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Lidamycin regulates p53 expression by repressing Oct4 transcription

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ABSTRACT

Antitumor antibiotic lidamycin (LDM) is widely used in the treatment of a variety of cancers. Here we demonstrated that LDM up-regulates the expression of the tumor suppressor p53 gene by repressing Oct4 transcription. We showed that low dose LDM-induced increase of p53 expression and *decrease of Oct4 expression* in P19 and HCT116-p53$^{+/+}$ cells. Knockdown of Oct4 expression by siRNA led to activation of p53 in both cell lines, whereas ectopical expression of Oct4 significantly inhibited p53 expression in P19 cells. LDM-induced p53 activation was blocked by ectopical *expression of Oct4.*

1. Introduction

Lidamycin (LDM, also known as C-1027) is one of the most potent members of the enediyne family, which can be dissociated into an apoprotein (LDP) and an active enediyne chromophore [1–3]. The labile chromophore is responsible for its cytotoxicity through its DNA damage activity [2,4], the non-covalently bound LDP serves as a vehicle to deliver the enediyne chromophore to tumor tissues [3]. Previous studies showed that LDM exhibited its anti-cancer effect through a variety of mechanisms in a dose-dependent manner, including chromosomal aberrations, telomere dysfunction, cell cycle arrest, and apoptosis [5,6]. A high dose (1 pM) of LDM induced unusual DNA damage response to double-strand breaks [4], whereas low dose (0.1 nM) of LDM induced cell cycle arrest in a p53-dependent manner [5]. However, the molecular mechanisms of p53 activation induced by low dose-LDM are still largely unknown.p53 functions as a transcription factor and coordinates in a wide variety of cellular processes. The steady-state level of p53 is usually kept low by its negative regulator HDM2 (mouse ortholog is mdm2) and HDMX (mouse ortholog is mdmX) [7]. However, p53 is stabilized and activated as a central mediator to respond to stimuli in a promoter-specific manner, including the induction of cell cycle arrest, senescence, differentiation, and apoptosis [7,8]. During embryonic stem (ES) cell differentiation, p53 directly suppresses the expression of several genes, such as the pluripotency factor Nanog [9] and expression of p53 is regulated by another pluripotency factor Oct4 [10].

Oct4, a homeodomain transcription factor of the POU family [11], plays a crucial role in the maintenance of self-renewal and pluripotency in ES cells, inner cell mass (ICM), and primordial germ cells [11,12]. In the process of ES cell self-renewal, the expression of Oct4 promotes cell cycle progression by suppressing its target gene p21, a cyclin-dependent kinase inhibitor [13]. In contrast, downregulation of Oct4 results in blocking of cell cycle progression followed by differentiation [13]. Oct4 has been proposed as a biomarker for cancer stem cell (CSC)-like cells [14]. Oct4 is detectable in a variety of cancers such as breast, bladder, and lung cancers [14,15]. Growing evidence shows that Oct4 participates in malignancy and is associated with CSCs and poor prognosis of human cancers [14,15]. Forced expression of Oct4 gene causes dedifferentiation of cancer cells, which acquire cancer stem cell phenotypes [16]. Repression of Oct4 expression promotes differentiation of human endometrial adenocarcinoma cells through upregulation of microRNA-145 [17]. Therefore, Oct4 may be a useful target in cancer stem cell therapy.

Our previous study showed that LDM inhibited cancer cell growth through downregulation of ES cell-like genes [18]. LDM activates p53 and p21 through dowregulating Oct4 and consequently induces differentiation of mouse EC cells P19 [19]. However, the molecular mechanism by which low-dose LDM regulates the Oct4-p53 pathway is still poorly understood. In this report, we demonstrated that LDM up-regulated p53 by repressing Oct4 transcription.

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2. Materials and methods

2.1. Chemicals

LDM [3,5] was provided by Dr. Yong-Su Zhen (Institute of Medicinal Biotechnology, Chinese Academy of Medical Science, Beijing, China). LDM (1 μM) were prepared in distilled water and then stored at −70 °C.

2.2. Cell culture

Mouse EC P19 cells and human colon cancer HCT116-p53$^{+/+}$ cells were maintained in Dulbecco's modified Eagle medium (DMEM, GIBCO/BRL) containing 10% fetal bovine serum (FBS, HyClone) and incubated at 37 °C.

Floating multicellular spheroids were described previously [20,21]. The CD133^{high}/CD44^{high} fraction of HCT116-p53$^{+/+}$ colon cancer cells was sorted by fluorescence activated cell sorting (FACS) analysis. Cells with the particular phenotype were seeded at a density 4×10^2/well on a 6-well tissue culture plate in serum-free MSCB medium (supplement with 20 ng/ml EGF, 10 ng/ml FGF-2, 10 ng/ml LIF and 25% Matrigel matrix). After 1 week in culture, fresh medium of normal culture (DMEM/F12 supplement with 10% FBS) was added and maintained for another week. Primary spheres were gently disaggregated by repeated pipetting and transferred into tissue culture flasks for further propagation and maintenance.

2.3. Cell cycle arrest

The cells (2×10^4) were seeded into 60 mm dishes. For synchronization, the cells were incubated in serum-free DMEM for 24 h, and then 10% FBS was added into the culture medium. After cells were exposed to LDM for 24 h, samples were collected and fixed with 500 μl of 70% ethanol at 4 °C for 1 h. Subsequently, cells were resuspended in PBS with RNase A (50 mg/ml) and PI (2 mg/ml) at 37 °C for 30 min. The stained cells were analyzed on a flow cytometer.

2.4. RNA isolation for PCR and real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). Two micrograms of RNA was subjected to reverse transcription by M-MLV reverse transcriptase (Promega, Madison, WI).

For real-time quantitative PCR, the analysis was performed with primers as previously described [18].

Mouse p53: (sense) 5′-GCAACTTCTAGAAACCCTGGGG-3′ and (antisense) 5′-TTGGGAAATGGAGGCCTGG-3′.

Human p53: (sense) 5′-GCGAGCACTGCCCAACAACA-3′ and (antisense) 5′-GGATCTGAAGGGTGAAATATTCT-3′.

Each 15 μl PCR mixture contained 1 μl of cDNA, 0.15 μl of each primer (400 nM), 6.2 μl of ddH2O and 7.5 μl of SYBR® Green Real-time PCR Master Mix (TOYOBO, Osaka, Japan). Relative mRNA expression levels of Oct4 and p53 were determined in triplicate on a SYBR® Green Real-Time PCR Detection System (Stratagene Mx3000P, USA) and normalized to actin levels.

2.5. RNA interference

Small interfering (si) RNAs targeting Oct4 in mouse and human were purchased from Ambion. P19 and HCT116-p53$^{+/+}$ cells were transfected for 6 h with LipofectAMINE 2000 (LF2000) according to the manufacturer's guidelines. After recovery in fresh cell culture medium, cells were transfected again at 24 h to maintain knockdown efficiency. Silencing was assessed by RT-PCR at 48 h and by Western blots at 72 h.

2.6. Generation of lentivirus for overexpressing Oct4 and P19 cell infection

The lentivirus overexpressing Oct4 was prepared and infected as previously described [19].

2.7. Western blot analysis

Cells were lysed with 1× lysis buffer (Promega) by centrifugation. Equal amounts of Proteins were electrophoresed on SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane (Pierce). The membranes were probed with primary antibodies. The blots were developed with goat anti-mouse IgG (IRDye 700; LI-COR) and goat anti-rabbit IgG (IRDye 800; LI-COR), and imaged on an infrared scanner (LI-COR). Antibodies to Oct4, p53, and GADPH were from Santa Cruz Biotechnology.

2.8. Luciferase assay

2.8.1. p53 transactivity luciferase reporter assay

Luciferase activity assays were performed using PG13-luc and MG15-luc reporter plasmids (Addgene) in HEK-293T cells. The cells were plated in a 24-well plate and split 24 h before transfection. Co-transfection of reporter plasmid was performed as previously described in [22]. After 24 h post-transfection, cells were treated with 0.1 nM LDM and cultured for another 24 h. The transfected-cells were variously treated and analyzed using a Dual-luciferase Reporter Assay kit (Promega). Results showed that the firefly luciferase activity normalized to *Renilla* luciferase activity.

2.8.2. Oct4 promoter reporter assay

A 2.2 kb genomic DNA encompassing the promoter region of *Oct4* in the pGL3 vector (Promega, Madison, WI) was constructed by insertion into HindIII and XhoI restriction enzyme sites upstream of luciferase. The transfection and luciferase activity detection procedures were the same as the p53 transactivity luciferase reporter assay.

2.9. ChIP assay

As described before, in vivo binding of the Oct4 with *p53* promoter or LDP with the Oct4 promoter was performed [23].

Mouse

- For Nanog (sense) 5′-TGTTTTAGTGTGGGTATGGGCC-3′ and (antisense) 5′-TGTGGTCCCTCCTCTTTC-3′.
- For Oct4 (sense) 5′-GGAACTGGGTGTGGGGAGGTTGTA-3′ and (antisense) 5′-AGCAGATTAAGGAAGGGCTAGGACGAGAG-3′.
- For Sox2 (sense) 5′-CCCTGTTCCAAGTCTCTTTCTGCTAGTCA-3′ and (antisense) 5′-CACCGATTTCAATCCAACACCATCATAG-3′.

Human

- For p53 (sense) 5′-CGGATTACTTGCCCTTACT and (antisense) 5′-AATCCAGGGAAGCGTGTC-3′.
- For Nanog (sense) 5′-GAGGATGCCCCCTAAGCTTTCCCTCCC-3′ and (antisense) 5′-CCTCCTACCCTACCCACCCCCTATTCTCCC-3′.
- For Oct4 (sense) 5′-GGGGAACCTGGAGGATGGCAAGCTGAGAAA-3′ and (antisense) 5′-GGCCTGGTGGGGGTGGGAGGAACAT-3′.
- For Sox2 (sense) 5′-GGATAACATTGTACTGGGAAGGGACA-3′ and (antisense) 5′-CAAAGTTTCTTTATTCGTATGTGTGAGCA-3′.

PCR products were resolved on a 1% agarose gel and quantified using the ImageQuant software on a Gel Detector (BioRad, USA).

2.10. Statistical analysis

The Student's *t*-test and ANOVA test were used for unvaried analysis. The statistical significance is defined by a two-tailed *P*-value of 0.05.

3. Results

3.1. Low-dose LDM activates p53 and suppresses Oct4 expression

Low dose of LDM induces cell cycle arrest in a p53-dependent manner [5]. Our previous results found that the binding of Oct4 to p53 promoter was attenuated by low-dose LDM treatment in P19 cells [19]. We then asked if Oct4 is involved in the LDM caused p53 response. To answer this question, the changes of p53 and Oct4 were examined in P19 cells after cells were exposed to low-dose LDM. We showed that the levels of p53 mRNA and protein increased in a time-dependent manner and Oct4 levels coincidently decreased when cells were treated with low dose LDM (Fig. 1A and C). Although the expression of Oct4 in HCT116 $p53^{+/+}$ cells is much lower than that in P19 cells (Fig. 1D), a similar expression pattern of p53 and Oct4 was observed in 3-D spheroids of HCT116 $p53^{+/+}$ cells after treatment with a low-dose LDM (Fig. 1B and D). Taken together, the activation of p53 is closely associated with the down-regulation of Oct4 induced by LDM in both mouse and human cancer cells.

To confirm that Oct4 directly regulates the expression of p53, Oct4 was silenced by siRNA in P19 and HCT116-$p53^{+/+}$ cells and p53 expression was evaluated. As shown in Fig. 1E–H, the level of p53 mRNA increased in Oct4 siRNA transfected cells in the presence or absence of LDM treatment, compared with that in control siRNA transfected cells (Fig. 1E). The protein level of p53 was also elevated when Oct4 was knocked down by siRNA or when cells were treated with low dose of LDM (Fig. 1G). Similar results were obtained in HCT116-$p53^{+/+}$ cells (Fig. 1F and H). We thus *speculated* that low dose of LDM may regulate p53 level by modifying Oct4 expression.

3.2. LDM-induced p53 activation is blocked by ectopical expression of Oct4

Next, we wanted to know if ectopical expression of Oct4 could block LDM-induced p53 change. To address this issue, we evaluated p53 levels when cells were treated with low dose LDM in P19-Oct4 cells in which Oct4 was ectopically expressed through a tet-on control system (Fig. 2). Overexpression of Oct4 was confirmed at mRNA and protein levels in P19-Oct4 cells (Fig. 2A–C). As shown in Fig. 2A and C, low dose LDM-induced p53 increase was blocked by ectopical expression of Oct4. In the meantime, cell cycle progress was promoted by ectopical expression of Oct4 in P19 cells (Fig. 2D). These results suggest that there may be some cause-effect regulation between transcription factor Oct4 and p53 genes, and that Oct4 may act as a repressor for p53 *transcription* in P19 EC cells.

3.3. LDM suppresses the transcription of Oct4 through directly binding of LDP to Oct4 promoter

Our previous results show that low-dose LDM not only induces G1/S cell cycle arrest and inhibits cell growth of P19, but also induces P19 cell differentiation through downregulation of transcription factor Oct4 [18,19]. To identify how LDM regulates the transcription of Oct4 gene, we analyzed the effect of LDM on mouse Oct4 promoter reporter vector pGL3-Oct4-promoter-luc. The pGL3-Oct4-promoter-luc plasmid was transiently co-transfected with pCMV-BK/Oct4 plasmid into 293T cells. Luciferase activity was evaluated after cells were treated with low-dose LDM (Fig. 3A). The result showed that the luciferase activity of the mouse Oct4 promoter reporter constructs was 5-fold higher than that of the empty vector. However, the luciferase activity of the mouse Oct4 promoter reporter was gradually reduced to nearly 50% when cells were treated with low-dose LDM, as compared with that in untreated 293T cells. To further confirm whether LDM directly binds to the Oct4 promoter, we performed a ChIP assay in P19 and HCT116-$p53^{+/+}$cells using an anti-LDP antibody (LDP is a component of LDM). We found that LDP bound to the promoter of Oct4 after treatment with LDM, but did not bind to the promoters of Sox2 or Nanog (Fig. 3B and C). The result indicates that LDP directly binds to the Oct4 promoter.

3.4. Low-dose LDM regulates p53 transcriptional activity through downregulation of Oct4

To assess whether Oct4 regulates p53 transcriptional activity in 293T cells, a luciferase reporter plasmid containing 13 copies of the p53-binding sites PG-13 was transfected into 293T cells and luciferase activity was evaluated after cells were treated with low-dose LDM. The luciferase reporter plasmid MG-15 which contains mutated p53-binding sites was used as a negative control. In agreement with our previous findings, Oct4 suppressed luciferase activity of PG-13 luciferase reporter. After cells were exposed to low-dose LDM, luciferase activity of PG-13 luciferase reporter was upregulated and Oct4 was downregulated. However, no activation of the MG15 luciferase reporter was observed under the same condition (Fig. 4A).

We previously found that the binding of Oct4 to *p53* promoter was attenuated by low-dose LDM treatment in P19 cells [19]. To confirm the effect of low-dose LDM on the binding of Oct4 to p53 promoter, ChIP was performed in HCT116 $p53^{+/+}$ cells after cells were exposed to low-dose LDM. Oct4 binding to its target gene p53 decreased gradually when cells were exposed to LDM for 24 h (Fig. 4B). The p53 activation through downregulation of Oct4 by low-dose LDM was accompanied with inhibition of cell growth and induction of cell cycle arrest in HCT116 $p53^{+/+}$ cells (Fig. 4C and D). However, although Oct4 expression was not detected in HT29 cancer cells, a similar pattern of cell growth and cell cycle arrest was observed after p53 was activated with low-dose LDM treatment (Fig. S1). These result indicated that multiple mechanisms exist in p53 activation by low dose LDM, and p53 activation though downregulation of Oct4 is one of the mechanisms.

4. Discussion

LDM has been shown to possess exceptional antitumor activity due to their unique ability of DNA damage in tumor cells [1,2,6]. It is currently being evaluated in clinical trials as a potential anticancer agent in China. The mechanisms of LDM are complicated and involve several biological processes in a dose-dependent manner, including cell cycle arrest, apoptosis, mitotic cell death, DNA damage and chromosome aberrations [5,6]. Low-dose LDM induced p53 activation in breast cancer cells [5]. Our previous study showed that p53 is regulated during LDM induced P19 cell differentiation [19]. In the present study, we demonstrated for the first time that LDM up-regulates p53 expression through repressing Oct4 transcription.

Transcription factor Oct-4 is expressed in pluripotent cells of ICM and epiblast, and plays a pivotal role in maintaining

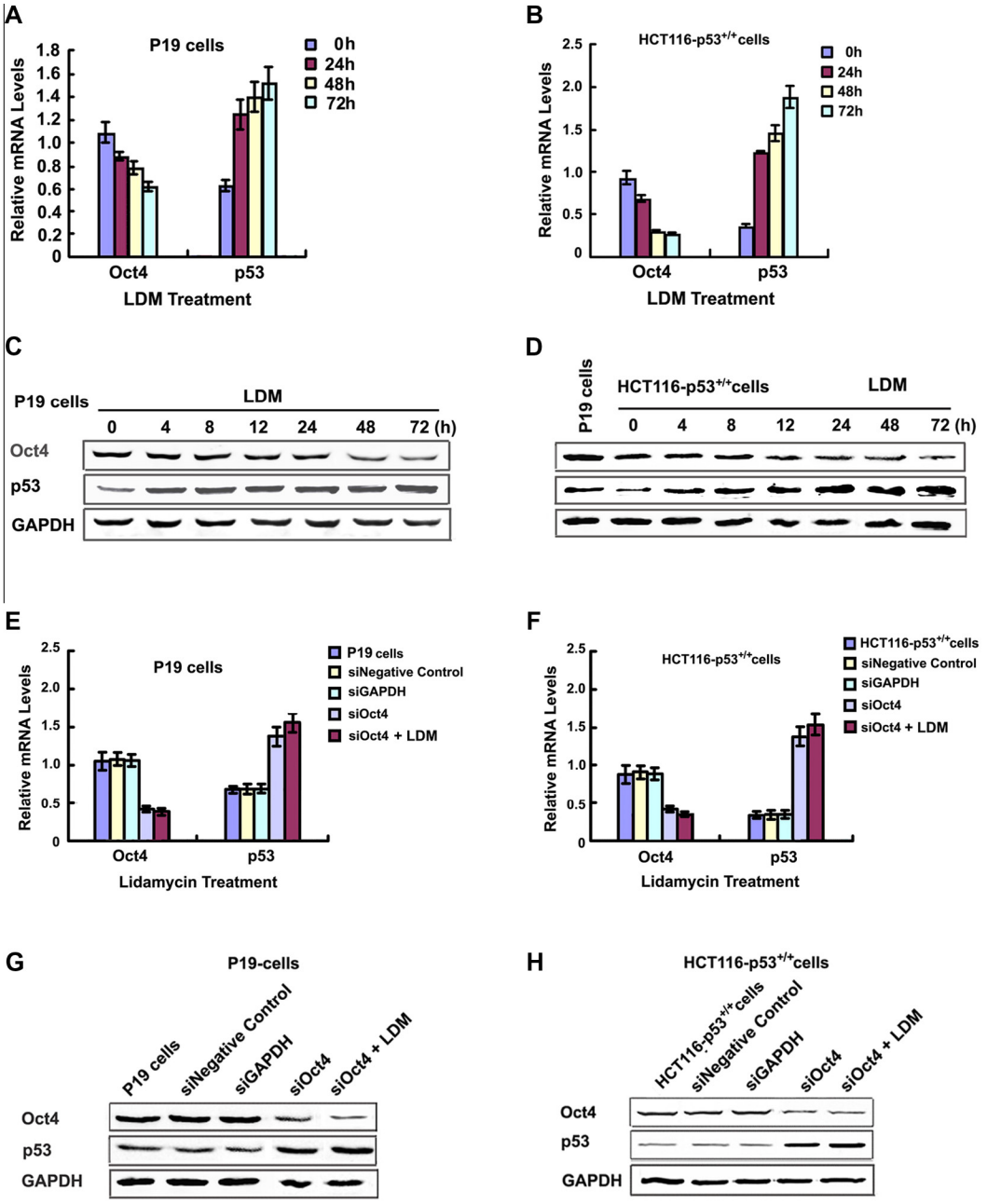

Fig. 1. LDM induced activation of p53 and suppression of transcription factor Oct4. After exposure of cells to 0.1 nM LDM in P19 and HCT116-p53$^{+/+}$cells for the indicated time, the transcripts of Oct4 and p53 were examined by real-time RT-PCR. Data represent mean ± SD (n = 3). (A) P19 cells. (B) HCT116-p53$^{+/+}$ cells. The proteins of Oct4 and p53 were examined by Western blot analysis. (C) P19 cells. (D) HCT116-p53$^{+/+}$ cells. Specific Oct4-knockdown resulted in the activation of p53 gene. Data represent mean ± SD (n = 3). The transcripts of Oct4 and p53 were examined by real-time RT-PCR. (E) P19 cells. (F) HCT116-p53$^{+/+}$ cells. The proteins of Oct4 and p53 were examined by Western blot. (G) P19 cells. (H) HCT116-p53$^{+/+}$ cells. A cell-forming sphere was generated as described in Section 2.2.

undifferentiated pluripotent state in ES and EC cells [11,24]. Oct-4 expression is associated with an undifferentiated phenotype of tumors and poor prognosis in cancer patients [14]. Expression of Oct4 is decreased and activation of p53 elongates the G1 phase of cell cycle by p21 induction when hESCs (human embryonic stem cells) were exposed to differentiating conditions [25]. Our result also showed that p53 activation by a low-dose LDM involved downregulation of Oct4 in P19 and HCT116-p53$^{+/+}$ cells. Therefore, we hypothesized that there exists a regulation between activation of p53 and suppression of Oct4 expression after treatment with LDM. Our experiments showed that Oct4 knockdown by siRNA resulted in p53 activation in P19 and HCT116-p53$^{+/+}$ cells. Note that there is no significantly difference in the activation of p53 between the Oct4 siRNA transfected-cells and the Oct4 siRNA transfected-cells with LDM treatment. Conversely, overexpression of Oct4 inhibited p53 expression and promoted cell cycle progression in P19 cells. In addition, LDM-caused p53 activation at low dose was blocked by ectopical expression of Oct4. Taken together, these results demonstrate that low-dose LDM induced activation of p53 is mediated by Oct4.

In this study, we found that p53 transcriptional activity on luciferase reporter plasmid PG-13 was blocked by ectopic expression of Oct4. Furthermore, ectopic expression of Oct4 also blocked LDM induced activation of p53 transcriptional activity. Thus, our data demonstrate that Oct4 is a negative regulator of p53 and low-dose LDM activates p53 transcriptional activity through repressing Oct4

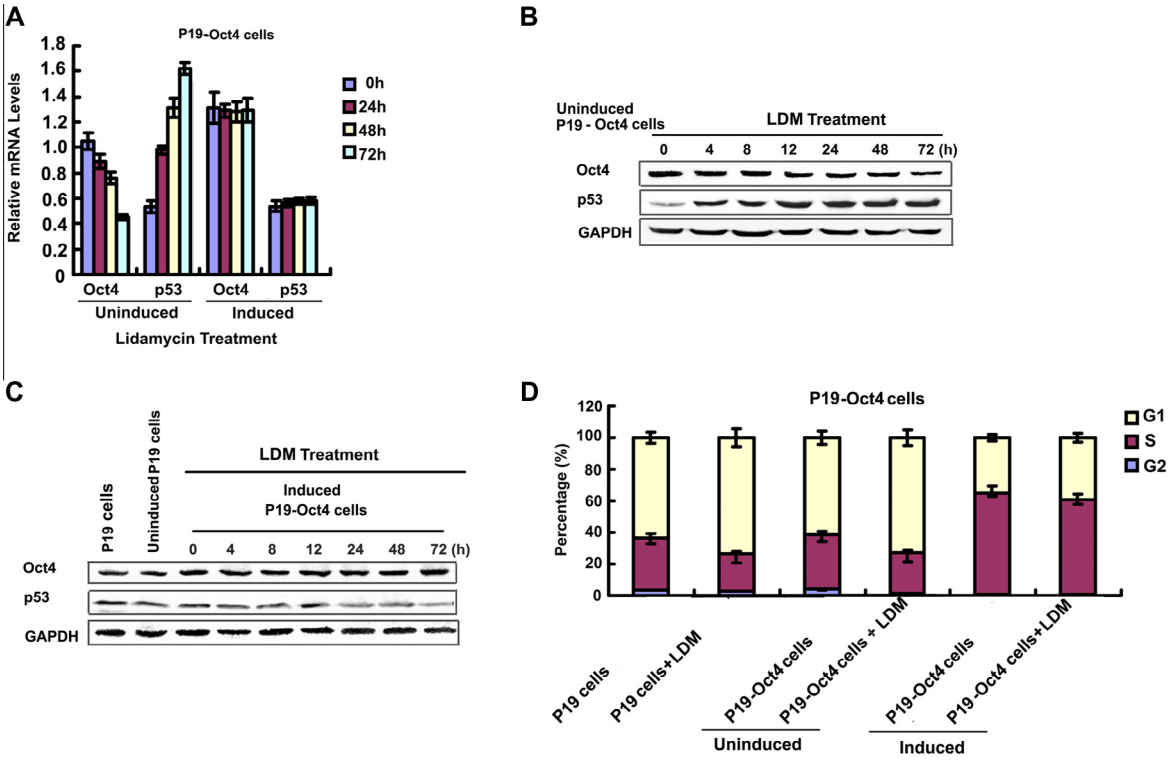

Fig. 2. Ectopical expression of Oct4 blocked activation of p53 in mouse EC P19 cells. The cells were treated with low-dose LDM in 72 h. Data represent mean ± SD (n = 3). (A) The transcripts of Oct4 and p53 were examined by real-time RT-PCR. (B) The proteins of Oct4 and p53 were examined in uninduced P19-Oct4 cells by Western blot. (C) The proteins of Oct4 and p53 were examined in induced P19-Oct4 cells by Western blot. (D) Ectopical expression of Oct4 promoted cell cycle progress.

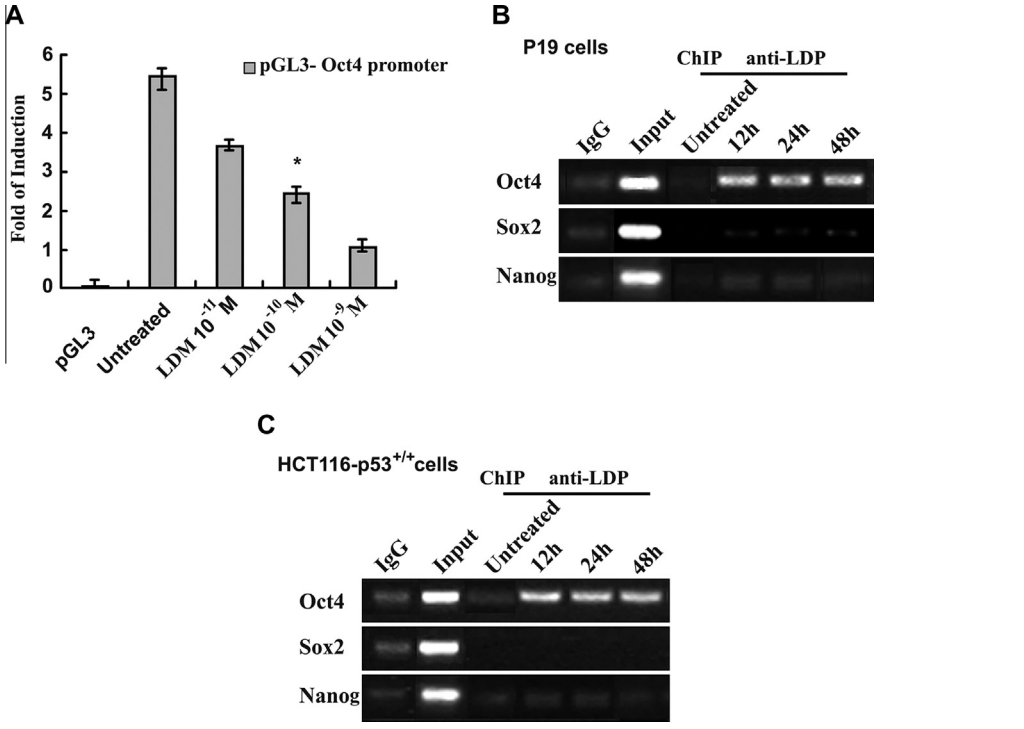

Fig. 3. LDM downregulated Oct4. (A) LDM inhibited Oct4 transcriptional activity in a dose-dependent manner. Oct4 promoter-driven luciferase activities were measured in 293 cells 48 h after LDM treatment with indicated dose. Data represent mean ± SD (n = 3), $^{*}p < 0.01$. ChIP analysis of LDM binding to Oct4 promoter in (B). Mouse EC P19 cells. (C) HCT116-p53$^{+/+}$cells. After LDM treatment at the indicated time, ChIP assay was performed using an antibody against LDP, followed by PCR with primers amplifying the promoter regions of *Oct4*, *Sox2*, and *Nanog*. PCR products were separated by agarose gel electrophoresis.

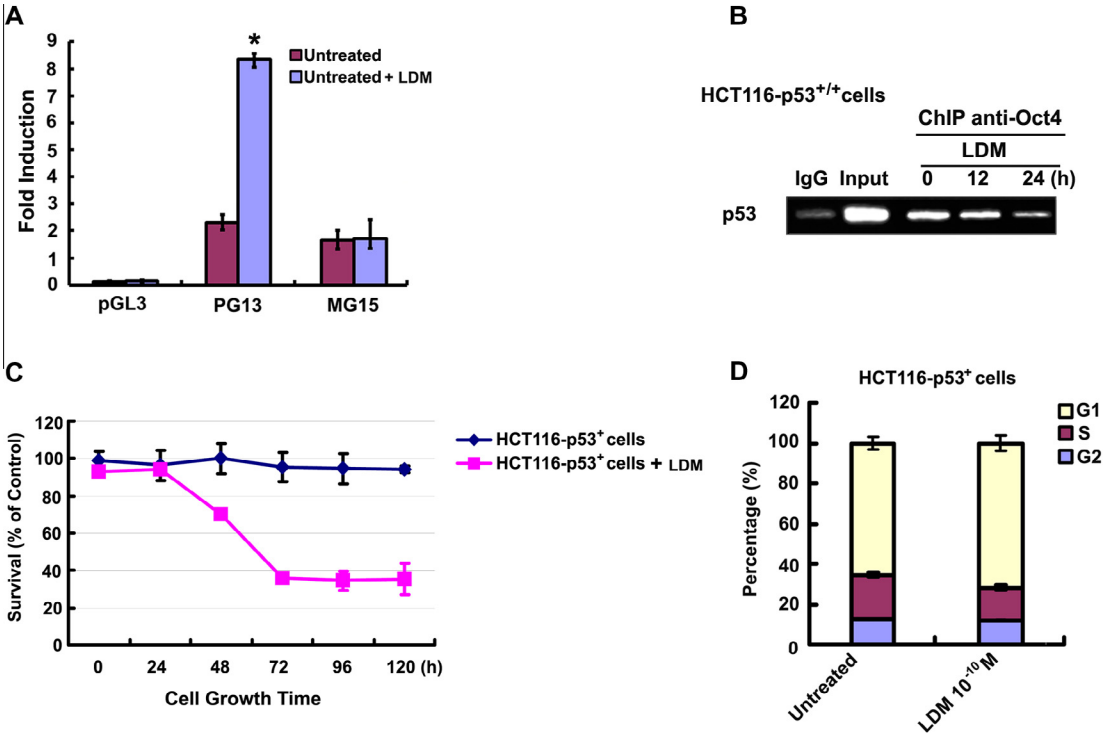

Fig. 4. LDM inhibited p53 transcriptional activity through the downregulation of Oct4 expression. (A) p53 transcriptional activity was measured by PG13-luciferase reporter assay in 293T cells. Data show normalized values by TK-*Renilla* luciferase. Data represent mean ± SD ($n = 3$), $^{*}p < 0.01$. (B) ChIP assay was performed by using an antibody against Oct4 in HCT116-p53$^{+/+}$ cells. The amount of precipitated DNA with control (−) or anti-Oct4 (+) antibody was examined by PCR with primers amplifying the promoter regions of *p53*. (C) Low-dose LDM inhibited growth of HCT116-p53$^{+/+}$ cells. (D) Inhibitory effect of low-dose LDM on cell cycle distribution was determined in HCT116-p53$^{+/+}$ cells by FACS analysis. The experiment was repeated 3 times.

expression in mouse EC P19 and human cancer cell HCT116-p53$^{+/+}$. It is previously found that p53 directly regulates Oct4 expression [22], which is in agreement with our results. Therefore, there may exist a reciprocal regulation loop between Oct4 and p53. We previously reported that LDM inhibits cell growth and induces differentiation of P19 cells through downregulation of Oct4 [18,19]. Therefore, we speculated that LDM probably regulates Oct4 expression at transcriptional level. As shown in Fig. 3, LDM regulates Oct4 transcription activity through the direct binding to the Oct4 promoter by LDP (apoprotein of LDM). LDM molecule contains an apoprotein and a chromophore. The former is served for a protecting protein and the latter manifests the ability to damage DNA strands by inducing sequence-specific double-strand DNA cleavage [1,2]. However, it is demonstrated that LDP has higher binding capacity in various human cancer tissues than in the corresponding normal tissues. And more importantly, when LDP was fused with a ligand oligopeptide to epithelial growth factor receptor (EGFR)/CD13, or CD20, the fusion proteins showed higher affinity and extremely potent cytotoxicity to targeted-cancer cells [26,27]. Our result demonstrated that LDP directly binds to the promoter of transcription factor Oct4. Therefore, LDP also served as a vehicle to deliver the enediyen chromophore to specific DNA sequence in cancer cells. Taken together, our results suggest that LDM activates p53 pathway through negative regulation of Oct4 at transcriptional level.

In conclusion, although there is a complicated network to regulate p53 transcriptional activity, our study reveals a novel mechanism for regulation of p53 transcription through suppression of Oct4 expression by a low-dose LDM. Because ES-like gene Oct4 is a promising target for cancer stem cell in cancer therapy [28], therefore, our findings suggest that LDM may be used for cancer treatment by targeting cancer stem cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.03.082.

References

[1] L. Yu, I.H. Goldberg, P.C. Dedon, Enediyne-mediated DNA damage in nuclei is modulated at the level of the nucleosome, J. Biol. Chem. 269 (1994) 4144–4151.
[2] Y.J. Xu, Y.S. Zhen, I.H. Goldberg, C1027 chromophore, a potent new enediyne antitumor antibiotic, induces sequence-specific double-strand DNA cleavage, Biochemistry 33 (1994) 5947–5954.
[3] L. Cai, H. Chen, Q. Miao, S. Wu, Y. Shang, Y. Zhen, Binding capability of the enediyne-associated apoprotein to human tumors and constitution of a ligand oligopeptide-integrated protein, J. Biotechnol. 144 (2009) 142–150.
[4] D.R. Kennedy, T.A. Beerman, The radiomimetic enediyne C-1027 induces unusual DNA damage responses to double-strand breaks, Biochemistry 45 (2006) 3747–3754.

[5] X. Liu, H. He, Y. Feng, M. Zhang, K. Ren, R. Shao, Difference of cell cycle arrests induced by lidamycin in human breast cancer cells, Anticancer Drugs 17 (2006) 173–179.
[6] Q.Y. He, Y.Y. Liang, D.S. Wang, D.D. Li, Characteristics of mitotic cell death induced by enediyne antibiotic lidamycin in human epithelial tumor cells, Int. J. Oncol. 20 (2002) 261–266.
[7] C. Dai, W. Gu, P53 post-translational modification: deregulated in tumorigenesis, Trends Mol. Med. 16 (2010) 528–536.
[8] E.S. Helton, X. Chen, P53 modulation of the DNA damage response, J. Cell. Biochem. 100 (2007) 883–896.
[9] T. Lin, C. Chao, S. Saito, S.J. Mazur, M.E. Murphy, E. Appella, Y. Xu, P53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression, Nat. Cell Biol. 7 (2005) 165–171.
[10] P.A. Campbell, C. Perez-Iratxeta, M.A. Andrade-Navarro, M.A. Rudnicki, Oct4 targets regulatory nodes to modulate stem cell function, PLoS One 2 (2007) e553.
[11] J. Nichols, B. Zevnik, K. Anastassiadis, H. Niwa, D. Klewe-Nebenius, I. Chambers, H. Scholer, A. Smith, Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4, Cell 95 (1998) 379–391.
[12] M. Pesce, H.R. Scholer, Oct-4: control of totipotency and germline determination, Mol. Reprod. Dev. 55 (2000) 452–457.
[13] J. Lee, Y. Go, I. Kang, Y.M. Han, J. Kim, Oct-4 controls cell-cycle progression of embryonic stem cells, Biochem. J. 426 (2010) 171–181.
[14] I. Ben-Porath, M.W. Thomson, V.J. Carey, R. Ge, G.W. Bell, A. Regev, R.A. Weinberg, An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors, Nat. Genet. 40 (2008) 499–507.
[15] S.H. Chiou, M.L. Wang, Y.T. Chou, C.J. Chen, C.F. Hong, W.J. Hsieh, H.T. Chang, Y.S. Chen, T.W. Lin, H.S. Hsu, C.W. Wu, Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation, Cancer Res. 70 (2010) 10433–10444.
[16] S.M. Kumar, S. Liu, H. Lu, H. Zhang, P.J. Zhang, P.A. Gimotty, M. Guerra, W. Guo, X. Xu, Acquired cancer stem cell phenotypes through Oct4-mediated dedifferentiation, Oncogene 31 (2012) 4898–4911.
[17] Y. Wu, S. Liu, H. Xin, J. Jiang, E. Younglai, S. Sun, H. Wang, Up-regulation of microRNA-145 promotes differentiation by repressing OCT4 in human endometrial adenocarcinoma cells, Cancer 117 (2011) 3989–3998.
[18] H.Y. Zhen, Q.H. He, Y.Z. Zhen, S.L. Wang, Y.N. Liu, W.H. Wu, X.Y. Zhang, A.L. Lu, L. Shen, Inhibition of mouse embryonic carcinoma cell growth by lidamycin through down-regulation of embryonic stem cell-like genes Oct4, Sox2 and Myc, Invest. New Drugs 29 (2011) 1188–1197.
[19] H.Y. Zhen, Q.H. He, Y. Li, J. Zhou, C. Yao, Y.N. Liu, L.J. Ma, Lidamycin induces neural differentiation of mouse embryonic carcinoma cells through down-regulation of transcription factor Oct4, Biochem. Biophys. Res. Commun. 421 (2012) 44–50.
[20] I.L. Botchkina, R.A. Rowehl, D.E. Rivadeneira, M.S. Karpeh Jr., H. Crawford, A. Dufour, J. Ju, Y. Wang, Y. Leyfman, G.I. Botchkina, Phenotypic subpopulations of metastatic colon cancer stem cells: genomic analysis, Cancer Genomics Proteomics 6 (2009) 19–29.
[21] G.I. Botchkina, E.S. Zuniga, M. Das, Y. Wang, H. Wang, S. Zhu, A.G. Savitt, R.A. Rowehl, Y. Leyfman, J. Ju, K. Shroyer, I. Ojima, New-generation taxoid SB-T-1214 inhibits stem cell-related gene expression in 3D cancer spheroids induced by purified colon tumor-initiating cells, Mol. Cancer 9 (2010) 192.
[22] H. Qin, T. Yu, T. Qing, Y. Liu, Y. Zhao, J. Cai, J. Li, Z. Song, X. Qu, P. Zhou, J. Wu, M. Ding, H. Deng, Regulation of apoptosis and differentiation by p53 in human embryonic stem cells, J. Biol. Chem. 282 (2007) 5842–5852.
[23] 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.
[24] H. Niwa, J. Miyazaki, A.G. Smith, Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells, Nat. Genet. 24 (2000) 372–376.
[25] A.K. Jain, K. Allton, M. Iacovino, E. Mahen, R.J. Milczarek, T.P. Zwaka, M. Kyba, M.C. Barton, P53 regulates cell cycle and microRNAs to promote differentiation of human embryonic stem cells, PLoS Biol. 10 (2012) e1001268.
[26] W. Sheng, Y. Shang, L. Li, Y. Zhen, An EGFR/CD13 bispecific fusion protein and its enediyne-energized analog show potent antitumor activity, Anticancer Drugs 25 (2014) 82–91.
[27] W. Sheng, Y. Shang, Q. Miao, Y. Li, Y. Zhen, Antitumor efficacy of the scFv-based fusion protein and its enediyne-energized analogue directed against epidermal growth factor receptor, Anticancer Drugs 23 (2012) 406–416.
[28] J.E. Trosko, From adult stem cells to cancer stem cells: Oct-4 Gene, cell–cell communication, and hormones during tumor promotion, Ann. N. Y. Acad. Sci. 1089 (2006) 36–58.