HHS Public Access

Author manuscript

Toxicol Appl Pharmacol. Author manuscript; available in PMC 2024 October 15.

Published in final edited form as:

Toxicol Appl Pharmacol. 2023 October 15; 477: 116688. doi:10.1016/j.taap.2023.116688.

NAT10 regulates the repair of UVB-induced DNA damage and tumorigenicity

Zizhao Yang[1,3], **Emma Wilkinson**[1,2,3], **Yan-Hong Cui**[1,3], **Haixia Li**[1], **Yu-Ying He**[1,*]

[1]Department of Medicine, Section of Dermatology, University of Chicago, Chicago, Illinois, USA

[2]Committee on Cancer Biology, University of Chicago, Chicago, Illinois, USA

[3]These authors contributed equally to this work

Abstract

Chemical modifications in messenger RNA (mRNA) regulate gene expression and play critical roles in stress responses and diseases. Recently we have shown that N^6-methyladenosine (m^6A), the most abundant mRNA modification, promotes the repair of UVB-induced DNA damage by regulating global genome nucleotide excision repair (GG-NER). However, the roles of other mRNA modifications in the UVB-induced damage response remain understudied. N4-acetylcytidine (ac4C) is deposited in mRNA by the RNA-binding acetyltransferase NAT10. This NAT10-mediated ac4C on mRNA has been reported to increase both mRNA stability and translation. However, the role of ac4C and NAT10 in the UVB-induced DNA damage response remains poorly understood. Here we show that NAT10 plays a critical role in the repair of UVB-induced DNA damage lesions through regulating the expression of the key GG-NER gene DDB2. We found that knockdown of NAT10 enhanced the repair of UVB-induced DNA damage lesions by promoting the mRNA stability of DDB2. Our findings are in contrast to the previously reported role of NAT10-mediated ac4C deposition in promoting mRNA stability and may represent a novel mechanism for ac4C in the UVB damage response. Furthermore, NAT10 knockdown in skin cancer cells decreased skin cancer cell proliferation *in vitro* and tumorigenicity *in vivo*.Chronic UVB irradiation increases NAT10 protein levels in mouse skin. Taken together, our findings

*Correspondence (Y.Y.H.): yyhe@uchicago.edu.

Present address for Dr. Zizhao Yang:
School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Conflict of interest statement

The authors declare no conflicts of interest.

Credit Author Statement

Y.Y.H. and Z.Y. conceived the project and designed the original studies. Z.Y. performed most of the experiments. E.W. performed the experiments on the effect of NAT10 knockdown on DDB2 levels in A431 cells and the effect of UVB on NAT10 levels. Y.H.C. performed the experiments on the effect of NAT10 knockdown on ac4C enrichment, NAT10 RIP, ac4C RIP. H.L. performed the immunofluorescence analysis of the effect of chronic UVB irradiation on NAT10 levels in mouse skin. E.W., Z.Y., and Y.Y.H. wrote the manuscript with the input from Y.H.C. and H.L. All authors approved the final manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

demonstrate a novel role for NAT10 in the repair of UVB-induced DNA damage products by decreasing the mRNA stability of DDB2 and suggest NAT10 as a potential novel target for preventing and treating skin cancer.

Keywords

N4-acetylcytidine mRNA modification; ac4C; NAT10; global genome nucleotide excision repair; GG-NER; UVB-induced DNA damage; DNA repair

Introduction

RNA modifications have been identified on every nucleotide and in various RNA species (Roundtree *et al.*, 2017; Frye *et al.*, 2018; Delaunay and Frye, 2019; Barbieri and Kouzarides, 2020; Cui *et al.*, 2022). To date, over 100 chemical modifications in RNA have been characterized (Roundtree *et al.*, 2017; Frye *et al.*, 2018; Delaunay and Frye, 2019; Barbieri and Kouzarides, 2020; Cui *et al.*, 2022). N4-acetylcytidine (ac4C) is an RNA cytidine modification that was initially characterized in *E. coli* $tRNA^{Met}$ (Stern and Schulman, 1978). With advances in mass spectrometry and sequencing technologies, ac4C has since been identified in 18S rRNA, mRNA, $tRNA^{Ser/Leu}$, and lncRNA (Stern and Schulman, 1978; Ito *et al.*, 2014a; Ito *et al.*, 2014b; Sharma *et al.*, 2015; Arango *et al.*, 2018; Zhang *et al.*, 2022b; Yu *et al.*, 2023). To date, ac4C is the only acetylation modification identified in eukaryotic RNA (Arango *et al.*, 2018). ac4C deposition in mRNA has been found in both coding and wobble sequences (Arango *et al.*, 2018). In the coding regions of genes, ac4C promotes mRNA stability and protein translation, while ac4C in wobble sites promotes translation stability(Arango *et al.*, 2018);

The only known ac4C writer is N-acetyltransferase 10 (NAT10), which contains both RNA binding and acetyltransferase functions (Montgomery *et al.*, 2016; Arango *et al.*, 2018; Sleiman and Dragon, 2019; Dalhat *et al.*, 2021b). NAT10 catalyzes ac4C deposition across all identified substrates in both humans and prokaryotes in a coenzyme A and ATP-dependent manner (Ito *et al.*, 2014a; Ito *et al.*, 2014b; Sharma *et al.*, 2015; Arango *et al.*, 2018; Sleiman and Dragon, 2019; Jin *et al.*, 2020; Levy *et al.*, 2020). As a tRNA demethylase, NAT10 also requires the adaptor protein THUMP (Sharma *et al.*, 2015; Luo *et al.*, 2023). Functionally, NAT10 has been widely studied across several cellular processes and diseases (Luo *et al.*, 2023; Xie *et al.*, 2023a). NAT10 has been found to contribute to the development and progression of several cancers including pancreatic cancer, colon cancer, gastric cancer, esophageal squamous cell carcinoma, multiple myeloma, and bladder cancer, all in an ac4C-dependent manner (Zhang *et al.*, 2021; Wang *et al.*, 2022; Wei *et al.*, 2022; Zhang *et al.*, 2022c; Zheng *et al.*, 2022b; Deng *et al.*, 2023; Liao *et al.*, 2023; Yu *et al.*, 2023; Zhang *et al.*, 2023a; Zong *et al.*, 2023). While NAT10 has been found to contribute to inflammation, human RNA transcription, HIV transcription, and microtubule stability, the ac4C-dependent role in these processes remains unclear (Shen *et al.*, 2009; Hicks *et al.*, 2017; Jean *et al.*, 2017; Zhang *et al.*, 2023b). In addition, NAT10 can act as a protein acetyltransferase, and contribute to cellular processes independent of its function as an RNA acetyltransferase, including both DNA repair (through regulation of PARP1, spindle

assembly and chromosome segregation) and the stress response (through regulation of p53) (Liu *et al.*, 2016; Zhang and Li, 2019; Zheng *et al.*, 2022a).

A major risk factor causing skin cancer is UVB radiation (DiGiovanna and Kraemer, 2012); UVB radiation can induce DNA lesions, including cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts (6–4 PPs) (Batty and Wood, 2000). Upon UVB-induced DNA damage, several cellular processes, including the global genome nucleotide excision repair (GG-NER) process, are activated (Batty and Wood, 2000; Aziz Sancar *et al.*, 2004; DiGiovanna and Kraemer, 2012). The process of GG-NER involves the sequential and temporal activation of xeroderma pigmentosum (XP) proteins, which function to recognize, remove, and repair the DNA damage (Batty and Wood, 2000; Riedl *et al.*, 2003; Aziz Sancar *et al.*, 2004; Cleaver *et al.*, 2009; DiGiovanna and Kraemer, 2012). More specifically, DDB2 (XPE), XPC, and XPA, recognize damaged DNA and recruit other NER factors; XPB and XPD unwind the damaged DNA; and XPF and XPG use their endonuclease capabilities to remove the damaged DNA lesions (Batty and Wood, 2000; Riedl *et al.*, 2003; Aziz Sancar *et al.*, 2004; Shah *et al.*, 2018). Together, the regulation and coordinated activation of these proteins are critical for resolving DNA damage and the loss-of-function of any of these proteins can contribute to mutagenesis and cancer (Aziz Sancar *et al.*, 2004; DiGiovanna and Kraemer, 2012).

However, the molecular mechanism by which UVB-induced DNA damage response is regulated remains poorly understood. Here we investigated the role of NAT10-mediated ac4C acetylation in UVB-induced DNA damage response and skin cancer tumorigenesis.

Materials and methods

Cell Culture and UVB irradiation

HaCaT (human keratinocyte, kindly provided by Dr. Fusenig), A431 (human skin squamous carcinoma), and HEK293T (human embryonic kidney) cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco), 100 U/mL penicillin as well as 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA). Cells were irradiated with UVB as described previously (Yang *et al.*, 2021).

Plasmid and Lentivirus Generation and Infection

NAT10 shRNA lentivirus plasmids as well as negative control plasmids (shNC) were obtained from Sigma, with catalog number: TRCN00000296354, TRCN00000296355, TRCN00000296411, TRCN0000035700 and TRCN0000035702, respectively. To generate the lentivirus, HEK293T cells were transfected with these lentiviral constructs, together with the pCMVdelta8.2 packaging plasmid and pVSV-G envelope plasmid using X-tremeGENE 9 (Roche, Manheim, Germany). Virus-containing supernatants were collected at 24–48 h after high-speed centrifugation. Target cells were transduced in the presence of polybrene (8 μg/mL) (Sigma-Aldrich, St. Louis, MO, USA) and selected with puromycin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1 μg/mL for 7 days.

siRNA Transfection

The following siRNAs were used: siRNA targeting DDB2 (sc-37799) and negative control (sc-37007) (Santa Cruz Biotechnology). A nucleofector (Amaxa, Gaithersburg, MD, USA) was used to transfect cells with siRNA according to the manufacturer's instructions.

Quantitative Real-Time PCR (qPCR)

Quantitative real-time PCR assays were performed using a CFX Connect real-time system (Bio-Rad, Hercules, CA, USA) with Bio-Rad iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). The threshold cycle number (CQ) for each sample was determined in triplicate then normalized to housekeeping control to determine relative mRNA expression.

ac4C RNA Dot Blot Assay

Total RNA was isolated using TRIzol following the manufacturer's instructions. RNA was then denatured by heating at 98 °C for 10 min, spotted on Amersham Hybond-N^+ membrane (GE Healthcare, Chicago, IL, USA), and subsequently UV cross-linked twice to the membrane. After drying, the membrane was blocked with 5% BSA (in 1× PBST) for 1 h and then incubated with a specific anti-ac4C antibody (Abcam, EPRNCI-184–128, ab252215, 1:1000) overnight at 4 °C. The membrane was then incubated with HRP-conjugated anti-rabbit IgG (Cell Signaling Technology, Beverly, MA, USA, 1:2000) for 1 h at room temperature and then developed with Thermo ECL SuperSignal Western Blotting Detection Reagent (Thermo Fisher Scientific, Waltham, MA, USA).

Immunoblotting

Protein extracts were obtained by washing cells once with cold PBS and then lysing cells in RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with a protease and phosphatase inhibitor mixture (Thermo Fisher Scientific, Waltham, MA, USA). Protein samples were then sonicated and spun down at 13,200 RPM for 15 min at 4 °C. After quantifying protein concentrations using the Pierce BCA assay (Thermo Fisher Scientific, Waltham, MA, USA), the samples were heated for 10 min at 70 °C. Protein abundance was analyzed by SDS–polyacrylamide gel electrophoresis followed by immunoblotting. The following antibodies were used: anti-NAT10 (Santa Cruz, sc-271770, 1:1000); anti-XPA (Santa Cruz, sc-28353, 1:1000); anti-XPB (Santa Cruz, sc-271500, 1:1000); anti-XPC (Santa Cruz, sc-74410, 1:800); anti-XPD (Proteintech, 10818–1-AP, 1:1000); anti-DDB2 (Santa Cruz, sc-81246, 1:500); anti-XPF (Santa Cruz, sc-398032, 1:500); anti-XPG (Proteintech, 11331–1-AP, 1:800); anti-GAPDH (Santa Cruz, sc-47724, 1:5000).

ac4C RNA Immunoprecipitation (RIP) Assay

100 μg total RNA was extracted using TRIzol following the manufacturer's instructions. Total RNA was diluted in 500 μL IP buffer (150 mM NaCl, 0.1% NP-40, 10 mM Tris, pH 7.4, 100 U RNase inhibitor) and incubated with an ac4C antibody (Abcam, EPRNCI-184–128, ab252215, 1:1000). The mixture was rotated for 4 h at 4 °C. Next Dynabeads® Protein A/G (Thermo Fisher Scientific, Waltham, MA) beads coated with BSA were added into the solution and the mixture was rotated for an additional 2 h at 4 °C. The bead mixture was

washed four times with IP buffer supplemented with RNAse inhibitor and the ac4C-bound RNA was eluted with elution buffer (5 mM Tris-HCL pH 7.5, 1 mM EDTA pH 8.0, 0.05% SDS, and 4.2 μl Proteinase K (20 mg/ml)). The final eluted RNA was concentrated using an RNA Clean & Concentrator-5 kit (Zymo Research, Irvine, CA). The input and IP samples were then subjected to qPCR analysis, as described in our previous works (Yang *et al.*, 2019).

NAT10 RNA Immunoprecipitation (RIP) Assay

The NAT10 RIP assay was carried out using the EZ-Magna RIP® RNA-Binding Protein Immunoprecipitation Kit (MilliporeSigma 17–701 US) following the manufacturer's instructions, with the NAT10 antibody (Santa Cruz Biotechnology, sc-271770). The DDB2 primer sequences were: Forward 5'- AGGACGCGATGGCTCCCAAGA −3'; Reverse 5'-TTCTGCTAGGACCGGAGCCCT −3', as used previously (Yang *et al.*, 2021).

Slot Blot Assay for CPD Repair Speed and Extent Determination

Genomic DNA was extracted from the cells collected at various time points after UVB exposure using the QIAamp DNA Mini Kit (51306, QIAGEN, Hilden, Germany) following the manufacturer's protocol. CPD levels were determined using immunoblotting with an anti-CPD antibody (TDM-2, Cosmo Bio Co., Koto-Ku, Tokyo, Japan, CAC-NM-DND-001, 1:4000). Methylene blue staining was used as a loading control for total DNA. To determine repair kinetics, percentage (%) repair was calculated by measuring the ratio of average optical density at the specified time points compared to zero hours. It is assumed that zero hours represents 100% of the CPD damage after the cells were exposed to UVB irradiation prior to CPD repair.

RNA Stability Assay

Cells were treated with the transcriptional inhibitor actinomycin D (2 μM) over a time course.Total RNA was isolated with an RNeasy plus mini kit (QIAGEN, Hilden, Germany). The HPRT1 housekeeping gene was used as a control.

Immunofluorescence

Immunofluorescence staining of mouse samples was performed as described previously (Yang *et al.*, 2021). Antibodies used are: an anti-NAT10 antibody (1:200, Proteintech Cat no. 13365–1-AP) and cytokeratin (1:100, Jackson immunoresearch Cat no. 706545148).

Tumorigenicity Assay in Immunocompromised Mice and UVB Irradiation

All animal procedures were approved by the University of Chicago institutional animal care and use committee (IACUC). Athymic nude mice were obtained from Harlan-Envigo. 1×10^6 A431 human squamous-cell carcinoma cells in PBS with or without NAT10 knockdown were injected subcutaneously into the right flanks of female mice (6–8 weeks of age). Tumor growth was monitored and measured with a caliper, and tumor volume was calculated using the following formula: $(mm^3) = d^2 \times D/2$, where "d and D" are the shortest and the longest diameters, respectively. For UVB irradiation, female SKH-1 hairless mice were irradiated as described previously (Yang *et al.*, 2021). The initial dose of UVB was 80 mJ/cm^2 for

the first week, followed by a weekly 10% increase until it reached 100 mJ/cm^2. Mice were irradiated with UVB every other day 3 times a week for 31 weeks. Skin or tumor samples were then collected.

Statistical Analyses

Statistical analyses were carried out using Prism 9 (GraphPad). Data are shown as mean ± SD as indicated and were analyzed by Student's *t*-test. A value of $p<0.05$ was considered significant in all cases.

Results

NAT10 knockdown increases the repair of UVB-induced DNA damage

To understand the role of NAT10 in UVB-induced DNA damage repair, we stably knocked down NAT10 using shRNA in a human keratinocyte cell line (HaCaT). A non-targeting control was transfected in parallel (shNC). Western Blotting was used to determine the efficiency of NAT10 knockdown (Figure 1A). We found that plasmids shNAT10–1 and shNAT10–2 resulted in the most robust knockdown effect on NAT10, and all subsequent experiments were performed using these cells. As expected, we also found, using RNA dot blotting, that ac4C levels were also decreased after NAT10 knockdown (Figure 1B).

We next sought to determine the functional role of NAT10 in keratinocytes. Several studies have suggested that NAT10 plays a role in cellular functions; however, the role of NAT10 in response to UVB-induced stress remains unclear(Liu *et al.*, 2018; Guerrero Llobet *et al.*, 2022). Although NAT10 has been previously found to play a role in response to DNA damage, as yet the role of NAT10 in response to UVB-induced DNA damage is not fully understood(Liu *et al.*, 2020; Xie *et al.*, 2023b). UVB damage produces cyclobutane pyrimidine dimers (CPDs) that are major tumorigenic DNA lesions(Batty and Wood, 2000; Aziz Sancar *et al.*, 2004; DiGiovanna and Kraemer, 2012; Balmus *et al.*, 2018). Using DNA slot blot assay to evaluate CPD repair efficiency in HaCaT cells with or without NAT10 knockdown, we found that knockdown of NAT10 in HaCaT cells significantly accelerated the UVB-induced CPD repair (Figure 1C and 1D). Taken together, our results suggest that NAT10 regulates DNA damage repair in response to UVB exposure.

NAT10 knockdown affects the expression of key GG-NER factors

We next sought to further elucidate the role of NAT10 in the repair of UVB-induced DNA damage. This repair process is complex and requires the temporal activation of GG-NER factors that identify and repair the DNA lesions(Batty and Wood, 2000; Riedl *et al.*, 2003; Aziz Sancar *et al.*, 2004). We thus sought to determine whether NAT10 regulates the expression of critical GG-NER factors. Interestingly, among these factors, we found that the protein levels of XPA and DDB2 increased after NAT10 knockdown, while the expression of other GG-NER factors such as XPB, XPC, XPD, XPF and XPG remained unchanged (Figure 2). These results suggest that NAT10 may have a critical, yet selective, role in regulating the expression of some key GG-NER factors.

NAT10 binds to the DDB2 gene and regulates DDB2 mRNA stability

Our lab has previously found that DDB2 expression could be modified by m^6A mRNA methylation, and the deposition of this modification on the DDB2 mRNA transcript plays a critical role in GG-NER (Yang *et al.*, 2021). Out of the two XP proteins that showed changes in expression upon NAT10 knockdown (XPA and DDB2), we decided to focus more deeply on whether NAT10-dependent ac4C deposition could control DDB2 expression, given our previous studies (Yang *et al.*, 2021). NAT10-mediated ac4C deposition in mRNA can regulate target mRNA stability and translation(Arango *et al.*, 2018). We therefore sought to elucidate the mechanism by which NAT10 regulates DDB2. Using qPCR analysis, we found that DDB2 mRNA levels were also increased upon NAT10 knockdown (Figure 3A). These results suggest that NAT10-dependent regulation of DDB2 may occur at the mRNA level.

Using an actinomycin D mRNA stability assay, we next sought to determine whether NAT10 regulates DDB2 mRNA stability. Indeed, knockdown of NAT10 increased DDB2 mRNA stability (Figure 3B). These results suggest that ac4C on DDB2 mRNA may decrease DDB2 mRNA stability, contrary to previous findings (Arango *et al.*, 2018). To further demonstrate that this is an ac4C-dependent effect on DDB2 mRNA, we used an RNA immunoprecipitation assay (RIP) to determine whether DDB2 is ac4C-modified and whether the DDB2 mRNA transcript binds to NAT10. Our findings indicated that the DDB2 transcript is indeed ac4C-modified (Figure 3C) and binds to NAT10 (Figure 3D). Furthermore, NAT10 knockdown increased DDB2 protein levels in A431 cells (Fig. 3E), consistent with our observation in HaCaT cells (Figure 2). These data demonstrate that the DDB2 gene is a target of NAT10-dependent ac4C acetylation and that the deposition of ac4C on the DDB2 mRNA transcript may decrease DDB2 mRNA stability.

DDB2 is a critical functional target of NAT10 in regulating the repair of UVB-induced DNA damage.

To better integrate the role of DDB2 in the NAT10-mediated UVB-induced DNA damage repair response, we knocked down DDB2 in HaCaT cells with NAT10 knockdown using siRNA (Figure 4A). We then evaluated the dynamics of CPD repair upon DDB2 knockdown and found that CPD repair was significantly impaired after DDB2 knockdown in NAT10 knockdown cells, compared to NAT10 knockdown alone (Figure 4B and 4C). These results suggest that the NAT10-dependent regulation of DDB2 may be a critical mechanism in the UVB-induced DNA damage response.

NAT10 knockdown inhibits skin cancer tumorigenicity

Finally, we sought to determine whether NAT10 plays an active role in skin tumorigenesis. To do so, we stably knocked down NAT10 in A431 human skin squamous carcinoma cells using shRNA (Figure 5A). We then performed a proliferation assay to determine the effect of NAT10 knockdown on A431 proliferation. We found that knockdown of NAT10 in A431 cells decreased tumorigenicity *in vitro* (Figure 5B). We also sought to determine the role of NAT10 in A431 tumorigenicity *in vivo* using xenograft models. Indeed, knockdown of NAT10 decreased A431 tumor volume and weight (Figure 5C and 5D). Furthermore, although we found acute UVB irradiation at 6 and 24 h decreases the NAT10 protein levels

in both HaCaT and A431 cells (Fig. 5E and F), chronic UVB irradiation increased NAT10 protein level in mouse skin (Fig. 5G), suggesting, suggesting that NAT10 increase is an early event in chronic UVB-induced skin tumorigenesis *in vivo*. Taken together, these data show that NAT10 is required for A431 skin cancer cell proliferation *in vitro* and tumorigenicity *in vivo*.

Discussion

ac4C mRNA modification is an emerging modification of interest and may be an important factor in regulating gene expression and cellular functions(Arango *et al.*, 2018; Jin *et al.*, 2020). NAT10 is the sole known writer that mediates the deposition of ac4C in mRNA(Stern and Schulman, 1978; Ito *et al.*, 2014a; Cai *et al.*, 2017; Arango *et al.*, 2018). NAT10-mediated ac4C in mRNA is believed to enhance mRNA stability and protein translation(Arango *et al.*, 2018). However, the context-dependent effects of ac4C on gene expression have yet to be fully elucidated. Our findings suggest a potentially novel role for NAT10 in regulating GG-NER in response to UVB-induced DNA damage and establish DDB2 as a downstream target of NAT10. In addition, NAT10 knockdown inhibits cell proliferation and tumor growth in mice. Furthermore, chronic UVB irradiation increases NAT10 levels in mouse skin. These findings demonstrate a critical new role of NAT10 and ac4C in UVB damage response and suggest a tumor-promoting role of NAT10 in skin cancer.

While previous studies have found that NAT10-mediated ac4C promotes target mRNA stability, we found that loss of NAT10 increased DDB2 mRNA stability(Arango *et al.*, 2018). We have several hypotheses to address this apparent contradiction. First, RNA modifications assume cell-type and context-specific effects, which can have disparate effects on gene expression under different conditions (Roundtree *et al.*, 2017; Frye *et al.*, 2018; Delaunay and Frye, 2019; Barbieri and Kouzarides, 2020). Therefore, it is possible that this effect on DDB2 mRNA stability may be keratinocyte-specific. Consistent with our hypothesis, *Zhu* et al. found that knockdown of NAT10 in mesenchymal stem cells led to increased mRNA stability of *Gremlin1* mRNA(Zhu *et al.*, 2021). Accordingly, m^6A deposition in mRNA has established roles in regulating diverse aspects of gene expression, including translation, decay, and nuclear processing(Roundtree *et al.*, 2017). It is therefore possible that ac4C has unique roles in regulating gene expression in specialized cell types distinct from its established function in regulating mRNA stability and protein translation.

Second, our studies did not establish where ac4C is located on the DDB2 mRNA transcript. There are currently no known ac4C consensus sequences. It is possible that ac4C deposition may be at an unconventional site on the DDB2 transcript, which may contribute to this unexpected effect on DDB2 mRNA stability. Further investigation into the deposition of ac4C and its role across specialized cell types and under different stress responses is therefore critical to characterize the cell-type and context-specific role of ac4C in gene expression. Finally, it is also possible that NAT10 may work in concert with other protein complexes to achieve such an effect on DDB2 mRNA stability. To address this question, future work is needed to identify NAT10-binding partners.

Our recent studies suggest that moderate changes in DDB2 protein abundance due to METTL14 or DDB2 manipulation alter the repair dynamics of UVB-induced DNA damage (Yang *et al.*, 2021), suggesting that DDB2 availability is critical for the GG-NER capacity. Here we found that DDB2 is a critical functional target for NAT10, as moderate DDB2 dose reduction in NAT10 knockdown cells is sufficient to reverse the effect of NAT10 knockdown. In addition to DDB2, we also observed changes in XPA expression upon NAT10 knockdown. Future investigation will elucidate how NAT10 and ac4C regulates XPA and whether such regulation is critical for NAT10's function in GG-NER.

Another interesting future direction involves the interplay between m^6A and ac4C on the DDB2 mRNA transcript. We previously found that m^6A deposition on the DDB2 transcript regulates its translation. In our current study we found that ac4C deposition regulates DDB2 mRNA stability(Yang *et al.*, 2021). Further studies are needed to determine whether these modifications cooperate or antagonize one another and how they contribute to the dynamic changes in DDB2 expression upon UVB exposure or other stresses.

In conclusion, our study identifies a novel role for NAT10 in the repair of UVB-induced DNA damage. Mechanistically, we show that knockdown of NAT10 increases DDB2 mRNA stability and binds to the DDB2 mRNA transcript and that the DDB2 transcript is ac4C modified. Functionally, we demonstrate that knockdown of DDB2 reverses the effect of NAT10 knockdown on CPD repair. Together, these findings suggest that the NAT10/ac4C/DDB2 axis is an important factor in the repair of UVB-induced DNA damage. Furthermore, we show that NAT10 plays a tumor-promoting role in skin cancer and is increased by chronic UVB irradiation. Taken together, our findings establish NAT10-mediated ac4C acetylation of DDB2 as a novel mechanism in the UVB-induced DNA damage process and suggest NAT10 as a potential preventive and therapeutic target for skin cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Ann Motten for a critical reading of the manuscript. This work was supported in part by NIH grants ES024373 (Y.Y.H.), ES030576 (Y.Y.H.), ES031693 (Y.Y.H.), the CACHET (NIH ES027792), the University of Chicago Comprehensive Cancer Center (NIH CA014599), the CTSA (NIH UL1 TR000430), and the University of Chicago Friends of Dermatology Endowment Fund.

References

Arango D, Sturgill D, Alhusaini N, Dillman AA, Sweet TJ, Hanson G, Hosogane M, Sinclair WR, Nanan KK, Mandler MD, Fox SD, Zengeya TT, Andresson T, Meier JL, Coller J, Oberdoerffer S, 2018. Acetylation of Cytidine in mRNA Promotes Translation Efficiency. Cell 175, 1872–1886.e1824. [PubMed: 30449621]

Sancar Aziz, Lindsey-Boltz Laura A., Ünsal-Kaçmaz Keziban, Linn S, 2004. Molecular Mechanisms of Mammalian DNA Repair and the DNA Damage Checkpoints. Annual Review of Biochemistry 73, 39–85.

Balmus G, Larrieu D, Barros AC, Collins C, Abrudan M, Demir M, Geisler NJ, Lelliott CJ, White JK, Karp NA, Atkinson J, Kirton A, Jacobsen M, Clift D, Rodriguez R, Sanger Mouse Genetics P,

Adams DJ, Jackson SP, 2018. Targeting of NAT10 enhances healthspan in a mouse model of human accelerated aging syndrome. Nat Commun 9, 1700. [PubMed: 29703891]

Barbieri I, Kouzarides T, 2020. Role of RNA modifications in cancer. Nature Reviews Cancer 20, 303–322. [PubMed: 32300195]

Batty DP, Wood RD, 2000. Damage recognition in nucleotide excision repair of DNA. Gene 241, 193–204. [PubMed: 10675030]

Cai S, Liu X, Zhang C, Xing B, Du X, 2017. Autoacetylation of NAT10 is critical for its function in rRNA transcription activation. Biochemical and biophysical research communications 483, 624–629. [PubMed: 27993683]

Chen L, Wang WJ, Liu Q, Wu YK, Wu YW, Jiang Y, Liao XQ, Huang F, Li Y, Shen L, Yu C, Zhang SY, Yan LY, Qiao J, Sha QQ, Fan HY, 2022. NAT10-mediated N4-acetylcytidine modification is required for meiosis entry and progression in male germ cells. Nucleic Acids Res 50, 10896–10913. [PubMed: 35801907]

Cleaver JE, Lam ET, Revet I, 2009. Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. Nature Reviews Genetics 10, 756–768.

Cui L, Ma R, Cai J, Guo C, Chen Z, Yao L, Wang Y, Fan R, Wang X, Shi Y, 2022. RNA modifications: importance in immune cell biology and related diseases. Signal Transduction and Targeted Therapy 7, 334. [PubMed: 36138023]

Cui Z, Xu Y, Wu P, Lu Y, Tao Y, Zhou C, Cui R, Li J, Han R, 2023. NAT10 promotes osteogenic differentiation of periodontal ligament stem cells by regulating VEGFA-mediated PI3K/AKT signaling pathway through ac4C modification. Odontology.

Dalhat MH, Altayb HN, Khan MI, Choudhry H, 2021a. Structural insights of human N-acetyltransferase 10 and identification of its potential novel inhibitors. Scientific Reports 11, 6051. [PubMed: 33723305]

Dalhat MH, Altayb HN, Khan MI, Choudhry H, 2021b. Structural insights of human N-acetyltransferase 10 and identification of its potential novel inhibitors. Sci Rep 11, 6051. [PubMed: 33723305]

Dalhat MH, Mohammed MRS, Ahmad A, Khan MI, Choudhry H, 2021c. Remodelin, a N-acetyltransferase 10 (NAT10) inhibitor, alters mitochondrial lipid metabolism in cancer cells. Journal of Cellular Biochemistry 122, 1936–1945. [PubMed: 34605570]

Dalhat MH, Mohammed MRS, Alkhatabi HA, Rehan M, Ahmad A, Choudhry H, Khan MI, 2022. NAT10: An RNA cytidine transferase regulates fatty acid metabolism in cancer cells. Clin Transl Med 12, e1045. [PubMed: 36149760]

Delaunay S, Frye M, 2019. RNA modifications regulating cell fate in cancer. Nature Cell Biology 21, 552–559. [PubMed: 31048770]

Deng M, Zhang L, Zheng W, Chen J, Du N, Li M, Chen W, Huang Y, Zeng N, Song Y, Chen Y, 2023. Helicobacter pylori-induced NAT10 stabilizes MDM2 mRNA via RNA acetylation to facilitate gastric cancer progression. Journal of Experimental & Clinical Cancer Research 42, 9. [PubMed: 36609449]

DiGiovanna JJ, Kraemer KH, 2012. Shining a Light on Xeroderma Pigmentosum. Journal of Investigative Dermatology 132, 785–796. [PubMed: 22217736]

Frye M, Harada BT, Behm M, He C, 2018. RNA modifications modulate gene expression during development. Science 361, 1346–1349. [PubMed: 30262497]

Guerrero Llobet S, Bhattacharya A, Everts M, Kok K, van der Vegt B, Fehrmann RSN, van Vugt M, 2022. An mRNA expression-based signature for oncogene-induced replication-stress. Oncogene 41, 1216–1224. [PubMed: 35091678]

Hao H, Liu W, Miao Y, Ma L, Yu B, Liu L, Yang C, Zhang K, Chen Z, Yang J, Zheng Z, Zhang B, Deng F, Gong P, Yuan J, Hu Z, Guan W, 2022. N4-acetylcytidine regulates the replication and pathogenicity of enterovirus 71. Nucleic Acids Research 50, 9339–9354. [PubMed: 35971620]

Hicks JA, Li L, Matsui M, Chu Y, Volkov O, Johnson KC, Corey DR, 2017. Human GW182 Paralogs Are the Central Organizers for RNA-Mediated Control of Transcription. Cell Rep 20, 1543–1552. [PubMed: 28813667]

Ito S, Akamatsu Y, Noma A, Kimura S, Miyauchi K, Ikeuchi Y, Suzuki T, Suzuki T, 2014a. A single acetylation of 18 S rRNA is essential for biogenesis of the small ribosomal subunit in Saccharomyces cerevisiae. J Biol Chem 289, 26201–26212. [PubMed: 25086048]

Ito S, Horikawa S, Suzuki T, Kawauchi H, Tanaka Y, Suzuki T, Suzuki T, 2014b. Human NAT10 is an ATP-dependent RNA acetyltransferase responsible for N4-acetylcytidine formation in 18 S ribosomal RNA (rRNA). J Biol Chem 289, 35724–35730. [PubMed: 25411247]

Jean MJ, Power D, Kong W, Huang H, Santoso N, Zhu J, 2017. Identification of HIV-1 Tat-Associated Proteins Contributing to HIV-1 Transcription and Latency. Viruses 9.

Jin G, Xu M, Zou M, Duan S, 2020. The Processing, Gene Regulation, Biological Functions, and Clinical Relevance of N4-Acetylcytidine on RNA: A Systematic Review. Mol Ther Nucleic Acids 20, 13–24. [PubMed: 32171170]

Levy MJ, Montgomery DC, Sardiu ME, Montano JL, Bergholtz SE, Nance KD, Thorpe AL, Fox SD, Lin Q, Andresson T, Florens L, Washburn MP, Meier JL, 2020. A Systems Chemoproteomic Analysis of Acyl-CoA/Protein Interaction Networks. Cell Chem Biol 27, 322–333.e325. [PubMed: 31836350]

Liao L, He Y, Li SJ, Yu XM, Liu ZC, Liang YY, Yang H, Yang J, Zhang GG, Deng CM, Wei X, Zhu YD, Xu TY, Zheng CC, Cheng C, Li A, Li ZG, Liu JB, Li B, 2023. Lysine 2-hydroxyisobutyrylation of NAT10 promotes cancer metastasis in an ac4C-dependent manner. Cell Res 33, 355–371. [PubMed: 36882514]

Liu HY, Liu YY, Yang F, Zhang L, Zhang FL, Hu X, Shao ZM, Li DQ, 2020. Acetylation of MORC2 by NAT10 regulates cell-cycle checkpoint control and resistance to DNA-damaging chemotherapy and radiotherapy in breast cancer. Nucleic Acids Res 48, 3638–3656. [PubMed: 32112098]

Liu P, Li F, Lin J, Fukumoto T, Nacarelli T, Hao X, Kossenkov AV, Simon MC, Zhang R, 2021. m6A-independent genome-wide METTL3 and METTL14 redistribution drives the senescence-associated secretory phenotype. Nature Cell Biology 23, 355–365. [PubMed: 33795874]

Liu X, Cai S, Zhang C, Liu Z, Luo J, Xing B, Du X, 2018. Deacetylation of NAT10 by Sirt1 promotes the transition from rRNA biogenesis to autophagy upon energy stress. Nucleic acids research 46, 9601–9616. [PubMed: 30165671]

Liu X, Tan Y, Zhang C, Zhang Y, Zhang L, Ren P, Deng H, Luo J, Ke Y, Du X, 2016. NAT 10 regulates p53 activation through acetylating p53 at K120 and ubiquitinating Mdm2. EMBO reports 17, 349–366. [PubMed: 26882543]

Luo J, Cao J, Chen C, Xie H, 2023. Emerging role of RNA acetylation modification ac4C in diseases: Current advances and future challenges. Biochem Pharmacol 213, 115628. [PubMed: 37247745]

Montgomery DC, Garlick JM, Kulkarni RA, Kennedy S, Allali-Hassani A, Kuo YM, Andrews AJ, Wu H, Vedadi M, Meier JL, 2016. Global Profiling of Acetyltransferase Feedback Regulation. J Am Chem Soc 138, 6388–6391. [PubMed: 27149119]

Riedl T, Hanaoka F, Egly J-M, 2003. The comings and goings of nucleotide excision repair factors on damaged DNA. The EMBO journal 22, 5293–5303. [PubMed: 14517266]

Roundtree IA, Evans ME, Pan T, He C, 2017. Dynamic RNA Modifications in Gene Expression Regulation. Cell 169, 1187–1200. [PubMed: 28622506]

Shah P, Zhao B, Qiang L, He Y-Y, 2018. Phosphorylation of xeroderma pigmentosum group C regulates ultraviolet-induced DNA damage repair. Nucleic acids research 46, 5050–5060. [PubMed: 29660033]

Sharma S, Langhendries J-L, Watzinger P, Kötter P, Entian K-D, Lafontaine DLJ, 2015. Yeast Kre33 and human NAT10 are conserved 18S rRNA cytosine acetyltransferases that modify tRNAs assisted by the adaptor Tan1/THUMPD1. Nucleic Acids Research 43, 2242–2258. [PubMed: 25653167]

Shen Q, Zheng X, McNutt MA, Guang L, Sun Y, Wang J, Gong Y, Hou L, Zhang B, 2009. NAT10, a nucleolar protein, localizes to the midbody and regulates cytokinesis and acetylation of microtubules. Experimental Cell Research 315, 1653–1667. [PubMed: 19303003]

Shrimp JH, Jing Y, Gamage ST, Nelson KM, Han J, Bryson KM, Montgomery DC, Thomas JM, Nance KD, Sharma S, Fox SD, Andressen T, Sinclair WR, Wu H, Allali-Hassani A, Senisterra G, Vedadi M, Lafontaine D, Dahlin JL, Marmorstein R, Walters MA, Meier JL, 2021. Remodelin

Is a Cryptic Assay Interference Chemotype That Does Not Inhibit NAT10-Dependent Cytidine Acetylation. ACS Med Chem Lett 12, 887–892. [PubMed: 34141066]

Sleiman S, Dragon F, 2019. Recent Advances on the Structure and Function of RNA Acetyltransferase Kre33/NAT10. Cells 8, 1035. [PubMed: 31491951]

Stern L, Schulman LH, 1978. The role of the minor base N4-acetylcytidine in the function of the Escherichia coli noninitiator methionine transfer RNA. J Biol Chem 253, 6132–6139. [PubMed: 355249]

Sturgill D, Arango D, Oberdoerffer S, 2022. Protocol for base resolution mapping of ac4C using RedaC:T-seq. STAR Protocols 3, 101858. [PubMed: 36595942]

Su R, Dong L, Li Y, Gao M, He PC, Liu W, Wei J, Zhao Z, Gao L, Han L, Deng X, Li C, Prince E, Tan B, Qing Y, Qin X, Shen C, Xue M, Zhou K, Chen Z, Xue J, Li W, Qin H, Wu X, Sun M, Nam Y, Chen C-W, Huang W, Horne D, Rosen ST, He C, Chen J, 2022. METTL16 exerts an m6A-independent function to facilitate translation and tumorigenesis. Nature Cell Biology 24, 205–216. [PubMed: 35145225]

Thomas JM, Briney CA, Nance KD, Lopez JE, Thorpe AL, Fox SD, Bortolin-Cavaille M-L, Sas-Chen A, Arango D, Oberdoerffer S, Cavaille J, Andresson T, Meier JL, 2018. A Chemical Signature for Cytidine Acetylation in RNA. Journal of the American Chemical Society 140, 12667–12670. [PubMed: 30252461]

Thomas JM, Bryson KM, Meier JL, 2019. Nucleotide resolution sequencing of N4-acetylcytidine in RNA. Methods Enzymol 621, 31–51. [PubMed: 31128786]

Tsai K, Jaguva Vasudevan AA, Martinez Campos C, Emery A, Swanstrom R, Cullen BR, 2020. Acetylation of Cytidine Residues Boosts HIV-1 Gene Expression by Increasing Viral RNA Stability. Cell Host Microbe 28, 306–312 e306. [PubMed: 32533923]

Wang G, Zhang M, Zhang Y, Xie Y, Zou J, Zhong J, Zheng Z, Zhou X, Zheng Y, Chen B, Liu C, 2022. NAT10-mediated mRNA N4-acetylcytidine modification promotes bladder cancer progression. Clin Transl Med 12, e738. [PubMed: 35522942]

Wei R, Cui X, Min J, Lin Z, Zhou Y, Guo M, An X, Liu H, Janz S, Gu C, Wang H, Yang Y, 2022. NAT10 promotes cell proliferation by acetylating CEP170 mRNA to enhance translation efficiency in multiple myeloma. Acta Pharm Sin B 12, 3313–3325. [PubMed: 35967285]

Wu Y, Cao Y, Liu H, Yao M, Ma N, Zhang B, 2020. Remodelin, an inhibitor of NAT10, could suppress hypoxia-induced or constitutional expression of HIFs in cells. Molecular and Cellular Biochemistry 472, 19–31. [PubMed: 32529496]

Xiang Y, Laurent B, Hsu C-H, Nachtergaele S, Lu Z, Sheng W, Xu C, Chen H, Ouyang J, Wang S, 2017. RNA m6A methylation regulates the ultraviolet-induced DNA damage response. Nature 543, 573–576. [PubMed: 28297716]

Xie L, Zhong X, Cao W, Liu J, Zu X, Chen L, 2023a. Mechanisms of NAT10 as ac4C writer in diseases. Molecular Therapy - Nucleic Acids 32, 359–368. [PubMed: 37128278]

Xie R, Cheng L, Huang M, Huang L, Chen Z, Zhang Q, Li H, Lu J, Wang H, Zhou Q, 2023b. NAT10 drives cisplatin chemoresistance by enhancing ac4C-associated DNA repair in bladder cancer. Cancer research 83, 1666–1683. [PubMed: 36939377]

Yang S, Wei J, Cui Y-H, Park G, Shah P, Deng Y, Aplin AE, Lu Z, Hwang S, He C, He Y-Y, 2019. m6A mRNA demethylase FTO regulates melanoma tumorigenicity and response to anti-PD-1 blockade. Nature Communications 10, 2782.

Yang Z, Yang S, Cui Y-H, Wei J, Shah P, Park G, Cui X, He C, He Y-Y, 2021. METTL14 facilitates global genome repair and suppresses skin tumorigenesis. Proceedings of the National Academy of Sciences 118, e2025948118.

Yu X-M, Li S-J, Yao Z-T, Xu J-J, Zheng C-C, Liu Z-C, Ding P-B, Jiang Z-L, Wei X, Zhao L-P, Shi X-Y, Li Z-G, Xu WW, Li B, 2023. N4-acetylcytidine modification of lncRNA CTC-490G23.2 promotes cancer metastasis through interacting with PTBP1 to increase CD44 alternative splicing. Oncogene 42, 1101–1116. [PubMed: 36792757]

Zhang H, Chen Z, Zhou J, Gu J, Wu H, Jiang Y, Gao S, Liao Y, Shen R, Miao C, Chen W, 2022a. NAT10 regulates neutrophil pyroptosis in sepsis via acetylating ULK1 RNA and activating STING pathway. Commun Biol 5, 916. [PubMed: 36068299]

Zhang H, Shan W, Yang Z, Zhang Y, Wang M, Gao L, Zeng L, Zhao Q, Liu J, 2023a. NAT10 mediated mRNA acetylation modification patterns associated with colon cancer progression and microsatellite status. Epigenetics 18, 2188667. [PubMed: 36908042]

Zhang L, Li DQ, 2019. MORC2 regulates DNA damage response through a PARP1-dependent pathway. Nucleic Acids Res 47, 8502–8520. [PubMed: 31616951]

Zhang X, Zeng J, Wang J, Yang Z, Gao S, Liu H, Li G, Zhang X, Gu Y, Pang D, 2022b. Revealing the Potential Markers of N(4)-Acetylcytidine through acRIP-seq in Triple-Negative Breast Cancer. Genes (Basel) 13.

Zhang Y, Deng Z, Sun S, Xie S, Jiang M, Chen B, Gu C, Yang Y, 2022c. NAT10 acetylates BCL-XL mRNA to promote the proliferation of multiple myeloma cells through PI3K-AKT pathway. Frontiers in Oncology 12.

Zhang Y, Jing Y, Wang Y, Tang J, Zhu X, Jin W-L, Wang Y, Yuan W, Li X, Li X, 2021. NAT10 promotes gastric cancer metastasis via N4-acetylated COL5A1. Signal Transduction and Targeted Therapy 6, 173. [PubMed: 33941767]

Zhang Z, Zhang Y, Cai Y, Li D, He J, Feng Z, Xu Q, 2023b. NAT10 regulates the LPS-induced inflammatory response via the NOX2-ROS-NF-κB pathway in macrophages. Biochim Biophys Acta Mol Cell Res, 119521. [PubMed: 37307924]

Zheng J, Tan Y, Liu X, Zhang C, Su K, Jiang Y, Luo J, Li L, Du X, 2022a. NAT10 regulates mitotic cell fate by acetylating Eg5 to control bipolar spindle assembly and chromosome segregation. Cell Death & Differentiation 29, 846–860. [PubMed: 35210604]

Zheng X, Wang Q, Zhou Y, Zhang D, Geng Y, Hu W, Wu C, Shi Y, Jiang J, 2022b. N-acetyltransferase 10 promotes colon cancer progression by inhibiting ferroptosis through N4-acetylation and stabilization of ferroptosis suppressor protein 1 (FSP1) mRNA. Cancer Communications 42, 1347–1336. [PubMed: 36209353]

Zhu Z, Xing X, Huang S, Tu Y, 2021. NAT10 Promotes Osteogenic Differentiation of Mesenchymal Stem Cells by Mediating N4-Acetylcytidine Modification of Gremlin 1. Stem Cells Int 2021, 8833527. [PubMed: 33953754]

Zong G, Wang X, Guo X, Zhao Q, Wang C, Shen S, Xiao W, Yang Q, Jiang W, Shen J, Wan R, 2023. NAT10-mediated AXL mRNA N4-acetylcytidine modification promotes pancreatic carcinoma progression. Experimental Cell Research 428, 113620. [PubMed: 37156457]

Highlights

- NAT10 regulates the repair of UV-induced DNA damage through regulating DDB2 expression
- NAT10 regulates the stability of the DDB2 mRNA
- The DDB2 transcript is ac4C modified and binds to NAT10
- NAT10 acts as a tumor-promoting factor in skin cancer and is induced by chronic UVB irradiation

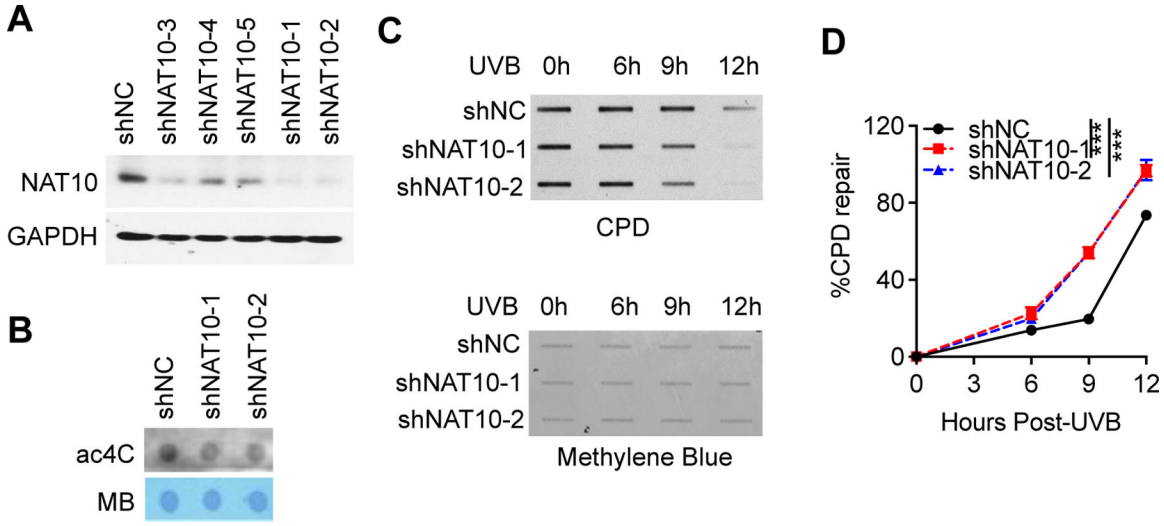

Figure 1.

NAT10 knockdown increased the repair of UVB-induced DNA damage. (A) Immunoblot analysis confirming NAT10 knockdown in HaCaT cells. (B) Dot blot analysis of the ac4C level in HaCaT cells with or without NAT10 knockdown. Methylene blue staining was used as the loading control. (C) DNA slot blot analysis of the levels of CPD at the indicated times post-UVB (20 mJ/cm^2) in HaCaT cells with or without NAT10 knockdown. Methylene blue staining was used as the loading control. (D) Quantification of C. Results are shown as Mean ± S.D. (n = 3). ***, $P < 0.001$, between comparison groups (9 h and 12 h); Student's *t*-test.

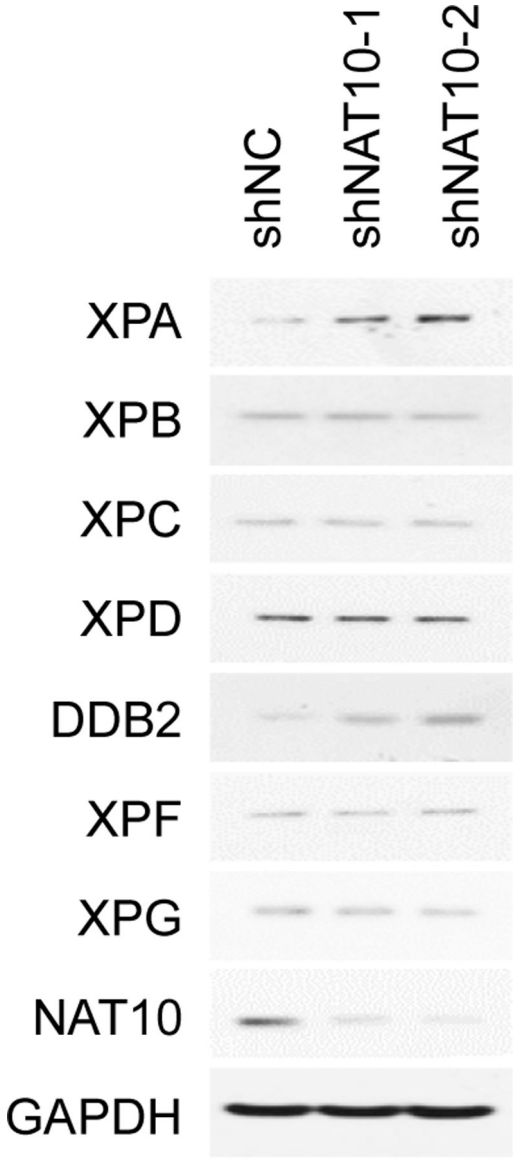

Figure 2.
NAT10 regulates the expression of the critical GG-NER factors. Immunoblot analysis of the protein levels of key GG-NER factors in HaCaT cells with or without NAT10 knockdown.

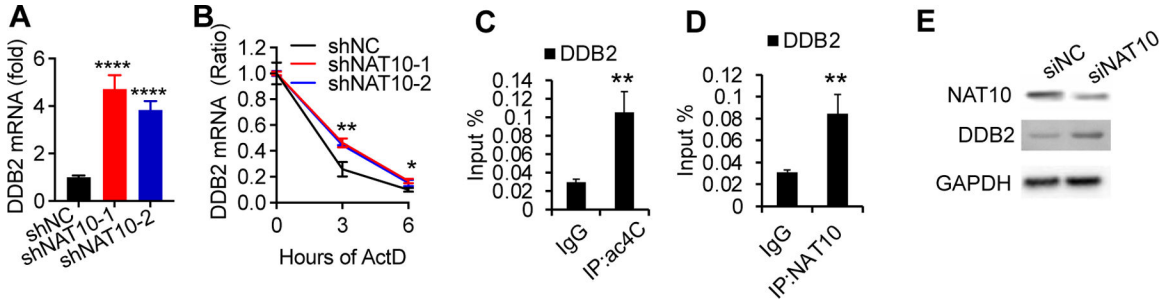

Figure 3.

NAT10 regulates DDB2 mRNA stability and binds to the DDB2 transcript and DDB2 is ac4C modified. (A) qPCR analysis of DDB2 mRNA levels in HaCaT cells with or without NAT10 knockdown. (B) qPCR analysis of the DDB2 mRNA stability in HaCaT cells with or without NAT10 knockdown treated with or without actinomycin D (ActD) for 0, 3, and 6 h. (C) qPCR analysis of the ac4C level of the DDB2 transcript following ac4C RNA immunoprecipitation (ac4C RIP). (D) qPCR analysis for binding of NAT10 to the DDB2 transcript following NAT10 RNA immunoprecipitation (NAT10 RIP). (E) Immunoblot analysis of the protein levels of key GG-NER factors in A431 cells with or without NAT10 knockdown. Results are shown as Mean ± S.D. (n=6 for A; n = 3 for B; n = 3 for C and D). *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$, compared with the shNC group (A and B) or the IgG group (C and D); Student's *t*-test.

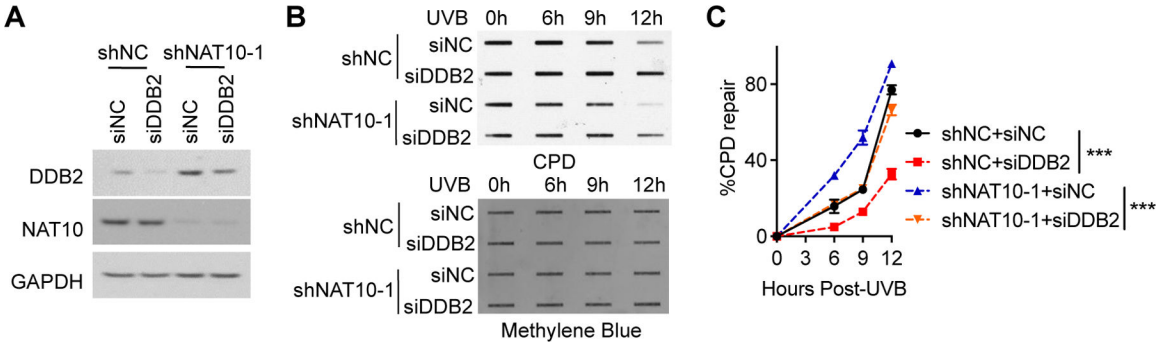

Figure 4.

The role of DDB2 in the effect of NAT10 knockdown on the repair of UVB-induced DNA damage through regulating DDB2. (A) Immunoblot analysis of DDB2 protein levels in HaCaT cells with or without NAT10 knockdown, DDB2 knockdown by siRNA, or in combination. (B) DNA slot blot analysis of the levels of CPD at the indicated times post-UVB (20 mJ/cm^2) in cells as in A. (C) Quantification of B. Results are shown as Mean ± S.D. (n = 3). ***, $P < 0.001$, between comparison groups (9 h and 12 h); Student's *t*-test.

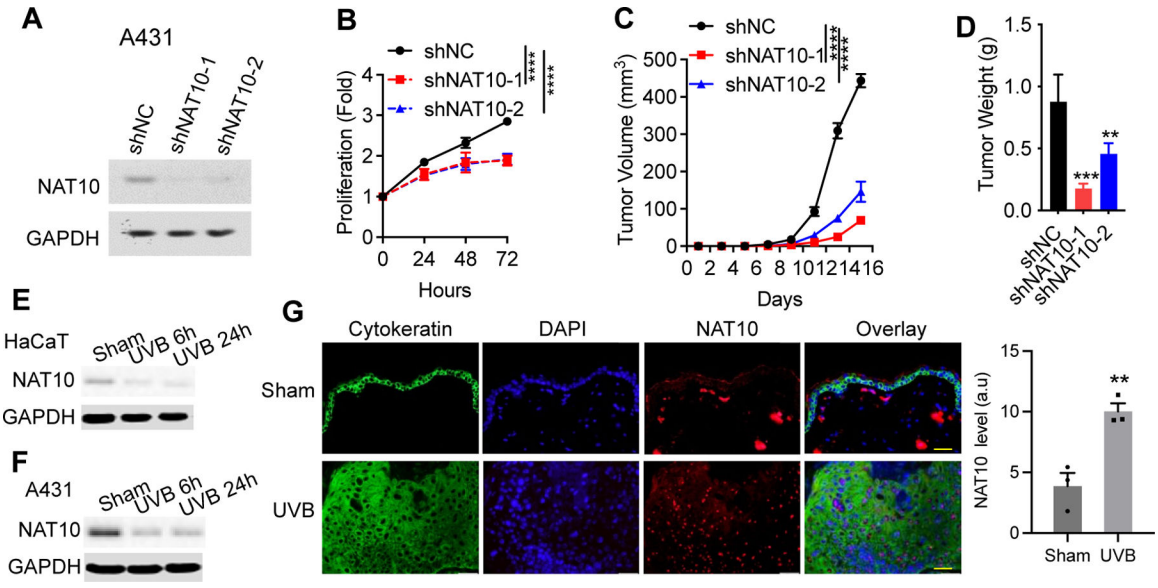

Figure 5. NAT10 knockdown inhibits tumorigenicity.

(A) Immunoblot analysis confirming NAT10 knockdown in A431 cells. (B) Proliferation analysis in cells as in A. (C and D) Tumor volume (C) and weight (D) in nude mice following subcutaneous inoculation of A431 cells with or without NAT10 knockdown. (E, F) Immunoblot analysis of the protein levels of NAT10 in HaCaT (E) or A431 (F) cells with or without UVB irradiation. (G) Immunofluorescence analysis and quantification in each cell of NAT10 (red) protein levels in sham and chronically UVB-irradiated mouse skin. Cytokeratin (green for epidermis) and DAPI (blue for nucleus) are used as counter stains. Scale bar: 200 μm. Results are shown as Mean ± S.D. (n=8 for B; n = 5 for C and D; n=3 for G). **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$, (48 h and 72 h for B; day 11, 13 and 15 for C), compared with the shNC group (B-D); Student's *t*-test.

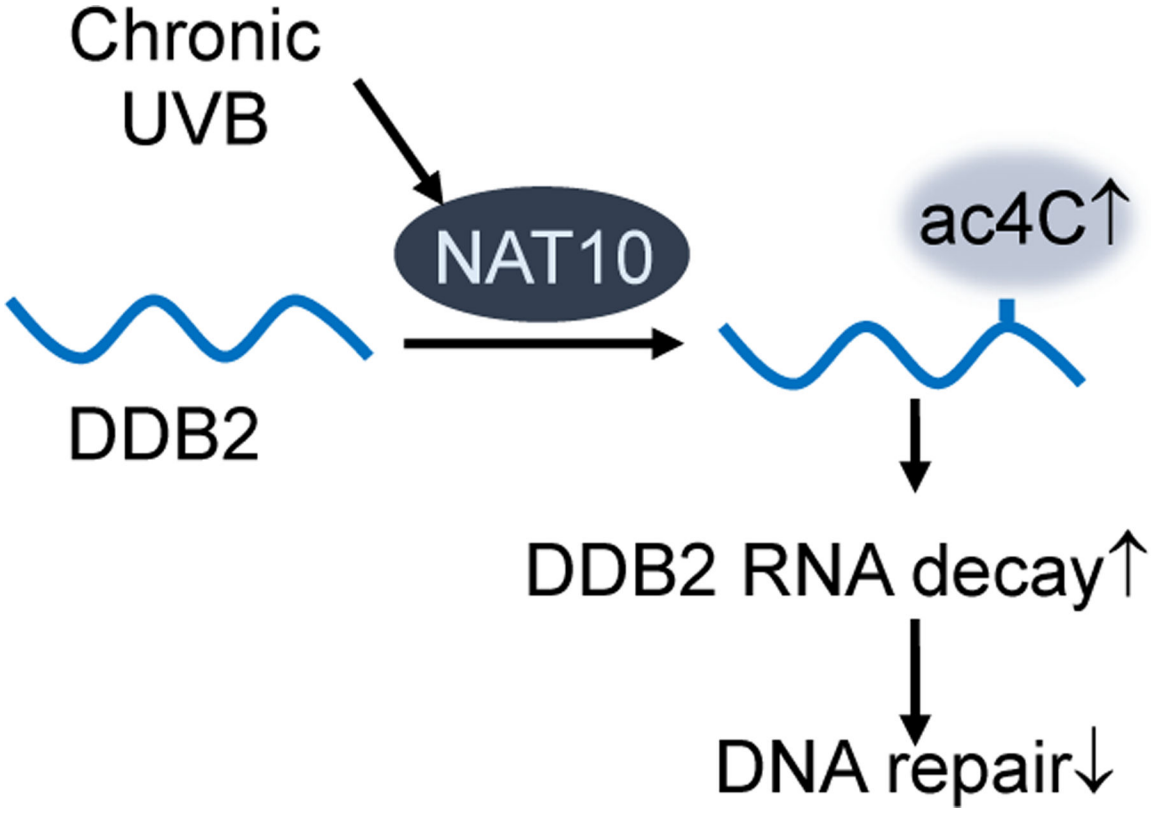

Figure 6.

Schematic summary of the role of NAT10 and ac4C mRNA modification in regulating the repair of UVB-induced DNA damage and tumorigenicity.