Dual Specificity Phosphatase 1/CL100 Is a Direct Transcriptional Target of E2F-1 in the Apoptotic Response to Oxidative Stress

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Abstract

E2F-1 mediates apoptosis through transcriptional regulation of its targets. We report here that E2F-1 acts as a direct transcriptional regulator of dual specificity phosphatase 1 (DUSP1; CL100), a threonine and tyrosine phosphatase that inhibits mitogen-activated protein (MAP) kinases. We found that DUSP1 is transcriptionally induced by ectopic E2F-1 expression and that extracellular signal-regulated kinase 1/2 are dephosphorylated in the presence of E2F-1 and DUSP1. E2F-1 mediates apoptosis in the cellular response to oxidative stress. DUSP1 levels are significantly increased in an E2F-1–dependent manner following oxidative stress but not other stresses examined. DUSP1 mediates the cellular response to oxidative stress. We found that E2F-1 binds to chromatin encompassing the DUSP1 promoter and greatly stimulates the promoter activity of the DUSP1 gene. In particular, E2F-1 physically binds to an E2F-1 consensus sequence and a palindromic motif in the DUSP1 promoter. Interestingly, E2F-1 is acetylated following oxidative stress. Our findings show that E2F-1 is a transcriptional activator of DUSP1 and that DUSP1 is a link between E2F-1 and MAP kinases. [Cancer Res 2007;67(14):6737–44]

Introduction

E2F-1 plays dual and opposing roles in tumorigenesis (1, 2). As an oncogene, E2F-1 promotes cell cycle progression and stimulates cell proliferation (3). E2F-1 binds to variants of the consensus sequence (4, 5), which exists in the promoters of a number of genes important for cell cycle progression (6, 7). E2F-1 is negatively regulated by Rb. When hypophosphorylated, Rb binds to E2F-1 and suppresses E2F-1–mediated transcriptional activation (8, 9). E2F-1 functions as a tumor suppressor in a tissue-specific manner (1, 2). Overexpression of E2F-1 can trigger apoptosis (10, 11). In addition, E2F-1–deficient mice exhibit defects in apoptosis and aberrant cell proliferation in some tissues (12). The mechanisms by which E2F-1 mediates apoptosis are largely unknown. Thus, identification of downstream targets of E2F-1 function in apoptosis may provide insight into the mechanisms involved. One of the significant targets identified thus far for E2F-1 function in apoptosis is the p53 homologue p73 (13, 14). E2F-1 regulates transcription of p73, which can induce apoptosis in the absence of p53 (15, 16). In addition, p14ARF, a tumor suppressor that regulates p53 stability, is a transcriptional target for E2F-1 in apoptotic signaling and tumor suppression (17, 18).

Reactive oxygen species may cause damage on lipid, protein, and DNA, and cells under oxidative stress initiate protective responses for repair or elimination of damaged cells (19–21). It has been reported that oxidative stress causes cell death by apoptosis in some lymphocyte cell lines (22). We have previously shown that p53 is required for the cellular apoptotic response to oxidative stress (23, 24). Because E2F-1 coordinates with p53 in apoptosis, it is possible that E2F-1 is also involved in mediating the cellular response to oxidative stress.

Using DNA microarray technology, we observed that dual specificity phosphatase 1 (DUSP1) is a potential target of E2F-1. DUSP1 (also called CL100, 3CH134, Erp, and MKP1) was originally cloned because of its strong response to hydrogen peroxide (25, 26). DUSP1 encodes a dual threonine/tyrosine phosphatase that specifically dephosphorylates and inactivates mitogen-activated protein (MAP) kinases (27). The MAP kinase cascade is a predominant pathway for cell growth and proliferation. The activity of MAP kinases is regulated by dual phosphorylation on their tyrosine and threonine residues. MAP kinases are activated via phosphorylation on threonine and tyrosine and are inactivated by a family of DUSP, which are induced in response to environmental stressors and growth factor stimulation. It was recently shown that DUSP1 plays a proapoptotic role in oxidative stress–induced cell death through inhibition of MAP kinases extracellular signal–regulated kinase (ERK)-1/2 in a neuronal cell line (28). It has been reported that DUSP1 is down-regulated in human ovarian and prostate cancers (29, 30). In this report, we found that DUSP1 is highly responsive to oxidative stress in an E2F-1–dependent manner, and we show that E2F-1 is a direct transcriptional regulator of DUSP1.

Materials and Methods

Constructs. The human DUSP1 promoter was amplified by PCR from human genomic DNA using the following primers: 5′-CAAGTCTTCCGGGGGCCCAAGACTAGAA-3′ (forward); 5′-TCGCAACACAGCCACAATGTCCCTTCCAGCAG-3′ (reverse). The promoter fragment (833 bp) was ligated into the pGL3-basic reporter (Promega), resulting in pGL3/DUSP1-luc. The DUSP1 promoter with a deletion of the E2F-1 consensus site (DUSP1Δ603-luc) was generated by PCR using a forward primer 5′-CCCCCAG-TAGTGGTGGTCTCAG-3′ and the same reverse as above. pGL3/DUSP1Δ603-luc was generated by changing the palindromic sequence from 5′-GGTGAGCT-CACC-3′ to 5′-GGTGAGCTCACAG-3′ using the QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer's protocol. The human DUSP1 expression vector was constructed using the vector pcDNA3.1 (Invitrogen). The coding region of human DUSP1 was amplified from a human cDNA library by PCR using the following primers: DUSP1-21up, 5′-GGCAGAAAGCAGCAAGGAAGCAG-3′ (forward); DUSP1-21dn, 5′-TCCAG-ATGGGATGGTGAAGACG-3′ (reverse). The resulting PCR product (1,200 bp) was subcloned into pcDNA3.1, resulting in pcDNA3/DUSP1. The primes for
generating the DUSP1siRNA vector are forward, 5'-CGTGCCCTCAAGAGAGATGGTGCTGAAGCGCACGTTTTTT-3', and reverse, 5'-AATTAAAAACGTCGGCCTTCACGACCACCTCTCTGAGATGTGGCTGCTGAAGCGCACGGCC-3'. The annealed oligos were cloned into the pSilencer 1.0-U6 siRNA expression vector (Ambion). A random sequence was ligated into the U6 vector as a scrambled control.

Gene transfection and luciferase assay. Plasmids were introduced into cells using Lipofectamine reagent (Invitrogen) according to the manufacturer's protocol. Luciferase reporters were transfected into MCF7 cells along with a pcMV-β-gal reporter plasmid to normalize the transfection efficiency. Cell extracts were processed using the Dual-Light kit (Tropix) according to the manufacturer's instructions. Luciferase activity was measured with a Berthold Autolumat LB953 Rack Luminometer. Luciferase values were normalized against β-galactosidase activity.

Terminal deoxynucleotidyl transferase biotin-DUTP nick end labeling. Exponentially growing cells in the chamber were treated with indicated conditions. The cells were fixed by 1% paraformaldehyde. The apoptotic cells were detected using ApopTag Fluorescein In Situ Apoptosis Detection Kit (Chemicon).

Electrophoretic mobility shift assay. Oligonucleotides were labeled with 32P by using T4 polynucleotide kinase and [γ-32P]ATP as described elsewhere (31). Human glutathione S-transferase (GST)-E2F-1 fusion protein was produced from growing E. coli BL21 transfected by pGEX-2T-E2F-1 and induced by isopropyl-L-thiol (32). Electrophoretic mobility shift assay (EMSA) was conducted as previously described (24). Briefly, 32P-labeled probes were mixed with 0.5 µg of purified recombinant GST-E2F-1 fusion protein in 20-µL DNA binding reaction buffer consisting of 20 mmol/L Tris-HCl (pH 7.5), 4% Ficoll-400, 2 mmol/L EDTA, 0.5 mmol/L DTT, and 0.2 mg of poly(deoxyinosinic-deoxycytidylic acid). For supershift, an anti-E2F-1 monoclonal antibody (Santa Cruz Biotechnology) was included in the reaction mixtures. The reaction mixtures were incubated at 4°C for 20 min, resolved by a 4% polyacrylamide gel, and used for autoradiography.

Chromatin immunoprecipitation. Growing cells were processed for chromatin immunoprecipitation with an anti-E2F-1 antibody and a chromatin immunoprecipitation assay kit (Upstate) according to the manufacturer's protocol. Cells were cross-linked by 1% formaldehyde and chromatin was sonicated. Samples were incubated with the anti-E2F-1 monoclonal antibody. The chromatin samples without addition of the antibody were used as negative controls and genomic DNA was used as a positive control for the PCR reaction (input). Immunocomplexes were precipitated with protein A beads, and DNA-protein cross-links were reversed by boiling for 30 min in 100 mmol/L Tris (pH 8.8), 10% β-mercaptoethanol, 4% SDS. The precipitated DUSP1 promoter was amplified by PCR using the primers spanning the potential E2F-1 binding site.

Results

Transcriptional regulation of DUSP1 by E2F-1. To determine whether E2F-1 can induce the expression of DUSP1 in vivo, we introduced a CMV-driven E2F-1 expression vector (pCMV/E2F-1) into MCF7, a breast cancer cell line with low levels of endogenous E2F-1 (data not shown). We obtained stable clones expressing ectopic E2F-1 through G418 selection and Western blot analysis of E2F-1 protein (data not shown). These clones were designated as MCF7/E2F-1 followed by clone numbers. If E2F-1 is a positive regulator of DUSP1, the levels of DUSP1 expression should be increased in cells expressing E2F-1. As shown in Fig. 1A, DUSP1 transcript is low in MCF7/pCMV (top, lane 1) and was largely increased in MCF7/E2F-1 clones (lanes 2 and 3). Correspondingly, the DUSP1 protein level is elevated in the presence of ectopic E2F-1 (Fig. 1B, lanes 2 and 3). These results indicate that DUSP1 transcription is regulated by E2F-1.

To determine the role of E2F-1 in oxidative cell death, we measured the cell viability under oxidative stress in relation with E2F-1 expression. As shown in Fig. 1C, MCF7 cells were mostly killed by H2O2 in the presence of ectopic E2F-1 whereas a large number of MCF7 cells survived under the same conditions in the absence of E2F-1. The nature of cell death is apoptosis, which is determined by terminal deoxynucleotidyl transferase biotin-DUTP nick end labeling (TUNEL) assay (Fig. 1D). These results suggest that E2F-1 is a mediator of cell killing following oxidative stress.

To determine whether DUSP1 is responsive to genotoxic stress and whether its response is dependent on E2F-1, mouse embryo fibroblasts (MEF; E2F-1−/− and E2F-1−/−) were treated with γ-irradiation, UV, serum deprivation, or H2O2. As shown in Fig. 2A, the expression of DUSP1 is low in E2F-1−/−MEFs and not induced by γ-irradiation (lanes 1 and 2). In addition, DUSP1 is not induced by either serum starvation or a moderate dose of UVC irradiation (Fig. 2A, lanes 4 and 5), which also causes cell cycle arrest (33). However, DUSP1 levels markedly increase following oxidative...
damage by H$_2$O$_2$ (Fig. 2A, lane 1 versus lane 3). Furthermore, the transcriptional response of DUSP1 to oxidative damage is completely diminished in E2F-1$^{-/-}$ MEFs (Fig. 2A, lane 3 versus lane 8). These observations suggest that DUSP1 is inducible by oxidative stress in an E2F-1-dependent manner and that E2F-1 selectively regulates DUSP1 in response to oxidative damage. To determine how E2F-1 selectively regulates DUSP1 expression specifically under oxidative stress, we first examined the levels of E2F-1 expression in E2F-1$^{-/-}$ MEFs. However, there is no change of E2F-1 protein in these cells following oxidative damage (data not shown). It was reported that acetylation of E2F-1 is associated with its function (34). We thus analyzed the status of acetylation of E2F-1 in MEFs using a specific antibody to acetylated lysine. As shown in Fig. 2B, there is an acetylated band corresponding to E2F-1 protein. This is confirmed by immunoprecipitation–Western blot analysis using an anti–E2F-1 antibody for immunoprecipitation, which was then blotted by the anti–acetylation antibody (Fig. 2C). These results indicate that E2F-1 is acetylated following oxidative stress, and this modification may contribute to selective regulation of DUSP1. The importance of E2F-1 in the cellular response to oxidative stress was further confirmed in Fig. 2D, in which E2F-1$^{-/-}$ MEFs were susceptible to H$_2$O$_2$ but E2F-1$^{+/+}$ MEFs were resistant to oxidative damage. These results suggest that E2F-1 is important for the cellular response to oxidative damage.

**Figure 2.** Induction of DUSP1 by E2F-1 in response to oxidative stress. A, levels of DUSP1 mRNA in MEFs under various conditions. Exponentially growing E2F-1$^{+/+}$ and E2F-1$^{-/-}$ MEFs were exposed to γ-rays (6 Gy), H$_2$O$_2$ (100 μmol/L), serum starvation (0.1% fetal bovine serum), or UV light (10 J/m$^2$) as indicated. The cells were harvested after 4 h and RNA from each group was fractionated on 1.2% formaldehyde agarose gel and transferred for Northern blotting with an [α-32P]dCTP–labeled DUSP1 cDNA probe. The blot was rehybridized with β-actin cDNA probe as loading control. B, Western blot analysis of acetylated E2F-1 in MEFs after oxidative damage. Exponentially growing E2F-1$^{+/+}$ MEFs were treated with H$_2$O$_2$ (100 μmol/L) for 24 h. Total proteins were extracted and resolved by 10% SDS-PAGE and transferred for Western blotting with an anti–acetylated lysine antibody. β-Actin was used as a loading control. C, immunoprecipitation/IP-Western blot analysis of acetylated E2F-1 in E2F-1$^{+/+}$ MEFs following H$_2$O$_2$ treatment. The antibody against E2F-1 was used to precipitate E2F-1 protein from cell lysates. Immunoprecipitates were separated by SDS-PAGE and immunoblotted with the anti–acetylated lysine antibody. D, requirement of E2F-1 for the cellular response to oxidative stress. Exponentially growing E2F-1$^{+/+}$ and E2F-1$^{-/-}$ MEFs were treated with H$_2$O$_2$ (100 μmol/L) for 24 h and scored for viable cells by trypan blue exclusion. Columns, mean of three independent experiments, each containing duplicate cultures; bars, SD.

**DUSP1 is a direct transcriptional target of E2F-1.** To understand the molecular basis of regulation of DUSP1 by E2F-1, we searched the regulatory region of the human DUSP1 gene in a database. The TATA box is located at −25 to −20. There is a variant of the E2F-1 consensus site between −594 and −587 (TTAGGGCC). Interestingly, there is a perfect 12- bp palindromic motif from −122 to −111 (5'-GGTGACGTACC'-3') in the DUSP1 promoter (Fig. 3A). We carried out a luciferase assay to determine whether the DUSP1 promoter is regulated by E2F-1. To create a luciferase reporter, the promoter of the human DUSP1 gene was amplified and ligated into a luciferase reporter vector, pGL3-basic (Promega), adjacent to a luciferase reporter gene, resulting in a reporter, referred to as DUSP1-luc. DUSP1d603-luc, a reporter without the E2F-1 consensus site, was also created. To examine luciferase activity, the luciferase reporters were transfected into MCF7 cells along with a pCMV vector as a control, or with an E2F-1 expression vector (pCMV/E2F-1). As shown in Fig. 3B, the DUSP1 promoter activity is low in the absence of E2F-1. The activity of the DUSP1-luc is greatly induced by E2F-1. As expected, the DUSP1 promoter is significantly activated by E2F-1. However, the activity of the DUSP1 promoter without the E2F-1 site is significantly decreased although it still remains active to some extent. To test whether the palindromic sequence is important for E2F-1 transactivity, we did a PCR-based site-directed mutagenesis to create a mutant form of DUSP1-luc, pGL3/DUSP1mt-luc, which contains a
C-to-G transition in the palindromic sequence. We observed that luciferase activity was also reduced greatly in the luciferase reporter containing a single mutated palindromic sequence in the presence of E2F-1. To further confirm the importance of this palindrome in the promoter activity, we then generated a 4-bp deletion of the palindrome and a complete deletion of the palindrome in the promoter, which are referred to as DUSP1p-mut-luc and DUSP1d203-luc, respectively. As shown in Fig. 3B, partial deletion of the palindrome leads to a significant reduction of luciferase activity, and complete deletion of the palindrome further reduces luciferase activity to the level of DUSP1d603-luc lacking the E2F-1 consensus site. These results suggest that E2F-1 regulates DUSP1 promoter activity likely through both the E2F-1 consensus site and this palindromic motif. To determine whether the palindromic sequence alone is sufficient for transactivity of a promoter by E2F-1, we ligated this palindrome upstream of a short fragment of the human Mad2 promoter in pGL3-basic luciferase plasmid and tested this reporter in a luciferase assay to examine its transactivity in the presence of E2F-1. We observed that the activity of the pGL3/PM-Mad2-luc is not increased in the presence of E2F-1 (data not shown). These results suggest that E2F-1 specifically binds to the DUSP1 promoter in vivo.

To determine whether E2F-1 binds to the variant of the E2F-1 consensus site in the DUSP1 promoter, we did EMSA to detect the association of E2F-1/GST fusion protein with the synthetic oligonucleotide containing the imperfect E2F-1 consensus sequence derived from the DUSP1 promoter. As shown in Fig. 4B, a shifted band was produced from incubation of E2F-1 protein (lane 2). The specificity of this binding was confirmed by competition and supershift by the anti–E2F-1 antibody. The shifted band was completely eliminated or reduced by the addition of a 50-fold or a 10-fold molar excess of the same unlabeled oligonucleotide (lanes 3 and 4). Furthermore, this band was supershifted by addition of the anti–E2F-1 monoclonal antibody in the
reaction (lane 5). Because the palindromic motif is necessary for the full induction of the DUSP1 promoter by E2F-1, it is possible that E2F-1 can also interact with this palindrome. To test this possibility, EMSA was carried out with synthetic oligonucleotides containing the palindromic sequences as probes (Fig. 4C, oligo list). The labeled probes were incubated with E2F-1/GST fusion protein. As shown in Fig. 4C, there is a clear retarded band of a protein-DNA complex resulting from its incubation with the 32P-labeled oligonucleotide (lane 2). To determine the specificity of the interaction, we did competition and supershift experiments. The E2F-1-30W complex formation was blocked by the cold oligonucleotide 30W (lane 3) but not by a nonspecific oligo NS30W (lane 4). Furthermore, this band was supershifted by the anti-E2F-1 antibody in the reaction (lane 5). Interestingly, E2F-1 failed to interact with a palindromic sequence containing a C-to-G conversion (30M, lane 7), which also reduces the promoter activity (Fig. 3B). As a negative control, this E2F-1/oligo complex was not supershifted by unrelated antibodies, anti-p21 and anti-c-Fos (Supplementary Fig. S1A, lanes 4 and 5). These results suggest that E2F-1 protein specifically binds to the palindromic motif in the promoter of the DUSP1 gene. To test whether this binding activity of E2F-1 is influenced by oxidative stress, we compared the complex formation between the palindrome and endogenous E2F-1 from MEFs in the absence or presence of H2O2. As shown in Supplementary Fig. S1B, endogenous E2F-1 forms a complex with the palindrome as a shifted band only under oxidative stress. This observation indicates that oxidative stress promotes binding of E2F-1 to the palindrome site in the DUSP1 promoter, which may lead to induction of DUSP1 expression, as shown in Fig. 2A.

Role of DUSP1 in the E2F-1–mediated cellular response to oxidative stress. DUSP1 is a dual specificity protein phosphatase that specifically dephosphorylates and inactivates MAP kinases.

Figure 4. Physical association of E2F-1 with the DUSP1 promoter in vivo and in vitro. A, detection of physical association of E2F-1 with the chromatin encompassing the DUSP1 promoter by chromatin immunoprecipitation (ChIP). MCF7 cells with or without ectopic E2F-1 were treated with 1% formaldehyde and cell lysates were sonicated. Cross-linked protein-chromatin complexes were immunoprecipitated by the anti–E2F-1 antibody. The DUSP1 promoter was amplified from either genomic DNA without chromatin immunoprecipitation (input) or the chromatin immunoprecipitation products by PCR using the primers corresponding to the DUSP1 promoter. PCR products were analyzed through gel electrophoresis. Bottom, PCR amplification of the human actin promoter from genomic DNA and the chromatin immunoprecipitation samples immunoprecipitated by the anti-p21 antibody. B, EMSA detection of binding of E2F-1 protein to oligonucleotides containing a putative E2F-1 consensus site. Purified GST-human E2F-1 fusion protein was incubated with 32P-labeled double-stranded oligonucleotides as listed. Competition with an unlabeled oligonucleotide is indicated. Supershift resulted from inclusion of the anti–E2F-1 antibody. Arrows, shifted and supershifted bands. Oligonucleotide sequences are listed as one strand (5′-3′).

Identification of a Palindrome as a Binding Site for E2F-1
To show the importance of DUSP1 in signaling oxidative cell
deat, we used the pSilencer 1.0-U6 siRNA vector to construct a
DUSP1siRNA, which is under the control of a U6 RNA polymerase
III promoter, to knockdown DUSP1 expression. The DUSP1siRNA
vector was introduced into MEFs and the pSilencer 1.0 U6
containing a random sequence (designated as U6) was used as a
control. Stable clones were selected by administration of hygro-
mycin B and further tested for DUSP1 expression by Northern
blotting. As shown in Fig. 5A, whereas its expression in MEF/U6
cells is increased following H₂O₂, DUSP1 remains unchanged in the
cells expressing DUSP1siRNA, indicating that the expression of
these DUSP1 siRNAs interferes with the transcription of DUSP1.
With these cells available, we examined the sensitivity of MEF/U6
and MEF/DUSP1siRNA to H₂O₂ (Fig. 5B). As anticipated, the cells
expressing DUSP1siRNA are resistant to oxidative stress by H₂O₂
whereas the majority of the control MEF/U6 cells are killed by the
same dosage of H₂O₂. These results confirm the importance of
DUSP1 in the cellular response to oxidative damage.

To determine whether DUSP1 mediates the cellular response to oxidative stress, we constructed a human DUSP1 expression vector by cloning a human DUSP1 cDNA containing the full coding sequence in frame into a mammalian expression vector driven by a human cytomegalovirus (CMV; pcDNA3/hygro, Invitrogen). The resulting DUSP1 expression vector, pcDNA3/DUSP1, or an empty vector, pcDNA3, was transfected into MCF7, a breast cancer cell line containing a low level of DUSP1. These cells were then selected with hygromycin B for isolating stable clones of MCF7/pcDNA3, designated as MCF7/pcDNA3, or MCF7/DUSP1, designated as MCF7/DUSP1-C9 and MCF7/DUSP1-C10. The expression of DUSP1 in these cells was examined by Northern blotting shown in Fig. 5C. As expected, phosphorylation of ERK1 and ERK2 is decreased in these MCF7/DUSP1 cells expressing DUSP1 (Fig. 5D). Because DUSP1 can dephosphorylate ERK1/2, E2F-1 may suppress the activity of ERK1/2 through up-regulation of DUSP1. Indeed, phosphorylation of ERK1/2 is decreased in MCF7/E2F-1 cells (Fig. 5D, lane 1 versus lanes 4 and 5). We chose MCF7/pcDNA3 and MCF7/DUSP1 cells for functional studies. We found that while MCF7/pcDNA3 cells are resistant to oxidative stress, these MCF7/DUSP1 cells are highly susceptible to cell killing by H2O2 treatment (Fig. 6A). The dead cells are stained positive by the TUNEL assay, suggesting that cells are killed by apoptosis (Fig. 6B). These results indicate that DUSP1 functions as a cell death mediator in the cellular response to oxidative stress. To show that DUSP1 suppresses tumor formation, we did experiments in vitro and in vivo. First, we compared the growth rate of MCF7 cells with or without DUSP1 under normal culture conditions. As expected, the growth rate of MCF7/DUSP1 cells is much lower than that of MCF7/pcDNA3 (Fig. 6C). Second, we examined the colony formation capability of MCF7/pcDNA3 and MCF7/DUSP1 cells in soft agar and then determined whether these cells form tumors in nude mice. We found that there is much less colony formation by the MCF7/DUSP1 cells than by control MCF7/pcDNA3 cells (Fig. 6D). Furthermore, the capability of MCF7/DUSP1 cells to form a tumor is significantly reduced in nude mice. As shown in Supplementary Table S1, whereas almost all the mice injected with MCF7/pcDNA3 produced tumors in 6 weeks, only one third of mice injected with MCF7/DUSP1 cells developed tumors. These results suggest that DUSP1 is capable of suppressing tumor formation in vitro.

Discussion

In this article, we show a new mechanism for E2F-1 to mediate cell death in response to oxidative damage. We report that E2F-1 is a transcriptional regulator of DUSP1 and that E2F-1 is required for the response of DUSP1 to oxidative stress. We show that E2F-1 is capable of regulating DUSP1 at the transcriptional level. We found that E2F-1 can bind to a variant of its consensus sequence in the promoter of the human DUSP1 gene. In addition, we described a new binding mechanism for E2F-1 function in apoptosis. We show that E2F-1 physically binds to a perfect palindromic motif in the promoter of DUSP1. E2F-1 physically interacts with the promoter in vivo, stimulates the promoter activity, and greatly increases transcription of DUSP1. Interestingly, oxidative stress modifies E2F-1 by acetylation and promotes E2F-1 binding to a palindromic motif for transcriptional regulation of DUSP1. It is known that acetylation of E2F-1 promotes transactivity of its target genes (34). Our results support this notion and further show that oxidative stress increases its binding capability. This evidence provides an explanation of how E2F-1 selectively regulates DUSP1 or other stress-inducible genes. The discovery of a palindrome motif as a novel E2F-1 binding site may lead to the identification of new target genes for E2F-1 functions. Although it is well known that E2F-1 functions as a mediator of cell death, the mechanism involved is largely unclear. The current model is that E2F-1 can regulate p73 and Apaf-1, both of which are mediators of apoptosis (15, 16, 36). Because DUSP1 can dephosphorylate MAP kinases and inactivate the cell survival pathway, E2F-1 may determine cell fate through regulation of a MAP kinase inhibitor. This cell death pathway is different from conventional mitochondrial apoptosis. Because DUSP1 is a cell death mediator and inhibitor of tumor growth, DUSP1 may be a downstream effector of E2F-1 function in controlling tumorigenesis. It was reported that DUSP1/C100 expression is down-regulated in advanced cancer and its reexpression decreases malignant potential (29, 30). Here we show that DUSP1 is a transcriptional target of E2F-1, and DUSP1 suppresses tumor formation in nude mice. Because DUSP1 is an inhibitor of MAP kinases, and MAP kinases are predominant factors for cell growth and proliferation, DUSP1 may shut down cell survival signaling through inhibition of MAP kinases, leading to cell death. Our results establish a functional link between E2F-1 and MAP kinases and provide insight into a new mechanism whereby E2F-1 controls a major cell survival pathway. Identification of the E2F-1/DUSP1/ERK axis may reveal a signaling pathway to kill cancer cells by chemotherapy.

References

4. Kovesdi I, Rechel B, Nevins JR. Identification of a transcriptional regulator of DUSP1 and that E2F-1 is required for the response of DUSP1 to oxidative stress. We show that E2F-1 is capable of regulating DUSP1 at the transcriptional level. We found that E2F-1 can bind to a variant of its consensus sequence in the promoter of the human DUSP1 gene. In addition, we described a new binding mechanism for E2F-1 function in apoptosis. We show that E2F-1 physically binds to a perfect palindromic motif in the promoter of DUSP1. E2F-1 physically interacts with the promoter in vivo, stimulates the promoter activity, and greatly increases transcription of DUSP1. Interestingly, oxidative stress modifies E2F-1 by acetylation and promotes E2F-1 binding to a palindromic motif for transcriptional regulation of DUSP1. It is known that acetylation of E2F-1 promotes transactivity of its target genes (34). Our results support this notion and further show that oxidative stress increases its binding capability. This evidence provides an explanation of how E2F-1 selectively regulates DUSP1 or other stress-inducible genes. The discovery of a palindrome motif as a novel E2F-1 binding site may lead to the identification of new target genes for E2F-1 functions. Although it is well known that E2F-1 functions as a mediator of cell death, the mechanism involved is largely unclear. The current model is that E2F-1 can regulate p73 and Apaf-1, both of which are mediators of apoptosis (15, 16, 36). Because DUSP1 can dephosphorylate MAP kinases and inactivate the cell survival pathway, E2F-1 may determine cell fate through regulation of a MAP kinase inhibitor. This cell death pathway is different from conventional mitochondrial apoptosis. Because DUSP1 is a cell death mediator and inhibitor of tumor growth, DUSP1 may be a downstream effector of E2F-1 function in controlling tumorigenesis. It was reported that DUSP1/C100 expression is down-regulated in advanced cancer and its reexpression decreases malignant potential (29, 30). Here we show that DUSP1 is a transcriptional target of E2F-1, and DUSP1 suppresses tumor formation in nude mice. Because DUSP1 is an inhibitor of MAP kinases, and MAP kinases are predominant factors for cell growth and proliferation, DUSP1 may shut down cell survival signaling through inhibition of MAP kinases, leading to cell death. Our results establish a functional link between E2F-1 and MAP kinases and provide insight into a new mechanism whereby E2F-1 controls a major cell survival pathway. Identification of the E2F-1/DUSP1/ERK axis may reveal a signaling pathway to kill cancer cells by chemotherapy.

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