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Original Article

Nampt is involved in DNA double-strand break repair

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Abstract

DNA double-strand break (DSB) is the most severe form of DNA damage, which is repaired mainly through high-fidelity homologous recombination (HR) or error-prone non-homologous end joining (NHEJ). Defects in the DNA damage response lead to genomic instability and ultimately predispose organs to cancer. Nicotinamide phosphoribosyltransferase (Nampt), which is involved in nicotinamide adenine dinucleotide metabolism, is overexpressed in a variety of tumors. In this report, we found that Nampt physically associated with CtIP and DNA-PKcs/Ku80, which are key factors in HR and NHEJ, respectively. Depletion of Nampt by small interfering RNA (siRNA) led to defective NHEJ-mediated DSB repair and enhanced HR-mediated repair. Furthermore, the inhibition of Nampt expression promoted proliferation of cancer cells and normal human fibroblasts and decreased β-galactosidase staining, indicating a delay in the onset of cellular senescence in normal human fibroblasts. Taken together, our results suggest that Nampt is a suppressor of HR-mediated DSB repair and an enhancer of NHEJ-mediated DSB repair, contributing to the acceleration of cellular senescence.

Key words Nampt, DNA-PKcs/Ku80, CtIP, DNA repair, cellular senescence

The DNA within our cells is constantly exposed to, attacked by, and damaged by a variety of DNA damaging agents, such as ultraviolet radiation, ionizing radiation, mutagenic agents, and reactive oxygen species[1,2]. To protect their DNA from these agents, human beings have evolved a highly efficient and complex system, the DNA damage response (DDR), to cope with damaged DNA. The DDR process includes the activation of cell cycle checkpoints to halt cell cycle progression and allow time for DNA repair, senescence or apoptosis when DNA damage is irreparable[3]. A defective DDR leads to DNA alterations, the accumulation of which ultimately results in genomic instability, the most significant cancer hallmark[4].

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Of the various forms of DNA damage, DNA double-strand break (DSB) is probably the most genotoxic[3]. DSBs are generated when the two complementary strands of the DNA double helix are broken simultaneously[2]. A single unrepaired DSB is sufficient to kill a mammalian cell. In mammalian cells, DSBs are mainly repaired by error-prone non-homologous end joining (NHEJ) and high-fidelity homologous recombination (HR)[3]. HR repair occurs in the S and G_2 phases of the cell cycle, due to its requirement of a homologous chain as a template to complete repair, whereas NHEJ repair joins the broken DNA together with no or simple processing of the DNA ends. Thus, HR-mediated DSB repair is essential for genomic integrity, whereas NHEJ-mediated DSB repair probably causes mutations.

CtBP-interacting protein (CtIP), the DNA endonuclease, is phosphorylated upon DNA damage (possibly by ATM/ATR), cooperates with the Mre11-Rad50-Nbs1 complex in processing DSB broken ends, and dissociates from breast cancer type 1 susceptibility protein (BRCA1)[5-7]. BRCA1 is essential in HR-mediated DSB repair through its direct interaction with PALB2. This interaction fine-tunes the DSB repair partly through its modulatory role in the PALB2-dependent loading of BRCA2-RAD51 repair machinery at DNA breaks[8]. Thus,

CtIP plays an important role in HR.

Ku80 (also known as Ku86, the 86 kDa subunit of the Ku antigen), a single-strand DNA-dependent ATP-dependent helicase, forms a heterodimer with Ku70 [9]. The Ku80/Ku70 dimer modulates NHEJ-mediated DSB repair through a DNA-dependent association with the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) to form the DNA-PK complex and with the LIG4-XRCC4 complex to re-ligate the broken ends[9].

Nicotinamide phosphoribosyltransferase (Nampt) is the rate-limiting enzyme in the mammalian nicotinamide adenine dinucleotide (NAD) salvage pathway, which is more efficient and quicker than that of *de novo* NAD synthesis [10]. Nampt catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide, which is an intermediate in the biosynthesis of NAD[11,12]. NAD serves as a substrate for protein modifications, including protein deacetylation and mono- and poly (ADP-ribosyl)ation [10]. Poly(ADP-ribosyl)ation is induced immediately after DNA damage[13]. The poly(ADP-ribose) polymerases (PARPs), mainly PARP1, catalyze the synthesis of poly (ADP-ribose) polymers (PAR) onto specific target proteins using NAD^+ as a substrate [14]. Recently, NAD-dependent deacetylation of CtIP by Sirt6 and HDACs has been demonstrated to facilitate HR-mediated DSB repair [15,16]. HDAC inhibitors regulate NHEJ-mediated DSB repair through modulation of Ku70 acetylation[17]. Taken together, NAD-dependent deacetylation of DDR factors constitutes an integral process in DSB repair. Thus, it is not surprising that Nampt is involved in tumorigenesis. It has been reported that Nampt is overexpressed in colorectal cancer[18], breast tumors[19], and prostate cancer[20].

In this study, we investigated a potential link between Nampt and DSB repair.

Materials and Methods

Cell lines, plasmids, siRNA oligos, and antibodies

The human cervical cancer HeLa cell line and human fibroblast WI38 cell line were purchased from the American Type Culture Collection (Rockville, MD). The green fluorescent protein (GFP) reporter system for HR-mediated DSB repair (DR-GFP U2OS cells), the GFP reporter system for NHEJ-mediated DSB repair (EJ5-GFP HEK293 cells), and the I-SceI expression construct were generous gifts from Jeremy Stark (City of Hope National Medical Center/Beckman Research Institute, Duarte, CA). All cell lines were cultured in DMEM medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT) and were grown at 37℃ in the presence of 5% CO_2.

The full-length cDNA of Nampt (GenBank accession number: NM_005746) was obtained from Open-Biosystems. The Nampt coding sequence was amplified by polymerase chain reaction (PCR) and cloned into pcDNA-3HA vector, resulting in pcDNA-HA-Nampt.

All siRNA oligo duplexes (OnTarget plus option) were purchased from Dharmacon (Lafayette, CO). The small interfering RNA (siRNA) directed against human Nampt was a mixture of four pre-designed OnTarget plus siRNA oligonucleotide duplexes (siNampt). The forward sequences of individual siRNA oligos were 5'-GGU AAGAAGUUUCCUGUUAUU-3' for si1Nampt; 5'-CAAAU UGGAUUGAGACUAUUU-3' for si2Nampt; 5'-UAACU UAGAUGGUCUGGAAUU-3' for si3Nampt; and 5'-CAAG CAAAGUUUAUUCCUAUU-3' for si4Nampt. The control siRNA oligo (siControl) sequence was 5'-CGUACGCGG AAUACUUCGAdTdT-3'.

The control short hairpin sequence (5'-tcgagcgTT CTCCGAACGTGTCACGTttgatatccgACGTGACACGTTC GGAGAAttttttccaaag-3') or the Nampt short hairpin sequence (5'-tcgagcgCAAGCAAAGUUUAUUCCUATttg atatccgUAGGAAUAAACUUUGCUUGttttttccaaag-3') was subcloned into the retroviral vector pMSCV-TMP. Sequences in upper cases are target sequences, whereas those in lower cases are linker sequences. The retroviral particles for shControl or shNampt were produced in the packaging cells PA317 as described previously[21].

Antibodies against Nampt (BL2122), HA (A190-208A), DNA-PKcs (BL2406), and CtIP (BL1913) were purchased from Bethyl Laboratories (Montgomery, TX). Mouse monoclonal antibodies against β-actin (clone AC15) and γ-H2AX were purchased from Sigma (St. Louis, MO) and Millipore (Billerica, MA), respectively. Peroxidase-conjugated secondary antibodies were from JacksonImmuno Research (West Grove, PA).

HR- or NHEJ-mediated DSB repair GFP reporter systems

The HR-mediated DSB repair assay was performed as described previously[22,23]. Briefly, DR-GFP U2OS cells were transfected with Nampt siRNA oligos twice within a 24-hour interval using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. At 24 h after the second siRNA transfection, the cells were transfected with I-SceI expression plasmid (pCBA Sce) using FuGENE (Roche). Two days later, GFP-positive cells were assayed by FACScan.

The NHEJ-mediated DSB repair assays in EJ5-GFP HEK293 cells were described previously[24]. Briefly, EJ5-GFP HEK293 cells with one copy of the EJ5-GFP reporter stably integrated into their genome were transfected with siNampt or siControl, and a second transfection was performed 24 h later with the I-SceI- expressing construct or an empty vector. Cells were harvested 72 h

after the second transfection, and the fraction of GFP-positive cells was determined by flow cytometry.

Immunoblotting and immunoprecipitation

Total cell lysates were extracted with NP-40 buffer [50 mmol/L Tris-HCl (pH 7.5), 400 mmol/L NaCl, 5 mmol/L EDTA, 0.5% NP-40] and protease inhibitor mixture (Roche). The supernatant was subsequently diluted with NP-40 buffer [50 mmol/L Tris-HCl (pH 7.5), 5 mmol/L EDTA, 0.5% NP-40] into 150 mmol/L NaCl lysate. Immunoprecipitation was performed using 2 μg of antibody for 1 mg of total cell lysate and protein A sepharose (Amersham) to pull down immunocomplexes. Precipitates were washed with NP-40 buffer (150 mmol/L NaCl). Precipitates or total cell lysates were resolved with 4%–18% gradient SDS-PAGE and transferred onto a nitrocellulose membrane. Blots on nitrocellulose were blocked with 5% nonfat milk in PBST (PBS with 0.05% Tween-20) and sequentially incubated with primary antibodies and horseradish peroxidase-conjugated secondary antibodies in 5% nonfat milk in PBST. Blots were washed with PBST after 1-hour incubation. The immunoreactive bands were visualized using Thermo Super Signal West Femto Substrate kit reagents and following the provided instructions.

Cell growth assay

U2OS or WI38 cells were transfected with siNampt or siControl with RNAiMAX. Cells were trypsinized 24 h after transfection and transferred into 6-well plates (5×10^4 cells per well for U2OS and 1×10^4 cells per well for WI38). The cell number was counted every day for 6 days (U2OS cells) or 5 days (WI38 cells), with 6 parallel wells being used at each time point.

Senescence-associated β-galactosidase (SA-β-gal) staining

WI38 cells at passage 39 were infected with shControl or shNampt retroviral particles. Infectants were cultured with puromycin (1 μg/mL) for 1 week and without puromycin for an additional week. Cells were processed for SA-β-gal staining at passage 46 as described by Dimri *et al*. [25]. Briefly, the cells were washed with PBS and fixed with 0.5% glutaraldehyde in PBS for 5 min at room temperature. After washing with PBS, the cells were incubated with a freshly prepared staining solution [1 mg/mL 5-bromo-4-chloro-3-indolyl-β-dgalactopyranoside (X-gal), 40 mmol/L citric acid/sodium phosphate (pH 6.0), 5 mmol/L potassium ferrocyanide, 5 mmol/L potassium ferricyanide, 15 mmol/L NaCl, and 2 mmol/L $MgCl_2$] at 37℃ for 16 h.

Results

Nampt physically associated with Ku80 and CtIP

To explore a potential link between the Nampt-dependent NAD synthesis pathway and DNA repair, we performed a large-scale immunoprecipitation in HeLa cells using the anti-Nampt antibody in combination with mass spectrometric analysis. As the size of Nampt is similar to that of the IgG heavy chain, the presumptive Nampt band was probably buried in the IgG mass. DNA-PKcs and Ku80, two key players in the NHEJ-mediated DSB repair, were found in the Nampt immunocomplex (Figure 1A). Co-immunoprecipitation assays confirmed that both endogenous DNA-PKcs and Ku80 were present in the Nampt immunocomplex and a screen for other candidates revealed that CtIP was present in the same complex in HeLa cells (Figure 1B). When expressed in U2OS cells, HA-tagged Nampt immunoprecipitated with endogenous DNA-PKcs/Ku80 and CtIP (Figure 1C).

Depletion of Nampt led to an increase of HR-mediated DSB repair efficiency

DNA-PKcs/Ku80 and CtIP are key proteins involved in the early steps of NHEJ and HR, respectively. Therefore, we reasoned that Nampt is involved in DSB repair. The efficiency of HR-mediated DSB repair was determined using a well-established reporter system stably integrated in DR-GFP U2OS cells. We found that inhibiting Nampt expression using a mixture of 4 siRNA oligo (siNampt) resulted in a 1.1-fold increase of GFP-positive cells in comparison to mock-depleted cells (siControl) (Figure 2A), and knockdown of Nampt expression by individual siRNAs derived from the mixture yielded similar results (Figure 2B). This result was consistent with the results of the genome-wide siRNA screen, in which the depletion of Nampt expression led to a 1.07-fold increase in HR-mediated DSB repair efficiency[26]. To rule out the possibility of off-target siRNA effects, we introduced a silent mutation in the siRNA target sequence in the Nampt cDNA to generate HA-res1-4Nampt, whose RNA could not be targeted by the corresponding siRNA oligo for degradation. We identified that HA-res4Nampt, when expressed in DR-GFP U2OS cells, could not be knocked down by si4Nampt (Figure 2C and data not shown). When HA-res4Nampt was stably expressed in DR-GFP U2OS cells, transfection with the si4Nampt did not result in an increase of HR-mediated DSB repair efficiency (Figure 2D). Taken together, these data demonstrate that Nampt suppresses HR-mediated DSB repair.

We also determined whether Nampt modulates NHEJ-mediated DSB repair. We used the GFP-based

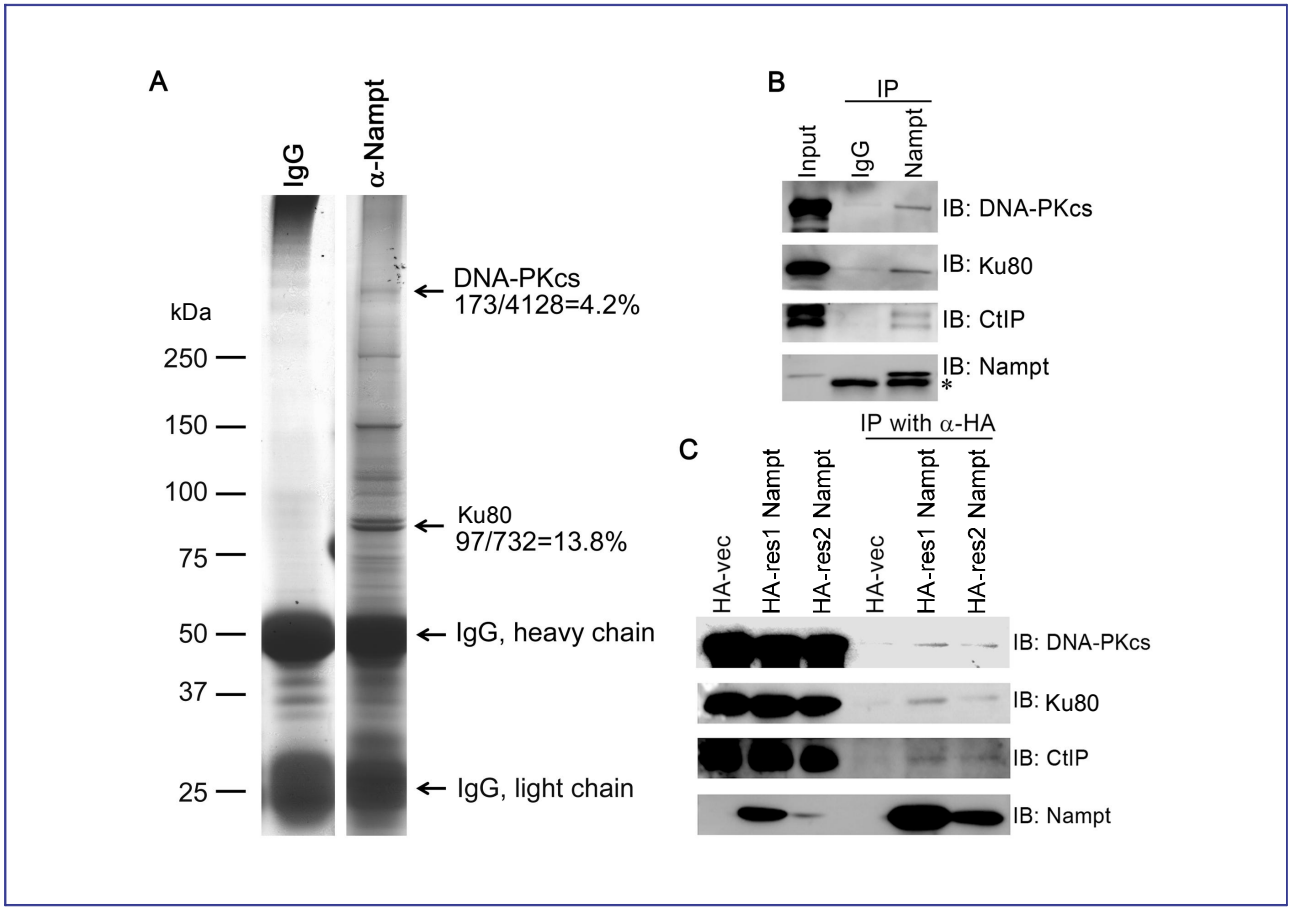

Figure 1. **Nampt associates with CtIP and DNA-PKcs/Ku80.** A, mass spectrometric analysis reveals that DNA-PKcs and Ku80 are present in the Nampt immunocomplex. The endogenous Nampt immunocomplex was pulled down from 50 mg of total cell lysates derived from log-phase HeLa cells using 50 μg of affinity-purified Nampt rabbit polyclonal antibodies or normal rabbit IgG as a negative control. Discrete bands were excised for mass spectrometric analysis. The numbers on the right side of the panel indicate the number of identified polypeptides divided by the number of predicted polypeptides equals the percent coverage of the polypeptides. B, endogenous Nampt associates with endogenous CtIP and DNA-PKcs/Ku80. Endogenous Nampt in HeLa cell lysate was immunoprecipitated with an anti-Nampt antibody, and the precipitated complex was resolved with 4%–8% gradient SDS-PAGE and immunoblotted with antibodies as indicated. *non-specific band. C, HA-Nampt pulls down endogenous CtIP and DNA-PKcs/Ku80. HA-Nampt is stably expressed in HeLa cells. Total cell lysate was extracted and immunoprecipitated with an anti-HA antibody and blotted with antibodies as indicated.

chromosomal reporter EJ5-GFP in HEK293 cells to measure total NHEJ-mediated DSB repair efficiency [24]. EJ5-GFP contains a promoter that is separated from a GFP-coding cassette by a puromycin gene flanked by two I-SceI sites in the same orientation. Once the puromycin gene is excised by the two I-SceI-induced DSBs, the promoter is joined to the rest of the expression cassette by NHEJ repair, leading to the restoration of the GFP gene. Therefore, the number of GFP-positive cells is a measure of NHEJ-mediated DSB repair. Inhibition of Nampt expression by the mixture of 4 siRNA oligos or an individual siRNA oligo reduced the percentage of GFP-positive cells to approximately 60% (Figure 2E), indicating that Nampt is an enhancer of NHEJ-mediated DSB repair.

Depletion of Nampt promoted cell proliferation

As we found that depletion of Nampt resulted in an increase of HR-mediated DSB repair and a decrease of NHEJ-mediated DSB repair, we reasoned that Nampt could regulate cell proliferation and senescence. Indeed, inhibition of Nampt expression by siRNA in U2OS cells (Figure 3A) or WI38 normal human fibroblasts (Figure 3B) promoted cell proliferation in the cell growth assays. When WI38 cells (passage 39) were retrovirally infected with shRNA, infectants were cultured until passage 46 before they were fixed and stained for β-galactosidase

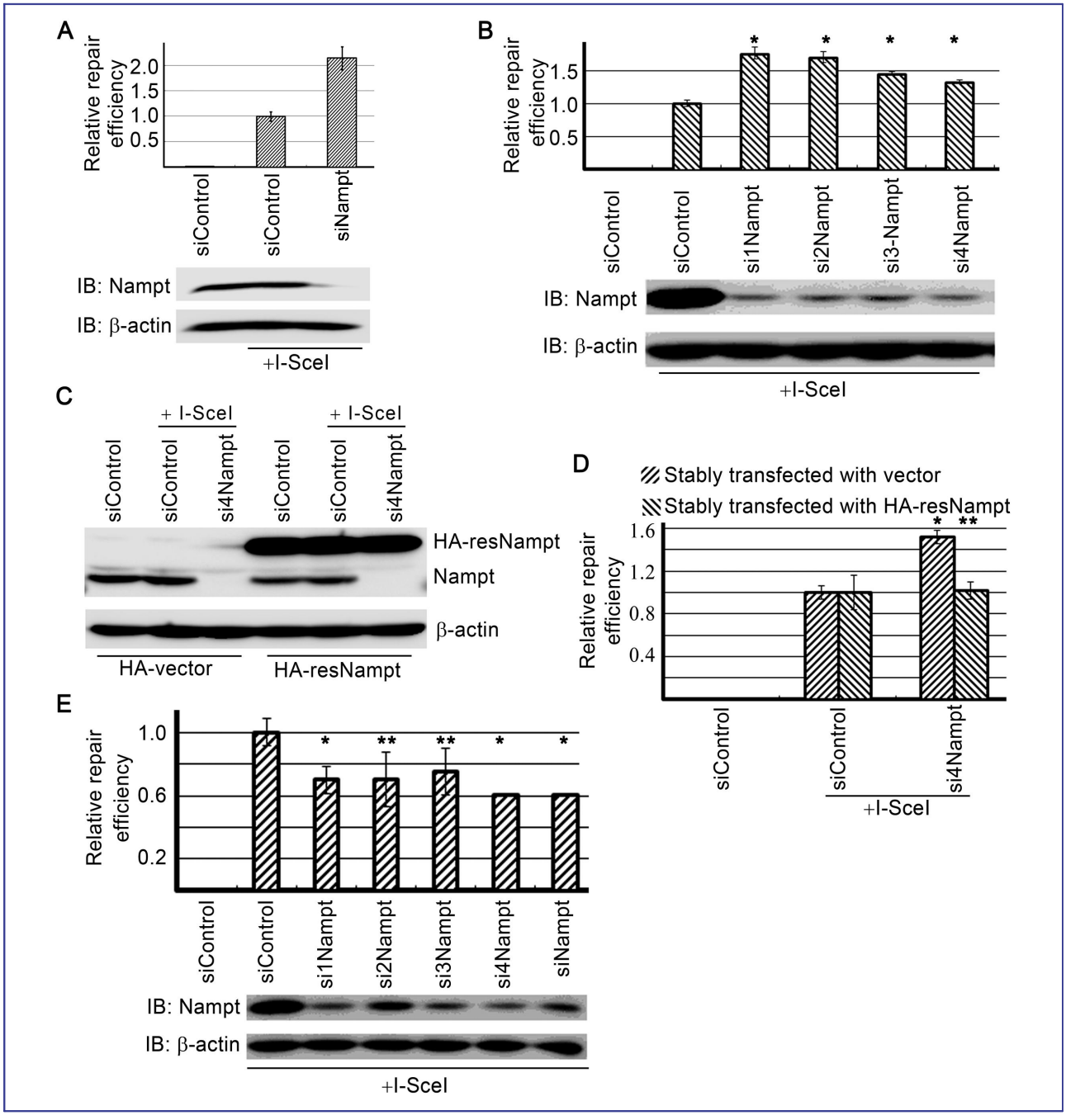

Figure 2. **Nampt suppresses HR-mediated DSB repair while promoting NHEJ-mediated DSB repair.** A and B, inhibition of Nampt expression increases HR-mediated DSB repair efficiency. DR-GFP U2OS cells were transfected with a mixture of 4 siRNA oligos (siNampt) (A) or individual siRNA oligos (si1-4Nampt) (B). Transfectants were additionally transfected with an I-SceI expression construct 24 h after siRNA transfection. Cells were harvested 48 h after the second transfection to quantify GFP-positive cells by FACS. C and D, expression of an siRNA-resistant form of Nampt rescues the HR-mediated DSB repair increase that resulted from the depletion of endogenous Nampt. DR-GFP U2OS cells were stably transfected with an empty HA vector or an HA-resNampt expression construct whose expression product is resistant to depletion by si4Nampt. Expression of endogenous Nampt was inhibited by si4Nampt (C). The relative HR-mediated DSB repair efficiency was determined by analyzing GFP-positive cells by FACS (D). E, depletion of Nampt expression decreases NHEJ-mediated DSB repair efficiency. EJ5-GFP 293 cells were first transfected with siNampt, si1-4Nampt, or a non-targeting control siControl, and 24 h later with an expression construct for I-SceI. Cells were harvested 72 h after the second transfection for immunoblotting with the antibodies as indicated (bottom panel) and flow cytometric analysis of GFP-positive cells (top panel). All values are presented as mean ± standard deviation (SD) of at least three independent experiments. $^{*}P < 0.05$, $^{**}P > 0.05$, vs. control cells.

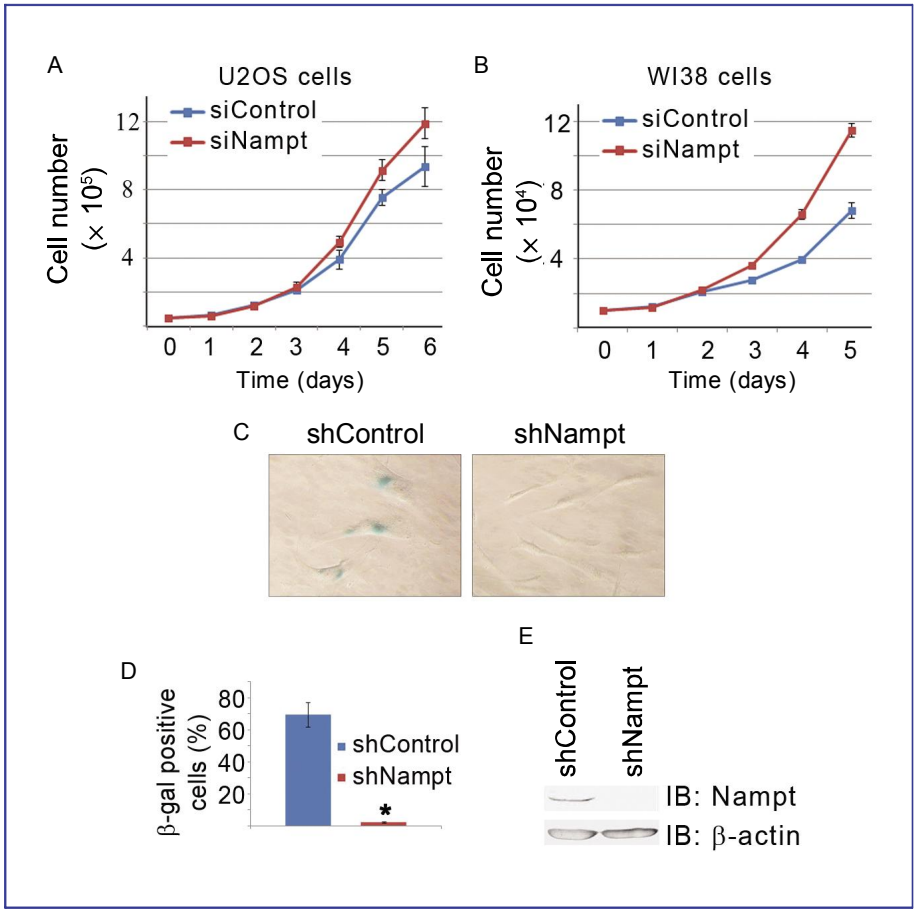

Figure 3. **Depletion of Nampt expression promotes cell proliferation and suppresses cell senescence.** A and B, inhibition of Nampt expression promotes cell growth. Nampt expression was inhibited by transfection with siNampt, and the cell number was determined every 24 h in U2OS cells (A) and WI38 cells (B). Six duplicate wells were used at each time point. C–E, inhibition of Nampt expression delays cell senescence. WI38 cells at passage 39 were infected with shNampt or shControl, cultured for 14 days, and stained for β-gal. Representative cells are shown in (C), and quantitative data are shown in (D). The status of Nampt expression is shown in (E). All values are presented as mean ± SD of at least three independent experiments. *P < 0.05, vs. control cells.

(β-gal). As shown in Figure 3C-E, depletion of Nampt expression resulted in a significant decrease in the percentage of β-gal-positive cells in comparison to the mock infectants. We thus conclude that Nampt plays an important role in suppressing cell proliferation and promoting cell senescence. This result is consistent with previous findings that Nampt is highly expressed in a variety of human tumors[2,13,25]. Therefore, Nampt inhibitors could be promising therapeutic approaches for cancer treatment.

Discussion

Nampt was first identified as pre-B-cell colony-enhancing factor (PBEF), a cytokine which acts on early B-lineage precursor cells by enhancing the effect of interleukin-7 and stem cell factor on pre-B-cell colony formation [27]. PBEF was later found to have Nampt activity[11,12]. Nampt is the rate-limiting enzyme in the NAD salvage pathway. NAD serves as a substrate for protein modifications, including protein deacetylation and mono- and poly-ADP-ribosylation, and plays important roles in a variety of life activities, such as metabolism, aging, and tumorigenesis [27]. In this report, we found that Nampt physically interacts with CtIP and DNA-PKcs/Ku80 and that siRNA-silenced Nampt expression promotes HR-mediated repair while inhibiting NHEJ-mediated repair. Given that NAD serves as a substrate for protein deacetylation and that deacetylation of CtIP and DNA-PKcs/Ku80/Ku70 facilitates DSB repair, we speculate that Nampt may control the local NAD pool adjacent to the DNA damage site, therefore modulating the acetylation status of key DDR factors, including CtIP and DNA-PKcs/Ku70/Ku80. Alternatively, Nampt may modulate the recruitment of DDR factors to the site of DNA damage, and this process is independent of Nampt enzymatic activity. Nonetheless, the molecular mechanism of Nampt involvement in DSB repair warrants further investigation.

Conclusion

We conclude that Nampt is a suppressor of HR-mediated DSB repair and an enhancer of NHEJ-mediated

DSB repair accelerating cell senescence.

Acknowledgments

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References

[1] Friedberg EC. Out of the shadows and into the light: the emergence of DNA repair. Trends Biochem Sci, 1995,20:381.
[2] Jackson SP. Sensing and repairing DNA double-strand breaks. Carcinogenesis, 2002,23:687–696.
[3] Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell, 2010,40:179–204.
[4] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell, 2011,144:646–674.
[5] Sartori AA, Lukas C, Coates J, et al. Human CtIP promotes DNA end resection. Nature, 2007,450:509–514.
[6] Matsuoka S, Ballif BA, Smogorzewska A, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science, 2007,316:1160–1166.
[7] Huertas P, Jackson SP. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. J Biol Chem, 2009,284:9558–9565.
[8] Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat Rev Cancer, 2012,12:68–78.
[9] Mladenov E, Iliakis G. Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. Mutat Res, 2011,711:61–72.
[10] Zhang LQ, Heruth DP, Ye SQ. Nicotinamide phosphoribosyltransferase in human diseases. J Bioanal Biomed, 2011,3:13–25.
[11] Martin PR, Shea RJ, Mulks MH. Identification of a plasmid-encoded gene from Haemophilus ducreyi which confers NAD independence. J Bacteriol, 2001,183:1168–1174.
[12] Rongvaux A, Shea RJ, Mulks MH, et al. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. Eur J Immunol, 2002,32:3225–3234.
[13] D'Amours D, Desnoyers S, D´Silva I, et al. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J, 1999,342:249–268.
[14] Min W, Wang ZQ. Poly(ADP-ribose) glycohydrolase (PARG) and its therapeutic potential. Front Biosci, 2009,14:1619–1626.
[15] Kaidi A, Weinert BT, Choudhary C, et al. Human SIRT6 promotes DNA end resection through CtIP deacetylation. Science, 2010,329:1348–1353.
[16] Robert T, Vanoli F, Chiolo I, et al. HDACs link the DNA damage response, processing of double-strand breaks and autophagy. Nature, 2011,471:74–79.
[17] Chen CS, Wang YC, Yang HC, et al. Histone deacetylase inhibitors sensitize prostate cancer cells to agents that produce DNA double-strand breaks by targeting ku70 acetylation. Cancer Res, 2007,67:5318–5327.
[18] Hufton SE, Moerkerk PT, Brandwijk R, et al. A profile of differentially expressed genes in primary colorectal cancer using suppression subtractive hybridization. FEBS Lett, 1999,463:77–82.
[19] Bae SK, Kim SR, Kim JG, et al. Hypoxic induction of human visfatin gene is directly mediated by hypoxia-inducible factor-1. FEBS Lett, 2006,580:4105–4113.
[20] Wang B, Hasan MK, Alvarado E, et al. NAMPT overexpression in prostate cancer and its contribution to tumor cell survival and stress response. Oncogene, 2011,30:907–921.
[21] Bai L, Deng X, Li J, et al. Regulation of cellular senescence by the essential caveolar component PTRF/Cavin-1. Cell Res, 2011,21:1088–1101.
[22] Pierce AJ, Johnson RD, Thompson LH, et al. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev, 1999,13:2633–2638.
[23] Chowdhury D, Xu X, Zhong X, et al. A PP4-phosphatase complex dephosphorylates gamma-H2AX generated during DNA replication. Mol Cell, 2008,31:33–46.
[24] Bennardo N, Cheng A, Huang N, et al. Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. PLoS Genet, 2008,4:e1000110.
[25] Dimri GP, Lee X, Basile G, et al. A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. Proc Natl Acad Sci U S A, 1995,92:9363–9367.
[26] Adamson B, Smogorzewska A, Sigoillot FD, et al. A genome-wide homologous recombination screen identifies the RNA-binding protein RBMX as a component of the DNA-damage response. Nat Cell Biol, 2012,14:318–328.
[27] Samal B, Sun Y, Stearns G, et al. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. Mol Cell Biol, 1994,14:1431–1437.