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RESEARCH ARTICLE

Methylation-mediated regulation of E2F1 in DNA damage-induced cell death

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Abstract

E2F1 promotes DNA damage-induced apoptosis and the post-translational modifications of E2F1 play an important role in the regulation of E2F1-mediated cell death. Here, we found that Set9 and LSD1 regulate E2F1-mediated apoptosis upon DNA damage. Set9 methylates E2F1 at lysine 185, a conserved residue in the DNA-binding domain of E2F family proteins. The methylation of E2F1 by Set9 leads to the stabilization of E2F1 and up-regulation of its proapoptotic target genes p73 and Bim, and thereby induces E2F1-mediated apoptosis in response to genotoxic agents. We also found that LSD1 demethylates E2F1 at lysine 185 and reduces E2F1-mediated cell death. The identification of the methylation/demethylation of E2F1 by Set9/LSD1 suggests that E2F1 is dynamically regulated by epigenetic enzymes in response to DNA damage.

Keywords: Methylation, signal transduction, apoptosis

Introduction

E2F family of transcription factors plays an essential role in both cell cycle regulation and cell death (1). During cell entry from G1 to S phase, E2F1 is activated once released from the Rb-E2F1 repressor complex and a series of genes responsible for transition into S phase are transcriptionally regulated (2). On the other hand, E2F1 also promotes apoptosis in response to DNA damage, and induces the expression of a set of proapoptotic genes including *p73* and *Bim* (3,4).

Many post-translational modifications have been shown to regulate E2F1's activity. For example, serine 31 phosphorylation of E2F1 by ATM/ATR stabilizes the E2F1 protein and promotes DNA damage-induced cell death (5). Acetylation of lysine 117, 120, 125 in E2F1 by PCAF can also up-regulate E2F1's activity in the process of cell death (6).

Recently, the regulation of lysine methylation by methyltransferases and demethylases has been demonstrated to play critical roles not only in the regulation of histone proteins but also in non-histone proteins (7). Set9, originally identified as a lysine methyltransferase of histone H3 lysine 4 (H3K4), regulates the biological function of non-histone proteins such as TAF10 (8), p53 (9), and DNMT1 (10) through lysine methylation. LSD1 is the first identified histone demethylase (11), but also regulates p53 and DNMT1 as well as the other non-histone proteins (12). For example, LSD1 decreases p53 transactivation through demethylating p53 protein at lysine 370 (13). LSD1 is also involved in the regulation of cell proliferation and LSD1 knockout mice are embryonic lethal (14). Taken together, the dynamic methylation and demethylation of non-histone proteins could be an important regulatory step in the control of diverse biological processes.

Here, we identified that Set9 methylates E2F1 at K185 and the methylation stabilizes E2F1 protein, thereby enhancing E2F1-induced apoptosis upon DNA damage. On the other hand, LSD1 demethylates E2F1 at lysine 185 and inhibits DNA damage-induced cell death. Our findings uncover a new mode of regulation of E2F1 and the process of apoptosis.

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Experimental procedures

Cell culture and transfections

U2OS, HCT116 p53$^{-/-}$, and 293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 5% CO_2 concentration. Cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol.

RNAi plasmids

ShLuc (TR3002) or shSet9 (TI306985) plasmids were purchased from Origene, Rockville, MD. ShE2F1 target sequenceis:GTCACGCTATGAGACCTCA.ShLSD1plasmid was a gift from Dr. Y. Shi (Harvard Medical School, Boston, MA).

Immunoprecipitation and western blotting

Immunoprecipitation and western blots were performed as described (15).

In vitro methylation assay

The 2 μg of the recombinant full-length GST-E2F1 protein or His-E2F1 DBD (DNA-binding domain) were incubated with 1.5 μg recombinant Set9 in the presence of 2 μCi ^{3}H-*S*-adenosyl methionine (SAM; PerkinElmer, Waltham, MA) or 0.1 μM SAM (Sigma, St. Louis, MO) in the reaction buffer (50 mM Tris–Cl pH 8.5, 5 mM $MgCl_2$, 0.8 mM dithiothreitol [DTT]) at 30°C for 1 h. The reaction products were loaded on SDS-PAGE, followed by autoradiography or western blotting with the K185me-E2F1-specific antibody.

In vitro demethylation assay

E2F1 protein was first methylated *in vitro* by Set9, then 2 μg of methylated products were incubated with 5 μg recombinant LSD1 in the demethylation assay buffer (50 mM HEPES/NaOH pH 8.0, 25% glycerol) at 37°C for 2 h. After the reaction, mixtures were subjected to electrophoresis on SDS-PAGE followed by western blotting or autoradiography.

Electrophoretic mobility shift assay

The 3′-biotin-labeled DNA probe was prepared by annealing complementary oligonucleotides synthesized by Invitrogen. The probe sequence is as described (16): GCGTTTGGCGCTTTGGCGCTTTGGCGC. Electrophoretic mobility shift assay (EMSA) was carried out using the LightShift Chemiluminescent EMSA Kit according to the manufacturer's protocol (Pierce, Rockford, IL).

Luciferase reporter assay

Bim-Luc was kindly provided by Dr. Q. Yu (Genome Institute of Singapore, Singapore). Luciferase assays were carried out using the Dual-Luciferase assay kit (Promega, Madison, WI).

Primers for real-time PCR:

p73 forward: AACGCTGCCCCAACCACGAG
p73 backward: GCCGGTTCATGCCCCCTACA
GAPDH forward: GAAGGTGAAGGTCGGAGTC
GAPDH backward: GAAGATGGTGATGGGATTTC.

Cell apoptosis assay

Cells were stained with Annexin V-APC (BD) followed by flow cytometry. For transient transfection, GFP was

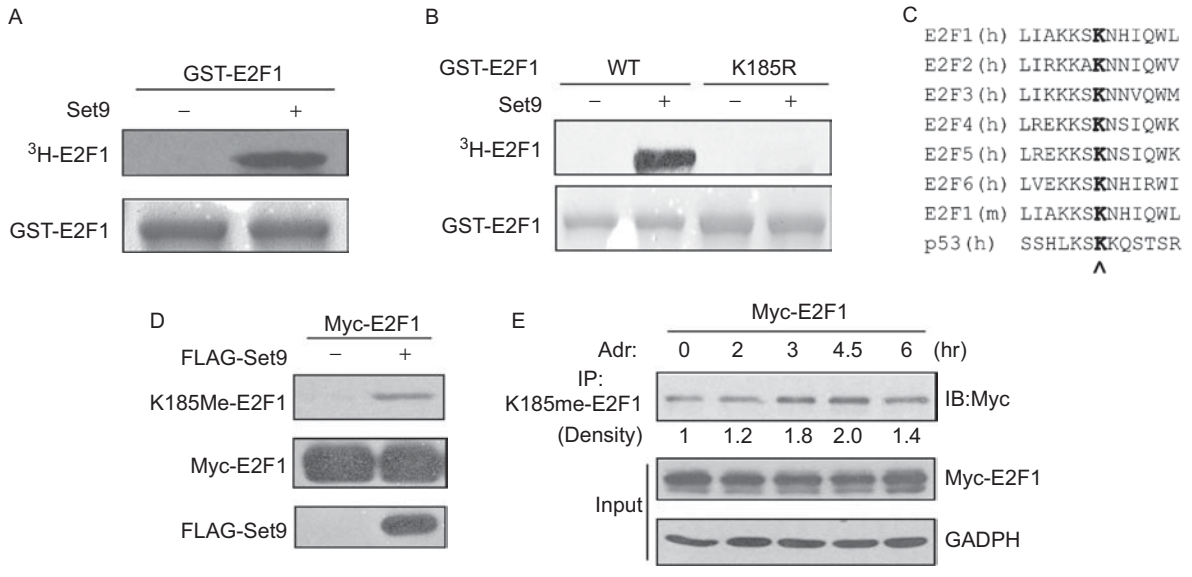

Figure 1. Set9 methylates E2F1 at K185 *in vitro* and *in vivo*. (A) Methylation assays were performed by incubating the recombinant Set9 and GST tagged E2F1 in the presence of ^{3}H-SAM. The reaction products were loaded on SDS-PAGE gels and methylation of E2F1 was assessed by autoradiography. Set9 methylates E2F1 *in vitro*. (B) *In vitro* methylation assay performed as in A. E2F1 K185 is the site methylated by Set9. (C) Comparison of the sequences surrounding the methylation site in the E2F family and p53. The methylated lysines are indicated in bold. (D) Lysates of 293T cells transfected with plasmids encoding Myc-E2F1 together with FLAG-Set9 or the control vector were immunoblotted with the methylation-specific (K185me) E2F1 antibody. Set9 methylates E2F1 at K185 *in vivo*. (E) K185me-E2F1-immunoprecipitates from cells transfected with Myc-E2F1 and treated with adriamycin for 0–6 h were immunoblotted with anti-Myc antibody.

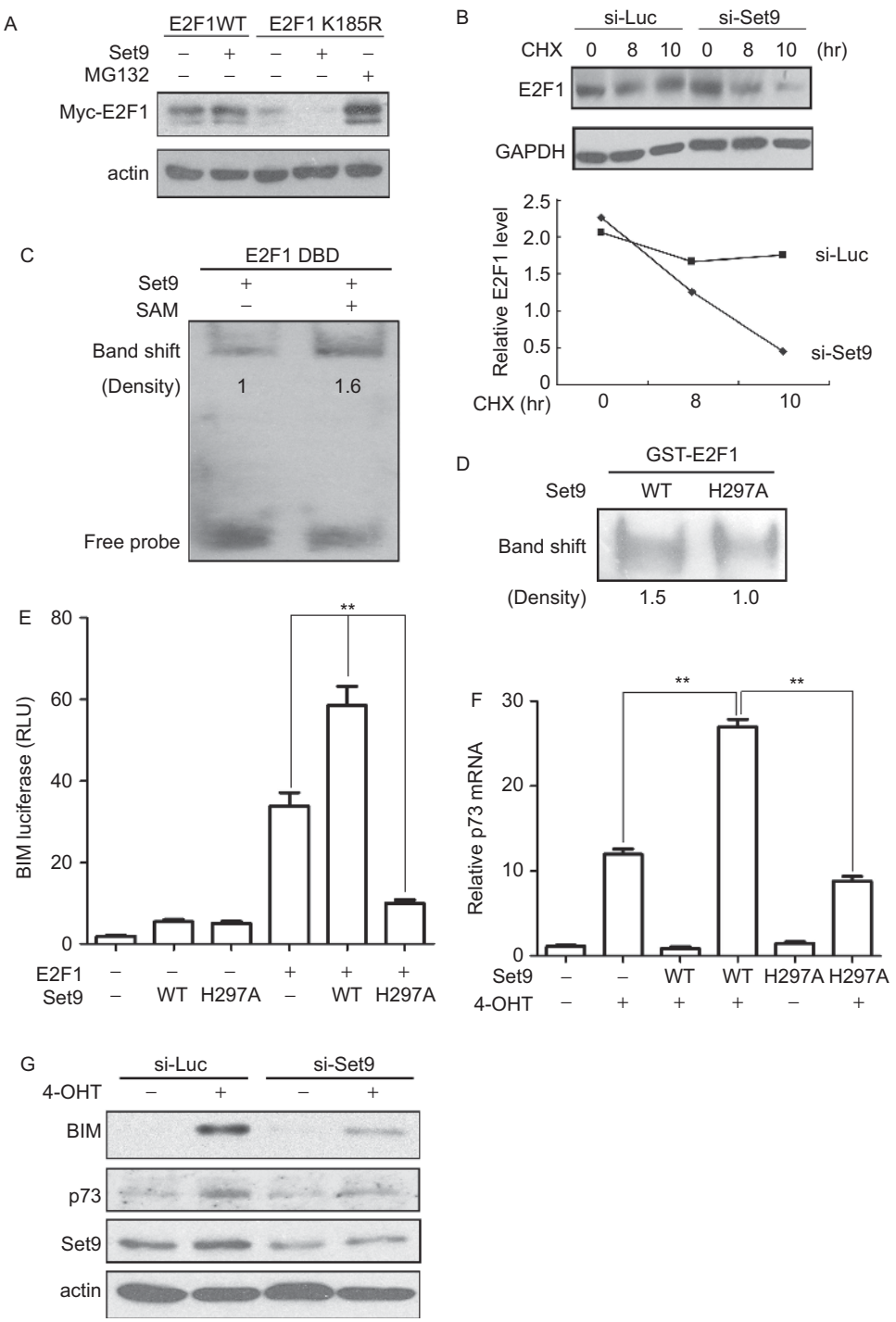

Figure 2. Set9 stabilizes E2F1 protein and increases E2F1 function. (A) Lysates of 293T cells transfected E2F1 WT or E2F1 K185R together with Set9 or the control vector and treated with or without MG132 were immunoblotted with the Myc or actin antibody, the latter to serve as a loading control. Set9 can stabilize E2F1 protein. (B) Lysates of HCT116 $p53^{-/-}$ cells stably transfected with shSet9 or the control shLuc and treated with cycloheximide (CHX) for the indicated hours were immunoblotted with the E2F1 antibody. Quantification of relative E2F1 protein levels shows Set9 knockdown destabilizes E2F1. (C) The electrophoretic mobility shift assay (EMSA) was performed using recombinant His-tagged E2F1 DBD (amino acids 92–195). (D) *In vitro* methylation was carried out by incubating the recombinant full-length GST-E2F1 protein with Set9 WT followed by electrophoretic mobility shift assay (EMSA) analysis as in (C). (E) 293T cells were transfected with plasmids encoding E2F1, Set9 WT, or Set9 H297A together with plasmids encoding the Bim-luciferase reporter gene and tk-renilla reporter. Shown are mean + SE of normalized firefly/renilla luciferase values. Set9 significantly increases E2F1-dependent Bim-luciferase reporter gene expression (*t*-test, $P<0.01$, $n=3$). (F) U2OS cells stably expressing inducible ER-E2F1 were transfected with Set9 WT or H297A. P73 mRNA level was analyzed by quantitative real-time PCR. Shown are the relative values normalized to GAPDH (*t*-test, $P<0.01$, $n=3$). (G) Cos7 cells stably expressing inducible ER-E2F1 were transfected with shLuc or shSet9 in the presence or absence of 4-OHT. Cell lysates were immunoblotted with the indicated antibody to BIM, p73, Set9, or actin. Set9 knockdown reduces E2F1-induced BIM and p73 expression.

co-transfected with the indicated plasmids and GFP-positive cells were sorted by FACS and analyzed for cell death.

Results

Set9 methylates E2F1 in vitro and in vivo

Set9 is the first identified lysine methyltransferase that can methylate non-histone proteins such as TAF10, p53, and DNMT1 as well as many other proteins (8–10). To test whether E2F1 is a substrate for Set9, we expressed recombinant E2F1 together with Set9 protein and performed a methylation assay *in vitro*. Set9 shows strong methyltransferase activity toward E2F1 (Figure 1A). To further delineate the methylation site, we mutated lysine 185, which is in a conserved Set9 methylation motif (Figure 1C), to arginine. As expected, K185R mutation abolished Set9-mediated E2F1 methylation (Figure 1B and 1C). Set9 is known as a monomethyltransferase on non-histone substrates (17). Therefore, we generated a polyclonal antibody that specifically recognized the monomethylated E2F1 at K185. Using this antibody, we found that overexpressed Set9 methylated E2F1 in 293T cells (Figure 1D). It has been shown that Set9 methylates p53 and activates p53 transcriptional activity in response to DNA damage (18). We next examined E2F1 methylation levels in cells at different time points after treatment with adriamycin, a drug that promotes DNA damage. The methylation levels of E2F1 gradually increased during the drug treatment (Figure 1E), suggesting that DNA damage induces the methylation of E2F1.

Methylation stabilizes E2F1 and augments its transcriptional activity

It has been shown that protein methylation plays an important role in protein stability (19). In our experiments, we found that methylation by Set9 increased the protein levels of E2F1 wild type (WT), but that Set9 failed to stabilize the mutant E2F1 (K185R) (Figure 2A). Furthermore, the protein levels of E2F1 K185R are much lower than that of E2F1 WT and the decrease can be rescued by treating with proteasome inhibitor MG132 (Figure 2A). Knockdown of Set9 dramatically decreased E2F1's half-life (Figure 2B). Taken together, E2F1 methylation at K185 by Set9 stabilizes E2F1 through inhibiting proteasome-dependent degradation.

K185 is a conserved site in the DNA-binding domain (DBD) of all the E2F family members. Therefore, we tested whether methylation affects E2F1's DNA-binding activity. In these experiments, E2F1 DBD (amino acids 92–195) or recombinant full-length E2F1 was first methylated *in vitro* and then followed by the EMSA. We found that methylation of E2F1 increased its DNA-binding activity (Figure 2C and 2D). Next, we examined whether methylation regulates E2F1's transactivity in cells. *Bim* is one of the proapoptotic Bcl-2 family members and known to be transcriptionally regulated by E2F1 (20). We found that overexpression of Set9 significantly increased the expression of *Bim* promoter-driven luciferase and Set9 H297A, which lacks methyltransferase activity, failed to induce *Bim* expression (Figure 2E). WT Set9, but not H297A Set9, also drastically induced E2F1-mediated expression of *p73*, another putative target of E2F1 during DNA damage-induced

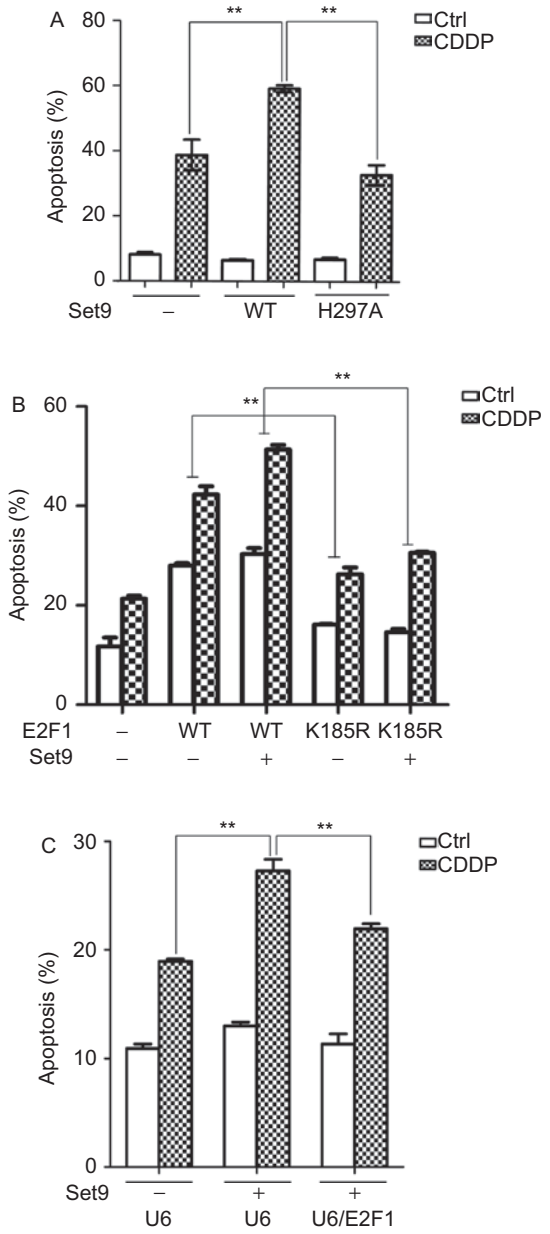

Figure 3. Methylation of E2F1 enhances E2F1-dependent cell death in U2OS cells. (A) U2OS cells stably expressing Set9 WT or H297A mutant were treated with CDDP (20 μm) or vehicle for 36 h. Cells death was analyzed with Annexin V staining followed by FACS (*t*-test, $P < 0.01$, $n = 3$). Set9 WT, not H297A, increased CDDP-induced cell death. (B) U2OS cells were transfected with GFP and E2F1 WT or K185R together with Set9 WT or H297A. Forty-eight hours after transfection, cells were treated with CDDP (20 μm) for 24 h. Cells death was assessed as in (B) after GFP sorting (*t*-test, $P < 0.01$, $n = 3$). K185 methylation of E2F1 is critical for Set9-mediated apoptosis upon DNA damage. (C) U2OS cells were co-transfected with Set9 and the E2F1 shRNA plasmid (U6/E2F1) or control vector (U6). Cells were treated with CDDP (20 μm) for 24 h and apoptosis was analyzed as in (B) (*t*-test, $P < 0.01$, $n = 3$). Set9 mediates apoptosis through E2F1.

apoptosis (Figure 2F). In other experiments, Set9 knockdown dramatically reduced E2F1-mediated expression of *Bim* and *p73* (Figure 2G). These findings suggest that Set9 stabilizes E2F1 protein and increases its activity through methylation of E2F1 at lysine 185.

Set9 enhances E2F1-dependent cell apoptosis

We next examined the role of methylation in E2F1-dependent apoptosis. Consistent with previous findings (9), we observed that Set9 WT, but not the Set9 H297A, increased apoptosis in U2OS cells induced by CDDP (cisplatin) treatment (Figure 3A). Expression of WT E2F1 also induced apoptosis, although K185R mutant E2F1 failed to increase cell death in U2OS cells (Figure 3B). Overexpression of Set9 significantly increased E2F1 WT- but not K185R-mediated apoptosis in response to DNA damage (Figure 3B), suggesting methylation at K185 is critical for E2F1's proapoptotic activity upon DNA damage. To further test whether E2F1 mediates Set9-induced cell death in response to DNA damage, we knocked down E2F1 by using a shE2F1 construct in Set9 overexpressed cells. E2F1 deficiency led to a reduction in Set9-mediated cell death in the presence of CDDP (Figure 3C). Taken together, the methylation of E2F1 by Set9 increased cell death induced by DNA damage.

It has been reported that Set9 increases DNA damage-induced cell death through methylating p53 and up-regulating p53 transactivity (9). However, loss of function of p53 is found in a variety of cancer cell lines, and E2F1 has been shown to induce apoptosis in a p53-independent manner (3,21,22). We therefore asked whether Set9 could enhance E2F1-mediated apoptosis in the absence of p53. As expected, overexpression of Set9 promotes DNA damage-induced cell death in HCT116 $p53^{-/-}$ cells (Figure 4A), and Set9 knockdown rendered cells resistant to CDDP-induced apoptosis (Figure 4B). Western blotting also showed that Set9 WT, not H297A Set9, increased the protein expression of E2F1's targets *p73* and *Bim* in HCT116 $p53^{-/-}$ cells after DNA damage (Figure 4C). Collectively, Set9 increases the proapoptotic function of E2F1 independently of p53.

To further characterize the function of Set9 in cell growth, we performed a colony-formation assay and we found that Set9 WT but not the H297A mutant inhibited cell growth in HCT116 $p53^{-/-}$ cells (Figure 4D), although knockdown of Set9 led to increased cell proliferation

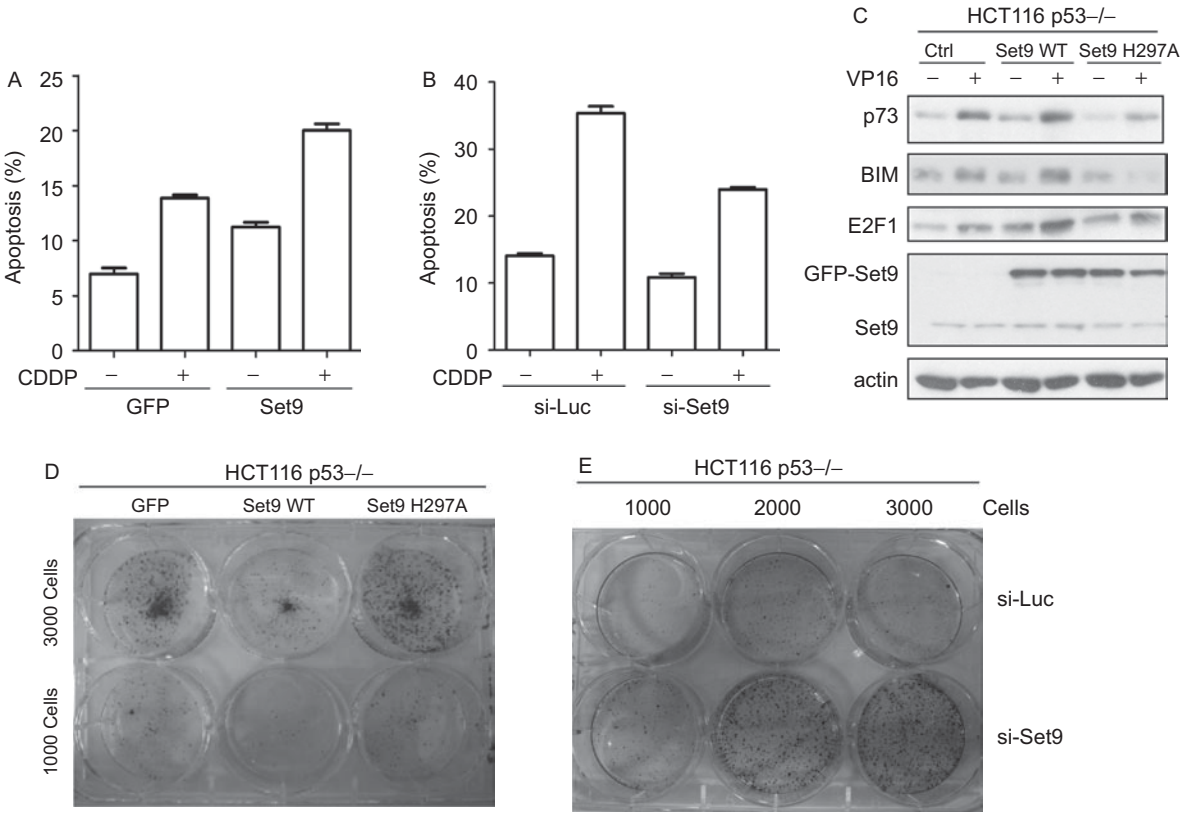

Figure 4. Set9 increases apoptosis in HCT116 p53 null cells. (A) HCT116 $p53^{-/-}$ cells stably expressing GFP or GFP-Set9 WT were treated with or without 50 μm CDDP for 24 h. Cell death was analyzed after Annexin V staining followed by FACS. Set9 significantly enhances apoptosis in HCT116 $p53^{-/-}$ cells (*t*-test, $P<0.01$, $n=3$). (B) HCT116 $p53^{-/-}$ cells stably transfected with shLuc or shSet9 were treated with or without CDDP. Cell death was analyzed as in (A). Set9 knockdown significantly reduces apoptosis in HCT116 $p53^{-/-}$ cells (*t*-test, $P<0.01$, $n=3$). (C) Lysates of HCT116 $p53^{-/-}$ cells stably transfected with GFP-Set9 WT, GFP-Set9 H297A, or the control vector and treated with or without 25 μm VP16 were immunoblotted with the indicated antibodies. Enzyme inactive (H297A) Set9 induces less expression of E2F1 target genes compared with Set9 WT. (D) Colony-formation assay was performed in HCT116 $p53^{-/-}$ cells stably transfected with GFP-Set9 WT, GFP-Set9 H297A, or the vector control. Set9 expression inhibits colony formation in the absence of p53. (E) HCT116 $p53^{-/-}$ cells were stably transfected with shLuc or shSet9 and colony-formation assay was performed as in (D). Set9 knockdown increases colony formation.

(Figure 4E). Taken together, Set9 inhibits cell growth and enhances E2F1 proapoptotic ability independently of p53.

LSD1 demethylates E2F1 and knockdown of LSD1 increased CDDP-induced cell death

Recently, LSD1 has been shown to demethylate non-histone proteins including p53 and DNMT1 (13,20). In our experiments, we found that LSD1 physically interacted with E2F1 (Figure 5A). We then examined the possibility of E2F1 demethylation by LSD1. We observed that LSD1 removed K185 methylation of E2F1 *in vitro* (Figure 5B and 5C). We also found that ectopically expressed LSD1 decreased E2F1 methylation levels in 293T cells (Figure 5D), although LSD1 knockdown significantly enhanced E2F1 methylation at K185 in cells (Figure 5E). Similar to the function of LSD1 in $p53^{+/+}$ cells (7), knockdown of LSD1 increased DNA damage-induced apoptosis in HCT116 $p53^{-/-}$ cells (Figure 5F). Taken together, LSD1 inhibits CDDP-induced apoptosis through demethylating E2F1 at lysine 185.

Discussion

In this study, we have uncovered a novel regulatory mechanism of E2F1 in DNA damage-induced cell death. Our findings indicate that E2F1 is methylated at K185 and stabilized by Set9, and Set9-mediated methylation of E2F1 enhances the proapoptotic ability of cells upon DNA damage in both p53 WT U2OS and p53 null HCT116 cells. We also found that LSD1 inhibits E2F1-mediated cell death in the presence of DNA damage by demethylating E2F1 at K185.

As a methyltransferase, Set9 methylates histone H3K4 *in vitro*, but the recombinant Set9 fails to target nucleosome for methylation (23). There have been several non-histone proteins reported to the date as the substrates for Set9 including TAF10, p53, estrogen receptor α (ERα), RelA, PCAF, and DNMT1, indicating Set9 may be an important methyltransferase for non-histone proteins. It has also been shown that Set9 regulates substrates through increasing protein stability (p53, ERα) or decreasing protein stability (DNMT1, RelA) (9,10,24–26). However, the molecular mechanism underlying Set9-mediated protein stability needs to be further investigated.

When this manuscript was under preparation, Kontaki and Talianidis have reported that in H1299 p53-deficient tumor cells, Set9 and LSD1 regulate DNA damage-induced cell death in a manner opposite to that in p53 WT cells, through the regulation of E2F1 stabilization (27). In this article, the authors suggested that Set9-mediated methylation of E2F1 at lysine 185 inhibits

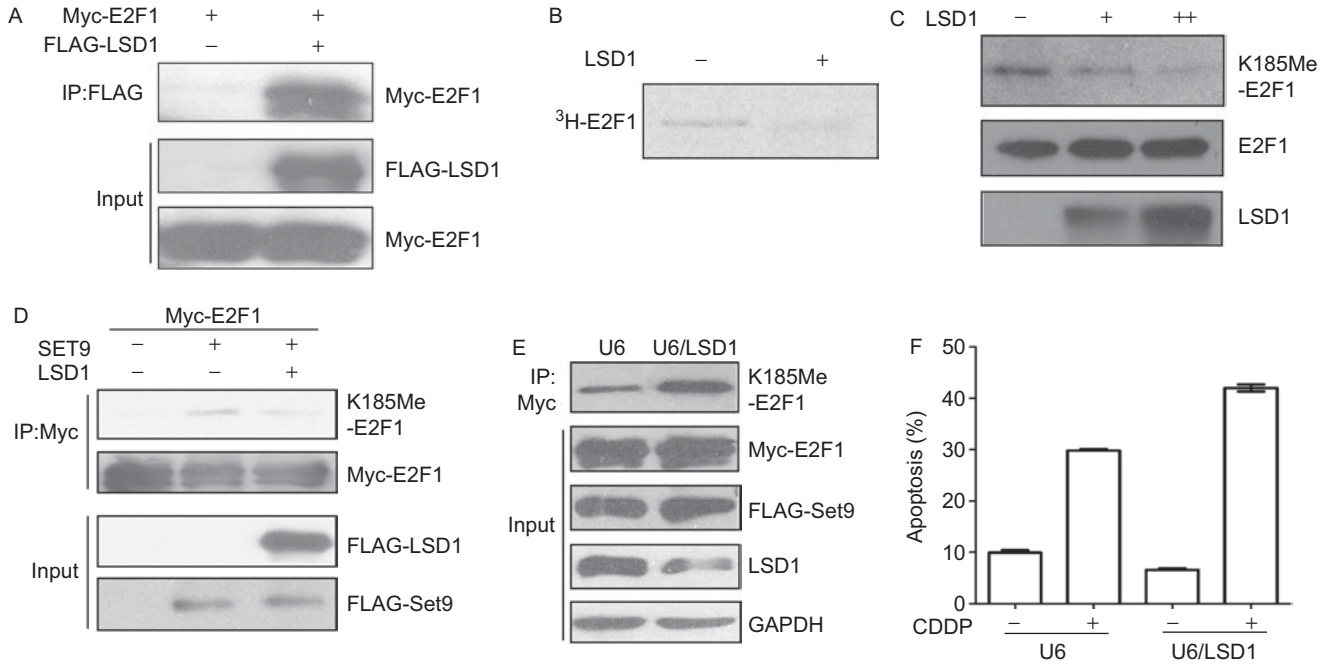

Figure 5. LSD1 demethylates E2F1 and LSD1 knockdown enhances CDDP-induced apoptosis. (A) FLAG-immunoprecipitates of 293T cells transfected with Myc-E2F1 and FLAG-LSD1 or the control vector were immunoblotted with the Myc antibody. E2F1 interacts with LSD1 *in vivo*. (B) The recombinant GST-E2F1 protein was methylated by Set9 in the presence of ^{3}H-SAM as in Figure 1A, and incubated with His-LSD1 in the demethylation buffer. The methylation of E2F1 was detected by autoradiograph. (C) *In vitro* demethylation assay was performed as in (B) and the methylation of E2F1 was analyzed by using the K185me-E2F1-specific antibody. LSD1 demethylates E2F1 *in vitro*. (D) Myc-immunoprecipitates of 293T cells transfected with Myc-E2F1 together with Set9 and LSD1 were immunoblotted with the K185me-E2F1 or Myc antibody. LSD1 can demethylate E2F1 *in vivo*. (E) Cells were transfected with Myc-E2F1 and Set9 together with the shLSD1 (U6/LSD1) plasmid or the control vector (U6). The Myc-immunoprecipitates were blotted with the K185me-E2F1 antibody. LSD1 knockdown increases E2F1 methylation. (F) HCT116 $p53^{-/-}$ cells stably transfected with the U6/LSD1 plasmid or control vector were treated with or without CDDP. Cell death was analyzed as in Figure 4B. Knockdown of LSD1 significantly increases cell death in HCT116 $p53^{-/-}$ cells (t-test, $P < 0.01$, $n = 3$).

acetylation and phosphorylation of E2F1 and promotes its ubiquitination and degradation, whereas LSD1 stabilizes E2F1 by demethylation. In our findings, we also found that Set9 methylates E2F1 at lysine 185 and LSD1 demethylates it. However, in contrast to their results, we observed that Set9-dependent methylation at lysine 185 stabilized E2F1 protein and enhanced E2F1-mediated cell death upon DNA damage in both p53 WT and p53 null cancer cells, although LSD1 inhibited E2F1 activity through demethylating E2F1. Even though the discrepancy might be due to the difference of cell types or DNA damage agents or the methods of cell death analysis, our data showed that Set9 overexpression induced DNA damage and cell death both in p53 WT (U2OS) and p53 null (HCT116 $p53^{-/-}$) cells, which is consistent with the biological function of Set9 reported in the previous studies (10,26,28). Furthermore, overexpression of Set9 significantly reduced colony formation in HCT116 $p53^{-/-}$ cells (Figure 4D), and Set9 knockdown increased colony formation (Figure 4E). Together, these findings suggest that Set9 retards cancer cell growth and promotes DNA damage-induced cell death. Additionally, LSD1 has been shown to function as a counterpart to Set9 in the regulation of Set9-methylated substrates. For example, LSD1 removes Set9-mediated DNMT1 methylation at K1096 and increases its activity (20). High expression of LSD1 has been found in prostate carcinoma, neuroblastoma, breast cancer, and colon cancer (29–32). LSD1 is also involved in the epithelial–mesenchymal transition (EMT) in malignant procession (33). Recently, high expression of LSD1 has been found in breast cancer tissue and LSD1 knockdown using siRNA or inhibition with small molecular inhibitors led to growth retardation of breast cancer cells (34). Conventional knockouts of LSD1 turn out to be embryonic lethal (14). Together, multiple lines of evidences indicate that LSD1 functions as a protumorigenesis protein and is highly correlated with cell proliferation and cancer progression.

Conclusions

In summary, our findings provide a new regulatory mechanism of E2F1 function in DNA damage-induced cell death, in which Set9 and LSD1, a pair of epigenetic modifiers, dynamically regulate E2F1 transcription activity in response to apoptotic stimuli. Our data implicate these molecules are potential pharmaceutical candidates for cancer intervention.

Acknowledgements

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Declaration of interest

All the authors claim no conflict of interest.

References

1. Rogoff HA, Kowalik TF. Life, death and E2F: linking proliferation control and DNA damage signaling via E2F1. Cell Cycle 2004, 3, 845–846.
2. Inoshita S, Terada Y, Nakashima O, Kuwahara M, Sasaki S, Marumo F. Regulation of the G1/S transition phase in mesangial cells by E2F1. Kidney Int 1999, 56, 1238–1241.
3. Stiewe T, Pützer BM. Role of the p53-homologue p73 in E2F1-induced apoptosis. Nat Genet 2000, 26, 464–469.
4. Zhao Y, Tan J, Zhuang L, Jiang X, Liu ET, Yu Q. Inhibitors of histone deacetylases target the Rb-E2F1 pathway for apoptosis induction through activation of proapoptotic protein Bim. Proc Natl Acad Sci USA 2005, 102, 16090–16095.
5. Lin WC, Lin FT, Nevins JR. Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. Genes Dev 2001, 15, 1833–1844.
6. Martínez-Balbás MA, Bauer UM, Nielsen SJ, Brehm A, Kouzarides T. Regulation of E2F1 activity by acetylation. EMBO J 2000, 19, 662–671.
7. Huang J, Berger SL. The emerging field of dynamic lysine methylation of non-histone proteins. Curr Opin Genet Dev 2008, 18, 152–158.
8. Kouskouti A, Scheer E, Staub A, Tora L, Talianidis I. Gene-specific modulation of TAF10 function by SET9-mediated methylation. Mol Cell 2004, 14, 175–182.
9. Chuikov S, Kurash JK, Wilson JR, Xiao B, Justin N, Ivanov GS, McKinney K, Tempst P, Prives C, Gamblin SJ, Barlev NA, Reinberg D. Regulation of p53 activity through lysine methylation. Nature 2004, 432, 353–360.
10. Estève PO, Chin HG, Benner J, Feehery GR, Samaranayake M, Horwitz GA, Jacobsen SE, Pradhan S. Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. Proc Natl Acad Sci USA 2009, 106, 5076–5081.
11. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 2004, 119, 941–953.
12. Nicholson TB, Chen T. LSD1 demethylates histone and non-histone proteins. Epigenetics 2009, 4, 129–132.
13. Huang J, Sengupta R, Espejo AB, Lee MG, Dorsey JA, Richter M, Opravil S, Shiekhattar R, Bedford MT, Jenuwein T, Berger SL. p53 is regulated by the lysine demethylase LSD1. Nature 2007, 449, 105–108.
14. Wang J, Scully K, Zhu X, Cai L, Zhang J, Prefontaine GG, Krones A, Ohgi KA, Zhu P, Garcia-Bassets I, Liu F, Taylor H, Lozach J, Jayes FL, Korach KS, Glass CK, Fu XD, Rosenfeld MG. Opposing LSD1 complexes function in developmental gene activation and repression programmes. Nature 2007, 446, 882–887.
15. Bi W, Xiao L, Jia Y, Wu J, Xie Q, Ren J, Ji G, Yuan Z. c-Jun N-terminal kinase enhances MST1-mediated pro-apoptotic signaling through phosphorylation at serine 82. J Biol Chem 2010, 285, 6259–6264.
16. Ohtani K, DeGregori J, Nevins JR. Regulation of the cyclin E gene by transcription factor E2F1. Proc Natl Acad Sci USA 1995, 92, 12146–12150.
17. Couture JF, Collazo E, Hauk G, Trievel RC. Structural basis for the methylation site specificity of SET7/9. Nat Struct Mol Biol 2006, 13, 140–146.
18. Ivanov GS, Ivanova T, Kurash J, Ivanov A, Chuikov S, Gizatullin F, Herrera-Medina EM, Rauscher F 3rd, Reinberg D, Barlev NA. Methylation–acetylation interplay activates p53 in response to DNA damage. Mol Cell Biol 2007, 27, 6756–6769.

19. Morgunkova A, Barlev NA. Lysine methylation goes global. Cell Cycle 2006, 5, 1308–1312.
20. Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, Su H, Sun W, Chang H, Xu G, Gaudet F, Li E, Chen T. The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet 2009, 41, 125–129.
21. Shu HK, Julin CM, Furman F, Yount GL, Haas-Kogan D, Israel MA. Overexpression of E2F1 in glioma-derived cell lines induces a p53-independent apoptosis that is further enhanced by ionizing radiation. Neuro-oncology 2000, 2, 16–21.
22. Palacios G, Talos F, Nemajerova A, Moll UM, Petrenko O. E2F1 plays a direct role in Rb stabilization and p53-independent tumor suppression. Cell Cycle 2008, 7, 1776–1781.
23. Wang H, Cao R, Xia L, Erdjument-Bromage H, Borchers C, Tempst P, Zhang Y. Purification and functional characterization of a histone H3–lysine 4-specific methyltransferase. Mol Cell 2001, 8, 1207–1217.
24. Subramanian K, Jia D, Kapoor-Vazirani P, Powell DR, Collins RE, Sharma D, Peng J, Cheng X, Vertino PM. Regulation of estrogen receptor alpha by the SET7 lysine methyltransferase. Mol Cell 2008, 30, 336–347.
25. Ea CK, Baltimore D. Regulation of NF-kappaB activity through lysine monomethylation of p65. Proc Natl Acad Sci USA 2009, 106, 18972–18977.
26. Yang XD, Huang B, Li M, Lamb A, Kelleher NL, Chen LF. Negative regulation of NF-kappaB action by Set9-mediated lysine methylation of the RelA subunit. EMBO J 2009, 28, 1055–1066.
27. Kontaki H, Talianidis I. Lysine methylation regulates E2F1-induced cell death. Mol Cell 2010, 39, 152–160.
28. Senanayake MD, Amunugama H, Boncher TD, Casero RA, Woster PM. Design of polyamine-based therapeutic agents: new targets and new directions. Essays Biochem 2009, 46, 77–94.
29. Mimasu S, Umezawa N, Sato S, Higuchi T, Umehara T, Yokoyama S. Structurally designed trans-2-phenylcyclopropylamine derivatives potently inhibit histone demethylase LSD1/KDM1. Biochemistry 2010, 49, 6494–6503.
30. Huang Y, Greene E, Murray Stewart T, Goodwin AC, Baylin SB, Woster PM, Casero RA Jr. Inhibition of lysine-specific demethylase 1 by polyamine analogues results in reexpression of aberrantly silenced genes. Proc Natl Acad Sci USA 2007, 104, 8023–8028.
31. Huang Y, Stewart TM, Wu Y, Baylin SB, Marton LJ, Perkins B, Jones RJ, Woster PM, Casero RA Jr. Novel oligoamine analogues inhibit lysine-specific demethylase 1 and induce reexpression of epigenetically silenced genes. Clin Cancer Res 2009, 15, 7217–7228.
32. Magerl C, Ellinger J, Braunschweig T, Kremmer E, Koch LK, Höller T, Büttner R, Lüscher B, Gütgemann I. H3K4 dimethylation in hepatocellular carcinoma is rare compared with other hepatobiliary and gastrointestinal carcinomas and correlates with expression of the methylase Ash2 and the demethylase LSD1. Hum Pathol 2010, 41, 181–189.
33. Lin T, Ponn A, Hu X, Law BK, Lu J. Requirement of the histone demethylase LSD1 in Snai1-mediated transcriptional repression during epithelial–mesenchymal transition. Oncogene 2010, 29, 4896–4904.
34. Lim S, Janzer A, Becker A, Zimmer A, Schüle R, Buettner R, Kirfel J. Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology. Carcinogenesis 2010, 31, 512–520.