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p53 Down-regulates SETDB1 gene expression during paclitaxel induced-cell death

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ABSTRACT

Paclitaxel (PTX) is a chemotherapeutic drug which induces tubulin stability and regulates expression of death related genes in human cancer cells. Its anticancer mechanism is well known, however its effects on chromatin remodeling factors are poorly understood. In this study, we examine if PTX affects expression of SETDB1 HMTase during cell death. PTX induces cell death via G2/M arrest in human lung cancer cells. PTX treatment induces the p53 protein, but down-regulates expression of SETDB1 at the transcriptional level as well as the protein level. SETDB1 promoter activity is increased to approximately 30-fold in normal condition, but the activity is significantly inhibited in the PTX treated group. In addition, p53 transfection inhibits SETDB1 promoter activity. The p53 protein directly binds to proximal region of the SETDB1 promoter, and H3K9me3 occupancy in this region also increased in the presence of p53. Immunoprecipitation experiment showed interaction of p53 and SUV39H1, suggesting that association of p53 and SUV39H1 is responsible for increased H3K9me3 occupancy and transcription repression of SETDB1. This result demonstrates that PTX down-regulates SETDB1 gene expression in a p53 dependent manner, and p53 might participate in heterochromatic repression on the promoter regions of SETDB1.

1. Introduction

Cancer is caused by epigenetic changes as well as genetic alteration in a single cell. Many critical pathways causing cancer have been studied, and chemotherapeutic drugs targeting these pathways are used to cure cancers [1,2]. Although their action mechanisms vary depending on of the drugs, chemotherapeutic drugs commonly generate extensive DNA fragmentation to induce cell death in cancer cells. However, considering that DNA is attached to a variety of histone proteins, decomposition of chromatin structure is a prerequisite for DNA fragmentation. Thus, a study on regulation of the chromatin remodeling factors by chemotherapeutic drugs was needed.

Paclitaxel (PTX) is a chemotherapeutic drug for many common malignancies. It has been used in the treatment of a number of human cancers including: ovarian cancer, breast cancer, NSCLC [3–5]. PTX-treated cells have defects in mitotic spindle assembly, and chromosome segregation, and then enter into cell death [6]. PTX has been found to induce nuclear translocation of FOXO3 through phosphorylated-c-Jun NH2-terminal kinase (JNK) in breast cancer cells [7]. It also affects gene expression of Bcl-2, and Bcl-xL resulting in the induction of apoptosis by p53-dependent or -independent mechanisms [8]. The expression of DEC1 and DEC2, differentiated embryonic chondrocyte gene, is up-regulated in PTX-treated MCF-7 cells [9]. Recent proteomic analysis has suggested that in PTX treated cells many contexts of gene expression are altered [10].

SETDB1 is a histone methyltransferase (HMTase) toward H3 lysine 9 (H3K9), and participates in heterochromatin compaction during cell differentiation, development, cell death, and carcinogenesis [11]. It recruits DNMTs, HDACs and KAP-1 to construct heterochromatin structure [12,13]. Another study has shown that SETDB1 expression is high in early stages of mouse brain development, implicating that there is an epigenetic role of SETDB1 in temporal and tissue-specific gene expression [14]. SETDB1 epigenetically regulates expression of target genes via dictating histone methylation on H3K9 [15]. Methylation of H3K9 is associated with correct chromosome condensation [16]. Acute application of cisplatin affects methylation status of H3K9 in neuroblastoma cells, implicating that H3K9 might be an important marker for cell death [17]. The repressive marker SETDB1

Abbreviations: PTX, paclitaxel; HMTase, histone methyltransferase; H3K9, histone H3 at lysine 9; ChIP, chromatin immunoprecipitation.

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contributes to heterochromatin formation, however currently the expression of SETDB1 by anticancer drugs is poorly understood.

Thus in this study, we examine SETDB1 gene expression and the molecular mechanism of SETDB1 regulation in PTX treated human lung cancer cells, We suggest that p53 might participate in heterochromatic repression by interacting with SUV39H1 on the promoter regions of SETDB1 genes.

2. Materials and methods

2.1. Cell cultures, paclitaxel, and antibodies

A549, H460, and H1299 cell lines were obtained from KCB (Korean Cell bank, Korea), and were cultured in RPMI 1640 containing 10% fetal bovine serum in a humidified incubator at 37 °C. PTX was purchased from Sigma Aldrich, and was dissolved as 1 mM stock in DMSO and stored at −20 °C. The antibodies were purchased from the following companies: SETDB1 (Abcam), SUV39H1 (Upstate), and β-actin (Sigma–Aldrich).

2.2. FACS analysis

Cells were seeded at 2×10^5 cells on 60 mm dishes, and treated with 500 nM PTX. Cells were harvested at the indicated times, and fixed with 70% ethanol. The fixed cells were stained with 50 μg/ml propidium iodide (Sigma–Aldrich) containing 10 μg/ml RNase (Invitrogen). DNA content was measured using a FACScan flow cytometer (Becton Dickinson), and the cell cycles were analyzed using CellQuest 3.1 DNA analysis software.

2.3. Western blot analysis

Cells were lysed with RIPA buffer [10 mM Tris–HCl (pH8.0), 1 mM EDTA, 140 mM NaCl, 1% Triton X-100, 0.1% deoxylcloate, 0.1% SDS] supplemented with Complete Mini protease inhibitor cocktail tablets (Roche). 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). Membranes were blocked with 5% skim milk for 1 h at room temperature. After washing, the membranes were incubated with appropriate primary antibodies. The membranes were washed and then incubated with the appropriate secondary antibodies for 1 h. The proteins were detected using a protein detection kit (Animal Genetic).

2.4. RT-PCR

Total RNA was isolated using a TRIZOL kit (Duchefa). Complementary DNA (cDNA) was synthesized with hexamer from total RNA. Conventional PCR was performed as described in a previous study [18]. cDNA was mixed with specific primer sets in 0.2 mM dNTP, 1 U Taq polymerase, buffer containing 1.5 mM $MgCl_2$ (Enzynomics, Seoul, Korea). The primer sequences were; SETDB1 S1 5′-TTA ACA CAG GCC CTG AAT TTC T-3′ SETDB1 AS1 5′-TAC CCC TGT GGG TAG ACA CTC T-3′, actin S1 5′-GTG GGG CGC CCC AGG CAC CAG GGC-3′, and actin AS1 5′-CTC CTT AAT GTC ACG CAC GAT TTC-3′. PCR reactions were carried out in a Perkin Elmer Thermal Cycler 9600 (Applied Biosystems). PCR products were resolved in 1% agarose gel.

2.5. Transient transfection, and luciferase assay

p53, KAP-1 expression plasmids, and pGL3- SETDB1-Luc reporter plasmids have been described in a previous study [19]. The promoter construct and plasmids were transfected into H1299 cells using 5 μl lipofectamine 2000 (Invitrogen). The cells were also co-transfected with the β-gal plasmid to normalize for transfection efficiency. Total cell lysates were prepared from cells 24 h post-transfection using 1× Passive Lysis Buffer (Promega), and were then assessed for firefly luciferase activity. Luminescence was monitored using a Glomax 96 microplate luminometer (Promega).

2.6. ChIP assay

The protocol describe in Shang et al. was used [20]. Briefly, H1299 cells were cross-linked with 1% formaldehyde. Cells were sonicated in lysis buffer [50 mM Tris–HCl (pH 8.0), 10 mM EDTA, 1% SDS]. The lysate was incubated with the appropriate antibody overnight, and washed sequentially with a low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris–HCl, 150 mM NaCl), high salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris–HCl, 500 mM NaCl), Lithium chloride buffer (0.25 M LiCl, 1% NP-40, 1 mM EDTA, 10 mM Tris–HCl) 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris–HCl, 150 mM NaCl). The beads were eluted with 1% SDS, 0.1 M NaHCO3 at 65 °C. A DNA extract was then used for PCR amplification. The primers used for PCR amplification of SETDB1 gene promoter were: p400SETDB1-S1; 5′-GCT GAG AAC GGA AAA CAA TC-3′, p400SETDB1-AS1; 5′-TGT GGA CAT TAC CTG AAG CC-3′, dis250 nSETDB1-S1; 5′-ACA CCA TGT TCA CGG ATC AG-3′, and dis250nSETDB1-S1; 5′-ACT GTC TTG ATT ACT GTA GC-3′.

2.7. Immunopreciptation

H1299 cells were transfected with a combination of indicated plasmids for 48 h and lysed with an RIPA buffer. For immunoprecipitation assays, 500 μg cell lysates were incubated with 2 μl appropriate antibody in 500 μl RIPA buffer for 3 h at 4 °C. Following the addition of 30 μl protein A/Gagarose beads, the reaction was incubated overnight at 4 °C while being rotated. The beads were washed four times in a RIPA buffer and then resuspended in a 20 μl SDS sample buffer, and then boiled for 5 min. The samples were then analyzed by Western blotting.

3. Results

3.1. PTX down-regulates SETDB1 during cell death via G2/M arrest

First, we checked whether PTX treatment induces G2/M arrest in human lung cancer cells. We treated A549 lung cancer cells with 500 nM PTX and performed FACS analysis. We confirmed that PTX treatment manifests G2/M arrest at 6 h, and induces cell death at 24 h (Fig. 1A). To examine expression of the SETDB1 by PTX, we treated A549 and H460 cells for 12 h with different concentrations of PTX namely 5, 50, and 500 nM. We found that SETDB1 protein level was down-regulated after PTX treatment (Fig. 1B). We obtained consistent results on the down-regulation of SETDB1 protein in a time dependent Western blot of the PTX treatment. PTX treatment also induced p53 expression at the molecular level, consistent with previous studies [21]. We then performed RT-PCR analysis to investigate if SETDB1 can be regulated at the transcriptional level. For this we treated A549 and H460 cells with various concentrations of PTX for 24 h, and found decreased SETDB1 RNA expression (Fig. 1C). This result implies that PTX down-regulates SETDB1 gene expression during cell death via G2/M arrest in human lung cancer cells.

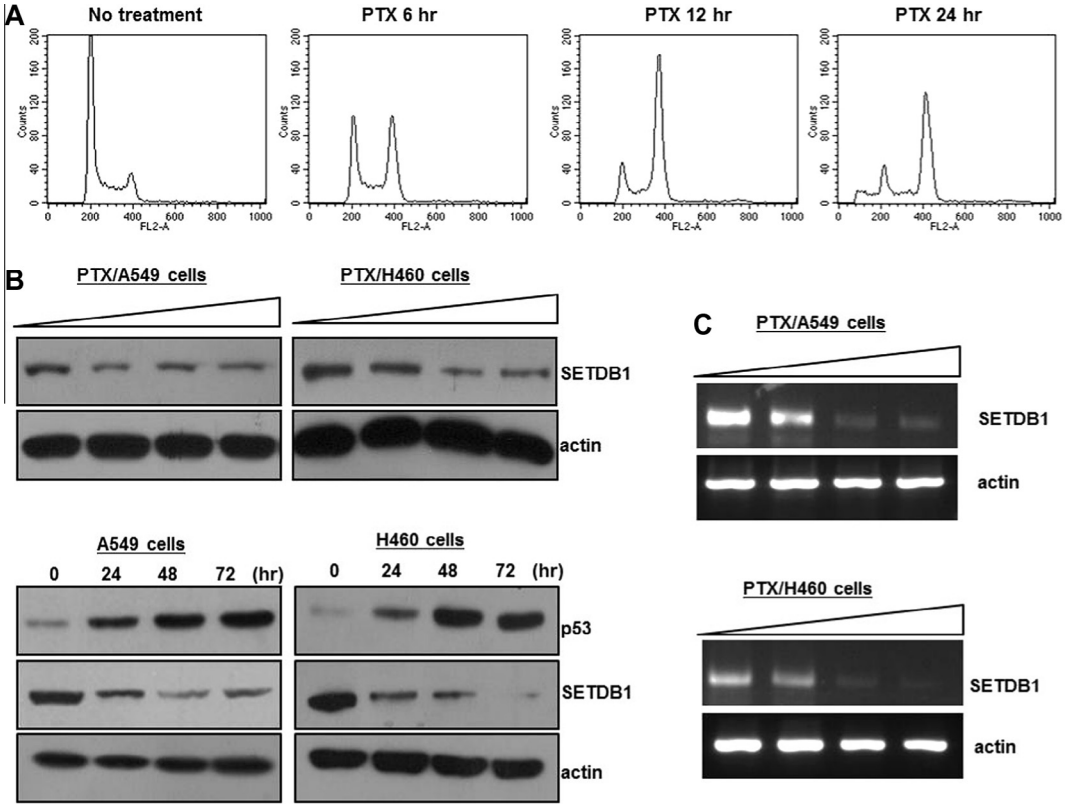

Fig. 1. FACS analysis and SETDB1 gene expression by PTX treatment in human lung cancer cells. (A) A549 cells were treated with PTX (500 nM) in time dependent manners. The cells were arrested into G2/M phase at 6 h, and finally went into cell death. (B) PTX was treated in dose or time dependent manners in A549 and H460 cells. Protein lysates were extracted, and subjected to Western blot. (C) RT-PCR analysis was performed in the PTX treated cells. Expression of SETDB1 was down-regulated at the transcriptional level.

3.2. PTX and p53 inhibit promoter activity of SETDB1

It is increasingly looking likely that PTX could regulate SETDB1 gene expression, so we performed promoter assay using pGL3-SETDB1-Luc. After pGL3-SETDB1-Luc was transfected into the A549 cells, we treated them with 500 nM PTX for 24 h, and performed a luciferase assay. SETDB1 promoter activity increased by approximately 30-fold compared to untreated cells, but the increased activity was significantly inhibited in the PTX treated group (Fig. 2A). When we analyzed the SETDB1 promoter with the TFSEARCH bioinformatics program (http://www.cbrc.jp/research/db/TFSEARCH.html), we focused on putative p53 binding sites in these regions. The p53 binding sites are mainly located in the proximal regions of the transcription start site (TSS), but found up to the 1500 kb upstream region. We therefore hypothesized that SETDB1 gene could be regulated by p53 that was induced by PTX. To

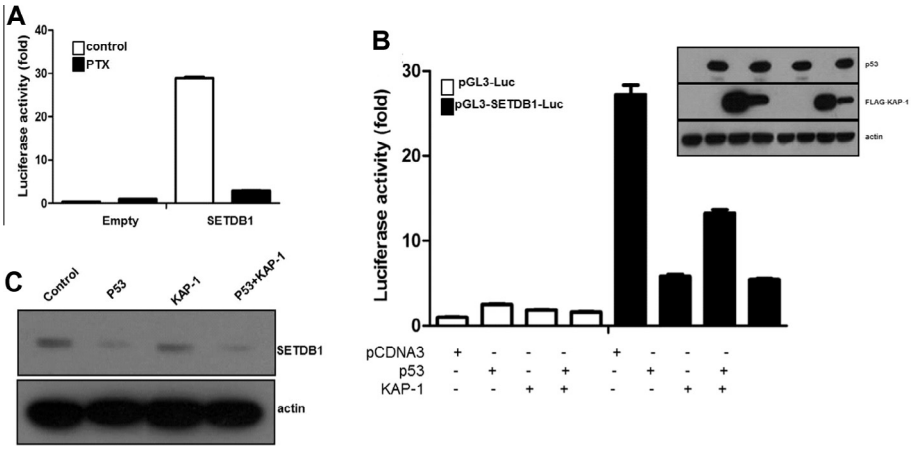

Fig. 2. Inhibition of SETDB1 promoter activity by PTX and p53. (A) The A549 cells were transfected with pGL3-basic-luc or pGL3-SETDB1-luc for 24 h, and PTX was treated for another 24 h. Cells were harvested and lyzed with luciferase assay buffer. SETDB1 promoter activity was increased 30-fold compared to pGL3-basic vector. However, PTX treatment significantly inhibits the SETDB1 promoter activity. (B) Luciferase assay was performed with p53 plasmid transfection. SETDB1 promoter activity was inhibited in the presence of p53. KAP-1, heterochromatin binding protein, also affected on SETDB1 promoter activity, but not much as p53. (C) We transfected p53 plasmid and performed Western blot analysis. The presence of p53 is correlated with the decreased SETDB1 expression.

examine whether p53 directly regulates SETDB1 promoter activity, we performed promoter assay using a plasmid containing human p53 cDNA in p53-null H1299 human lung cancer cells. SETDB1 promoter activity was severely inhibited by p53 transfection, as in the PTX treatment (Fig. 2B). KAP-1, heterochromatin binding protein, also inhibited SETDB1 promoter activity, but not much as p53. Western blot analysis showed that the presence of p53 is correlated with decreased SETDB1 expression, implicating that SETDB1 gene expression could be directly regulated by the p53 protein (Fig. 2C).

3.3. p53 Binds to SETDB1 promoter region and induces H3K9me3 occupancy

Next, we investigated if p53 binds to promoter regions of SETDB1. We prepared primer set for proximal region which contains putative p53 binding sites, and also prepared primer set for distal region that does not contain p53 binding sites (Fig. 3A). We transfected p53 plasmid and confirmed p53 expression in p53-null H1299 cells (Fig. 3B). ChIP analysis was performed to examine p53 binding on the SETDB1 promoter region. We found that p53 protein binds to proximal region of the SETDB1 promoter, but not to distal regions; this is consistent evidence on direct transcriptional regulation of SETDB1 by p53 (Fig. 3C). We also examined histone methylation status in this region using a histone methylation antibody. Interestingly, H3K9me3 occupancy increased with the presence of p53, whereas H3K4me3 decreased in the same conditions. This means that p53 can bind to promoter regions of SETDB1, and also changes the H3K9me3 status in this region.

3.4. p53 Interacts with SUV39H1

Therefore, one of H3K9me3-HMTases might be responsible for increased H3K9me3 occupancy at the SETDB1 promoter region, and might associate with p53 protein for SETDB1 expression. We are to examine the interaction between p53 and SUV39H1 or SETDB1 [11,22]. We performed transfection experiment using both p53 and SUV39H1 plasmids into H1299 cells, and checked exogenous expression of two proteins using Western blot (Fig. 4A). When we performed an IP-WB experiment with total lysates, we easily detected interaction of p53 and SUV39H1. We also examined possible interaction between p53 transfection and endogenous SETDB1. However, we found no interaction between p53 and SETDB1 (Fig. 4B). This result implicates that p53 interacts with SUV39H1, and then the increased H3K9me3 caused by SUV39H1 influences transcription repression of SETDB1.

4. Discussion

Our previous study showed that DZNep, inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase, regulates SETDB1 gene expression [19]. For that reason, we have examined if SETDB1 might be one of the important target HMTases for several anticancer drugs. Our present study demonstrates that PTX down-regulates the expression of SETDB1. We suggest that PTX-induced p53 protein could be responsible for SETDB1 gene expression at the transcriptional level. Moreover, we found that p53 directly binds to the proximal region of SETDB1 promoter, and also influences the amount of H3K9me3 occupancy in this region. The interaction of p53 and SUV39H1 might act on the promoter region of SETDB1. These results suggest that PTX-induced p53 epigenetically represses SETDB1 gene expression by forming a repressive complex with SUV39H1.

Previous studies have shown that PTX treatment induces p53 in many cancer cells, which triggers cell cycle arrest and/or apoptosis [21]; and p53 induces p21, Bax and MDM2 at the transcription level, whereas p53 can decrease Bcl-2 expression to maximize anticancer effects [24]. Diverse mechanisms have been proposed for p53 mediated activation or repression of the target gene expression [25]. One proposes that p53 is capable of transactivation and transrepression in a binding sequence specific manner [26]. SETDB1 contains several putative p53 binding sites in its promoter region and we confirmed that p53 directly binds to proximal region of SETDB1 promoter. However, it is hard to explain why there are differences in p53 binding specificity between transactivation and transrepression. Instead, p53 transcription could be determined by the binding of other activators or repressors, other types of cellular stress, or the interaction with cell-type specific transacting factors. Our study suggests that p53 interacts with SUV39H1, and the increased H3K9me3 resulting from SUV39H1 influences transcription repression of SETDB1. There is a plausible possibility that SETDB1 gene expression could be regulated by Sin3B/HDAC1 complex, because p53 and SUV39H1 interact with HDAC1 [23]. Although we only explain regulation of SETDB1 by p53 in this study, there are many signaling pathways for SETDB1 gene expression depending on transcription factors, cells or tissue types.

There have been reports that the SETDB1 gene is amplified in many types of cancers including lung carcinoma and melanoma, as well as many primary tumors [27,28]. Therefore, SETDB1 could be oncogenic, but at the same time has potential as a new therapeutic target protein. There are several published papers on regulation of HMTases by anticancer drugs. DZNep targets stability of the EZH2 protein, the HMTase component in PRC2 complex, and

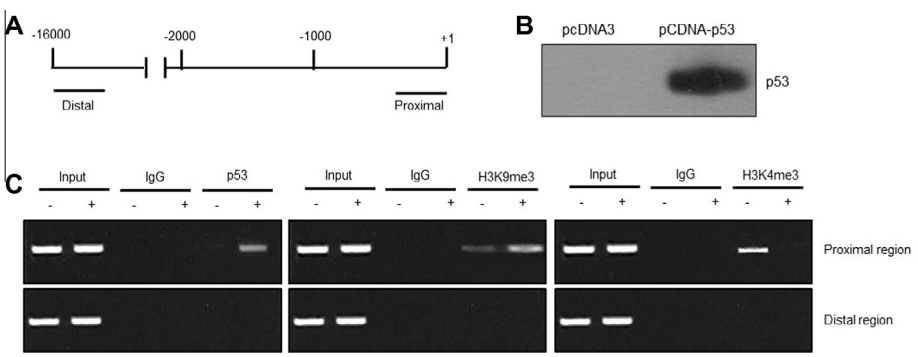

Fig. 3. Binding of p53 on SETDB1 promoter region and induced H3K9me3 occupancy. (A) Primer sets for ChIP analysis were selected at the proximal and distal regions. (B) p53 plasmid was transfected into H1299 cells and checked by Western blot. (C) After ChIP analysis using p53 antibody, p53 binding was detected in the proximal regions, but not in the distal regions. ChIP analysis using histone methylation antibodies was also examined. H3K4me3 was disappeared and H3K9me3 was appeared in the presence of p53.

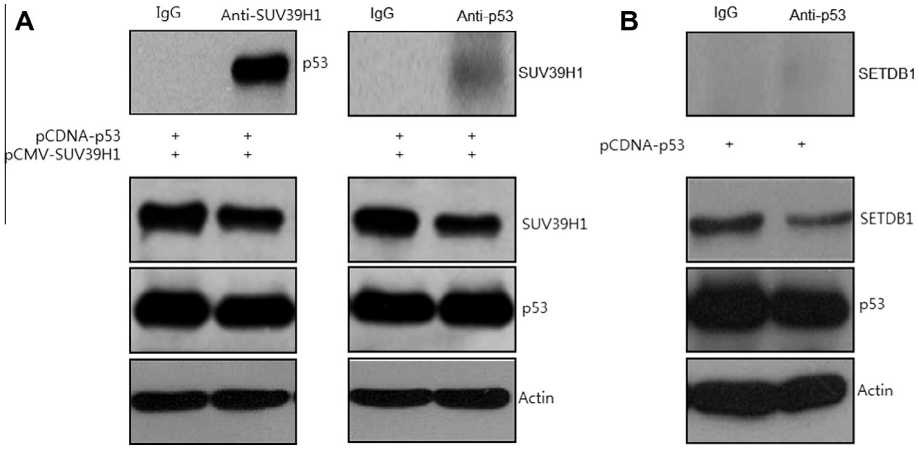

Fig. 4. Interaction of p53 and SUV39H1. (A) pCDNA-p53 and pCMV-SUV39H1 were transfected into H1299 cells and subjected to IP-WB experiment. p53 Protein was detected in IP with SUV39H1 antibody, and SUV39H1 protein was reversely detected after IP using p53 antibody. (B) Interaction between p53 and SETDB1 was examined in p53 transfection. SETDB1 was not detected in immunoprecipitation using the p53 antibody.

it reduces the amount of H3K27me3 [29]. A recent paper showed that doxorubin, anthracycline antibiotic, down-regulates SUV39H1 expression via p53 dependent pathway [30]. SUV39H1 silencing decreased H3K9me3 levels on promoters of target genes and enhanced the p53 apoptotic response. This implicates that HMTases are common targets for anticancer drugs. Interestingly, anticancer drugs that targeted HMTases are correlated with heterochromatin markers such as H3K9me3 or H3K27me3. This means that down-regulation of HMTases, including SETDB1, can result in a change of the chromatin structure, and could facilitate the access of protein complex inducing DNA fragmentation. Therefore, a novel mechanism for anticancer drugs could target heterochromatin related HMTases to enable DNA fragmentation. We believe that decomposition of chromatin structure needs to occur before DNA fragmentation can happen. Cell death related genes could be induced during down-regulation of SETDB1, and these genes could be regulated by histone methylation in the nucleosome residing in their promoter regions. This would indicate the probability that a part of PTX-induced genes is negatively regulated by SETDB1 expression.

In conclusion, our studies demonstrate that SETDB1 gene expression is dictated by p53 dependent repressor complex on its promoter region. It is also worth noting that this is the first report indicating that SETDB1 gene expression is regulated by p53 tumor suppressor.

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References

[1] Y. Wang, Y. Li, X. Liu, W.C. Cho, Genetic and epigenetic studies for determining molecular targets of natural product anticancer agents, Curr. Cancer Drug Targets 13 (2013) 506–518.
[2] B. Pang, X. Qiao, L. Janssen, A. Velds, T. Groothuis, R. Kerkhoven, M. Nieuwland, H. Ovaa, S. Rottenberg, O. van Tellingen, J. Janssen, P. Huijgens, W. Zwart, J. Neefjes, Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin, Nat. Commun. 4 (2013) 1908.
[3] G. Bocci, A. Di Paolo, R. Danesi, The pharmacological bases of the antiangiogenic activity of paclitaxel, Angiogenesis 16 (2013) 481–492.
[4] C. Best, D. Calianese, K. Szulak, G. Cammarata, G. Brum, T. Carbone, E. Still, K. Higgins, F. Ji, W. Di, H. Wanebo, Y. Wan, Paclitaxel disrupts polarized entry of membrane-permeable C6 ceramide into ovarian cancer cells resulting in synchronous induction of cell death, Oncol. Lett. 5 (2013) 1854–1858.
[5] P.M. Soetekouw, J.N. Timmer-Bonte, M.A. van der Drift, F. van Leeuwen, M. Wagenaar, L. van Die, J. Bussink, V.C. Tjan-Heijnen, Safety and efficacy of sequential chemotherapy with carboplatin plus gemcitabine followed by weekly paclitaxel in advanced non-small cell lung cancer, Int. J. Clin. Oncol. 18 (2013) 988–996.
[6] S. Singh, A.K. Dash, Paclitaxel in cancer treatment: perspectives and prospects of its delivery challenges, Crit. Rev. Ther. Drug Carrier Syst. 26 (2009) 333–372.
[7] A. Sunters, P.A. Madureira, K.M. Pomeranz, M. Aubert, J.J. Brosens, S.J. Cook, B.M. Burgering, R.C. Coombes, E.W. Lam, Paclitaxel-induced nuclear translocation of FOXO3a in breast cancer cells is mediated by c-jun NH2-terminal kinase and akt, Cancer Res. 66 (2006) 212–220.
[8] G. Tudor, A. Aguilera, D.O. Halverson, N.D. Laing, E.A. Sausville, Susceptibility to drug-induced apoptosis correlates with differential modulation of bad, bcl-2 and bcl-xL protein levels, Cell Death Differ. 7 (2000) 574–586.
[9] Y. Wu, F. Sato, U.K. Bhawal, T. Kawamoto, K. Fujimoto, M. Noshiro, S. Morohashi, Y. Kato, H. Kijima, Basic helix-loop-helix transcription factors DEC1 and DEC2 regulate the paclitaxel-induced apoptotic pathway of MCF-7 human breast cancer cells, Int. J. Mol. Med. 27 (2011) 491–495.
[10] D. Pineiro, V.M. Gonzalez, M. Salinas, M. Elena Martin, Analysis of the protein expression changes during taxol-induced apoptosis under translation inhibition conditions, Mol. Cell. Biochem. 345 (2010) 131–144.
[11] 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.
[12] E. Bolderson, K.I. Savage, R. Mahen, V. Pisupati, M.E. Graham, D.J. Richard, P.J. Robinson, A.R. Venkitaraman, K.K. Khanna, Kruppel-associated box (KRAB)-associated co-repressor (KAP-1) ser-473 phosphorylation regulates heterochromatin protein 1beta (HP1-beta) mobilization and DNA repair in heterochromatin, J. Biol. Chem. 287 (2012) 28122–28131.
[13] H. Hashimoto, P.M. Vertino, X. Cheng, Molecular coupling of DNA methylation and histone methylation, Epigenomics 2 (2010) 657–669.
[14] S.L. Tan, M. Nishi, T. Ohtsuka, T. Matsui, K. Takemoto, A. Kamio-Miura, H. Aburatani, Y. Shinkai, R. Kageyama, Essential roles of the histone methyltransferase ESET in the epigenetic control of neural progenitor cells during development, Development 139 (2012) 3806–3816.
[15] L.E. Lundberg, P. Stenberg, J. Larsson, HP1a, su(var)3–9, SETDB1 and POF stimulate or repress gene expression depending on genomic position, gene length and expression pattern in *Drosophila melanogaster*, Nucleic Acids Res. 41 (2013) 4481–4494.
[16] 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.
[17] 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.
[18] J.A. Park, K.C. Kim, Expression patterns of PRDM10 during mouse embryonic development, BMB Rep. 43 (2010) 29–33.
[19] J.K. Lee, K.C. Kim, DZNep, inhibitor of S-adenosylhomocysteine hydrolase, down-regulates expression of SETDB1 H3K9me3 HMTase in human lung cancer cells, Biochem. Biophys. Res. Commun. 438 (2013) 647–652.
[20] Y. Shang, X. Hu, J. DiRenzo, M.A. Lazar, M. Brown, Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription, Cell 103 (2000) 843–852.
[21] P. Giannakakou, R. Robey, T. Fojo, M.V. Blagosklonny, Low concentrations of paclitaxel induce cell type-dependent p53, p21 and G1/G2 arrest instead of mitotic arrest: molecular determinants of paclitaxel-induced cytotoxicity, Oncogene 20 (2001) 3806–3813.
[22] S. Rea, F. Eisenhaber, D. O'Carroll, B.D. Strahl, Z.W. Sun, M. Schmid, S. Opravil, K. Mechtler, C.P. Ponting, C.D. Allis, T. Jenuwein, Regulation of chromatin

structure by site-specific histone H3 methyltransferases, Nature 406 (2000) 593–599.
[23] N. Bansal, R. Kadamb, S. Mittal, L. Vig, R. Sharma, B.S. Dwarakanath, D. Saluja, Tumor suppressor protein p53 recruits human Sin3B/HDAC1 complex for down-regulation of its target promoters in response to genotoxic stress, PLoS One 6 (2011) e26156.
[24] T. Miyashita, J.C. Reed, Tumor suppressor p53 is a direct transcriptional activator of the human bax gene, Cell 80 (1995) 293–299.
[25] Y. Yu, J. Wang, W. Khaled, S. Burke, P. Li, X. Chen, W. Yang, N.A. Jenkins, N.G. Copeland, S. Zhang, P. Liu, Bcl11a is essential for lymphoid development and negatively regulates p53, J. Exp. Med. 209 (2012) 2467–2483.
[26] A. Mirza, Q. Wu, L. Wang, T. McClanahan, W.R. Bishop, F. Gheyas, W. Ding, B. Hutchins, T. Hockenberry, P. Kirschmeier, J.R. Greene, S. Liu, Global transcriptional program of p53 target genes during the process of apoptosis and cell cycle progression, Oncogene 22 (2003) 3645–3654.
[27] M. Rodriguez-Paredes, A. Martinez de Paz, L. Simo-Riudalbas, S. Sayols, C. Moutinho, S. Moran, A. Villanueva, M. Vazquez-Cedeira, P.A. Lazo, F. Carneiro, C.S. Moura, J. Vieira, M.R. Teixeira, M. Esteller, Gene amplification of the histone methyltransferase SETDB1 contributes to human lung tumorigenesis, Oncogene (2013) 1–7.
[28] 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) 1–12.
[29] 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.
[30] S.K. Mungamuri, E.K. Benson, S. Wang, W. Gu, S.W. Lee, S.A. Aaronson, P53-mediated heterochromatin reorganization regulates its cell fate decisions, Nat. Struct. Mol. Biol. 19 (2012) 478–484 (S1).