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Generation of an antibody recognizing a set of acetylated proteins, including subunits of BAF complexes

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ABSTRACT

The aim of this study was to generate an antibody specific to Ki-67 acetylated at lysine 3180, whose existence was reported in an acetylome study (Scholz, C., B.T. Weinert, S.A. Wagner, P. Beli, Y. Miyake, J. Qi, L.J. Jensen, W. Streicher, A.R. McCarthy, N.J. Westwood, S. Lain, J. Cox, P. Matthias, M. Mann, J.E. Bradner, and C. Choudhary. 2015). Rabbits were immunized with a synthetic acetylated peptide corresponding to acetylated lysine 3180 of Ki-67 and the residues flanking it. The obtained antibody, referred to as Ab3180 in this study, was affinity purified with the antigen peptide and characterized. Immunoblot analysis of cell extracts using Ab3180 revealed that this antibody unexpectedly recognized a set of acetylated proteins unrelated to Ki-67. Ab3180-recognizable proteins were immunoprecipitated from cell extracts in a stringent condition and identified by mass-spec analysis as subunits of BAF (mammalian SWI/SNF) chromatin remodeling complexes. The unique specificity of Ab3180 will allow this antibody to be a useful tool for analyzing the acetylation of BAF complexes and its significance to the formation/function of BAF complexes.

1. Introduction

Ki-67 has long been used as a cell proliferation marker [1], but its actual cellular functions are just being discovered in recent years. As imagined from its localization on the periphery of mitotic chromosomes, Ki-67 has been revealed to support the architecture of mitotic chromosomes [2–4] and also prevent chromosomes from being stacked together via its surfactant-like nature [5]. Although we and others have shown that the C-terminal region of Ki-67 (LR domain: LRD) is essential for its chromosomal targeting [5,6], the molecular mechanism on which Ki-67 executes its functions remains poorly understood.

Lysine acetylation is a reversible post-translational modification, which plays important regulatory roles in various biological processes. Inspired particularly by the discovery that acetylation of SMC3, an ATPase subunit of cohesin complex, is essential for the establishment of sister chromatid cohesion [7–9], a theory was put forward that the activity of Ki-67 might be regulated by acetylation as well. A search of the literature was done and a quantitative acetylome study [10] showing the existence of acetylation of Ki-67 within the LRD was found. The study identified lysine 3180 of Ki-67 (long form, UniProt ID: P46013-1) as one of the sites hyperacetylated upon treatment of cells with pan-sirtuin inhibitor nicotinamide (NAM).

The original aim of this study was to generate antibodies specific to Ki-67 acetylated at lysine 3180. Unfortunately, the antibody generated did not perform as expected; instead, it turned out to recognize BAF (mammalian SWI/SNF) chromatin remodeling complexes. BAF complexes are known to play pivotal roles in regulating genomic architecture and topology [11]. BAF complexes are also involved in gene expression and in non-transcriptional events such as DNA repair [12], sister chromatid cohesion [13], and DNA decatenation in anaphase [14]. BAF complexes are multimeric molecular assemblies of 10–15 subunits and are classified into three non-redundant final-form assemblies: canonical BAF (cBAF), polybromo-associated BAF (PBAF), and non-canonical BAF (ncBAF) [15]. All complexes contain a catalytic ATPase subunit, either BRG1 (SMARCA4) or BRM (SMARCA2), and core subunits such as BAF155 (SMARCC1) and BAF60A (SMARCD1). The other subunits present define the identity of each complex. The study of BAF complexes has been attracting extraordinary attention since over 20% of all cancers present a mutation in at least one of BAF subunits [16,17]. For developing therapeutic opportunities against cancers driven by BAF complex perturbation, basic research to clarify the working principle of BAF, both in normal and cancerous cells, has been compiled [18]. The antibody developed and characterized in this study (Ab3180) will serve as a unique probe for monitoring the acetylation status of BAF complexes. Through studies utilizing Ab3180 in the future, it is expected that the regulation of the formation/function of BAF complexes, which is mediated by acetylation, will be revealed.

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2. Materials and methods

2.1. Generation and purification of Ab3180

Ab3180 was developed in rabbits against the synthetic acetylated peptide GGQKSAKacVLMQNQ-C (in which ac indicates an acetylated residue), which was conjugated via its C-terminally designed Cysteine to keyhole limpet hemocyanin. The peptide GGQKSAKVLMQNQ corresponds to the residues 3174–3186 of human Ki-67 (long form, UniProt ID: P46013-1). The resulting sera were absorbed thoroughly with the HiTrap NHS-activated HP column (GE Healthcare, Chicago, IL) conjugated with the corresponding non-acetylated peptide (GGQKSAKVLMQNQ-C) and then subjected to the HiTrap NHS-activated HP column conjugated with the antigen acetylated peptide. After washing the column extensively with phosphate-buffered saline (PBS), the trapped antibodies were eluted from the column with 0.1 M glycine (pH 3.0), immediately neutralized with the appropriate amount of 1 M tris (hydroxymethyl)aminomethane (Tris; pH 8.0) and dialyzed against 50% (v/v) glycerol in PBS.

2.2. Cell culture

HCT116 cells and its derivatives were grown in Dulbecco's modified Eagle's medium (DMEM; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) supplemented with 10% fetal bovine serum. The cell line Ki-67-mACl (#2), HCT-116 based cell line in which mini auxin-inducible degron (mAID) plus mClover are knocked in at the C-terminal end of Ki-67, was described previously [3]. When indicated, cells were treated with 20 mM NAM (Sigma-Aldrich, St Louis, MO) and/or 0.5 mM indol 3-acetic acid (IAA; Tokyo Chemical Industry, Tokyo, Japan) for 4 h. For the experiment shown in Fig. 3E, HCT116 cells were transfected with GL2 siRNA (5'-CGUACGCGGAAUACUUCGATT-3' [19]) or BRG1 siRNA (5'-GGGUACCCUCAGGACAACATT-3' [20]) using Lipofectamine RNAi MAX Transfection Reagent (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions for 48 h.

2.3. Immunoblotting of cellular extracts and immunofluorescence

Cells were washed with ice-cold PBS supplemented with 0.3 mM phenylmethylsulfonyl fluoride (PMSF), collected by centrifugation, and snap-frozen in liquid nitrogen. Cell pellets were resuspended in buffer B [20 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 0.1% NP-40, 1 mM dithiothreitol (DTT), Complete protease inhibitor Mixture (Roche, Basel, Switzerland), and PhosSTOP (Roche)] supplemented with 0.25 units/mL Benzonase (Novagen, Madison, WI), kept on ice for 30 min, mixed with the same volume of 4 × concentrated sample buffer [250 mM Tris-HCl pH 6.8, 8% sodium dodecyl sulfate (SDS), 40% glycerol, 0.02% Bromophenol Blue and 0.1 M DTT], and heated at 95 °C for 3 min. The denatured protein samples were electrophoretically separated on a SuperSep Ace 5–20% gradient gel (Wako, Osaka, Japan) and blotted onto an Immobilon-P membrane (Merck Millipore, Billerica, MA). The following antibodies were used as primary antibodies at the indicated dilutions or concentrations: rabbit Ab3180 (1 μg/mL), rabbit anti-Ki-67 (1:1000, sc-15402; Santa Cruz Biotechnology, Dallas, TX), and mouse anti-β-tubulin (1:5000, AC-15; Sigma). For Fig. 1B, Ab3180 was preincubated with 10-time excess peptide (GGQKSAKVLMQNQC or GGQKSAKacVLMQNQC) by weight at room temperature for 2 h before use. The following antibodies were used as secondary antibodies at the indicated dilutions: goat anti-mouse-IgG conjugated to horseradish peroxidase (HRP) (1:3000, 170–6516; Bio-Rad, Hercules, CA), and goat anti-rabbit-IgG conjugated to HRP (1:3000, 170–6515; Bio-Rad) antibodies. Protein bands were visualized by chemiluminescence using Immobilon Western (Merck Millipore, Billerica, MA). Immunofluorescence was carried out as described previously [4] using Ab3180 as a primary antibody at 5 μg/ml.

2.4. Generation of Ki-67-KO HCT116

Annealed DNA oligonucleotides with sequences corresponding to the guide RNA (5'-CCAAUCCUAGAGCGCGUUUC-3') appropriate for cleaving the genome with Cas9 at the site 165-bp downstream of the start codon of MKi67 (NC_000010.11) were inserted into pX330 (Addgene plasmid #42230; [21]) to generate pMT709. The 1614-bp human genomic fragment around the start codon of MKi67 was PCR-amplified from genomic DNA using GoTaq DNA polymerase (Promega, Madison, WI) with primers P831 (5'-AAGTACCAAGGTGAACAAAG-3') and P832 (5'-GTTTTCGTTTGAATTGGGCG-3'). The PCR fragment was cloned into the pCRII vector (Invitrogen, Carlsbad, CA) to generate pMT710. At the position just downstream of the start codon within pMT710, mAID-NeoR cassette [22] was inserted via Gibson assembly (NEB, Ipswich, MA) to generate the homology-directed repair constructs pMT711. For generating Ki-67-KO HCT116, HCT116 cells were transfected with pMT709 and pMT711 using FuGene HD (Promega) and selected with 700 μg/mL G418 (09380-44; Nacalai Tesque, Kyoto, Japan). Clones in which the genome had been edited as designed in both alleles were selected via genomic PCR using KOD-plus-Neo (TOYOBO, Osaka, Japan) with appropriate primer sets. The loss of Ki-67 from cells was finally validated by immunoblotting and immunofluorescence analyses carried out as described previously [3].

2.5. Immunoprecipitation (IP)

Cells were lysed in buffer B supplemented with 0.25 units/mL Benzonase (Novagen) for 30 min on ice. The lysate was cleared by centrifugation at 21,500 × *g* for 10 min at 4 °C. The supernatant was incubated with antibodies (control antibodies or Ab3180) coupled to Dynabeads Protein A (Thermo Fisher Scientific) for 1 h on ice with occasional agitation. Typically, 4 x 10^6 cells were lysed with 300 μl of lysis buffer. This amount of each lysate was subjected to IP with 10 μg of antibodies coupled to 40 μl of Dynabeads Protein A. The beads were washed three times with buffer B using a magnet. For the final wash, sample tubes were replaced with new ones to reduce contamination by proteins bound nonspecifically to the tubes. The beads were further washed sequentially with buffer B250, buffer B500, and buffer B1000: buffers identical to buffer B except for the concentration of NaCl (250, 500, and 1000 mM, respectively). Washings were collected, and the proteins therein were recovered by trichloroacetic acid (TCA) precipitation as W250, W500, and W1000 fractions. The proteins still bound to beads were dissolved by boiling the beads with 4 × concentrated sample buffer for 3 min and retrieved using a magnet as R1000 (Fig. 2) or stringent IP fractions (Fig. 3A–B and D). For the preparation of R1000 fraction in Fig. 2B, Dynabeads Protein A beads coupled with Ab3180 was preincubated with 10-time excess peptide (GGQKSAKacVLMQNQC or GGQKSAKVLMQNQC) by weight at room temperature for 2 h before the incubation with cell lysates. For the IP in Fig. 3C, SDS was added to the cell lysate and all wash buffers to 0.1%. Immunoblot analysis of those IP fractions were performed as described above using the following primary antibodies: rabbit anti-ARID1A/BAF250A (1:1000, D2A8U; Cell Signaling), mouse anti-BRG1 (1:1000, sc-17796; Santa Cruz Biotechnology), rabbit anti-SMARCC2/BAF170 (1:1000, D809V; Cell Signaling), rabbit anti-SMARCC1/BAF155 (1:1000, D7F8S; Cell Signaling), and mouse anti-Acetylated-Lysine mAb (1:1000, Ac-K-103; Cell Signaling).

2.6. Identification of Ab3180-recognizable proteins by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Proteins immunoprecipitated with Ab3180 in a stringent condition were electrophoretically separated on a SuperSep Ace 5–20% gradient gel (Wako) and stained using ProteoSilver Plus Sliver Stain Kit (Sigma). Each gel band of interest was excised and cut into small pieces. After washing and destaining the gel pieces according to the manufacturer's

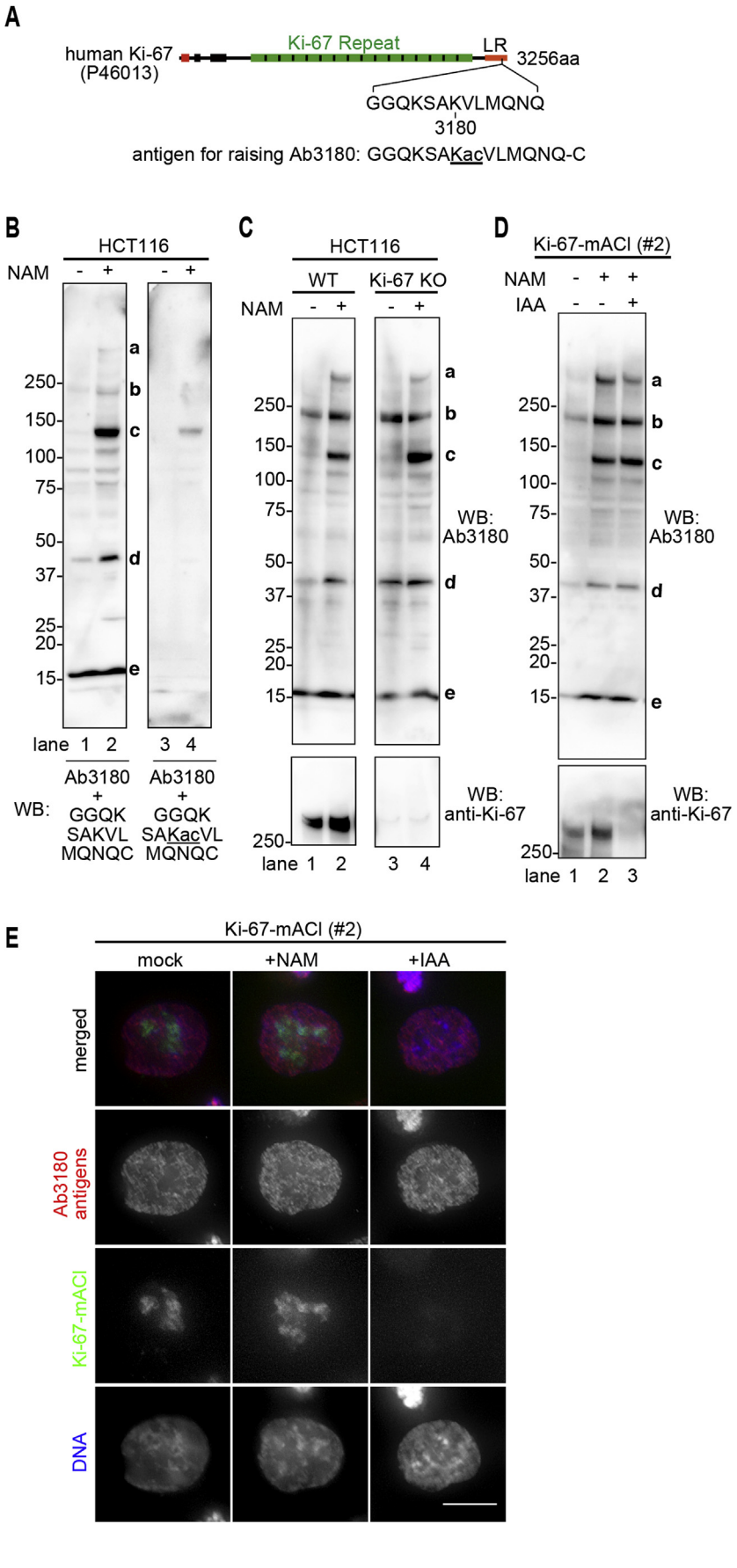

Fig. 1. Ab3180 recognizes a set of proteins in cells treated with NAM. (A) An acetylated peptide used as an antigen for developing Ab3180. The peptide corresponds to the residues 3174–3186 of human Ki-67 (long form, P46013-1). The lysine residue in the middle of the peptide (K3180) has been shown to become strongly acetylated upon treatment of cells with NAM, a pan-sirtuin inhibitor [10]. (B–D) Immunoblot analysis of cell extracts with Ab3180. (B) Benzonase extracts of mock- or NAM-treated HCT116 were analyzed. Ab3180 was pre-incubated with 10-time excess peptide (GGQKSAKVLMQNQC or GGQKSAKacVLMQNQC) by weight at room temperature for 2 h before use. (C) Benzonase extracts of mock- or NAM-treated HCT116 (wild type: WT) and its derivative (Ki-67 knock-out: Ki-67-KO) were analyzed. (D) Benzonase extracts of Ki-67-mACl (#2) cell mock-treated, treated with NAM, or treated with NAM plus IAA were analyzed. (B–D) A specific set of proteins (marked with bold letters **a**-**e**) were clearly detected. The absence (C, lanes 3–4) or degradation (D, lane 3) of endogenous Ki-67 was verified by immunoblot with anti-Ki-67 antibody. (E) Representative immunofluorescence images of Ki-67-mACl cells mock-treated, treated with NAM, or treated with IAA. Cells were stained for Ab3180 antigens. Ki-67-mAID-mClover (Ki-67-mACl) was detected via the fluorescence of mClover. DNA was counterstained with Hoechst 33342. Bar, 10 μm.

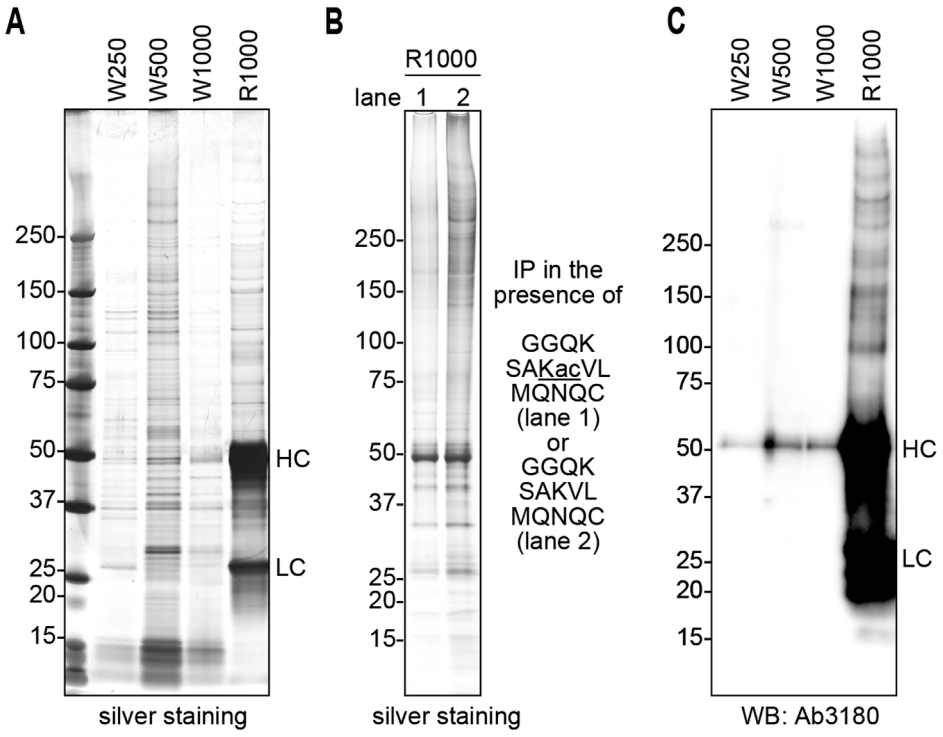

Fig. 2. Immuno-purification of proteins recognized by Ab3180. (A) IP of Ab3180-recognizable proteins from NAM-treated HCT116-cell extracts in a stringent condition. Immunoprecipitate that had been prepared in a buffer containing 150 mM NaCl was washed with buffer containing increasing concentration of NaCl (250 mM, 500 mM, and 1 M). Washings were collected, and proteins therein were recovered by TCA precipitation. Those proteins denoted as W250, W500 and W1000 and the proteins remaining on the IP beads after washing with buffer containing 1 M NaCl (R1000) were separated with 5–20% SDS-PAGE and analyzed by silver staining. (B) The R1000 fraction was prepared in the same way as (A) except that the IP resins coupled with Ab3180 had been preincubated with 10-time excess peptide (GGQKSAKacVLMQNQC or GGQKSAKVLMQNQC) by weight at room temperature for 2 h before use. (C) The same set of protein fractions to (A) was analyzed by immunoblot using Ab3180. HC, heavy chain of immunoglobulin; LC, light chain of immunoglobulin.

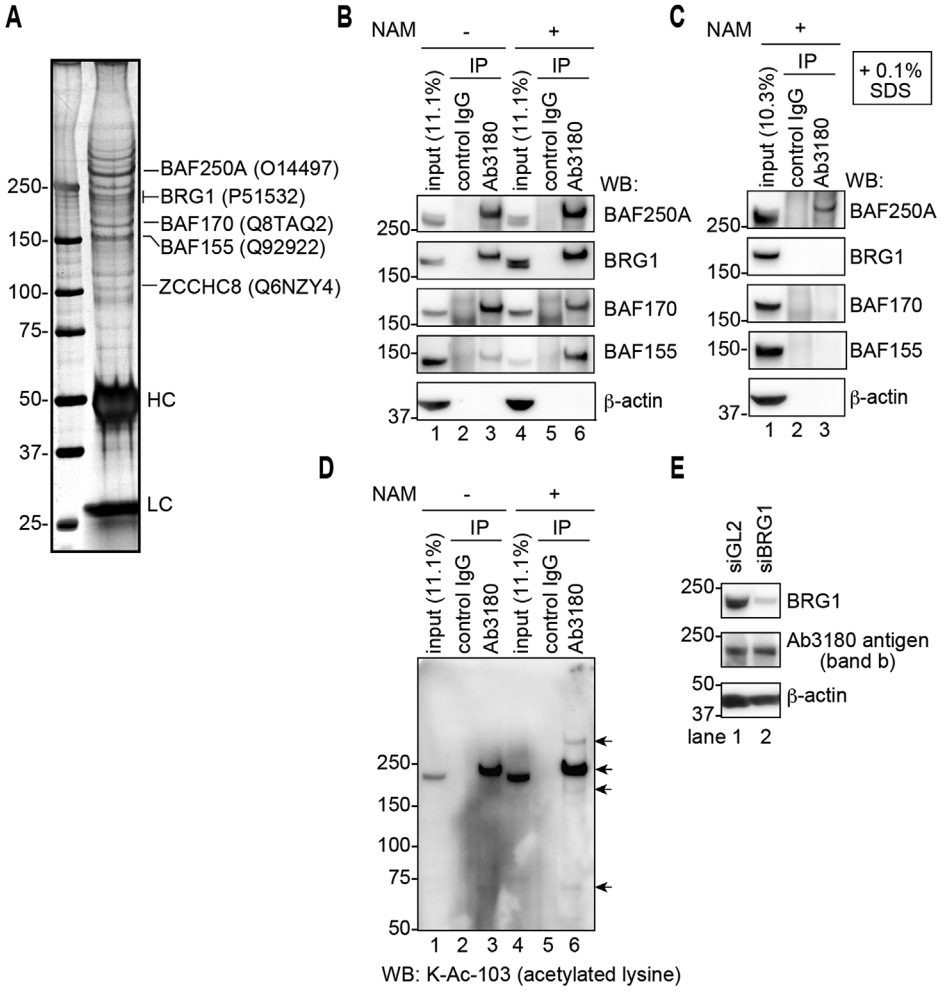

Fig. 3. Ab3180 recognizes BAF chromatin remodeling complexes. (A) Identification of Ab3180-recognizable proteins by mass-spec analysis. The immunoprecipitate with Ab3180 was prepared in a stringent condition as shown in Fig. 2 and conspicuous proteins seen in a silver-stained gel were processed for identification by mass-spec analysis. Names of proteins identified with a Mascot score higher than 450 were displayed with the UniProt IDs in parentheses. HC, heavy chain of immunoglobulin; LC, light chain of immunoglobulin. (B–D) Immunoblot analysis of the stringent IP fractions obtained from mock- or NAM-treated HCT116-cell extracts with control antibodies or Ab3180. In (C), 0.1% SDS was additionally added through the procedure. The blots were probed with indicated antibodies. In (D), acetylated proteins found in the stringent IP fractions were marked with arrows. (E) Immunoblot analysis of the extracts of HCT116 cells transfected with control siRNA (siGL2) or siRNA specific to BRG1 (siBRG1). The signal intensity of band **b**, one of the Ab3180 antigens seen in immunoblotting around 200 kDa, was not altered upon the substantial depletion of BRG1.

protocol, cysteine residues were reduced by DTT and alkylated with iodoacetamide. The proteins were digested with modified trypsin (V5111, Promega), and then the resulting peptides were subjected to LC-MS/MS. LC-MS/MS analysis was performed using Advance nanoLC (Bruker-Michrom, Auburn, CA) and LTQ linear ion trap mass spectrometer (Thermo Fisher Scientific) equipped with a NANO-HPLC capillary column C18 (0.075 mm ID x 150 mm length, 3 μm particle size, Nikkyo Technos, Tokyo, Japan) using a linear gradient (25 min, 5–35% CH_3CN/0.1% formic acid) at a flow rate of 300 nL/min. The resulting MS and MS/MS data were searched against the Swiss-Prot database using MASCOT software (Matrix Science, London, United Kingdom).

3. Results and discussion

3.1. Antibody developed against a synthetic acetylated peptide (Ab3180) recognized a set of proteins in HCT116 cells treated with NAM

A polyclonal antibody, referred to as Ab3180, was developed against a synthetic acetylated peptide (GGQKSAKacVLMQNQ) whose amino acid sequence is derived from human Ki-67 (Fig. 1A). The lysine residue in the middle of the peptide tract (K3180) was reported to be acetylated by certain acetyltransferase counteracting with NAM-sensitive deacetylase such as sirtuins [10]. Ab3180 was absorbed thoroughly with the corresponding non-acetylated peptide, and then affinity purified with the antigenic acetylated peptide. Ab3180 was not monospecific and detected multiple proteins in HCT116 cells by immunoblotting (Fig. 1B, lanes 1–2). Among five obvious protein bands (marked with bold letters **a**-**e**), the signal intensity of proteins **a**-**d** was clearly increased when cells were treated with NAM, suggesting that the acetylation level of those proteins was elevated upon inhibiting sirtuins. Those protein bands mostly disappeared by the competition with the antigen peptide (Fig. 1B, lanes 3–4) but not with the corresponding non-acetylated peptide (Fig. 1B, lanes 1–2), indicating that Ab3180 had the ability to recognize a set of acetylated proteins specifically. Because of the similarity in a NAM-responsive elevation of acetylation level and also in migration position in a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, it was assumed that the signal of protein **a** was derived from Ki-67 acetylated at K3180. To test this assumption, a Ki-67 knock-out (KO) cell line generated by CRISPR/Cas9 was utilized (Fig. 1C, lanes 3–4). Even in the complete absence of Ki-67 in the cell line, the immunoblot signal of protein **a** was not lost (Fig. 1C, lane4), indicating that the signal was derived from a protein unrelated to Ki-67, contradicting the assumption made. The next cell line utilized was Ki-67-mACl (#2), a HCT-116 based cell line in which mAID plus mClover are knocked in at the C-terminal end of Ki-67 [3] (Fig. 1D). In this cell line, the same set of proteins (proteins **a**-**e**) were clearly recognized by Ab3180 as well. Even when Ki-67-mACl (Ki-67 fused to mAID plus mClover) was induced to be degraded upon the treatment of cells with IAA, the immunoblot signal of protein **a** was not lost (Fig. 1D, lane 3), indicating again that the signal was derived from a protein unrelated to Ki-67.

In addition to these immunoblot analyses (Fig. 1C and D), immunofluorescence of Ki-67-mACl (#2) cells with Ab3180 was performed (Fig. 1E). Ab3180 antigens were found mainly in cellular nuclei but did not show clear co-localization with Ki-67-mACl, the majority of which was localized at nucleoli (Fig. 1E, first column). Somewhat unexpectedly, the staining pattern and intensity were not altered upon the treatment of cells with NAM (Fig. 1E, second column [+NAM]). Importantly, the localization of Ab3180 antigens was not largely affected by the loss of Ki-67-mACl upon the treatment of cells with IAA (Fig. 1E, third column [+IAA]). These observations indicated that Ab3180 majorly recognized proteins other than Ki-67 also in immunofluorescence.

Overall, it can be concluded that Ab3180 recognized a set of proteins that were most likely being acetylated. Although the possibility that Ab3180 did indeed recognize Ki-67 acetylated at K3180 as originally intended cannot be excluded, other proteins are recognized more dominantly from crude cellular extracts or in cells.

3.2. Ab3180 recognized BAF chromatin remodeling complexes

The study goal was changed to the identification of proteins recognized by Ab3180. To this end, it was decided that a simple strategy combining IP and mass-spec (MS) analysis should be employed. IP was carried out using Ab3180 from benzonase extracts of NAM-treated HCT116 cells. In the first trial where the immunoprecipitate was washed with buffer containing a physiological concentration of NaCl (150 mM), its protein composition was too complex to determine which proteins were recognized directly by Ab3180 (data not shown). Many proteins were supposed to be co-immunoprecipitated via forming certain protein complexes. Aiming to remove those proteins from the immunoprecipitate, it was washed sequentially with buffers containing an increasing concentration of NaCl (250, 500, 1000 mM). As shown in Fig. 2A, bunches of proteins were collected in the wash fractions (W250, W500, and W1000) and proteins of relatively simple composition remained on the IP beads (R1000). The complexity of proteins recovered in the R1000 fraction was significantly decreased by the preincubation of the IP beads with the antigen peptide but not with corresponding unacetylated peptide (Fig. 2B), indicating the specificity of the process as a whole. Immunoblot analysis of the set of fractions shown in Fig. 2A with Ab3180 (Fig. 2C) revealed that the proteins recognized by Ab3180 were efficiently collected in the fraction R1000.

Since succeeding in recovering proteins which seem to be recognized directly by Ab3180 with minimum contamination of co-immunoprecipitated proteins (Fig. 2), the next step was to molecularly identify those proteins by MS analysis. Five proteins were credibly identified with Mascot scores higher than 450 (Fig. 3A). Interestingly, four of them (BAF250A, BRG1, BAF170, BAF155) were subunits of BAF (mammalian SWI/SNF) chromatin remodeling complexes. The specific IP of those four proteins with Ab3180 was confirmed by immunoblotting probed with antibodies to each protein (Fig. 3B). The reason why the immunoprecipitated proteins showed slower migration in the gel than those in the crude cell lysate was not clear at present. Since this IP experiment was carried out in a stringent condition (using a buffer containing 1 M NaCl), it can be speculated that the BAF complexes were not maintained and each subunit of BAF complexes was precipitated with Ab3180 independently of other subunits. To test whether it is the case, the IP experiment was repeated in the presence of 0.1% SDS. In this condition where much of protein complex formation is supposed to be destroyed, only BAF250A was precipitated with Ab3180 (Fig. 3C). Thus, unlike my speculation, it is more likely that, in the experiment shown in Fig. 3B, BAF complexes kept in shape even in the presence of 1 M NaCl were precipitated with Ab3180 through the direct recognition of BAF250A by Ab3180. Considering the procedure of generation and purification of Ab3180, and the fact that certain proteins immunoprecipitated with Ab3180 in stringent condition were indeed recognized by an anti-acetylated lysine antibody (Fig. 3D, lanes 3 and 6), BAF250A in the same IP fraction (Fig. 3B, lanes 3 and 6) were supposed to be acetylated as well.

Finally, the possible identity between proteins detected by Ab3180 in immunoblots (Fig. 1B–D) and subunits of BAF complexes were examined. Considering the resemblance in the mobility in SDS-PAGE gels, BRG1 was suspected to be identical to protein **b** seen in Fig. 1B–D. To test if this was the case, an immunoblot with Ab3180 was performed for the extracts of cells from which BRG1 was knocked down using siRNA specific to BRG1 (Fig. 3E). Although BRG1 was successfully reduced judging from the immunoblot with an anti-BRG1 antibody (upper panel), there was no concurrent decrease in the signal intensity of protein **b** (middle panel). Thus, contradictory to the surmise posited above, the protein **b** is likely to be a protein different from BRG1.

3.3. Perspectives

Although BRM, a catalytic ATPase subunit of BAF complexes, was reported to be acetylated in its C terminus to limit BAF function [23], further studies focusing on the implication of acetylation of BAF subunits have not been performed so far to my knowledge. Fortuitously, the antibody developed and characterized in this study (Ab3180) recognizes BAF complexes within which BAF250A is likely being acetylated. Mapping the acetylated site(s) of BAF250A precisely with the aid of Ab3180 could be a good start point towards understanding the significance of acetylation of BAF subunits. Since some subunits of BAF complexes (BRG1, BRM1, PBRM1, BRD7, and BRD9) contain bromodomain(s), a "reader" of acetylated lysine, it is intriguing to test the possibility that the assembly and/or function of BAF complexes might be regulated via recognition of acetylated residue(s) of one subunit by bromodomain(s) of another subunit. From the view point of effective use of Ab3180 for analyzing the functional control of BAF, it is intriguing as well to characterize the property (for instance biochemical composition) of BAF complexes containing acetylated BAF250A, whose specific preparation might be possible solely by IP with Ab3180 and never with commercial antibodies against BAF components.

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CRediT authorship contribution statement

Masatoshi Takagi: Conceptualization, Investigation, Writing - review & editing.

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