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The HDAC inhibitor depsipeptide transactivates the p53/p21 pathway by inducing DNA damage

Haiying Wang[a,1], Wen Zhou[a,1], Zhixing Zheng[a], Ping Zhang[a], Bo Tu[a], Qihua He[b], Wei-Guo Zhu[a,*]

[a] Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Biochemistry and Molecular Biology, Peking University Health Science Center, 38 Xueyuan Road, Beijing 100191, China
[b] Department of Pathology and Biomedical Teaching Laboratory Center, Peking University Health Science Center, Beijing 100191, China

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ABSTRACT

Histone deacetylase (HDAC) inhibitors have been proven to be effective therapeutic agents to kill cancer cells through inhibiting HDAC activity or altering the structure of chromatin. As a potent HDAC inhibitor, depsipeptide not only modulates histone deacetylation but also activates non-histone protein p53 to inhibit cancer cell growth. However, the mechanism of depsipeptide-induced p53 transactivity remains unknown. Here, we show that depsipeptide causes DNA damage through induction of reactive oxygen species (ROS) generation, as demonstrated by a comet assay and by detection of the phosphorylation of H2AX. Depsipeptide induced oxidative stress was confirmed to relate to a disturbance in reduction-oxidation (redox) reactions through inhibition of the transactivation of thioredoxin reductase (TrxR) in human cancer cells. Upon treatment with depsipeptide, p53 phosphorylation at threonine 18 (Thr18) was specifically induced. Furthermore, we also demonstrated that phosphorylation of p53 at Thr18 is required for p53 acetylation at lysine 373/382 and for p21 expression in response to depsipeptide treatment. Our results demonstrate that depsipeptide plays an anti-neoplastic role by generating ROS to elicit p53/p21 pathway activation.

1. Introduction

Post-translational modifications (PTMs) of histone tails are associated with changes in histone structure and thereby affect gene expression [1,2]. Histone acetylation, which directly relates to the activation of gene transcription, is the most extensively studied PTM [3,4]. Two classes of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs), determine the acetylation status of histones [5]. Increases in histone acetylation and decreases in histone deacetylation are well associated with the expression of tumor suppressor genes, which is thus beneficial for cancer treatment [6].

Because HDACs have been identified as molecular targets in cancer therapy [7], HDAC inhibitors (HDACi) are an effective therapeutic agent to kill cancer cells by either inhibiting HDAC activity or altering gene transcription to cause growth arrest, differentiation and/or apoptosis of tumor cells [8,9]. Histone deacetylases are classified into three classes according to their structure and cellular localization [10]. Depsipeptide, (also known as FK228 or FR901228) is a more potent inhibitor of class I HDACs than other HDAC inhibitors, such as trichostatin A (TSA) and sodium butyrate [11], and depsipeptide is a promising anticancer agent for both mono- and polytherapy [10,12]. In addition to inducing histone acetylation, an activity common to all HDAC inhibitors, depsipeptide shows other biological functions through varying mechanisms of action [10]. For example, depsipeptide induces acetylation of non-histone proteins, such as p53 and Hsp90 [13,14]. Depsipeptide also cooperates with nucleoside analogs, such as 5-aza-2′-deoxycytidine (5-aza-CdR) and cytarabine (Ara-C), to activate transcription and to induce apoptosis. Apoptosis probably results from suppressing the repair of 5-aza-CdR induced DNA damage [15]. In addition, we previously found that depsipeptide is a DNA demethylating agent [16], and this activity has prompted the exploration of depsipeptide in a much broader scientific context. However, the precise molecular mechanism of its anticancer effect remains obscure. Based on these factors, it is essential to

Abbreviations: Ara-C, cytarabine; ATM, ataxiatelangiectasia-mutated; ATR, ATM- and Rad3-related; CAT, catalase; CHK2, the checkpoint kinase 2; CK1, casein kinase 1; DHE, dihydroethidium; DNA-PK, DNA-dependent serine/threonine protein kinase; Grx, glutaredoxin; HAT, histone acetyltransferases; HDACi, HDAC inhibitors; HDACs, histone deacetylases; L-NAC, L-N-acetylcysteine; PTMs, post-translational modifications; ROS, reactive oxygen species; SOD, superoxide dismutase; Trx, thioredoxin; TrxR, thioredoxin reductase; TSA, trichostatin; VRK1, vaccinia-related kinase 1; VRK2, vaccinia-related kinase 2.

* Corresponding author. Tel.: +86 10 82802235; fax: +86 10 82805079.
E-mail address: zhuweiguo@bjmu.edu.cn (W.-G. Zhu).

[1] These authors contributed equally to this work.

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understand the mechanism of the action of depsipeptide in human cancer cells.

Almost all HDAC inhibitors induce a significant increase in the expression of p21 [10,17,18], a protein that arrests the cancer cell cycle in the G1 phase. Interestingly, although p21 is a critical target of p53, HDAC inhibitor-induced p21 expression has been reported to be independent of p53 activation [19–21], however, we previously found that depsipeptide induces p21 expression by activating the p53 pathways [13]. Unlike other HDAC inhibitors, such as TSA, which induce p53 acetylation only by working with ionizing radiation or nicotinamide, an inhibitor of class III HDACs [22], depsipeptide alone, without cooperation from other agents, can induce acetylation of p53 at K373/382 and significantly increase p53 transactivity and p21 expression [13]. These differences between depsipeptide and other HDAC inhibitors in activating p53 or p21 expression lead us to further investigate the mechanism by which depsipeptide activates p53 activity.

As a transcription factor, depending on the nature of the stress, p53 can induce expression of many different downstream genes including p21, GADD45, and Bax to elicit various responses, such as cell cycle arrest, apoptosis, and DNA repair [23–25]. p53 exists in a latent conformation in unstressed cells because p53's C-terminal tail allosterically interacts with its core DNA binding domain and negatively regulates its sequence-specific DNA binding transcription activation [26]. Upon stress, the interaction and negative regulation is abolished by p53's posttranslational modifications, such as phosphorylation and acetylation [27]. The preeminent post-modification is the direct phosphorylation of p53. Phosphorylations at the p53C terminus such as Ser315 and Ser392 are reported to regulate the oligomerization state and sequence-specific DNA binding ability of p53 [28,29]. In addition, phosphorylation and acetylation modification of p53 are interrelated [30]. Phosphorylation of p53 is important for efficient binding with CBP/p300 and PCAF complexes. For example, phosphorylation at N-terminal serines, such as Ser15, Ser33, and Ser37 has been reported to recruit p300/CBP and PCAF to induce p53 acetylation in response to DNA damage [31,32]. Phosphorylation of p53 at Ser20 or Thr18 can stabilize the p300–p53 complex and induce p53 acetylation [33]. Recently, it was reported that p53 C-terminal phosphorylation also modulates C-terminal acetylation in response to DNA damage [34]. Our study also found that p53 phosphorylation at Ser15 in turn results in acetylation of p53 at Lys320 and Lys373/Lys382 through p300 and CBP respectively, which are directly responsible for 5-aza-CdR induced p21 expression [35].

In the present study, we conducted experiments to test how depsipeptide activates p53 transactivity and demonstrated that depsipeptide induces DNA damage by enhancing the production of reactive oxygen species (ROS), an effect that results from inhibiting the expression of thioredoxin reductase (TrxR). Subsequently, the accumulated ROS induces DNA damage, which in turn induces p53 phosphorylation at Thr18 and p53 acetylation at K373/382 as well as an increase in p21 expression.

2. Materials and methods

2.1. Cell culture and chemical treatments

Human lung cancer cell lines A549 and H1299 and the human colon cancer cell line HCT116 were grown in RPMI 1640 supplemented with 10% fetal bovine serum (heat inactivated at 56 °C for 45 min) and the appropriate amount of penicillin/streptomycin in a 37 °C incubator with a humidified, 5% CO_2 atmosphere. The HDAC inhibitor depsipeptide, at 0.05 or 0.1 μM, was added to these cells for 0 to 12 h, and cells were then harvested for experiments.

2.2. Western blotting

Protein expression was detected by Western blotting as previously described. Cells were lysed with lysis buffer [50 mM Tris–HCl, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 0.1% Igepal CA-630, and a mixture of protease inhibitors (Roche Diagnostics, Mannheim, Germany)]. Equal amounts of protein per sample (100–150 μg) were size-fractionated by 6–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The antibodies used were anti-p21 (F-5; Santa Cruz), anti-p53 (DO-1; Santa Cruz), anti-p300 (H-272; Santa Cruz), anti-β-actin (Santa Cruz), anti-acetyl-p53 (Lys373 and -382; Upstate), anti-phosp-p53 (Ser15, Ser20, Ser37, Ser392 and Thr18, Cell Signaling), anti-Trx, anti-TrxR (Santa Cruz) and anti-H3 (Cell Signaling) antibodies.

2.3. Alkaline comet assay to detect DNA strand breaks

The alkaline comet assay, also known as the single-cell gel electrophoresis assay, was performed as described previously [36]. In brief, fully frosted microscope slides were covered with 110 μl of 0.5% normal melting agarose at 60 °C. The slides were immediately covered with cover slips and were then maintained at 4 °C for 15 min to allow the agarose to solidify. About 10^5 cells (depsipeptide treated or untreated) in 40 μl of PBS were mixed with an equal amount (40 μl) of 1% lower melting agarose to form a cell suspension. After the cover slips were gently removed from the slides, the cell suspensions were pipetted onto the first agarose layer, spread and covered with cover slips, and maintained at 4 °C for 15 min to allow solidification. After removal of the cover slips, the slides were immersed in freshly prepared cold lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10.0, 1% sodium sarcosinate) with 1% Triton X-100 for 40 min at 4 °C. The slides were then placed in a horizontal gel electrophoresis tank filled with fresh electrophoresis solution (1 mM Na_2EDTA, 300 mM NaOH, pH 13.0) for 10 min. The slides were then rinsed twice in Tris buffer (0.4 M Tris–HCl, pH 7.5) for 15 min (to neutralize the excess alkali) at 4 °C. The slides were then stained with 75 μl of propidium iodide (5 μg/ml) for 30 min.

The slides were examined, and pictures were taken under a fluorescence microscope (TCS; Leica, Mannheim, Germany). To score the percentage of DNA in the comet tail, the CometScore automatic comet assay system was used (TriTek Corp, USA). The percentage of comet tail area (DNA tail area/total DNA area) and the comet tail length (from the center of the DNA head to the end of the DNA tail) were analyzed for 50 cells per slide.

2.4. ROS detection

Dihydroethidium (DHE), a redox sensitive fluorescent probe for superoxide, was used to detect ROS accumulation. After treatment, cells were incubated with 5 μM DHE (Sigma) for 30 min, after which they were washed, and ROS generation images were obtained by confocal laser microscopy and analyzed by an image analysis system (Q550CW; Leica).

2.5. Site-directed mutagenesis

Constructs for two p53 mutants (p53S37A and p53T18A) were generated using a site-directed mutagenesis kit (QuikChange; Stratagene, La Jolla, CA). A wild-type p53 expression vector (pCI-neo with full-length p53 cDNA) [37] was used as the mutagenesis template. The following primers were used for the mutagenesis: p53-S37A-up, 5′-CTG TCC CCC TTG CCG G(T)CC CAA GCA ATG GAT G-3′; p53-S37A-down, 5′-CAT CCA TTG CTT GGG C(A)CG GCA AGG GGG ACA G-3′; p53-T18A-up, 5′-CCC CTC TGA GTC AGG AAG(A) CAT TTT CAG ACC TAT G-3′; and p53-T18A-down, 5′-CAT AGG TCT