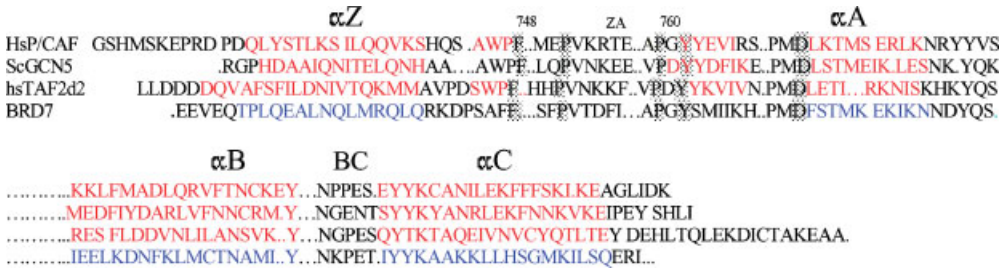

Fig. 1. Structure-based sequence alignment of BRD7. The sequences were aligned based on the experimentally determined structures of three bromodomains, highlighted in red. The predicted secondary structures in BRD7 bromodomain are in blue. Bromodomain are grouped on the basis of the predicted structural similarities. Residue numbers of the P/CAF bromodomain are indicated above its sequence. The conserved residues in ZAloop are shaded.

can bind to the acetylated histone through bromodomain.

Subcelluar Co-Localization Between BRD7 and Acetylated Histone H3

To study the interaction between BRD7 and acetylated histone H3 in vivo, confocal microscopy technique was first used to reveal the subcellular localization of BRD7 with acetylated histone H3. COS-7 cells were transiently transfected with wild type or bromodomain-deleted BRD7 mutant. At 24 h post-transfection, cells were collected and incubated with anti-myc antibody and anti-acetylated histone H3 antibody, then appropriate secondary antibodies conjugated with fluorescence were used to recognize the primary antibodies respectively. Bromodomain-deleted BRD7 mutant still localized in the nucleus but its localization became more granular compared with wild type (Fig. 2A). It was found that ectopic BRD7 protein co-localized with acetylated histone H3 in nucleus (Fig. 2B) and BRD7 bromodomain deletion mutant co-localization with acetylated histone H3 was not observed (Fig. 2C), which indicated the bromodomain may be essential for the co-localization of BRD7 with acetylated histone H3.

Chip Analysis of the Binding of BRD7 or BRD7 Bromodomain Deletion Mutant With Acetylated H3

In order to detect whether BRD7 can recognize acetylated histone H3 in vivo, Chip assay was performed. COS-7 cells were transiently transfected with wild-type or bromodomain-deleted BRD7 mutant, cell extracts were precipitated with anti-myc antibody to collect ectopic wild type or mutant BRD7 protein. Then Western-blot was performed by anti-acetylated histone H3 antibody to detect whether acetylated histone H3 exists in the complex containing BRD7 protein. Results showed that endogenous acetylated histone H3 can be precipitated by ectopic BRD7 protein in COS-7 cells transfected with wild-type BRD7, while not in bromodomain-deleted BRD7 mutant transfected cells (Fig. 3), which confirmed the bromodomain is essential for the binding of BRD7 with acetylated histone H3.

The Bromodomain of BRD7 Binding to Histone3 Peptides Acetylated at Lys14 In Vitro

To confirm the binding of BRD7 with acetylated-H3 in vitro and the binding site of BRD7 on acetylated histone H3, peptide binding assays using biotinylated histone tail peptides non-acetylated, mono-acetylated, and di-acetylated at specific lysine residues were carried out. As shown in Figure 4A, the results indicated the BRD7 strongly bound to histone H3 of acetylated at Lys14, compared with the weak binding with non-acetylated histone H3 peptide and acetylated at Lys9. We also observed that bromodomain-deleted BRD7 mutant lost the binding capacity with the acetylated histone H3 peptide (Fig. 4B). These findings provide evidence that BRD7 can specifically bind to histone H3 acetylated at Lys14 and the bromodomain is required for this binding in vitro.

TSA Treatment Alters Salt Solubility of BRD7

Salt solubility has been used to monitor binding of proteins to the chromatin compartment. TSA is inhibitor of histone deacetyltransferase (HDAC) and can increase the acetylation level of chromatin. Differential salt extraction experiment was used to know whether TSA

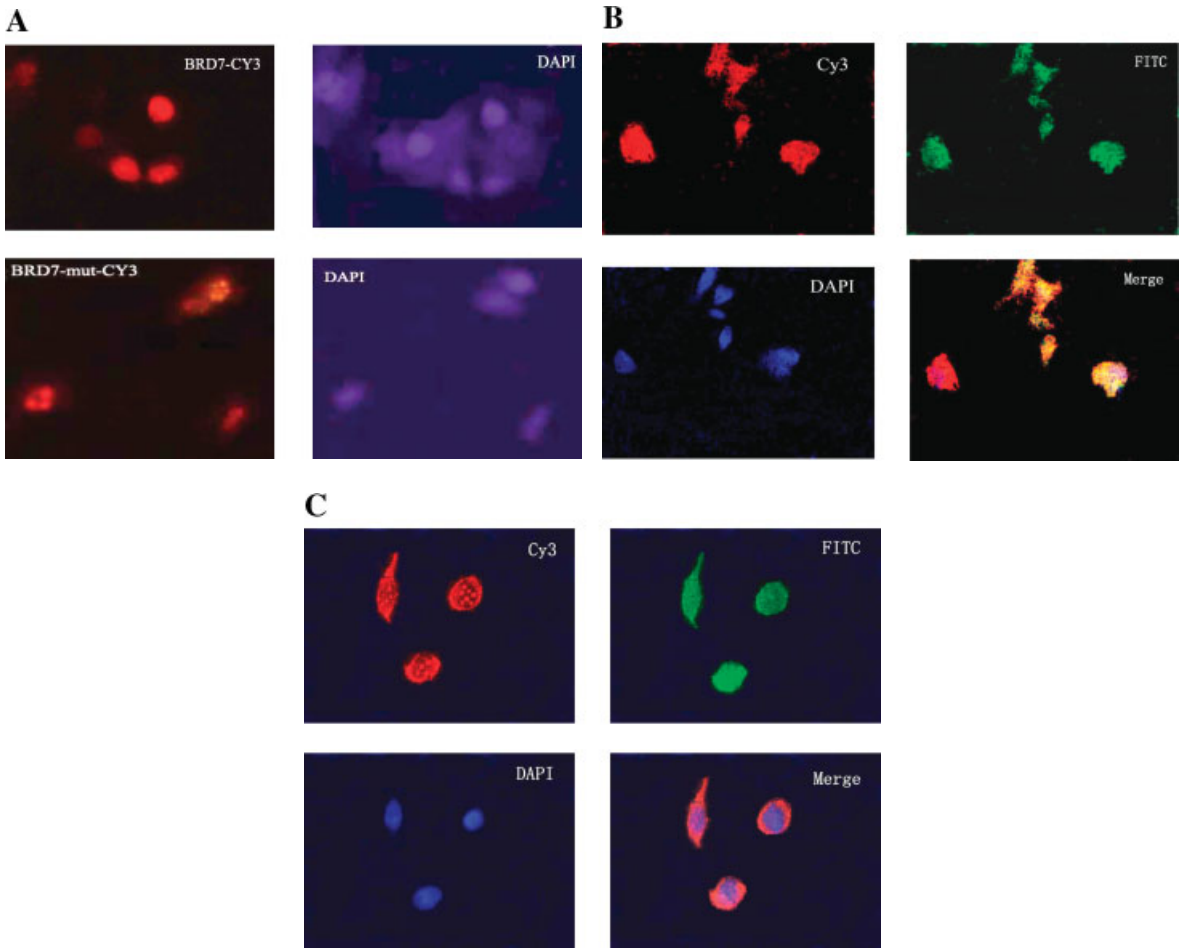

Fig. 2. Sub-celluar co-localization between BRD7 and acetylated histone H3. **A**: The localization of wild-type BRD7 and mutant BRD7 proteins in COS-7 cells. **B**: The co-localization of wild-type BRD7 and acetylated histone H3 in COS-7 cells. **C**: The co-localization of mutant BRD7 and acetylated histone H3 in COS-7 cells. Confocal micrographs depict the binding of BRD7 with acetylated histone H3 in COS-7 cells transiently expressed pCMV-BRD7 or pCMV-BRD7-mutant. The BRD7 expression is shown in red and acetylated histone H3 is identified by a secondary antibody conjugated to FITC(green), DNA is stained with DAPI (blue). The yellow color indicated protein co-localization. The merge image is an overlay of the three channels. Bars, 25 μm.

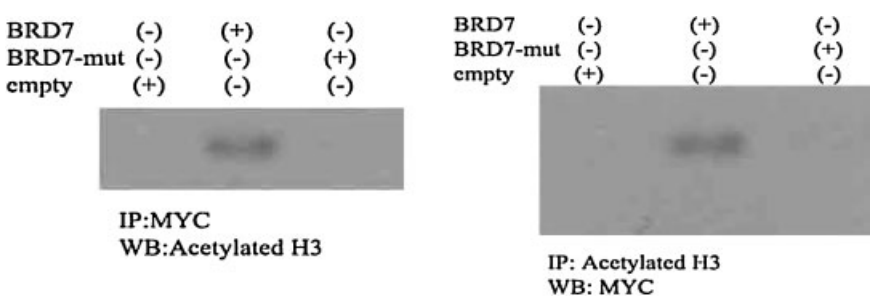

Fig. 3. Chip(chromosome immunoprecipitation) analysis of the binding of BRD7 or the bromodomain deleted BRD7 with the endogenous acetylated histone H3. Extracts from cells transfected with BRD7 or BRD7-mutatant and empty vector were immunoprecipitation with the antibodies indicated above the panels. Precipitates were detected by immunoblot analysis using the indicated antibodies.

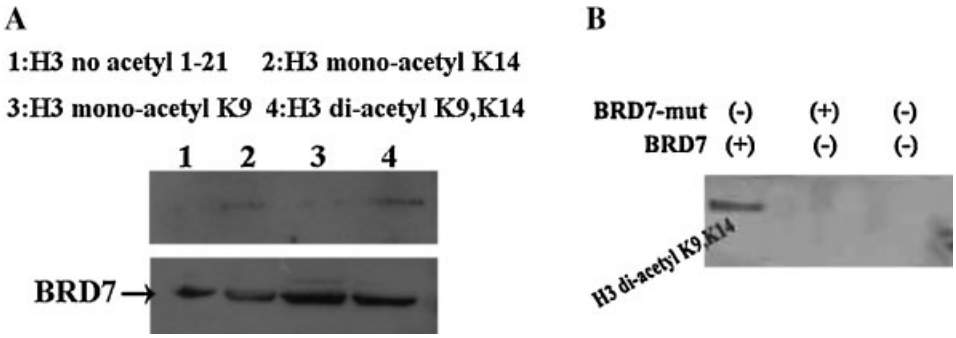

Fig. 4. The interaction between BRD7 or mutant BRD7 and acetylated histone H3 by using histone tail peptides. **A**: Wild-type (WT) BRD7 was transiently transfected into COS-7 cells. Twenty-four hours post-transfection, extracts was prepared and subjected to peptide pull-down assay using beads bound to either no-modified, mono-acetylated, and di-acetylated (Ac) histone H3 N-terminal tails. Proteins retained on the beads were then analyzed by Western blotting using an anti-myc antibody. **B**: Wild-type (WT) and mutant BRD7 proteins were expressed in COS-7 cells. After incubation with dia-acetylated (Ac) histone H3 N-terminal tails, Proteins retained on the beads were detected by Western-blot.

treatment could affect the binding capacity of BRD7 with acetylated chromatin. COS-7 cells were transiently transfected with BRD7 or bromodomain-deleted mutant. Nuclear extracts were prepared and then eluted with increasing concentrations of NaCl. The eluted fractions were tested for BRD7 protein with Western-blots (Fig. 5). In untreated BRD7 transfected cells, BRD7 was first eluted at 200 mM NaCl, followed by peak elution at around 400 mM. While in TSA treated BRD7 transfected cells, BRD7 was not detected until the NaCl concentration increased to 300 mM, with the peak elution at 400 mM. For bromodomain-deleted mutant transfected cells, BRD7 mutant was detected with 100 mM NaCl and there was no obvious change in the elution profile after TSA treatment. TSA treatment did not change their elution profiles, indicating that bromodomain deletions reduce the interaction with acetylated chromatin and that TSA did not affect the interaction. Acetylation level of histone was higher in TSA treated cells than in non-treated cells, which is consistent with the effect of TSA. The increase of salt concentration needed for efficient elution of BRD7 from nuclear extraction suggests that higher acetylated chromatin can in favor of binding with chromatin for BRD7 and bromodomain is required for the effect.

BRD7 has no HAT Activity

It is well known that many bromodomain proteins possess histone acetyltransferase (HAT) activity, although not all HATs contain a bromodomain. In order to know whether BRD7 protein has HAT activity, COS-7 cells were transiently transfected with BRD7 expression plasmid. As positive control, cells were treated with TSA. After 24 h post-transfection, Western-blot was performed with antibody against acetylated histone H3 to detect the acetylated level of histone3. BRD7 expression was confirmed by western-blots with antibody against myc. No obvious change was observed in the level of acetylated histone H3 between BRD7-transfected cells and control cells. However, an obvious increase of the acetylated histone H3 level was observed in positive

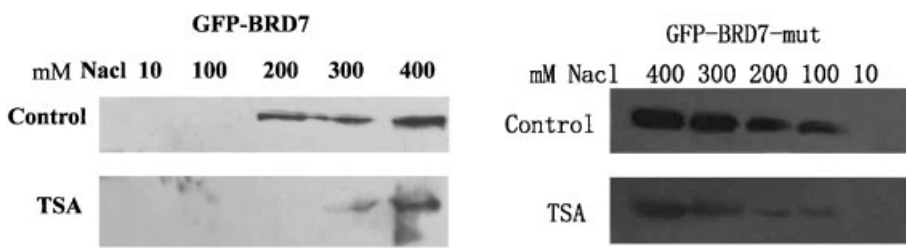

Fig. 5. Differential salt extraction experiment analysis solubility of BRD7 or BRD7 mutant in differential salt extraction. The nuclear extracts from cells transfected with BRD7 or mutant BRD7 by treated with TSA or without TSA treatment were washed with increasing concentrations of NaCl, and the Eluted proteins were detected by Western-blot with antibody against GFP.

control (Fig. 6). Taken together, this indicates that the ectopic expression of BRD7 cannot change the aceylation level of histone H3.

Loss of Inhibition of E2F3 Promoter Activity by BRD7 Bromodomain Deletion Mutant

In previous study, we found that BRD7 could inhibit E2F3 promoter activity. In this study, we further detect whether BRD7 can bind directly on the promoter of E2F3 by Chip assay. Nuclear extracts were prepared from BRD7 transfected cells or control cells and complex containing BRD7 protein was collected. Then PCR was used to detect whether E2F3 promoter existed in the complex. Results showed that there was no E2F3 promoter product in the BRD7-precipitated complex, confirming that E2F3 is not the direct transcription target of BRD7(data not shown). Considering most bromodomain proteins participated in transcriptional regulation in indirect way, it is possible that bromodomain mediated binding of BRD7 with acetylated chromatin may be the mechanism of transcriptional regulation of BRD7 on E2F3. To detect that, E2F3 reporter gene was co-transfected with BRD7 or bromodomain-deleted BRD7 mutant and we found that this mutant lost the ability to inhibit E2F3 promoter activity (Fig. 7).

The Bromodomain is a Essential for the Inhibition of Progression From G1 to S

From previous study, we know that BRD7 inhibits cell cycle G1 to S progression by transcriptionally regulating some cell cycle related genes, including E2F3. Combined with the above data, we want to know whether bromodomain is the basis of the effect of BRD7 on cell cycle. HNE1 cells transfected with wild type and mutant BRD7 were collected and cell cycle profiles were analyzed by Flow Cytometry according to DNA content. In HNE1 cells and control vector transfected cells, 49.85% and 52.13% of the cells were in G1 phase, while in BRD7 transfected cells, 59.13% cells were in G1 phase, showing an inhibition of G1–S progression after BRD7 expression and it was consistent with our previous result. In contrast, 49.45% cells in G1 phase in cells transfected with bromodomain-deleted BRD7 mutant, showing no difference with control cells (Fig. 8). These results indicate that the bromodomain-deleted BRD7 mutant lost the ability to inhibit G1 to S phase progression, which may be the result of the loss of transcriptional regulation of cell cycle targets, including E2F3.

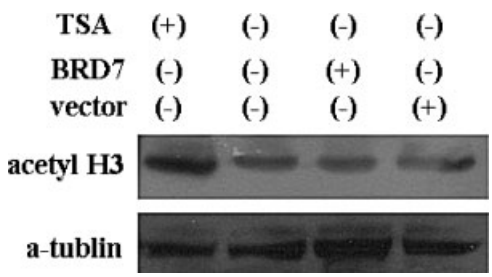

Fig. 6. Ectopic expression of BRD7 can not change the acetylated level of histones H3. Extracts from cells treated with TSA, or transfected with BRD7 and empty vector were detected by anti-acetylated histone H3. The a-tublin was used as internal control.

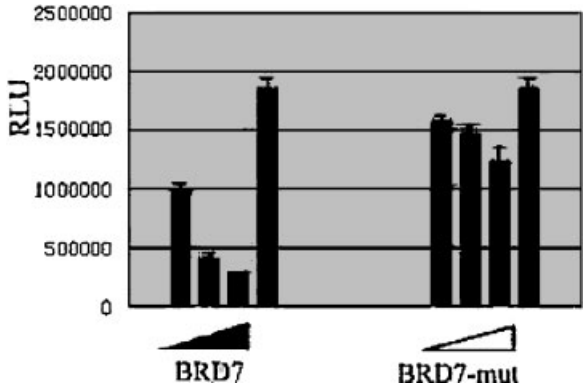

Fig. 7. BRD7 mutation in bromodomain loss ability to inhibition of E2F3 promoter activity. COS-7 cells on 6-well plates were co-transfected with E2F3 promoter-driven luciferase reporter (1.5 μg) and increasing amounts of wildly-type BRD7 or mutant BRD7 (0.5, 1.0, and 1.5 μg). Luciferase assays were performed in triplicate. A variation in transfection efficiency was normalized by the B-galactosidase activity under control of the sv40 B-galactosidase vector.

DISCUSSION

Histone N-terminals are platform of signal transduction implicated in transcriptional regulation. Extra-cellular signals cause diverse post-translation modifications of histone terminals by complex signal transduction pathways, which is of importance in signal-dependent chromatin remodeling and transcriptional regulation. That is the so called “histone code” hypothesis [Turner, 2000]. “Histone code” has attracted amounting attention due to the identification of some protein domains specifically recognizing particular post-translational modifications of histones. It revolutionizes the function of histone: histone is not just one

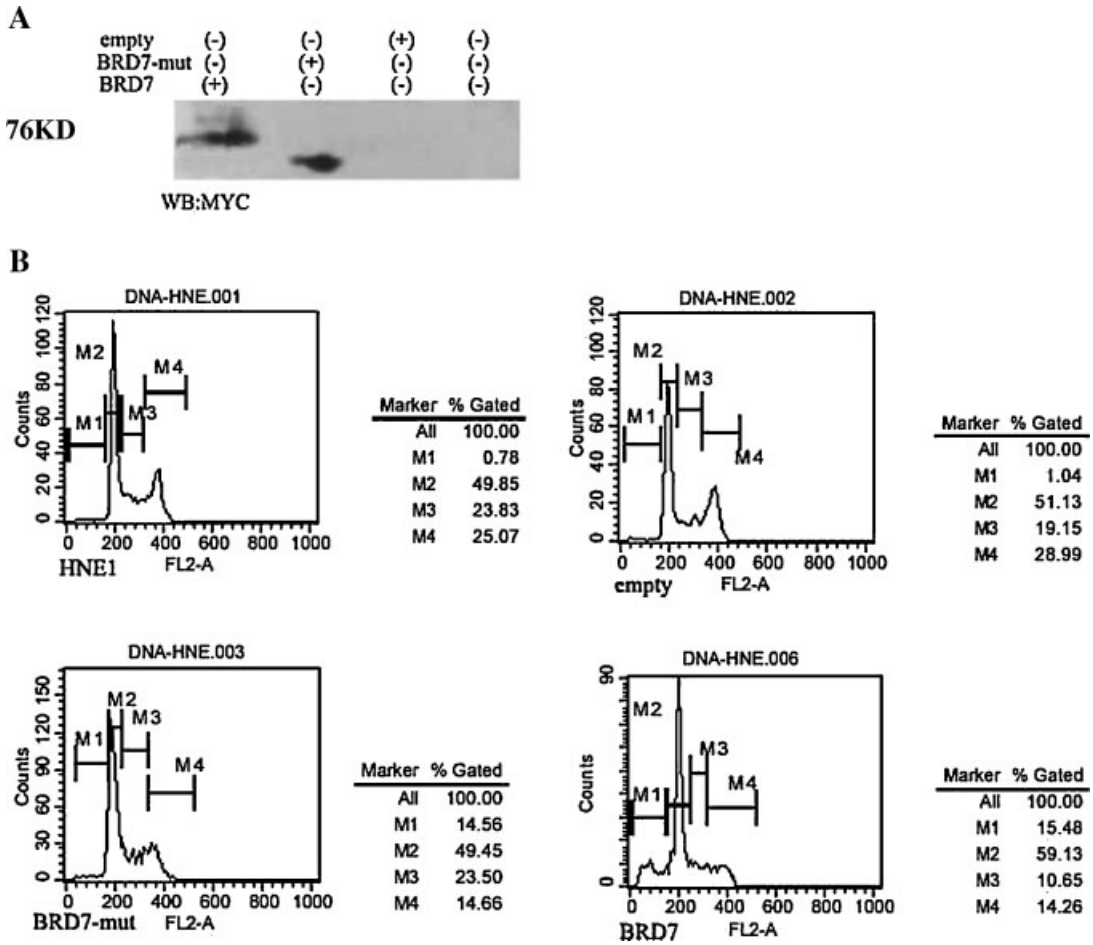

Fig. 8. Effect of mutant BRD7 on cell cycle in HNE1 cells. **A**: HNE1 cells were transfected with empty vector, pCMV-BRD7 and pCMV-mutant BRD7. After 48 h, Western-blot was performed to detect the expression of BRD7 and mutant BRD7. **B**: Cells were harvested at 48 h, stained with PI, and analyzed by flowcytometry (M2 · G0/G1 · M3 · S · M4 · G2/M).

passive structure to wrap DNA, but plays a positive role in transcriptional regulation.

Bromodomain is an evolutionally conserved module and can recognize acetylated lysines on N-terminals of histones [Appella and Anderson, 2001; Anup et al., 2003; Brooks and Gu, 2003; Dornan et al., 2003; Fulco et al., 2003; North et al., 2003; Yamaguchi et al., 2004]. So, bromodomain proteins are emerging as important regulators of chromatin remodeling and modification of complexes, but much remains to be learned about their binding determinants and their utilization in transcriptional regulation. Different bromodomains recognize different sites of acetylated histones, resulting in distinct histone codes.

BRD7 is the first identified bromodomain gene associated with Nasopharygeal Carcinoma (NPC). Previous studies indicated that BRD7 may participate in transcriptional regulation and could inhibit cell cycle progression by regulating some cell cycle related genes [Zhou et al., 2004]. In the present research, we studied the molecular mechanism of BRD7 involved in transcriptional regulation, as well as the role of bromodomain in this function.

Computer based sequence analysis showed that BRD7 shared part sequence similarity with three other described bromodomain proteins in the bromodomain ZA loop and BC loop region that are critical for the binding of bromodomain with acetylated chromatin. Then confocal microscopy experiment was used to detect the subcellular co-localization of BRD7 with acetylated histones. Results showed that wild-type BRD7 co-localized with acetylated histone H3 in the nucleus, while no co-localization between bromodomain-deleted BRD7 mutant and acetylated

histone H3 was observed. Though the Bromodomain-deleted mutant of BRD7 still displayed nuclear localization, its localization pattern became more granular compared with wild type BRD7, indicating the sub-nuclear localization of the BRD7 may be changed. Acetylated histones are associated with active transcription regulation, and the co-localization of BRD7 with acetylated histone H3 indicates that BRD7 is involved in transcriptional regulation, while bromodomain deletion impairs this ability. To provide evidence about the interaction of BRD7 with acetylated chromatin in vivo and in vitro, Chip and peptide binding assay were carried out. Both experiments demonstrated that BRD7 could bind specifically to acetylated H3 at Lys14 and the bromodomain is essential for this binding. We further used the differential salt extraction assay to determine whether TSA treatment can affect the ability of BRD7 binding to acetylated histone H3. The results showed that TSA treatment could increase the NaCl concentration that was required to elute BRD7 from nuclear extract, while effect on the bromodomain-deleted mutant was not observed, suggesting salt solubility of BRD7 was markedly reduced upon increased histone acetylation and this change also required bromodomain. These above results provide convincing evidence that BRD7 can bind specifically to acetylated H3 at Lys14 and bromodomain is essential for this binding.

Combined with our previous finding that BRD7 can regulate some cell cycle related genes including E2F3 and inhibit cell cycle from G1 to S progression in NPC cell line, we want to know how BRD7 regulates its target gene E2F3 and whether the binding of BRD7 with acetylated chromatin contributes to this effect. Chip experiment was used to reveal whether BRD7 could bind directly to the promoter region of E2F3. We did not detect E2F3 promoter by PCR in the BRD7-containing nuclear extract, indicating BRD7 may regulate E2F3 transcription in an indirect way. It was further found that the bromodomain deleted BRD7 failed to regulate E2F3 promoter activity and inhibited cell cycle progression, suggesting bromodomain is required for regulating E2F3 and cell cycle progression. Combined with our finding that bromodomain mediates the binding of BRD7 with acetylated chromatin, it is possible that BRD7 regulate the transcription of its targets by binding to acetylated chromatin through bromodomain, although more convincing and direct evidence is needed to confirm this.

More and more evidences have shown that bromodomain genes are of importance in transcriptional regulation and cellular events, including cell proliferation and cell differentiation. Their genetic alterations can lead to disorders including cancer. It is known that several bromodomain genes participate in carcinogenesis, such as, the bromodomain protein CBP [Shikama et al., 1997] is the tumor suppressor gene responsible for Rubinstein–Taybi syndrome [Petrij et al., 1995]. In addition, the bromodomain genes TIF1 [Douarin et al., 1995], RFG7 [Klugbauer and Rabes, 1999], BRG1 [Dunaief et al., 1994], RING [Denis and Green, 1996], and P/CAF [Yang et al., 1996] have all been implicated in the development of cancer. BRD7 is the first described bromodomain gene involved in NPC and function studies showed that it could inhibit cell growth in NPC cells. Our present study demonstrated that BRD7 binds to acetylated histone H3, which it contributes to the transcriptional regulation of its target genes. Therefore, down-regulation of BRD7 in NPC tissues and derived cell lines raises the possibility that transcriptional dysregulation is one of the mechanism implicated in the development of NPC.

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