Biochemical and Biophysical Research Communications 438 (2013) 647–652

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

DZNep, inhibitor of S-adenosylhomocysteine hydrolase, down-regulates expression of SETDB1 H3K9me3 HMTase in human lung cancer cells

Ju-Kyung Lee, Keun-Cheol Kim *

Medical and Bio-Material Research Center, Department of Biological Sciences, College of Natural Sciences, Kangwon National University, Chuncheon 200-701, Republic of Korea

ARTICLE INFO

Article history:
Received 26 July 2013
Available online 8 August 2013

Keywords:
DZNep
SETDB1
H3K9me3
EZH2
H3K27me3
Lung cancer cells

ABSTRACT

3-Deazaneplanocin A (DZNep), an epigenetic anticancer drug, leads to the indirect suppression of S-adenosyl methionine-dependent cellular methylations by inhibiting S-adenosyl homocystein (AdoHcy) hydrolase. Although it is well known that DZNep targets the degradation of EZH2 protein, H3K27me3 HMTase, there are still uncertainties about the regulation of other types of HMTases during cell death. In this study, we describe that SETDB1 gene expression was regulated by DZNep treatment in human lung cancer cells. We confirm that DZNep induced growth inhibition and increased the dead cell population of lung cancer cells. DZNep treatment affected histone methylations, including H3K27me3 and H3K9me3, but not H3K4me3. Reduced levels of H3K27me3 and H3K9me3 were related with the decreased EZH2 and SETDB1 proteins. Real time PCR analysis showed that SETDB1 gene expression was decreased by DZNep treatment, but no effect was observed for EZH2 gene expression. We cloned the promoter region of SETDB1 and SUV39H1 genes, and performed luciferase assays. The promoter activity of SETDB1 gene was down regulated by DZNep treatment, whereas no effect on SUV39H1 promoter activity was observed. In conclusion, we suggest that DZNep regulates not only on H3K27me3 HMTase EZH2, but also H3K9 HMTase SETDB1 gene expression at the transcription level, implicating that the mechanism of action of DZNep targets multiple HMTases during the death of lung cancer cells.

1. Introduction

Therapy targeting epigenetic machinery has been recently developed with the goal of curing various tumors. 3-Deazaneplanocin A (DZNep) is the cyclopentanyl analog of 3-deazaadenosine, and leads to the indirect suppression of S-adenosyl methionine-dependent cellular methylation by inhibiting S-adenosyl homocysteine (AdoHcy) hydrolase [1]. The potential use of DZNep exerts a variety of biological effects, such as gene activation, cellular differentiation, immunological modulation, cell death and antiviral activity [2–4].

The epigenetic implication of DZNep was discovered to inhibit H3K27me3 (trimethylation of histone H3 at lysine 27) and to induce apoptosis in cancer cells [5]. DZNep targets a decrease of EZH2 (enhancer of zeste homolog 2) protein level, which is responsible for H3K27me3, as a HMTase component of polycomb repressor complex 2 (PRC2) [6]. PRC2 complex contains SUZ12 (suppressor of zeste 12), and EED (embryonic ectoderm development) as well as EZH2, and DZNep treatment depleted the PRC2 component proteins through degradation [7]. Overexpression of EZH2 has been associated with a number of cancers, including melanoma, lymphoma, and breast and prostate cancers [8–10]. PRC2 complex plays an important role during development by regulating gene silencing [11]. EZH2 Knock down or DZNep treatment reactivates TXNIP, inhibits thioredoxin activity, and increases reactive oxygen species (ROS), leading to apoptosis in acute myeloid leukemia (AML) [6]. DZNep also increases apoptosis associated genes, like FBXO32, p16, p21 and p27 mRNA level, the altered expression of which is correlated with EZH2 depletion [12,13]. The anticancer effect of DZNep was also confirmed in breast cancer, hepatocelluar carcinoma, and lung cancers [14–16].

However, considering that DZNep is an AdoHcy hydrolase inhibitor, which leads to the indirect inhibition of S-adenosylmethionine (AdoMet) – dependent reactions, it is surprising that the effects of DZNep on cancer cells were found to be relatively specific to EZH2. DZNep also inhibits H4K20 methylation, but does not affect H3K9 methylation during cell death of breast cancer cells [5]. A recent report suggested that DZNep also caused a global

Abbreviations: DZNep, 3-Deazaneplanocin A; AdoHcy, S-adenosyl homocystein; AdoMet, S-adenosyl-methionine; H3K9me3, trimethylation of histone H3 at lysine 9; H3K27me3, trimethylation of histone H3 at lysine 27; EZH2, enhancer of zeste homolog 2; PRC2, polycomb repressor complex 2; SUZ12, suppressor of zeste 12; EED, embryonic ectoderm development; MTT, dimethyl thiozolyl-2′,5′-diphenyl-2-H-tetrazolium bromide.

* Corresponding author. Fax: +82 33 251 3990.
E-mail address: kckim@kangwon.ac.kr (K.-C. Kim).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.07.128

decrease in most histone modifications in MCF7 cells, but not H3K9me3 and H3K37me3, implicating that DZNep is effective in decreasing histone modifications with both repressive and active chromatin markers, in a non-selective manner [8,17]. Therefore, the molecular mechanisms of DZNep might be more expansive than our current knowledge base.

In this study, we aim to examine the possibility that DZNep regulates other types of histone methylations during cell death. Finally, we found that DZNep might be non-selective to EZH2, but affect the expression of H3K9me3-HMTases, SETDB1.

2. Materials and methods

2.1. Cell, DZNep, antibodies

H1299, H460 and A549 (human lung epithelial carcinoma) cell lines were obtained from KCB (Korean Cell bank), and were cultured in RPMI 1640 in a humidified incubator at 37 °C. DZNep was purchased from Santa Cruz (Santa Cruz, CA) and was dissolved as 10 mM in DMSO and stored at −20 °C until further use. Antibodies were purchased from the following companies; EZH2 (Abcam, ab3748), SETDB1 (Abcam, ab12317), SUV39H1 (Upstate, 05-615), H3K4me3 (Upstate, 07-745), H3K9me3 (Abcam, ab8898), H3K27me3 (Upstate, 07-449), and ß-actin (Sigma–aldrich, A5441).

2.2. MTT assay

Growth inhibitory effect was measured using an MTT (dimethyl thiozolyl-2′,5′-diphenyl-2-H-tetrazolium bromide) assay. MTT was purchased from Sigma–Aldrich, and was dissolved in phosphate-buffered saline (PBS) after filtration through a 0.2 μm filter. Cells were seeded at 2000 cells/well in 96-well plates. The next day, cells were treated with various concentrations of DZNep, and were incubated in MTT solution for 2 h at 37 °C. MTT solution was then carefully removed and replaced with 100 μl of DMSO to dissolve formazan crystals. Absorbance was measured using a microplate reader (Bio-Rad, Hercules, CA) at a wavelength of 570 nm. This experiment was conducted in triplicate.

2.3. FACS analysis

Cells were seeded at 2×10^5 cells at 60 mm dishes, and treated with 10 μM DZNep. After 24 h, cells were harvested, and fixed with 70% ethanol. The fixed cells were stained with 50 μg/ml propidium iodide (PI; Sigma–aldrich) containing 10 μg/ml RNase. DNA content was measured using a FACScan flow cytometer (Becton Dickinson, Mountain view, CA), and the cell cycles were analyzed using CellQuest 3.1 DNA analysis software.

2.4. Histone extraction

Histone proteins were extracted from the nuclear extract as previously described [18]. Briefly, an equal volume of cold 0.2 M H_2SO_4 was added to nuclei, and the supernatant was then collected by centrifugation at 12,000 rpm for 15 min at 4 °C, and was added to 100% trichloro acetic acid (TCA). Histones were precipitated, and were washed with 0.05 M HCl. After centrifugation, the histone pellet was dried at room temperature, and was used for western blot analysis.

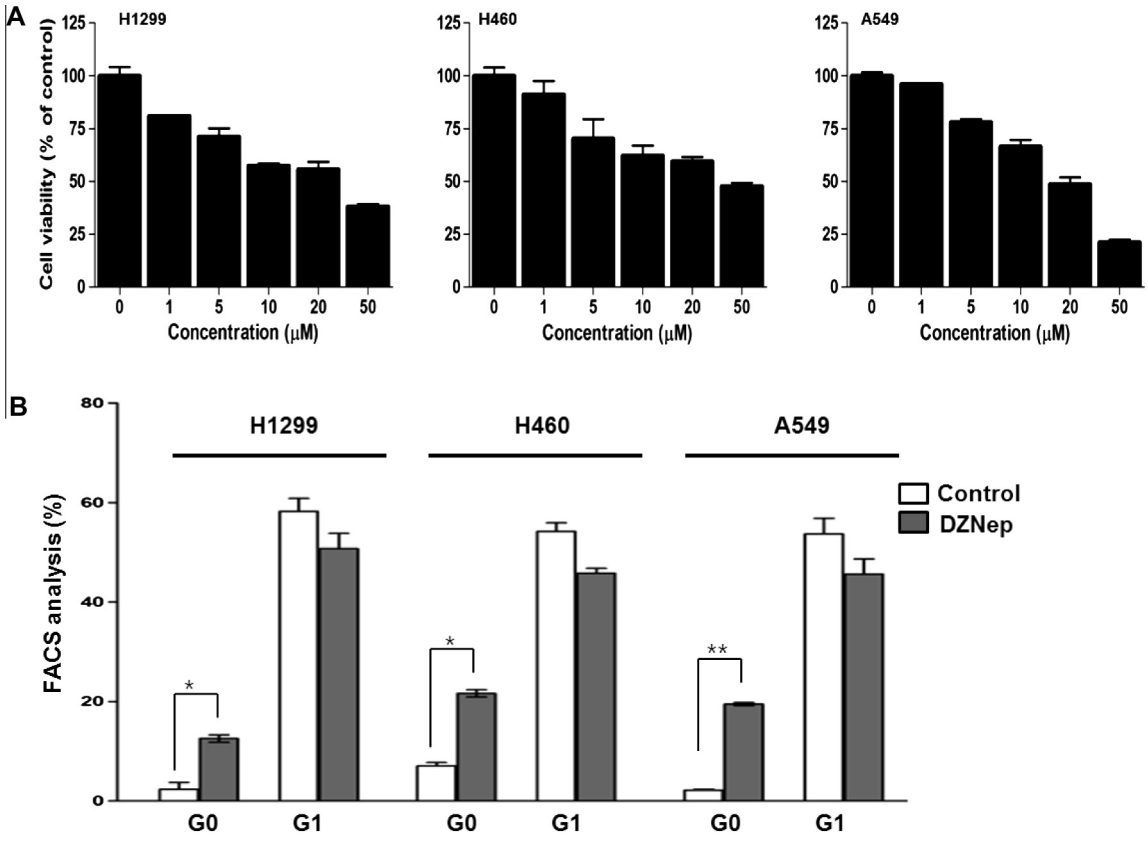

Fig. 1. Growth inhibitory effect of DZNep in human lung cancer cells. (A) Cells were treated for 48 h with 1, 5, 10, 20 or 50 μM DZNep. The cells were assayed by MTT assay. (B) Cells were treated with 10 μM DZNep for 48 h, and were stained with propidium iodide and subjected to FACS analysis. The graph showed the percentage of G0 and G1 cells. $^{*}p > 0.05$, $^{**}p > 0.001$.

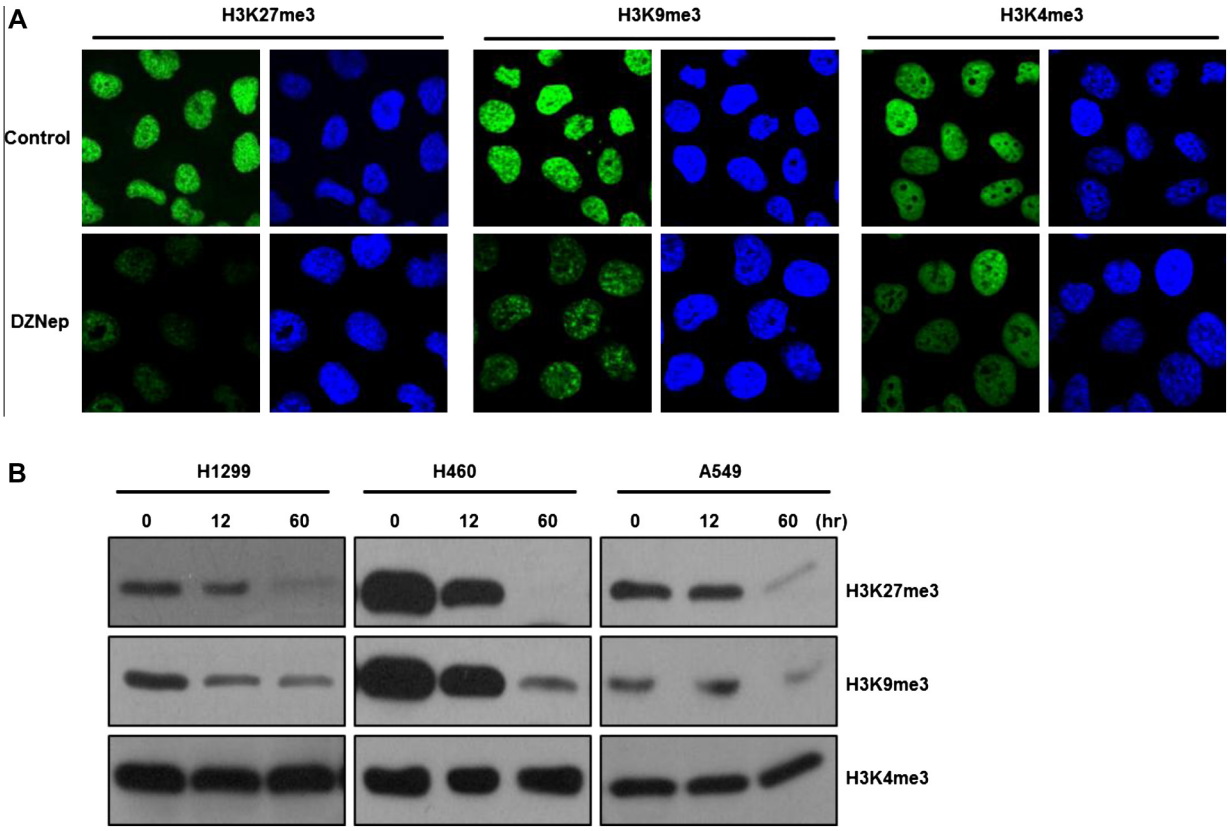

Fig. 2. Down regulation of H3K27me3 and H3K9me3 by DZNep treatment. (A) H1299 cells were plated on the coverslips and treated with 10 μM DZNep for 24 h. Cells were immunostained with H3K27me3, H3K9me3, or H3K4me3 antibody (green) and were then counterstained with DAPI (blue). (B) Histone proteins were extracted at the indicated times after 10 μM DZNep treatment. The lysates were subjected to western blot analyses using the indicated histone antibodies. (For interpretation of color in Fig. 2, the reader is referred to the web version of this article.)

2.5. Western blot analysis

Cells were lysed for 30 min at 4 °C in lysis buffer (10 mM Tris–HCl, pH 8.0, containing 140 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, 1% Igepal CA-630, 0.1% SDS) supplemented with Complete Mini protease inhibitor cocktail tablets (Roche, Indianapolis, IN). Total protein was quantified using the bicinchoninic acid (BCA) assay. Protein lysates were resolved in acrylamide gel and were transferred to a PVDF membrane (Millipore cooperation). Membranes were blocked with 5% skim milk for 1 h at room temperature. After washing, the membranes were incubated with appropriate primary antibodies. Membranes were washed and then incubated with the appropriate secondary antibodies for 1 h at room temperature. The proteins were detected using an Amersham ECL Plus kit (Animal Genetics, Inc.) according to the manufacturer's instruction.

2.6. Immunostaining

Cells were cultured on glass coverslips, and were treated with DZNep for 24 h. The coverslips were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton-X 100. The coverslips were blocked with 5% skim milk and were then incubated with primary antibodies for 3 h at room temperature. After washing, the coverslips were incubated with AlexaFluor488-conjugated goat anti-mouse or goat anti-rabbit secondary antibodies. Nuclei were briefly stained with 0.5 μg/ml DAPI (4′,6′-diamidino-2-phenylindole hydrochloride), washed with PBS, mounted, and examined using confocal microscopy.

2.7. Real-time PCR

Total RNA was extracted by Trizol reagent. Approximately 1–2 μg of total RNA was used to make cDNA using Superscript II reverse transcriptase (Life technologies Inc., Gatithersburg, MD). Quantitative real-time RT-PCR was performed using an ABI Prism 7000 RT-PCR thermocycler (Applied Biosystems, Carlsbad, CA). PCR reactions were performed with 10 pM of each primer and 10 μl SYBR Green Supermix in a total volume of 20 μl. Dissociation was performed according to a melting program. The threshold cycle (Ct) was calculated for each gene using the associated Sequence Detection software. GAPDH was used as an endogenous control for normalization. Gene expression was defined from the threshold cycle (Ct), and relative expression levels were calculated by using the 2-ΔΔCt method. The primers sets were as follows: EZH2 (S) 5′-GGA CGG CTC CTC TAA CCA TG-3′ (AS) 5′-TGC TTG GTG TTG CAC TGT GC-3′ SETDB1 (S) 5′-TTA ACA CAG GCC CTG AAT TTC T-3′ (AS) 5′-TAC CCC TGT GGG TAG ACA CTC T-3′ SUV39H1 (S) 5′-GCG TAT CCT CAA GCA GTT CC-3′ (AS) 5′-CTA CAG TGA TGC GTC CCA G-3′ GAPDH (S) 5′-GTA TTG GGC GCC TGG TCA C-3′ (AS) 5′-CTC CTG GAA GAT GGT GAT GG-3'.

2.8. Luciferase assay

Approximately 2 kb promoter regions of SETDB1 and SUV39H1 genomic sequences were identified from Ensembl Genome Browser (http://www.ensembl.org/index.html) and were cloned into the pGL3-Luc vector. The promoter construct was transfected into H1299 cells on a 60 mm plate, using 5 μl Lipofectamine 2000 (Invitrogen, Carlsbad, CA). The cells were also co-transfected with the ß-gal plasmid to normalize for transfection efficiency. Total cell

lysates were prepared from cells 24 h post-transfection using 1X Passive Lysis Buffer (Promega, Carlsbad, CA), and were then assessed for firefly luciferase activity. Luminescence was monitored using a Glomax 96 microplate luminometer (Promega, Fitchburg, WI).

3. Results

3.1. Increased cell death by DZNep in human lung cancer cells

MTT assays were used to screen the effective concentration of DZNep on H1299, A549, and H460 human lung cancer cells. The cells were treated in 96-well plates for 48 h with DMSO or DZNep at concentrations of 1 μM, 5 μM, 10 μM, 20 μM or 50 μM. DZNep induced a growth inhibitory effect in dose dependent manners in human lung cancer cells (Fig. 1A). As the growth inhibitory effect of DZNep was reliably detected from treatment of 5 μM and IC_{50} was approximately 10 μM, we used a concentration of 10 μM for further studies. To examine whether DZNep affected cell cycle phases, we also performed FACS analysis. We could not find distinct cell cycle arrest by treatment with 10 μM DZNep in lung cancer cells. Instead, DZNep resulted in a slight increase in the accumulation of sub-G1 phase of the cell cycle (Fig. 1B). These effects were more severe in A549 cells than in H1299 or H460 cells. This result implicated that DZNep induces growth inhibition and cell death in a dose-dependent manner in human lung cancer cells.

3.2. Effect of DZNep on H3K9me3

To examine the plausible regulation of DZNep on several types of histone methylations, we screened the levels of mono-, di-, or tri-methylations of H3K4 or H3K9, as well as H3K27me3. Immunostaining analysis showed that the level of H3K27me3 was decreased by treatment of DZNep in H1299 cells. It was detected as a dispersed pattern of H3K9me3 in control H1299 cells. Surprisingly, H3K9me3 was decreased in the nucleus of DZNep treated cells, as well as being detected as distinct dotted pattern. However, H3K4me3 was not changed (Fig. 2A). We also performed immunostaining on mono- and di- methylations of H3K9 and H3K4, but could not detect any decreased methylation of those

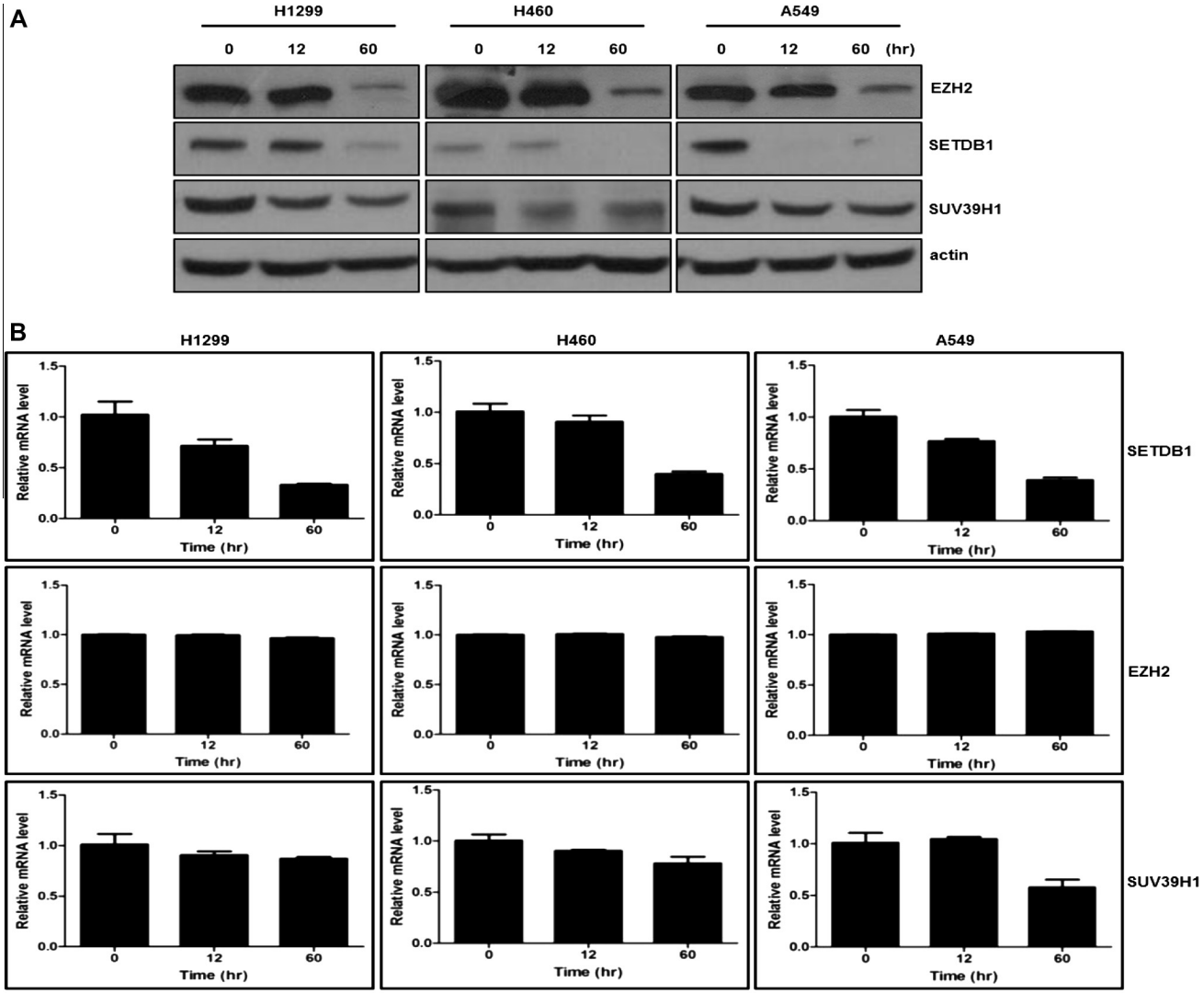

Fig. 3. Down regulation of EZH2 and SETDB1 proteins by DZNep treatment. (A) After DZNep treatment, total protein lysates were extracted at the indicated times. Western blot analysis was performed to examine the expression of EZH2, SETDB1, or SUV39H1. β-actin was used as the loading control. (B) Cells were treated with 10 μM DZNep during the indicated times. Total RNA was extracted, and was used for cDNA synthesis. Real time PCR was performed to examine the expression of EZH2, SETDB1, or SUV39H1. The gene expression pattern was compared with GAPDH expression.

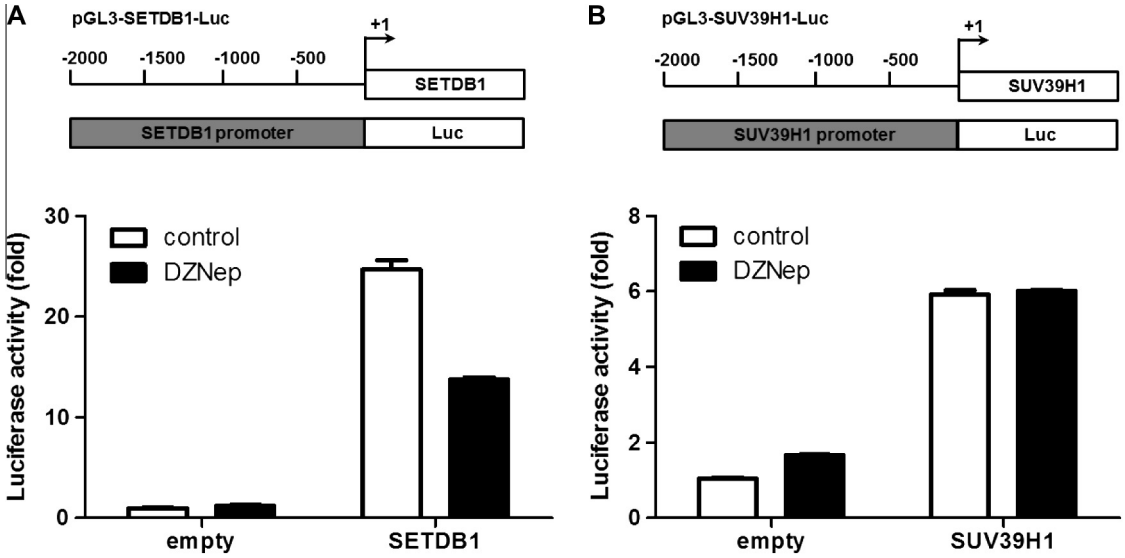

Fig. 4. Down regulation of promoter activity of SETDB1 gene by DZNep treatment. Putative promoter regions of (A) SETDB1 and (B) SUV39H1 genes were clone into pGL3-Luc vector. The plasmids were transfected into H1299 cells for 12 h, and were followed by DZNep treatment for a further 12 h. Luminescence was monitored using a Glomax 96 microplate luminometer.

(data not shown). We treated DZNep for 60 h, and performed western blot analysis to clarify the decreased H3K9me3. H3K27me3 was decreased from 12 to 60 h after DZNep treatment. H3K9me3 showed the same decreased pattern with H3K27me3 (Fig. 2B). Therefore, this result implicates that epigenetic targets of DZNep are diverse on histone methylations, and H3K9me3 might be a target for DZNep in human lung cancer cells.

3.3. Regulation of DZNep on SETDB1

We analyzed whether DZNep affects the protein level of H3K9me3 related HMTases. There are at least two reported H3K9me3 HMTases, SETDB1 and SUV39H1, which contribute to heterochromatin formation and gene silencing by interacting with other proteins [19,20]. SETDB1 or EZH2 were not changed at 12 h, but disappeared at 60 h (Fig. 3A). The protein level of SUV39H1 had no change in DZNep treatment. To investigate whether decreased protein levels of SETDB1 or EZH2 resulted from transcriptional level, we performed quantitative real time-PCR analysis. DZNep treatment has no effect on EZH2 expression up to 60 h in all cells. However, DZNep distinctly reduced SETDB1 gene expression at 12 h, and SETDB1 gene was continuously decreased for 60 h, compared to the gene expression of EZH2 (Fig. 3B). SUV39H1 gene expression was not changed by DZNep treatment in H1299 and H460 cells. This result implicates that the decreased SETDB1 protein by DZNep resulted in transcriptional regulation.

3.4. Down regulation of promoter activity SETDB1 gene by DZNep

To confirm whether DZNep affect the promoter activity of SETDB1 gene, we identified the putative SETDB1 promoter region using the Ensembl genome browser (http://www.ensembl.org) website, and cloned approximately 2 Kb upstream of transcriptional start site into pGL3-Luc vector. After the reporter plasmid was transfected into H1299 cells, we treated DZNep for 12 h. SETDB1 promoter activity was increased 25-fold, but this increase was halved by DZNep treatment (Fig. 4A). We also cloned the putative promoter region of SUV39H1 gene and performed luciferase assay. SUV39H1 promoter activity was increased 6-fold, which was relatively little activation compared to SETDB1 promoter activity (Fig. 4B). In addition, DZNep has no change on SUV39H1 promoter activity. This result consistently implicates that DZNep treatment regulates the promoter activity of SETDB1 gene.

4. Discussion

DZNep indirectly inhibits cellular methylation by limiting the available methyl donor groups [21]. The anticancer effect of DZNep is exerted by inhibiting histone methyltransferases or affecting the capping of mRNA in mediating under-methylation of mRNAs [22]. In this study, DZNep treatment induced cell death in human lung cancer cells, although there was a difference on the extent of the inhibitory effect, depending on cell types. The decreased levels of H3K27me3 and EZH2 protein were consistent with previous reports of groups, indicating that the depletion of EZH2 is achieved via proteolytic degradation [5]. In addition, the methylation level of H3K9me3 was also shown to be reduced by DZNep treatment in this study. Miranda et al. suggested that DZNep globally inhibits both repressive and active histone methylation markers on H3K27, H3K9, H3K20, and H3K4, although they did not show decreased H3K9me3 in their analysis [8]. Our novel data show that SETDB1 was reduced by DZNep treatment, among H3K9me3 HMTases. Although we do not have a plausible answer to this discrepancy with previous other reports, this might be due to the complexity of DZNep action shown in various human cancer cells types. Interestingly, SETDB1 seems to be regulated at the transcription level by DZNep, implicating a possible novel function of DZNep, in regulating the gene expression of some HMTases.

SETDB1 is a KAP-1-associated HMTase in two hybrid screening, and contributes to the HP1-mediated silencing of euchromatic genes [20]. SETDB1 also enhances accessibility onto damaged sites of repair factors during DNA damage double strand break DNA repair [23]. SETDB1 is up-regulated in immortalized bronchoepithelial cells, and increased levels of SETDB1 result in the transcriptional dysregulation of target genes [24]. Although the exact mechanism of DZNep is still under investigated in terms of gene expression regulation, the possibility exists that many factors may form a repressive complex at the promoter regions of SETDB1 gene. Recently, it is reported that EZH2 produces microRNA to maximize oncogenic functions, and requires further HMTase activity [25]. We can postulate that depletion of EZH2 by DZNep treatment

induces a down-regulation of SETDB1 at the transcription level by micro RNA network. It was also reported that DZNep activates a novel transcript that is located further downstream from the promoter containing a CpG island, such as KRT7 [8]. This implicates that DZNep might be repressed or activated by affecting gene expression machinery. As in the case of EZH2, SETDB1-mediated H3K9me3 participates in transcription repression pathways via chromatin remodeling [26].

There are some evidences that H3K9me3 might be an important factor in damaged DNA [23]. H3K9me3 is reduced by anticancer drugs, such as cisplatin or doxorubicin, implicating that H3K9me3 mediates multidrug resistance and spreading of the anticancer effect [27,28]. This study showed that DZNep downregulates H3K9me3 and H3K27me3, which are typical methylation types shown in repressive chromatin structures. These repressive marks contribute to heterochromatin formation, and therefore, are indispensable for fundamental cellular processes, including gene silencing [29]. A cooperative decreased level of both H3K9me3 by SETDB1 and H3K27me3 by EZH2 is likely linked to chromatin relaxation in DZNep treatment conditions and the promotion of cell death.

In conclusion, DZNep targets H3K9 HMTase-SETDB1 gene at the transcription level, as well as H3K27 HMTase-EZH2 at the post-translational level. Therefore, DZNep inhibits multiple repressive chromatin markers to induce cell death.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF 2011-0006047), the Ministry of Education, Science and Technology (the Regional Research Universities Program/Medical and Bio-Materials Research Center), and basic research program of Kangwon National University (C1009848-01-01).

References

[1] P.K. Chiang, G.L. Cantoni, Perturbation of biochemical transmethylations by 3-deazaadenosine in vivo, Biochem. Pharmacol. 28 (1979) 1897–1902.
[2] D.L. Mayers, J.A. Mikovits, B. Joshi, I.K. Hewlett, J.S. Estrada, A.D. Wolfe, G.E. Garcia, B.P. Doctor, D.S. Burke, R.K. Gordon, Anti-human immunodeficiency virus 1 (HIV-1) activities of 3-deazaadenosine analogs: Increased potency against 3'-azido-3'-deoxythymidine-resistant HIV-1 strains, Proc. Natl. Acad. Sci. USA 92 (1995) 215–219.
[3] Z. Xie, C. Bi, L.L. Cheong, S.C. Liu, G. Huang, J. Zhou, Q. Yu, C.S. Chen, W.J. Chng, Determinants of sensitivity to DZNep induced apoptosis in multiple myeloma cells, PLoS ONE 6 (2011) e21583.
[4] R.K. Gordon, K. Ginalski, W.R. Rudnicki, L. Rychlewski, M.C. Pankaskie, J.M. Bujnicki, P.K. Chiang, Anti-HIV-1 activity of 3-deaza-adenosine analogs. inhibition of S-adenosylhomocysteine hydrolase and nucleotide congeners, Eur. J. Biochem. 270 (2003) 3507–3517.
[5] J. Tan, X. Yang, L. Zhuang, X. Jiang, W. Chen, P.L. Lee, R.K. Karuturi, P.B. Tan, E.T. Liu, Q. Yu, Pharmacologic disruption of polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells, Genes Dev. 21 (2007) 1050–1063.
[6] J. Zhou, C. Bi, L.L. Cheong, S. Mahara, S.C. Liu, K.G. Tay, T.L. Koh, Q. Yu, W.J. Chng, The histone methyltransferase inhibitor, DZNep, up-regulates TXNIP, increases ROS production, and targets leukemia cells in AML, Blood 118 (2011) 2830–2839.
[7] S.R. Choudhury, S. Balasubramanian, Y.C. Chew, B. Han, V.E. Marquez, R.L. Eckert, (-)-Epigallocatechin-3-gallate and DZNep reduce polycomb protein level via a proteasome-dependent mechanism in skin cancer cells, Carcinogenesis 32 (2011) 1525–1532.
[8] T.B. Miranda, C.C. Cortez, C.B. Yoo, G. Liang, M. Abe, T.K. Kelly, V.E. Marquez, P.A. Jones, DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation, Mol. Cancer Ther. 8 (2009) 1579–1588.
[9] K. Holm, D. Grabau, K. Lovgren, S. Aradottir, S. Gruvberger-Saal, J. Howlin, L.H. Saal, S.P. Ethier, P.O. Bendahl, O. Stal, P. Malmstrom, M. Ferno, L. Ryden, C. Hegardt, A. Borg, M. Ringner, Global H3K27 trimethylation and EZH2 abundance in breast tumor subtypes, Mol. Oncol. 6 (2012) 494–506.
[10] L.G. Pellakuru, T. Iwata, B. Gurel, D. Schultz, J. Hicks, C. Bethel, S. Yegnasubramanian, A.M. De Marzo, Global levels of H3K27me3 track with differentiation in vivo and are deregulated by MYC in prostate cancer, Am. J. Pathol. 181 (2012) 560–569.
[11] I. Aldiri, M.L. Vetter, PRC2 during vertebrate organogenesis: a complex in transition, Dev. Biol. 367 (2012) 91–99.
[12] W. Fiskus, Y. Wang, A. Sreekumar, K.M. Buckley, H. Shi, A. Jillella, C. Ustun, R. Rao, P. Fernandez, J. Chen, R. Balusu, S. Koul, P. Atadja, V.E. Marquez, K.N. Bhalla, Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells, Blood 114 (2009) 2733–2743.
[13] W. Fiskus, R. Rao, R. Balusu, S. Ganguly, J. Tao, E. Sotomayor, U. Mudunuru, J.E. Smith, S.L. Hembruff, P. Atadja, V.E. Marquez, K. Bhalla, Superior efficacy of a combined epigenetic therapy against human mantle cell lymphoma cells, Clin. Cancer Res. 18 (2012) 6227–6238.
[14] T. Chiba, E. Suzuki, M. Negishi, A. Saraya, S. Miyagi, T. Konuma, S. Tanaka, M. Tada, F. Kanai, F. Imazeki, A. Iwama, O. Yokosuka, 3-deazaneplanocin A is a promising therapeutic agent for the eradication of tumor-initiating hepatocellular carcinoma cells, Int. J. Cancer 130 (2012) 2557–2567.
[15] A. Hayden, P.W. Johnson, G. Packham, S.J. Crabb, S-adenosylhomocysteine hydrolase inhibition by 3-deazaneplanocin A analogues induces anti-cancer effects in breast cancer cell lines and synergy with both histone deacetylase and HER2 inhibition, Breast Cancer Res. Treat. 127 (2011) 109–119.
[16] J. Puppe, R. Drost, X. Liu, S.A. Joosse, B. Evers, P. Cornelissen-Steijger, P. Nederlof, Q. Yu, J. Jonkers, M. van Lohuizen, A.M. Pietersen, BRCA1-deficient mammary tumor cells are dependent on EZH2 expression and sensitive to polycomb repressive complex 2-inhibitor 3-deazaneplanocin A, Breast Cancer Res. 11 (2009) R63.
[17] R.A. Varier, H.T. Timmers, Histone lysine methylation and demethylation pathways in cancer, Biochim. Biophys. Acta 2011 (1815) 75–89.
[18] J.A. Park, A.J. Kim, Y. Kang, Y.J. Jung, H.K. Kim, K.C. Kim, Deacetylation and methylation at histone H3 lysine 9 (H3K9) coordinate chromosome condensation during cell cycle progression, Mol. Cells 31 (2011) 343–349.
[19] A. Vaquero, M. Scher, H. Erdjument-Bromage, P. Tempst, L. Serrano, D. Reinberg, SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation, Nature 450 (2007) 440–444.
[20] D.C. Schultz, K. Ayyanathan, D. Negorev, G.G. Maul, F.J. Rauscher 3rd., SETDB1: A novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins, Genes Dev. 16 (2002) 919–932.
[21] P.K. Chiang, P.D. Burbelo, S.A. Brugh, R.K. Gordon, K. Fukuda, Y. Yamada, Activation of collagen IV gene expression in F9 teratocarcinoma cells by 3-deazaadenosine analogs. indirect inhibitors of methylation, J. Biol. Chem. 267 (1992) 4988–4991.
[22] X. Jiang, J. Tan, J. Li, S. Kivimae, X. Yang, L. Zhuang, P.L. Lee, M.T. Chan, L.W. Stanton, E.T. Liu, B.N. Cheyette, Q. Yu, DACT3 is an epigenetic regulator of wnt/beta-catenin signaling in colorectal cancer and is a therapeutic target of histone modifications, Cancer Cell 13 (2008) 529–541.
[23] A. Mund, T. Schubert, H. Staege, S. Kinkley, K. Reumann, M. Kriegs, L. Fritsch, V. Battisti, S. Ait-Si-Ali, A.S. Hoffbeck, E. Soutoglou, H. Will, SPOC1 modulates DNA repair by regulating key determinants of chromatin compaction and DNA damage response, Nucleic Acids Res. 40 (2012) 11363–11379.
[24] H. Watanabe, K. Soejima, H. Yasuda, I. Kawada, I. Nakachi, S. Yoda, K. Naoki, A. Ishizaka, Deregulation of histone lysine methyltransferases contributes to oncogenic transformation of human bronchoepithelial cells, Cancer Cell Int. 8 (2008). 15-2867-8-15.
[25] I.A. Asangani, B. Ateeq, Q. Cao, L. Dodson, M. Pandhi, L.P. Kunju, R. Mehra, R.J. Lonigro, J. Siddiqui, N. Palanisamy, Y.M. Wu, X. Cao, J.H. Kim, M. Zhao, Z.S. Qin, M.K. Iyer, C.A. Maher, C. Kumar-Sinha, S. Varambally, A.M. Chinnaiyan, Characterization of the EZH2-MMSET histone methyltransferase regulatory axis in cancer, Mol. Cell 49 (2013) 80–93.
[26] S. Cho, J.S. Park, Y.K. Kang, Dual functions of histone-lysine N-methyltransferase Setdb1 protein at promyelocytic leukemia-nuclear body (PML-NB): Maintaining PML-NB structure and regulating the expression of its associated genes, J. Biol. Chem. 286 (2011) 41115–41124.
[27] K. Tabata, H. Sakai, R. Nakajima, R. Saya-Nishimura, K. Motani, S. Okano, Y. Shibata, Y. Abiko, T. Suzuki, Acute application of cisplatin affects methylation status in neuroblastoma cells, Oncol. Rep. 25 (2011) 1655–1660.
[28] M. Toth, I.M. Boros, E. Balint, Elevated level of lysine 9-acetylated histone H3 at the MDR1 promoter in multidrug-resistant cells, Cancer Sci. 103 (2012) 659–669.
[29] A.I. Shevchenko, S.V. Pavlova, E.V. Dementyeva, S.M. Zakian, Mosaic heterochromatin of the inactive X chromosome in vole microtus rossiaemeridionalis, Mamm. Genome 20 (2009) 644–653.