NAT10 promotes cell proliferation by acetylating CEP170 mRNA to enhance translation efficiency in multiple myeloma

Rongfang Wei\textsuperscript{a,c,\dagger}, Xing Cui\textsuperscript{d,\dagger}, Jie Min\textsuperscript{c,\dagger}, Zigen Lin\textsuperscript{c}, Yanyan Zhou\textsuperscript{c}, Mengjie Guo\textsuperscript{c}, Xiaojuan An\textsuperscript{c}, Hao Liu\textsuperscript{c}, Siegfried Janz\textsuperscript{e}, Chunyan Gu\textsuperscript{a,c,*}, Hongbo Wang\textsuperscript{b,f,*}, Ye Yang\textsuperscript{c,*}

\textsuperscript{a}Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, Nanjing 210022, China
\textsuperscript{b}School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China
\textsuperscript{c}School of Medicine & Holistic Integrative Medicine, Nanjing University of Chinese Medicine, Nanjing 210023, China
\textsuperscript{d}Department of Hematology, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan 250014, China
\textsuperscript{e}Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI 53226, USA
\textsuperscript{f}Bohai Rim Advanced Research Institute for Drug Discovery, Yantai 264003, China

Received 15 October 2021; received in revised form 1 December 2021; accepted 16 December 2021

KEY WORDS
Multiple myeloma; Target; NAT10; Acetylation; CEP170; Chromosomal instability; Translation; Remodelin

Abstract Multiple myeloma (MM) is still an incurable hematologic malignancy, which is eagerly to the discovery of novel therapeutic targets and methods. N-acetyltransferase 10 (NAT10) is the first reported regulator of mRNA acetylation that is activated in many cancers. However, the function of NAT10 in MM remains unclear. We found significant upregulation of NAT10 in MM patients compared to normal plasma cells, which was also highly correlated with MM poor outcome. Further enforced NAT10 expression promoted MM growth \textit{in vitro} and \textit{in vivo}, while knockdown of NAT10 reversed those effects. The correlation analysis of acetylated RNA immunoprecipitation sequencing (acRIP-seq) and ribosome profiling sequencing (Ribo-seq) combined with RIP-PCR tests identified centrosomal protein 170 (CEP170) as an important downstream target of NAT10. Interfering CEP170 expression in
1. Introduction

Multiple myeloma (MM) is still an incurable hematological malignancy, especially the high-risk subgroup patients are characterized by high-proliferation feature and they have no significant improvement of outcome even treated with the advanced novel drugs including proteasome inhibitors, immune modulators et al.12. Furthermore, the prognostic stratification and therapeutic evaluation systems for MM also lack specific molecular indicators. Thus, seeking for novel diagnostic marker and potential therapeutic target for MM, especially for high-risk MM is of extreme urgency. Increasing studies demonstrate the importance of epigenetic regulation on MM malignancy, such as DNA methylation, histone acetylation and Epi-microRNAs. Here we focus on N-acetyltransferase 10 (NAT10) mRNA modification, which is originally presented in tRNA and rRNA and recently investigated in multiple types of cancer mRNA, not yet in MM.

N-Acetyltransferase 10 (NAT10) is the first identified acetyltransferase on ac4C mRNA modification containing 1025 amino acids with a molecular weight of 116 kD. There are three conserved domains in NAT10 protein, including the N-terminal acylase domain (GNAT family), an ATP/GTP binding motif and an ATPase domain. The acetylation site of NAT10 is lysine residue at 426 (K426), and acetylation of K426 is necessary for NAT10 activating rRNA transcription. NAT10 catalyzes mRNA acetylation within coding sequences (CDS) to improve mRNA stability and increase translation efficiency. It has been reported that NAT10 is involved in multiple cancers, such as epithelial ovarian cancer, hepatocellular carcinoma, breast cancer, colorectal cancer, acute myeloid leukemia, and acute myeloid leukemia. However, a large fraction of these studies describes other functions of NAT10 out of non-mRNA acetyltransferase. For instance, NAT10 acetylates p53 at K120 and stabilizes p53 at protein level by counteracting MDM2 action leading to inhibition of cellular proliferation in human colorectal carcinomas. NAT10 and SIRT1 mediate the acetylation of CCDC84 protein at lysine 31 in Hela cells, and the acetylated CCDC84 promotes HsSAS-6 binding to APC/CCdh1 to regulate centrosome duplication. The function of NAT10 in MM and the specific association between mRNA ac4C modification and MM pathogenesis are still poorly elaborated.

In this study, we first demonstrated the contributing role of NAT10 to MM growth in clinical cohorts and primary samples combined with laboratory works in vitro and in vivo. Furthermore, we discovered NAT10 catalyzing ac4C modification to enhance translation efficiency in MM cells and identified CEP170 as the major and functional downstream target of NAT10. In addition, inhibition of NAT10 by remodelin suppressed MM growth in vitro and in vivo. Taken together, we disclose that NAT10 is a potentially promising diagnostic marker and therapeutic target for MM.

2. Materials and methods

2.1. Gene expression profiling

The Gene expression profiling (GEP) cohorts were collected from GEO database as previous described. Total therapy 2 (TT2, GSE2658), TT3 (GSE2658), and the assessment of proteasome inhibition for extending remission (APEX, GSE9782) were employed. The Dutch-Belgian Cooperative Trial Group for Hematology Oncology Group-65 (HOVON65) trials was collected from GSE19784.

2.2. Antibodies and reagents

The antibodies were used as following, NAT10 (13365-1-AP, Proteintech), CEP170 (18899-1-AP, Proteintech), PARP (9542 S, Cell Signaling Technology), cleaved caspase-3 (9661 S, Cell Signaling Technology), ac4C (ab252215, Abcam), rabbit IgG (7074, Cell Signaling Technology), β-actin (60008-1-lg, Proteintech), Ki67 (AF0198, Affinity), α-tubulin (ab7291, Abcam), BrdU mAb (MI-11-3, MBL), secondary antibodies included goat anti-rabbit IgG(H + L) HRP (FMS-Rb01, Femacs) or mouse (S0002, Affinity). Remodelin was purchased from CENpharm. Puromycin was from AEPxBIO (B7587), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, M8180) was purchased from Solarbio. Actinomycin D was purchased from MCE (MedChemExpress, HY-17559), 5′-Bromo-uridine (BrU) was purchased from Sigma-Aldrich (850187). Lipofectamine Transfection Reagent (40802ES02), TREasy Total RNA Extraction Reagent (19201ES60), complementary DNA synthesis superMix (11123ES10), and SYBR Green Master Mix (11198ES03) were purchased from Yeasen.

2.3. Cell lines and culture

Human MM cell lines, CAG, KMS28PE, OPM2 and 293 T cells, were donated by Prof. Siegfried Janz (Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI, USA) and 5TMM3VT murine myeloma cell was provided by Prof. Wen Zhou (Central South University, Changsha, China). The cells were cultured in RPMI-1640 (01-100-1ACS, Biological Industries, Israel). 293 T cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, 01-052-1ACS, Biological Industries, Israel). All media were supplemented with 10% fetal bovine serum (FBS, 04-001-1ACS, Biological Industries, Israel), 100 U/mL penicillin and 100 μg/mL streptomycin.
100 μg/mL streptomycin (03-031-1B, Biological Industries, Israel). All cells were cultured in humidified 5% CO2 incubators at 37 °C.

2.4. The patient sample collection and immunohistochemistry analysis (IHC)

The posterior superior iliac spine of MM patients was chosen as the biopsy point, followed by disinfecting the skin, spreading a drape and anesthetizing with 2% lidocaine. Next, a bone marrow biopsy needle was used to pierce vertically and inserted the needle clockwise for 1–2 mm. Then the needle tube clockwise was withdrawn, the bone marrow tissue was obtained and placed in the fixative followed by the process of pathology milling. IHC was performed as described previously23. Immunostaining was performed on paraffin tissue sections. The main process was as follows. Slides were incubated with anti-NAT10 or anti-Ki67 overnight at 4 °C. Afterwards, the secondary antibody was applied and kept for 45 min at 37 °C, followed by dropping SABC at 37 °C for 30 min, 3,3’-diaminobenzidine (DAB) coloring, and finally counterstaining with hematoxylin. The study protocol was approved by the Human Research Ethics Committees of the Affiliated Hospital of Nanjing University of Chinese Medicine (Ethics number: KY2018005). All patients provided written informed consent for their bone marrow tissue samples to be used for research.

2.5. Plasmids and transfection

The plasmids containing the Homo NAT10 cDNA were purchased from TranSheepBio. The NAT10 coding sequence (NM_024662.3) was cloned into the lentiviral vector, pTSB carrying Flag tag. Lentiviruses containing the cDNA were obtained by cotransfection of the pTSB-NAT10 with packaging vectors (PSPAX.2 and PMD2.G) into 293 T cells in accordance with the protocol of Lipofectamine Transfection Reagent. The virus supernatant was harvested after 48 h, concentrated, and stored at −80 °C. MM cells were transfected with lentivirus containing NAT10 cDNA to yield NAT10-OE MM cells. Transfected cells were selected with puromycin treatment. Transduction efficiency was determined by Western blotting.

2.6. Transient transfection

Small interfering RNA (siRNA) was transfected into MM cells by using electrotransmitter (BTX). The specific process was as follows. BTXpress Cytoporation Media T4 (47-0003, BTX) was used to resuspend cells in 1 × 10³/mL, added siRNA to a final concentration of 100 nmol/L, and transferred to electric shock cup after mixed and transfection finally. Electric transfer parameters were determined as following: square wave, voltage 960 V, duration 0.1 ms, number of pulses 2, pulse interval 1.0 s and concentration of 100 nmol/L, and transferred to electric shock cup.

2.7. Western blotting

Western blotting assay was performed as described previously23. Anti-β-actin was used as a loading control for total protein.

2.8. Cell proliferation and viability assay

Cell viability was detected as described previously23 by using MTT assay according to the manufacturer’s protocol (Solarbio).

2.9. MM xenografts

CAG wild type (WT) cells and CAG NAT10-OE cells (2 × 10⁶) were injected subcutaneously into the left and right flank abdominal, respectively, of 6–8-weeks old NOD/SCID mice. In all cases, tumor diameter was measured with calipers. Once the tumor diameter reached 15 mm, mice were sacrificed, and tumor tissues were collected, weighted, and photographed. All animal work was performed in accordance with government-published recommendations for the Care and Use of Laboratory animals and guidelines of Institutional Ethics Review Boards of Nanjing University of Chinese Medicine (Ethics Registration No. 201905A003).

2.10. STMM3VT myeloma mouse model

Approximately 1 × 10⁶ STMM3VT cells were injected through the orbital vein into C57BL/KaLwRij mice from Harlan Laboratories (n = 8 per group). The mice were randomly divided into two groups (Control and Remodelin). On Day 3 after cell injection, intraperitoneal injection of remodelin (5 mg/kg) was performed twice weekly in remodelin group.

2.11. Flow cytometry analysis of cell cycle and apoptosis

Flow cytometry analysis of cell cycle and apoptosis was performed as described previously23. Flow cytometry equipped with Guava easyCyte System (Merck Millipore, Darmstadt, Germany) was applied to detect cell cycle and apoptosis.

2.12. ac4C detection by dot blot

Dot blot was performed using anti-ac4C antibody as described previously24. At first, 5 μg of RNA with denaturation solution (deionized formamide:37% formaldehyde:MOPS buffer = 66:21:13, v/v/v) was denatured in 65 °C for 5 min, followed by immediately placed on ice for 1 min and loaded onto Hybond-N+ membranes. The membranes were crosslinked twice with 150 ml/cm² in the UV254 nm Stratalinker 2400 (Stratagene). Afterwards, the membranes were stained with 0.02% methylene blue solution for 10 min, rinsed the background with DEPC water and scanned as an internal reference.

2.13. NAT10 immunoprecipitation

CAG NAT10-OE cells were utilized for NAT10 immunoprecipitation analysis. The procedure was performed as previously described2. Per each IP, 2.5 μg of anti-NAT10 antibody or 2.5 μg of rabbit IgG control were used.
2.14. Real-time PCR

Total RNA was extracted by using TRIeasy. Complementary DNA was synthesized by using reverse transcription kit according to instruction. Real-time quantitative PCR was performed with SYBR Green master Mix. Primer sequences were listed at Supporting Information Table S1.

2.15. Acetylated RNA immunoprecipitation and sequencing (acRIP-seq)

CAG WT and CAG NAT10-OE cells were used for acRIP-seq analysis. The procedure of acRIP-seq was performed as previously described. All data analysis and processing were performed by Guangzhou Epibiotek Co., Ltd. (Guangzhou, China).

2.16. Ribosome profiling and sequencing (Ribo-seq)

CAG WT and CAG NAT10-OE cells were used for Ribo-seq analysis. The procedure of Ribo-seq was performed as previously described. All data analysis and processing were applied by Guangzhou Epibiotek Co., Ltd.

2.17. RNA decay assay

MM cells were treated with mRNA transcription inhibitor actinomycin D (5 μg/mL) (HY-17559, MCE) for 0, 1, 2, and 3 h. Then, the total mRNA was isolated and used for qRT-PCR to quantify the relative abundance of CEP170 mRNA (relative to 0 h), and 18 S rRNA was used as internal control.

2.18. 5'-Bromo-uridine (BrU) immunoprecipitation chase-deep RT-qPCR (BRIC RT-qPCR)

BRIC RT-qPCR was performed as described previously. Briefly, cells were incubated at 37 °C in the presence of 150 μmol/L 5’-bromo-uridine (BrU; 850187, Sigma–Aldrich) for 24 h in a humidified incubator with 5% CO2. The cells were collected at indicated time points after replacing BrU containing medium with BrU-free medium. Total RNA was isolated by using TRIeasy. BrU-labeled total RNA (12 μg) was denatured by heating at 80 °C for 1 min and then added to the anti-BrdU mAb conjugated beads containing 2 μg of anti-BrdU mAb (MI-11-3, MBL). The mixture was incubated at 4 °C overnight with rotation. After immunoprecipitation, elution of RNA was carried out by adding 300 μL of TRIeasy directly to the mixture. BrU-labeled RNA was extracted by the TRIeasy method and then used for RT-qPCR.

2.19. Immunofluorescent staining and confocal microscopy

The cells were fixed with 4% paraformaldehyde, permeabilized with PBS containing 0.1% Triton X-100, and blocked with 4% BSA. After overnight incubation with primary antibodies at 4 °C, the slides were incubated with corresponding secondary antibodies. The images were captured by using a confocal microscope (TCS SP8, Leica, Germany).

2.20. Giemsa staining

Giemsa staining was conducted by using Wright–Giemsa stain (Wuhan Servicebio technology Co., Ltd., Wuhan, China) according to the manufacturer’s instructions. Briefly, the slides were stained for 5 min with solution A and next 5 min with solution B. Then the slides were washed with phosphate buffer (pH 6.8) and air dried.

2.21. TUNEL assay

The tunnel assay was performed by using TUNEL Apoptosis Detection Kit (Alexa Fluor 640; 40308, Yeasen) according to the manufacturer’s instructions. The images were captured by using a confocal microscope (TCS SP8, Leica, Germany).

2.22. Statistical analysis

All values were expressed as means ± standard deviation (SD). Two-tailed Student’s t-test and one-way analysis of variance (ANOVA) (≥ three groups) were used to determine significance between experimental groups. The Kaplan–Meier method was used to evaluate the correlation of NAT10 expression with myeloma patient survival. In all cases, significance was defined as *P < 0.05.

3. Results

3.1. NAT10 expression is elevated in MM, which confers poor survival of MM patients

To examine the expression of NAT10 in MM, we interrogated the gene expression profiling (GEP) dataset of normal plasma (NP) cells, monoclonal gamopathy of undetermined significance (MGUS) and MM bone marrow plasma cells. The analysis showed that NAT10 expression was significantly higher in plasma cells from MM (n = 351) patients than MGUS (n = 44) and NP (n = 22) cells (GSE2658, Fig. 1A). We further detected NAT10 expression in MM patient bone marrow tissues (n = 20) compared with normal controls (n = 10) by IHC at protein level. Consistently, IHC staining intensity score presented elevated NAT10 in bone marrow from MM patients compared with normal controls (Supporting Information Fig. S1A). In addition, Ki67, a key marker of proliferation, was highly in accord with NAT10 expression (Fig. 1B and Fig. S1B). These results indicated that NAT10 expression was increased in MM cells and associated with abnormal proliferation of MM. In addition, we correlated NAT10 expression with patient outcome in four independent MM cohorts with over 1000 samples. It was worth noting that MM patients with increased NAT10 expression had poor survival in four cohorts, TT2 (Fig. 1C), APEX (Fig. 1D), TT3 (Fig. 1E) and HOVON65 (Fig. 1F). Taken together, NAT10 expression is elevated in MM patients and correlated with poor survival, indicating that NAT10 may be a biomarker and potential therapeutic target of MM.

3.2. NAT10 is a driver gene for MM malignancy in vitro and in vivo

To verify NAT10 acting as a driver gene for MM malignancy, we overexpressed NAT10 in MM cells (CAG and KMS28PE) via lentivirus and knocked down NAT10 by siRNA, which were validated by RT-PCR (Fig. 2A) and Western blotting (Fig. 2B, Supporting Information Fig. S2A and S2B). MTT assay showed that cellular proliferation capacity was enhanced by elevated NAT10 relative to WT cells, and weakened by siNAT10 compared
with negative control (NC, Fig. 2C), suggesting that NAT10 boosted MM cell proliferation in vitro. To further extend these observations in vivo, we injected $2 \times 10^6$ CAG WT cells (left flank) and CAG NAT10-OE cells (right flank) subcutaneously to NOD/SCID mice ($n = 6$). Thirty-two days later, NAT10-OE cells generated larger tumors visually compared with WT cells (Fig. 2D), while the mean volume and weight of NAT10-OE tumors were also significantly higher than that of WT tumors statistically (Fig. 2E). Western blotting and IHC assays confirmed that NAT10 was increased in NAT10-OE tumors compared with WT tumors (Supporting Information Fig. S3A). In addition, we examined the correlation of NAT10 and Ki67 in xenograft tumors. The results showed that the expressions of NAT10 and Ki67 in NAT10-OE tumor tissues were significantly higher than that of WT tumors statistically (Fig. 2E). Western blotting and IHC assays confirmed that NAT10 was increased in NAT10-OE tumors compared with WT tumors (Supporting Information Fig. S3A). In addition, we examined the correlation of NAT10 and Ki67 in xenograft tumors. The results showed that the expressions of NAT10 and Ki67 in NAT10-OE tumor tissues were significantly higher than that of WT tumor tissues (Fig. S3B), and the expression of Ki67 was highly correlated with NAT10 expression (Fig. 2F). Collectively, we assume that NAT10 is a driver gene in MM and enforced expression of NAT10 promotes MM proliferation both in vitro and in vivo.

3.3. NAT10 regulates cycle distribution and promotes cellular proliferation in MM cells

To identify the mechanism responsible for NAT10 promoting MM malignancy, we performed RNA-seq in CAG and KMS28PE cells. Volcano plots showed that there were 1578 upregulated and 2529 downregulated genes in CAG NAT10-OE cells compared with CAG WT cells, as well as 849 upregulated and 1099 downregulated genes in KMS28PE NAT10-OE cells compared with KMS28PE WT cells (Fig. 3A). The intersection of all upregulated 67 genes or down-regulated 132 genes in the two pairs of cells was shown in the Venn diagrams (Fig. 3B). Further Gene ontology (GO) analysis presented that NAT10 was highly associated with cell proliferation and cell cycle signaling pathway (GSE155417, Fig. 3C), which was consistent with the data in Fig. 2. Next, we adopted flow cytometry to detect cell cycle distribution. An increase in G2/M phase fraction was observed with NAT10 overexpression (Fig. 3D) and correspondingly a decrease of cellular G2/M phase fraction was presented with siNAT10 (Fig. 3E) in MM cells. CDK4/6 are key initiators of the G1-to-S phase transition, while inhibition of CDK4/6 leads to G1 arrest of the cell cycle. Therefore, we tested whether NAT10 could affect the expression of CDK4 and CDK6 in MM cells. The results showed that the expressions of CDK4 and CDK6 were upregulated by increased NAT10 (Fig. 3F, Supporting Information Fig. S4A and S4B) and decreased by siNAT10 (Fig. 3G, Fig. S4C and S4D) at protein level. Additionally, siNAT10 upregulated the expression of cleaved-PARP and cleaved-caspase 3 (Fig. 3H, Fig. S4E and S4F). In summary, NAT10 regulates cell cycle distribution and promotes cellular proliferation in MM.

3.4. NAT10 acetylates mRNA to modulate progression of MM cells

Since NAT10 is characterized as acetyltransferase, we continued to investigate whether NAT10 promoted MM cell proliferation depending on the acetyltransferase catalytic function. With the
evidence showing that NAT10 acetylates mRNA, we detected mRNA acetylation level in MM cells via dot blot assay. The results indicated that increased ac4C was in accordance with overexpressed NAT10, while silencing NAT10 expression resulted in decreased ac4C acetylation in MM cells (Fig. 4A). Therefore, it might be concluded that NAT10 enhanced mRNA acetylation in MM cells. As to determine the NAT10 targets of ac4C, we performed the acRIP-seq assay (Fig. 4B) in CAG WT and CAG NAT10-OE cells to assess the distribution and switch of ac4C in the transcriptome. Typical CXX motif of ac4C peaks is shown in Fig. 4C, indicating that the quality of acRIP-seq assay was guaranteed. Interestingly, ac4C peaks mainly appeared within CDS and 3’ untranslated region (3’UTR) in MM cells (Fig. 4D), suggesting that NAT10 might modulate the expression of mRNA directly. Volcano plot illustrated the genes of significant changes with upregulated (red) and downregulated (blue) mRNA acetylation under the condition of NAT10 overexpression (Fig. 4E). GO analysis revealed that mRNA acetylation was associated with gene expression, translation, cell cycle and so on. (Fig. 4F). Here, we infer that NAT10 accelerates MM cells progression via catalyzing mRNA acetylation within CDS and promoting translation.

3.5. NAT10 acetylates CEP170 mRNA to enhance translation efficiency

The decay of mRNA and translation are intricately associated with each other. For instance, the decrease in mRNA stability is manifested as the decrease in translation, while the decrease in translation weakens the stability of mRNA. In this study, we focused on the signaling pathway of NAT10-regulated mRNA translation. To further explore the relationship between mRNA acetylation and translation, Ribo-seq (Fig. 5A), a translationomics research technology was utilized to detect the enhanced translational gene by NAT10-mediated mRNA acetylation in MM cells. According to the alignment position of ribosome footprints (RFs) on the genome, RFs were divided into four categories: CDS, 5’UTR, 3’UTR, Others. Generally, RFs were mostly distributed in the CDS area, but less in the UTR area (Fig. 5B). To screen the translation level of genes that undergo mRNA acetylation, we combined analyses of acRIP-seq and Ribo-seq, and the above four groups were divided based on mRNA acetylation level and translation level (GSE155917; Fig. 5C). Next, acRIP-seq and Ribo-seq analyses showed that 180 genes (45 genes in CDS region) were elevated at both mRNA acetylation and translation level in NAT10-OE cells compared with WT cells (Fig. 5D).

To further identify the downstream factors of NAT10, RT-PCR was performed to validate the upregulated candidate genes in both of the acRIP-seq and Ribo-seq analyses at the levels of acetylation and translation. CEP170 was increased evidently in NAT10-OE cells compared with WT cells (Fig. 5E) and decreased after NAT10 was interfered by siRNA in MM cells (Fig. 5F). In addition, ac4C peaks of CEP170 in group of NAT10-OE IP demonstrated significant increased enrichment.
compared with WT IP (Fig. 5G), indicating that NAT10 promoted CEP170 mRNA acetylation. Subsequently, RIP followed by RT-qPCR assay confirmed that NAT10 bound with CEP170 mRNA (Fig. 5H). Western blotting analysis showed that CEP170 expression was increased upon NAT10 overexpression and reduced by NAT10 siRNA at protein level (Fig. 5I, Supporting Information Fig. S5A and S5B). RNA decay assay presented a relatively higher stability of CEP170 transcripts in NAT10-OE cells compared with WT cells (Fig. 5J). BRIC RT-qPCR indicated that NAT10 significantly prolonged the half-life of CEP170 mRNA (Fig. 5K). Above data suggest that CEP170 may serve as an important target of NAT10, which upregulates CEP170 mRNA acetylation to stabilize CEP170 and enhances its translation efficiency.

3.6. NAT10 promotes chromosomal instability (CIN) to accelerate MM progression by interacting with CEP170

Even though we have identified that CEP170 is a downstream of NAT10, there is still no sufficient evidence showing that NAT10 influences MM cell proliferation via regulating CEP170. Therefore, we interfered CEP170 in NAT10-OE cells (Fig. 6A and Supporting Information Fig. S6) and overexpressed CEP170 in siNAT10 cells (Supporting Information Fig. S7A). MTT assays indicated that silencing CEP170 with siRNA significantly suppressed cellular proliferation in NAT10-OE cells compared with WT cells (Fig. 6B). Western blotting analysis confirmed that siNAT10 promoted apoptotic protein expression. All data are displayed as mean ± SD; *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 3  NAT10 regulates cycle distribution and promotes cellular proliferation in MM cells. (A) Volcano plots showed genes with upregulated (red) and downregulated (blue) expression upon NAT10 overexpression. (B) Venn diagram of two pairs of MM cells with common upregulated or downregulated genes. (C) Pathway enrichment analysis of RNA-seq data revealed that NAT10 was correlated to cell proliferation and cell cycle. (D, E) Flow cytometry analysis displayed that NAT10 overexpression increased G2/M phase fraction (D) and siNAT10 decreased G2/M phase fraction (E) in MM cells (n = 3). (F, G) Western blotting assays indicated that NAT10 upregulated CDK4 and CDK6 expression (F) and siNAT10 decreased CDK4 and CDK6 expression (G) in MM cells. (H) Western blotting analysis confirmed that siNAT10 promoted apoptotic protein expression. All data are displayed as mean ± SD; *P < 0.05, **P < 0.01, ***P < 0.001.
cells, we overexpressed CEP170 in CAG and OPM2 cells and interfered NAT10 expression with siRNA in CEP170-OE cells, as indicated by Western blotting (Fig. 6D). MTT assay results showed that CEP170 indeed promoted MM cell proliferation and silencing NAT10 with siRNA inhibited this effect in CEP170-OE cells (Fig. 6E). CEP170 is a centrosome protein as a marker for mature centrioles that is responsible for centrosome microtubule anchoring. Abnormal centrosome amplification leads to CIN. Our previous work identified that CEP170 significantly increased chromosomal plate width and decreased mitotic bipolar spindle length, suggesting that CEP170 was associated with MM CIN. Then, we conducted IF staining for α-tubulin and DAPI and found that elevated CEP170 compromised the integrity of the centrosome and bipolar shape of the spindle (Fig. 6F). Giemsa staining demonstrated that overexpression of CEP170 profoundly promoted an increase in the number of multiple nuclear cells as well as the separation error rate in MM cells (Fig. 6G and H). Here, we propose that NAT10 promotes CIN to accelerate MM progression by interacting with CEP170.

3.7. Remodelin impedes MM cell growth in vitro and prolongs the survival of 5TMM3VT mice in vivo

We further investigated if NAT10 could be a potent therapeutic target in MM by importing remodelin, a selective inhibitor of NAT10. Remodelin can correct nuclear architecture and attenuate senescence, which is lethal to ameliorat laminothries. We employed remodelin to evaluate the effect of NAT10 inhibition on MM cell growth, apoptosis and cell cycle. MTT assay showed that remodelin suppressed MM cell growth apparently compared with the untreated WT cells (Fig. 7A). Flow cytometry analysis indicated that remodelin strongly induced cell cycle arrest (Fig. 7B) and cell apoptosis (Fig. 7C). In addition, inhibition of NAT10 by remodelin increased the expression of cleaved-PARP and cleaved-caspase 3, and decreased the expression of CDK4 and CDK6 in MM cells (Fig. 7D, Supporting Information Fig. S8A–S8D). To further validate the effect of remodelin inhibiting NAT10 in vivo, we used the xenograft model of MM. It was showed that remodelin significantly inhibited the growth of xenograft tumors (Fig. 7E, Supporting Information Fig. S9A–S9C). Moreover, remodelin increased the expressions of cleaved-PARP and cleaved-caspase 3, and decreased the expressions of CDK4 and CDK6 in vivo (Fig. S9D). TUNEL assays also confirmed that remodelin induced cell apoptosis in xenograft tumors (Fig. 7F). In addition, 5TMM3VT mouse model presented that remodelin significantly extended the survival period of myeloma mice (Fig. 7G) relative to the untreated control mice in vivo. All the data above demonstrate that targeting NAT10 by remodelin can retard the development of MM in vitro and in vivo, indicating that NAT10 acts as a potential therapeutic target in MM.
4. Discussion

Although the development of novel proteasome inhibitors and other treatment strategies have greatly improved the survival of MM patients, the acquired drug resistance and malignant proliferation eventually lead to relapse and poor outcome\(^\text{36}\). Our group is committed to focus on discovery and identification of novel MM therapeutic targets and developing corresponding target-therapies. In this study, we investigated a novel target NAT10 in MM, which could acetylate \(\text{CEP170}\) mRNA to enhance the translation of \(\text{CEP170}\) in regulating MM cell growth and survival.

NAT10 functions as an oncogene in human cancers and participates in cell proliferation and migration\(^\text{9,10,12}\). Originally, many studies of NAT10 focused on the protein-acetyltransferase activity. For instance, NAT10 acetylates MORC2 at K767 to regulate DNA damage-induced G2 checkpoint mediated by MORC2 in breast cancer\(^\text{17}\). NAT10 activates p53 via acetylating p53 at K120 and counteracting MDM2 action in colorectal cancer\(^\text{16}\). Recently, NAT10 is reported to acetylate mRNA\(^\text{4}\), while ac4C is the first acetylation event in mRNA, all of which are catalyzed by NAT10 or its homologs\(^\text{38}\). The ac4C is closely related to several human diseases, as the ac4C content in the urine of patients is significantly higher than that of healthy people, including gestational diabetes\(^\text{39}\), interstitial cystitis\(^\text{40}\) and cancers\(^\text{41-43}\). Especially, ac4C is of great importance in diagnosis and treatment of cancers\(^\text{41-43}\). Analysis of ac4C function includes promoting protein translation, affecting RNA stability and alternative splicing, and regulating gene expression\(^\text{6,44,45}\). The ac4C peaks are enriched in the third codon encoding amino acid to improve the efficiency and accuracy of mRNA translation in human HeLa cells\(^\text{6,45}\). The modification of ac4C is involved in

---

Figure 5  NAT10 acetylates \(\text{CEP170}\) mRNA to enhance translation efficiency. (A) Schematic of Ribo-seq. (B) RFs were mostly distributed in the CDS area. (C) Quadrant diagram for combined analysis of acRIP-seq and Ribo-seq depicted the levels of mRNA acetylation and translation. (D) Hot map of genes with co-upregulated acetylation and translation efficiency in NAT10-OE cells. (E) qPCR tested the expression of the candidate genes in CAG WT and NAT10-OE cells \((n = 3)\). (F) \(\text{CEP170}\) mRNA level was downregulated in siNAT10 cells. (G) ac4C peaks of \(\text{CEP170}\) in CAG WT and NAT10-OE IP samples \((n = 3)\). (H) NAT10 immunoprecipitation followed by RT-qPCR disclosed that \(\text{CEP170}\) interacted with NAT10 \((n = 3)\). (I) \(\text{CEP170}\) was positively correlated with NAT10 at protein level in MM cells. (J) RT-qPCR was performed to detect \(\text{CEP170}\) mRNA stability in CAG and OPM2 cells with the addition of actinomycin D \((5 \mu\text{g/mL})\) \((n = 3)\). (K) BRIC RT-qPCR was conducted to measure the half-life of \(\text{CEP170}\) mRNA in CAG and OPM2 cells \((n = 3)\). All data are displayed as mean ± SD; \(*P < 0.05, **P < 0.01, ***P < 0.001\).
regulating gene expression via increasing stability and translation, possibly through its impact on mRNA decoding efficiency. Additionally, NAT10 acetylates multiple cytidines on HIV-1 RNAs to ac4C and depletion of ac4C by drug treatment or mutagenesis decreases the stability of HIV-1 transcripts leading to the reduction of viral replication. However, the role of ac4C modification and the function of NAT10 in either protein-acetyltransferase activity or acetylation of mRNA in MM warrant further research.

In present study, we first demonstrated that NAT10 expression was significantly correlated with poor survival of MM patients and increased NAT10 promoted MM growth in vitro and in vivo. Considering the crucial function of NAT10 in acetylating mRNA, we examined the mRNA-acetyltransferase activity of NAT10 in MM cells by using advanced high-throughput RNA sequencing techniques. The acRIP-seq was performed to delineate detailed mechanism on how NAT10 promoted MM cell proliferation. We found that ac4C regulated by NAT10 was associated with translation. To deeply explore the influence of ac4C on mRNA translation and the key downstream targets of NAT10, we combined the Ribo-seq data with acRIP-seq data to screen the potential targets with upregulated ac4C enrichment and translation efficiency. Finally, CEP170 was identified as the most significant downstream target of NAT10, and following studies confirmed that CEP170 expression was positively regulated by NAT10.

CEP170, localizing to centrosomes as well as spindle microtubules, is involved in microtubule organization and microtubule assembly. CEP170 serves as a marker for centriole maturation during the cell cycle and contributes to distinguish distinct mechanisms causing centrosome amplification, which is modulated by TBK1 to regulate microtubule dynamics and mitotic progression in cancer cells. Notably, NAT10 is associated with nuclear membrane and plays a vital role in mitosis. Thus, we inferred that NAT10 might regulate CEP170 during cell mitosis to influence MM progression. Our data show that NAT10 directly bound with CEP170 mRNA and promoted CEP170 mRNA acetylation in MM cells. We also found that reducing CEP170 expression significantly attenuated the cellular growth acceleration and G2/M phase fraction caused by elevated NAT10. These results suggest that...
NAT10 acetylated CEP170 mRNA to enhance CEP170 translation efficiency and then promoted malignant progression of MM.

To validate that NAT10 might be targetable in MM, we used a selective inhibitor of NAT10 remodelin in this study. Remodelin has been reported to ameliorate Hutchinson—Gilford progeria syndrome (HGPS) cellular defects by inhibiting NAT10 and restore nuclear shape of HGPS-derived patient cells via microtubule reorganization. Remodelin inhibits cell proliferation and migration, and induces cell cycle arrest or apoptosis in various cancer cells. In present study, we detected the effect of remodelin on MM in vitro and in vivo. Our data show that remodelin suppressed MM cell proliferation, induced cell cycle arrest and apoptosis in vitro and improved the survival of MM mice in vivo. Intriguingly, NAT10 is the only identified lysine acetyltransferase (KAT), and remodelin is the most potent and stable analog of KAT inhibitor 4-(4-chlorophenyl)-2-(2-cyclopentylidenehydrazinyl) thiazole to inhibit NAT10 KAT activity up to now. Applying remodelin as the core for discovery or synthetization of more effective compounds to inhibit the activity of NAT10 is worthy of further exploration.

5. Conclusions

Our work demonstrates the carcinogenesis role of NAT10 in promoting MM malignancy and dug up a novel mechanism of NAT10 acetylatng CEP170 mRNA to enhance CEP170 translation efficiency in MM. It may be concluded that NAT10 is a promising prognosis marker and potential therapeutic target for MM.

Acknowledgments

This work was supported by National Key R&D Program of China (2020YFA0509400) (to Ye Yang); National Natural Science Foundation of China 81970196 (to Chunyan Gu) and 82073885 (to Ye Yang); Natural Science Foundation of Jiangsu Province (China) BK20200097 (to Chunyan Gu); National Natural Science Foundation of China 82073888 (to Hongbo Wang); the Science and Technology Support Program for Youth Innovation in Universities of Shandong (China) (2019KJMS009) (to Hongbo Wang); Bohai rim Advanced Research Institute for Drug Discovery (China) (LX211011) (to Hongbo Wang); Jiangsu Postgraduate Research and Practice Innovation Program (China) KYCX21_1769 (to Rongfang Wei).

Author contributions

Ye Yang, Chunyan Gu and Hongbo Wang designed and conceived the experiments. Rongfang Wei and Xing Cui developed methodology and conducted most of the experiments. Jie Min analyzed and processed the sequencing data. Zigen Lin and Yanyan Zhou developed all animal experiments. Mengjie Guo, Xiaojuan An and...
Hao Liu acquired the data. Rongfang Wei drafted the manuscript. Chunyan Gu and Ye Yang reviewed and edited the manuscript. Siegfried Janz and Hongbo Wang provided technical or material support.

Conflicts of interest
The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2022.01.015.

References
1. Gu C, Lu T, Wang W, Shao M, Wei R, Guo M, et al. RFWD2 induces cellular proliferation and selective proteasome inhibitor resistance by mediating P27 ubiquitination in multiple myeloma. Leukemia 2021;35:1803–7.
2. Gao M, Bai H, Jethava Y, Wu Y, Zhu Y, Yang Y, et al. Identification and characterization of tumor-initiating cells in multiple myeloma. J Nail Cancer Inst 2020;112:507–15.
3. Caprio C, Sacco A, Giustini V, Roccoaro AM. Epigenetic aberrations in multiple myeloma. Cancers (Basel) 2020;12:2996.
4. Sas-Chen A, Thomas JM, Matzov D, Taoka M, Nance KD, Nir R, et al. Dynamic RNA acetylation revealed by quantitative cross-evolutionary mapping. Nature 2020;583:638–43.
5. van de Donk N, Pawlyn C, Yong KL. Multiple myeloma. Mol Biol Cell 2021;32:407–27.
6. Aragon D, Sturgill D, Alhussain N, Dillman AA, Sweet TJ, Hanson G, et al. Acetylation of cytidine in mRNA promotes translation efficiency. Cell 2018;175:872–86.
7. Lv J, Liu H, Wang Q, Tang Z, Hou L, Zhang B. Molecular cloning of a novel human gene encoding histone acetyltransferase-like protein involved in transcriptional activation of IERTERT. Biochem Biophys Res Commun 2003;311:506–13.
8. Cai S, Liu X, Zhang C, Xing B, Du X. Autoacetylation of NAT10 is critical for its function in rRNA transcription activation. Biochem Biophys Res Commun 2017;483:624–9.
9. Tan TZ, Miow QH, Huang RV, Wong MK, Ye J, Lau JA, et al. Functional genomics identifies five distinct molecular subtypes with clinical relevance and pathways for growth control in epithelial ovarian cancer. EMBO Mol Med 2013;5:1051–66.
10. Tan Y, Zheng J, Liu X, Lu M, Zhang C, Xing B, et al. Loss of nucleolar localization of NAT10 promotes cell migration and invasion in hepatocellular carcinoma. Biochem Biophys Res Commun 2018;499:1032–8.
11. Zhang X, Liu J, Yan S, Huang K, Bai Y, Zheng S. High expression of N-acetyltransferase 10: a novel independent prognostic marker of worse outcome in patients with hepatocellular carcinoma. Int J Clin Exp Pathol 2015;8:14765–71.
12. Zhang X, Chen J, Jiang S, He S, Bai Y, Zhu L, et al. N-Acetyltransferase 10 enhances doxorubicin resistance in human hepatocellular carcinoma cell lines by promoting the epithelial-to-mesenchymal transition. Oxid Med Cell Longev 2019;2019:7561879.
13. Ma R, Chen J, Jiang S, Lin S, Zhang X, Liang X. Up regulation of NAT10 promotes metastasis of hepatocellular carcinoma cells through epithelial-to-mesenchymal transition. Am J Transl Res 2016;8:4215–23.
14. Li Q, Liu X, Jin K, Lu M, Zhang C, Du X, et al. NAT10 is upregulated in hepatocellular carcinoma and enhances mutant p53 activity. BMC Cancer 2017;17:605.
15. Wu J, Zhu H, Wu J, Chen W, Guan X. Inhibition of N-acetyltransferase 10 using remodelin attenuates doxorubicin resistance by reversing the epithelial–mesenchymal transition in breast cancer. Am J Transl Res 2018;10:256–64.
16. Liu X, Tan Y, Zhang C, Zhang Y, Zhang L, Ren P, et al. NAT10 regulates p53 activation through acetylating p53 at K120 and ubiquitinating Mdm2. EMBO Rep 2016;17:349–66.
17. Zhang H, Hou W, Wang HL, Liu HJ, Jia XY, Zheng XZ, et al. GSK3beta-regulated N-acetyltransferase 10 is involved in colorectal cancer invasion. Clin Cancer Res 2014;20:4717–29.
18. Liu Z, Liu X, Li Y, Ren P, Zhang C, Wang L, et al. miR-6716-5p promotes metastasis of colorectal cancer through downregulating NAT10 expression. Cancer Manag Res 2019;11:5317–32.
19. Liang P, Hu R, Liu Z, Miao M, Jiang H, Li C. NAT10 upregulation indicates a poor prognosis in acute myeloid leukemia. Curr Probl Cancer 2020;44:100491.
20. Wang T, Zou Y, Huang N, Teng J, Chen Y. CCDC84 acetylation oscillation regulates centrosome duplication by modulating HsSAS-6 degradation. Cell Rep 2019;29:2078–91.
21. Gu C, Yang Y, Sompallae R, Xu H, Tompkins VS, Holman C, et al. FOXM1 is a therapeutic target for high-risk multiple myeloma. Leukemia 2016;30:873–82.
22. Broyl A, Hose D, Lokhorst H, de Kegn Y, Peeters J, Jauch A, et al. Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. Blood 2010;116:2543–53.
23. Li F, Liu Z, Sun H, Li C, Wang W, Ye L, et al. PCC208017, a novel small-molecule inhibitor of MARK3/MARK4, suppresses glioma progression in vitro and in vivo. Acta Pharmac Sin B 2020;10:289–300.
24. Sinclair WR, Arango D, Shrimp JH, Zengeya TT, Thomas JM, Montgomery DC, et al. Profiling cytidine acetylation with specific affinity and reactivity. ACS Chem Biol 2017;12:2922–6.
25. Imamachi N, Tani H, Mizutani R, Inamura K, Irie T, Suzuki Y, et al. BRIC-seq: a genome-wide approach for determining RNA stability in mammalian cells. Methods 2014;67:55–63.
26. Li K, You I, Wu Q, Meng W, He Q, Yang B, et al. Cyclin-dependent kinases-based synthetic lethality: evidence, concept, and strategy. Acta Pharmac Sin B 2021;11:2738–48.
27. Yuan K, Wang X, Dong H, Min W, Hao H, Yang P. Selective inhibition of CDK4/6: a safe and effective strategy for developing anticancer drugs. Acta Pharmac Sin B 2021;11:30–54.
28. Zi J, Han Q, Gu S, McGrath M, Kane S, Song C, et al. Targeting NAT10 induces apoptosis associated with enhancing endoplasmic reticulum stress in acute myeloid leukemia cells. Front Oncol 2020;10:598107.
29. Hanson G, Coller J. Codon optimality, bias and usage in translation and mRNA decay. Nat Rev Mol Cell Biol 2018;19:290–30.
30. Zhang W, Yang SL, Yang M, Herrlinger S, Shao Q, Collar JL, et al. Modeling microcephaly with cerebral organoids reveals a WDR62–CEP170–KIF2A pathway promoting cilium disassembly in neural progenitors. Nat Commun 2019;10:2612.
31. Guariguatti G, Duncan PI, Sterhofer YD, Holmström T, Duensing S, Nigg EA. The forkhead-associated domain protein Cep170 interacts with Polo-like kinase 1 and serves as a marker for mature centrioles. Mol Biol Cell 2005;16:1095–107.
32. El-Karim EA, Hagos EG, Ghabel AM, Yu B, Yang VW. Krüppel-like factor 4 regulates genetic stability in mouse embryonic fibroblasts. Mol Cancer 2013;12:89.
33. Croessmann S, Wong HY, Zabransky DJ, Chu D, Mendonca J, Guarguaglini G, et al. CHEK1 and FOXM1 are therapeutic targets for high-risk multiple myeloma. Leukemia 2016;30:873–82.
resistance to DNA-damaging chemotherapy and radiotherapy in breast cancer. *Nucleic Acids Res* 2020;48:3638–56.

38. Sharma S, Langhendries JL, Wätzinger P, Köttter P, Entian KD, Lafontaine DL. Yeast Kre33 and human NAT10 are conserved 18S rRNA cytosine acetyltransferases that modify tRNAs assisted by the adaptor Tan1/THUMP1. *Nucleic Acids Res* 2015;43:2242–58.

39. Law KP, Han TL, Mao X, Zhang H. Tryptophan and purine metabolites are consistently upregulated in the urinary metabolome of patients diagnosed with gestational diabetes mellitus throughout pregnancy: a longitudinal metabolomics study of Chinese pregnant women part 2. *Clin Chim Acta* 2017;468:126–39.

40. Parsons CL, Shaw T, Berecz Z, Su Y, Zupkas P, Argade S. Role of urinary cations in the aetiology of bladder symptoms and interstitial cystitis. *BJU Int* 2014;114:286–93.

41. Szymańska E, Markuszewski MJ, Markuszewski M, Kaliszán R. Altered levels of nucleoside metabolite profiles in urogenital tract cancer measured by capillary electrophoresis. *J Pharm Biomed Anal* 2010;53:1305–12.

42. Zhang T, Wu X, Ke C, Yin M, Li Z, Fan L, et al. Identification of potential biomarkers for ovarian cancer by urinary metabolic profiling. *J Proteome Res* 2013;12:505–12.

43. Li H, Qin Q, Shi X, He J, Xu G. Modified metabolites mapping by liquid chromatography-high resolution mass spectrometry using full scan/all ion fragmentation/neutral loss acquisition. *J Chromatogr A* 2019;1583:80–7.

44. Castello A, Fischer B, Eichelbaum K, Horos R, Beckmann BM, Strein C, et al. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell* 2012;149:1393–406.

45. Dominissini D, Rechavi G. N 4-acetylation of cytidine in mRNA by NAT10 regulates stability and translation. *Cell* 2018;175:1725–7.

46. Tsai K, Jaguva Vasudevan AA, Martinez Campos C, Emery A, Swanstrom R, Cullen BR. Acetylation of cytidine residues boosts HIV-1 gene expression by increasing viral RNA stability. *Cell Host Microbe* 2020;28:306–12.

47. Welburn JP, Cheeseman IM. The microtubule-binding protein Cep170 promotes the targeting of the kinesin-13 depolymerase Kil2b to the mitotic spindle. *Mol Biol Cell* 2012;23:4786–95.

48. Mazo G, Soplop N, Wang WJ, Uryu K, Tsou MF. Spatial control of primary ciliogenesis by subdistal appendages alters sensation-associated properties of cilia. *Dev Cell* 2016;39:424–37.

49. Fillai S, Nguyen J, Johnson J, Haura E, Coppola D, Chellappan S. Tank binding kinase 1 is a centrosome-associated kinase necessary for microtubule dynamics and mitosis. *Nat Commun* 2015;6:10072.

50. Lingle WL, Barrett SL, Negron VC, D’Assoro AB, Boeneman K, Liu W, et al. Centrosome amplification drives chromosomal instability in breast tumor development. *Proc Natl Acad Sci U S A* 2002;99:1978–83.

51. Iftekhar A, Berger H, Bouznad N, Heuberger J, Boccellato F, Dohrnt U, et al. Genomic aberrations after short-term exposure to colibactin-producing *E. coli* transform primary colon epithelial cells. *Nat Commun* 2021;12:10033.

52. Akimova E, Gassner FJ, Schubert M, Rehband S, Arzt C, Raasscher S, et al. SAMHD1 restrains aberrant nucleotide insertions at repair junctions generated by DNA end joining. *Nucleic Acids Res* 2021;49:2598–608.

53. Pihan GA, Wallace J, Zhou Y, Dossy SJ. Centrosome abnormalities and chromosome instability occur together in pre-invasive carcinomas. *Cancer Res* 2003;63:1398–404.

54. Chi YH, Haller K, Peloponese Jr JM, Jeang KT. Histone acetyltransferase hALP and nuclear membrane protein hsSUN1 function in de-condensation of mitotic chromosomes. *J Biol Chem* 2007;282:27447–58.

55. Gassmann R, Henzing AJ, Earnshaw WC. Novel components of human mitotic chromosomes identified by proteomic analysis of the chromosome scaffold fraction. *Chromosoma* 2005;113:385–97.

56. Balmus G, Larrieu D, Barros AC, Collins C, Abrudan M, Demir M, et al. Targeting of NAT10 enhances healthspan in a mouse model of human accelerated aging syndrome. *Nat Commun* 2018;9:1700.

57. Oh TI, Lee YM, Lim BO, Lim JH. Inhibition of NAT10 suppresses melanogenesis and melanoma growth by attenuating microphthalmia-associated transcription factor (MITF) expression. *Int J Mol Sci* 2017;18:1924.