E2FBP1/DRIL1, an AT-Rich Interaction Domain-Family Transcription Factor, Is Regulated by p53

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Abstract

E2FBP1/DRIL1 is an AT-rich interaction domain DNA-binding protein and is ubiquitously expressed in various tissues. It has been shown that Bright, the mouse orthologue of E2FBP1/DRIL1, exhibits sequence-specific DNA binding and regulates immunoglobulin transcription. Here we show a novel connection between E2FBP1/DRIL1 and the p53 tumor suppressor, a key regulator of growth arrest or apoptosis in response to cellular stress. We found a putative p53-binding site, which specifically responded to p53, in the second intron of the E2FBP1/DRIL1 gene. E2FBP1/DRIL1 was induced by p53 and up-regulated following DNA damage caused by UV radiation or doxorubicin treatment in a manner dependent on endogenous p53. The ectopic expression of E2FBP1/DRIL1 induced growth arrest in U2OS cells expressing normal p53, but not Saos-2 cells lacking p53. These results suggest that E2FBP1/DRIL1 may play a role in growth suppression mediated by p53.

Introduction

E2FBP1 was originally isolated as a cDNA encoding a protein that interacts with E2F-1, a member of the E2 transcription factor family (1). It was also independently discovered as DRIL1 (2), a human orthologue of murine Bright [B-cell regulator of immunoglobulin heavy-chain (IgH) transcription] (3), and Drosophila dead ringer (DRI) (4), both of which encode a conserved DNA-binding domain termed AT-rich interaction domain (ARID). In addition to the core ARID, these proteins share a broader homologous region that extends outside the core ARID, thus leading to the classification of these proteins as the extended ARID (eARID) subfamily (5).

E2FBP1/DRIL1 exhibits overall 79% identity and 83% similarity to Bright (2). Bright has been shown to act as a B cell-specific, matrix associating region-binding protein that transactivates the IgH enhancer and regulates B cell-specific gene expression (3, 6, 7). Bright binds to specific AT-rich sequences that are important for nuclear matrix association within matrix associating regions located in the IgH gene (3). Although Bright has been reported to specifically express in differentiating and mature B cells (3, 8), it has been shown that E2FBP1/DRIL1 is ubiquitously expressed in all tissues examined (2), suggesting that E2FBP1/DRIL1 might participate in various biological processes.

Here, we report a novel connection between E2FBP1/DRIL1 and the p53 tumor suppressor, a key regulator in a wide range of cellular processes, including cell cycle arrest and apoptosis (9–11). We provide evidence that E2FBP1/DRIL1 is a novel p53-target that may play a role in growth arrest mediated by p53.

Results

E2FBP1 Is Identical to DRIL1

E2FBP1 was originally isolated as a cDNA encoding an E2F-binding protein from an expression library of the human embryonal carcinoma cell line NEC14 using a GST-E2F-1 fusion protein as a probe (1). A search of the Genbank database revealed that the E2FBP1 matched DRIL1 (2), but the reported E2FBP1 cDNA lacked a 5’ portion of DRIL1. We later sequenced another E2FBP1 cDNA clone that was 700 bp longer than the previous clone (1). This full-length cDNA contained the additional 5’-sequence, confirming that these two genes are identical (termed E2FBP1/DRIL1 in this report).

E2FBP1/DRIL1 Is a Potential Target for p53

The ubiquitous expression of E2FBP1/DRIL1 raised the possibility that E2FBP1/DRIL1 might be involved in universal cellular processes. To examine the regulation of E2FBP1/DRIL1, we searched the genomic sequence of the E2FBP1/DRIL1 gene (2) and found a putative p53-binding site in its second intron (Fig. 1A). We thus examined whether the putative p53-binding site represents a functional p53-binding site. Gel mobility shift assays using a probe containing the putative p53-binding sequence detected a shift band in extracts of p53-deficient Saos-2 cells infected with an adenovirus expressing wild-type p53 (Ad-p53) (Fig. 1B). The band was competed with an unlabeled competitor containing the putative

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1K. Oda, unpublished results.
p53-binding sequence (p53BSWT), but by neither a mutant form of this sequence (p53BSMUT) nor a non-specific oligonucleotide (NS). The presence of p53 in the gel shift complex was confirmed by an antibody supershift assay using anti-p53 monoclonal antibodies. In addition, p53 binds to the wild-type sequence (p53BSWT) in a manner that is dependent on p53 expression (Fig. 1C, left panel) but not to the mutant version (p53BSMUT) that lacks the p53 consensus site (Fig. 1C, right panel). To determine whether this sequence specifically responds to p53, we inserted one copy of the oligonucleotide corresponding to the potential p53-binding site upstream of the SV40 minimum promoter of a luciferase reporter gene (p53BSWT-Luc). The reporter plasmid was transfected into Saos-2 along with a plasmid expressing wild-type p53. The expression of wild-type p53 caused 16-fold activation of the reporter (Fig. 1D). That this is a direct effect involving the putative p53-binding site in the reporter was demonstrated by the fact that point mutations of the p53 recognition sequence (p53BSMUT-Luc) eliminated the p53-mediated stimulation of the promoter activity. These results indicate that a functional p53-binding site is located in the second intron of the E2FBP1/DRIL1 gene, which is supported by the fact that there are an increasing number of p53-target genes that possess potential p53-binding sequences in their first or second introns (12–15).

We next examined whether endogenous E2FBP1/DRIL1 is induced by p53. U2OS cells were infected with Ad-p53. Analysis by reverse transcription (RT)-PCR showed that E2FBP1/DRIL1 mRNA expression was induced and that it peaked at 18 h after infection (Fig. 2A). Likewise, the levels of E2FBP1/DRIL1 protein after Ad-p53 infection were also examined in Saos-2 cells, which lack wild-type p53. Fig. 2B shows that E2FBP1/DRIL1 levels were significantly induced by the ectopic expression of p53.

**FIGURE 1.** Identification of a functional p53-binding site in the E2FBP1/DRIL1 gene. A. Schematic diagram of genomic structure of the E2FBP1/DRIL1 gene. Exons are boxed and numbered. The position of a potential p53-binding site is indicated and its sequence is shown below the p53 consensus binding sequence: R, purine; Y, pyrimidine; W, A or T. Mismatches are indicated by asterisks. B and C, p53 specifically binds to a potential p53-binding site in the E2FBP1 gene. Gel mobility shift assays were performed with cell extracts prepared from Ad-p53-infected Saos-2 cells and 32P-labeled double-stranded oligonucleotides containing the potential p53-binding site (p53BSWT) (B and C, left panel) or its mutant (p53BSMUT) (C, right panel) in intron 2 of the E2FBP1/DRIL1 gene. Anti-p53 antibodies Pab421 and Pab1801 were added as indicated. Competition assays were performed by adding a 100-M excess of unlabeled p53BSWT, p53BSMUT, or non-specific (NS) oligonucleotides to gel shift reaction mixtures. Arrows, complexes specific to p53. Asterisks, non-specific bands. D, The putative p53-binding site of E2FBP1/DRIL1 specifically responds to p53 in luciferase assays. Saos-2 cells were transfected with either p53BSWT-Luc or p53BSMUT-Luc, together with pCMV-p53 or a control cytomegalovirus (CMV) plasmid. Luciferase was assayed 48 h later. Data are averages of three independent experiments, each performed in duplicate.

**FIGURE 2.** Induction of endogenous E2FBP1/DRIL1 by p53. A, RT-PCR analysis of E2FBP1/DRIL1 in Ad-p53-infected U2OS cells. U2OS cells were infected with Ad-p53 at 50 FFU/cell and harvested at the indicated time. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplified under the same RT-PCR conditions verified equal loading. B, Induction of E2FBP1/DRIL1 protein by p53. Saos-2 cells infected with Ad-p53 and harvested 48 h later. Lysates were immunoprecipitated followed by Western blotting with anti-E2FBP1 (N-20) antibody.
Induction of E2FBP1/DRIL1 Following DNA Damage in a p53-Dependent Manner

DNA damage activates p53-mediated transcription (16–18). If E2FBP1/DRIL1 plays a role in the p53 regulatory pathway, DNA damage should also induce endogenous E2FBP1/DRIL1. We therefore examined the expression levels of E2FBP1/DRIL1 protein at different time points following UV radiation or exposure to doxorubicin in cells expressing wild-type p53. Northern blotting analysis revealed that E2FBP1/DRIL1 mRNA was induced by UV radiation in A549 cells (Fig. 3A). Furthermore, the expression of E2FBP1/DRIL1 protein is also up-regulated following UV radiation in human foreskin fibroblasts (HFFs) measured by Western blotting (Fig. 3B). The examination of E2FBP1/DRIL1 induction in U2OS cells over shorter time intervals revealed that UV radiation increased E2FBP1/DRIL1 expression within 4 h in a fashion similar to that of p53, followed by an increase of p21 

\[ \text{p53} \] 

indicated proteins. E2FBP1/DRIL1 expression was analyzed as in Fig. 2B. Cell extracts were analyzed by Western blotting with antibodies to the indicated proteins. E2FBP1/DRIL1 expression was analyzed as in Fig. 2B.

E2FBP1/DRIL1 Can Induce Growth Arrest

To further examine the role of E2FBP1/DRIL1 in the p53 regulatory pathway, we used colony formation assays to determine whether E2FBP1/DRIL1 alone suppresses tumor growth. We transfected an E2FBP1 expression vector containing the neomycin-resistance gene into U2OS and Saos-2 cells. The transfected cells were cultured in the presence of G418 for 2–3 weeks. As shown in Fig. 4A, the transfection of E2FBP1/DRIL1 substantially suppressed the growth of U2OS cells. In contrast, growth was not suppressed in p53-deficient Saos-2 cells, indicating that the growth suppression is cell-line specific and thus unlikely to be a consequence of non-specific cytostatic effects caused by E2FBP1/DRIL1 overexpression.

We next examined whether E2FBP1/DRIL1 inhibits DNA synthesis by BrdUrd incorporation. U2OS cells were co-transfected with plasmids expressing E2FBP1/DRIL1 and β-gal. Double immunofluorescence staining with anti-BrdUrd and β-gal antibodies revealed that BrdUrd incorporation was significantly inhibited in cells transfected with E2FBP1/DRIL1 (Fig. 4, B and C). On the other hand, significant characteristics of apoptotic phenotypes or terminal deoxynucleotidyl transferase-mediated nick end labeling-positive cells were not evident in the transfected cells under this condition (Fig. 4C; data not shown). Indeed, cell cycle analysis revealed that the induction of an adenovirus expressing E2FBP1/DRIL1 increased G1 population in U2OS cells without an apparent induction of apoptosis as indicated by the absence of cells with sub-G1 population in the infected cells (Fig. 4D). In agreement with the fact that E2FBP1/DRIL1 can induce G1 arrest, endogenous Rb protein decreased and became underphosphorylated in a manner dependent on E2FBP1/DRIL1 expression, whereas the p53 levels were not changed by E2FBP1/DRIL1, which is consistent with the idea that E2FBP1/DRIL1 acts
downstream of p53. These results indicate that E2FBP1/DRIL1 can induce G1 arrest in U2OS but not in p53-deficient Saos-2 cells and suggest that E2FBP1/DRIL1 may play a role in growth suppression mediated by p53.

Discussion

The eARID proteins constitute a subfamily of the ARID family of sequence-specific DNA-binding proteins that are involved in various biological processes. Here we show that the eARID protein, E2FBP1/DRIL1, is involved in transcriptional regulation mediated by the p53 tumor suppressor. We found a putative p53-binding site, which specifically responded to p53, in the second intron of the E2FBP1/DRIL1 gene. E2FBP1/DRIL1 expression is activated by p53 and is also induced following DNA damage in a manner dependent on endogenous p53. Furthermore, our results (Fig. 4, D and E) suggest that, in addition to the transcriptional activation of the E2FBP1/DRIL1 gene, endogenous p53 may also play a role in the stabilization of the protein. However, the basis of an increase of the mRNA in the p53−/− cells is unknown at present. It is possible that there is a feedback mechanism that up-regulates E2FBP1/DRIL1 mRNA by sensing the protein levels in the cell. In this regard, E2FBP1/DRIL1 mRNA might be up-regulated to compensate for low levels of the protein in the p53−/− cells.

We also show that the ectopic expression of E2FBP1/DRIL1 can suppress tumor cell growth in cells expressing wild-type p53 but not in cells lacking p53, suggesting that E2FBP1/DRIL1 may participate in tumor growth suppression mediated by p53. The mechanisms by which E2FBP1/DRIL1 induces the growth suppression remain unknown. The previous studies on Bright imply that E2FBP1/DRIL1 functions as a sequence-specific DNA-binding factor that regulates gene expression. The fact that p53 mediates tumor cell growth via transcriptional activation of genes inducing cell cycle arrest, such as p21Waf1/Cip1 and 14-3-3σ, suggests the possibility that E2FBP1/DRIL1 might also regulate such genes to induce growth arrest. Furthermore, the inability of E2FBP1/DRIL1 to suppress the growth of p53-deficient Saos-2 cells suggests that E2FBP1/DRIL1 by itself cannot suppress cell growth in the absence of wild-type p53 and that E2FBP1/DRIL1 requires the cooperative activity of p53 to exert its function. In support of this possibility, recent studies imply a functional link between E2FBP1/DRIL1 and p53. The closely related Bright co-localizes with the promyelocytic leukemia (PML) nuclear bodies and binds the Sp100 family protein, a component of PML nuclear bodies (6). Furthermore, p53 also co-localizes with the PML nuclear bodies and physically interacts with PML, which is critical for p53-dependent apoptosis after γ-irradiation (6, 20, 21).

Accumulating evidence has demonstrated that eARID subfamily of DNA-binding proteins is involved in diverse biological processes. The mouse orthologue Bright regulates B cell-specific gene expression and Drosophila DRI is involved in various developmental processes as a transcriptional activator or repressor and its loss-of-function mutations result in embryonic lethality (22, 23). Furthermore, E2FBP1/DRIL1 can associate with E2F-1, an E2F transcription factor important for progression through the G1-to-S phase transition, and cooperate with...
E2F-1 to activate E2F-dependent transcription (1). Recently, Peep et al. (24) demonstrated that E2FBP1/DRIL1 can bypass activated RAS-induced senescence in primary murine fibroblasts by deregulating the Rb/E2F1 pathway. Similar to that observed with E2F1 (25), however, the authors also mentioned that E2FBP1/DRIL1 induces premature senescence in primary human cells, possibly resulting from the deregulation of the Rb/E2F1 pathway, which appears to have different effects on murine and human cells. Evidence that E2FBP1/DRIL1 is involved in the p53 pathway and can induce growth arrest, yet we used human tumor cells in this study, together with an important role of p53 in cellular senescence (26), provides another explanation for senescence-associated cell cycle arrest induced by E2FBP1/DRIL1 in human cells. The involvement of E2FBP1/DRIL1 in both p53 and Rb/E2F1 pathways further supports the notion that E2FBP1/DRIL1 participates in various biological processes probably in cooperation with other transcription factors, such as p53 or E2F1, and thereby exerts different functions depending on such cooperating factors.

**Materials and Methods**

**Cell Culture and Transfection**

HFFs (Clontech Laboratories, Palo Alto, CA), U2OS, Saos-2, A549 (RIKEN Cell Bank, Tsukuba, Japan), and HOC313 cells were maintained in DMEM supplemented with 10% FCS. Human colorectal carcinoma HCT116 p53+/+ and p53−/− cells (19) were cultured in McCoy’s 5A medium (Life Technologies, Inc., Rockville, MD) with 10% FCS. Cells were transfected using the FuGENE6 transfection reagent (Roche Diagnostics, Basel, Switzerland) according to the manufacturer’s instructions.

**Sequencing**

pHβ-Ap-E2FBP1 containing a 2.3-kb E2FBP1 cDNA was provided by K. Oda. Both strands of the clone were sequenced using the Dye terminator Cycle Sequencing Ready Reaction kit provided by K. Oda. Both strands of the clone were sequenced using the Applied Biosystems automatic sequencer (Applied Biosystems) according to the manufacturer’s instructions.

**Antibodies**

The anti-E2FBP1 (N-20) polyclonal antibody was generated by immunizing rabbits with a peptide corresponding to the NH2-terminal amino acid residues 2–21 of E2FBP1/DRIL1 conjugated to keyhole limpet hemocyanin (KLH) and purified by affinity chromatography. The anti-E2FBP1 (243–593) rabbit polyclonal antibody against GST-E2FBP1 has been described (1). Monoclonal anti-p53 (DO-1 and Pab1808) and polyclonal anti-p21 (C-19) and Rb (C-15) antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA. Monoclonal anti-p53 (Pab421) and anti-β-gal (GAL-40) antibodies were purchased from Oncogene Research Products, Boston, MA and Sigma-Aldrich, St. Louis, MO, respectively. The polyclonal anti-β-gal antibody was purchased from Cappel, Aurora, OH.

**Plasmid Construction**

E2FBP1 cDNA was removed from the pHβ-Ap-E2FBP1 vector by SalI and BamH1 digestion and inserted into the XhoI and BamH1 sites of pcDNA3.1 (Invitrogen Life Technologies, Carlsbad, CA) resulting in pCMV-E2FBP1. E2FBP1p53BSWT-Luc and E2FBP1p53-BSMUT-Luc were created by inserting double-stranded oligonucleotide pairs, p35BSWT (5′-CTAGCGGACCGTGGGAGCATGCTA-3′ and 5′-GATCATGGCaTGTCCAGGCGTCA-3′) corresponding to the putative p53-binding sequence in intron 2 of E2FBP1/DRIL1 gene and p53BSMUT (5′-CTAGCGGACACTCTGGAAGAATGCTA-3′ and 5′-GATCATGGCATTTTCCCAGAGTGTTCCG-3′) corresponding to a mutant version of the putative p53-binding sequence into the pGL3-Luc vector (Promega, Madison, CA) digested with Nell-Bgl/I. The constructs were confirmed by DNA sequencing.

**Recombinant Adenoviruses and Infection**

The Ad-p53 virus was obtained from RIKEN Gene Bank, Tsukuba, Japan and Adx1w1 control virus has been described (27). Xpress epitope-tagged E2FBP1 was generated by PCR amplification of E2FBP1 cDNA (5′ primer, 5′-GGCGGATCCATGAAAACACTAGCGTGATTGG-3′; 3′ primer, 5′-TGCGGATGCTGCGGTTAAGGTCTAGAATA-3′) and subsequent cloning into the PCR-bluntII-TOPO vector (Invitrogen). The BamH1 and XbaI fragments from the resulting clone were then ligated into the BamH1-XbaI-digested pEF4/I- His vector (Invitrogen) to construct pEF/His-E2FBP1. pAxCAE2FBP1 was created by subcloning the blunt-ended BsaI-XbaI fragment, which contains Xpress-tagged E2FBP1 cDNA isolated from pEF4/His-E2FBP1, into the SwaI site of the cosmid pAxCAw1 (Takara). Recombinant E2FBP1 adenovirus (Ad-E2FBP1) was generated by in vitro homologous recombination in 293 cells with pAxCAE2FBP1 using the adenovirus Expression Vector Kit (Takara, Otsu, Japan) according to the manufacturer’s instructions. Viruses were propagated in 293 cells and virus stocks were prepared as described (28). Viral titers were determined by indirect immunofluorescent assays using the anti-72K serum as described (29). Cells were infected at the indicated multiplicity of infection in serum-free MEM for 60 min brief agitation every 15 min. After infection, the medium was replaced with culture medium.

**Gel Mobility Shift Assay**

Whole cell extracts were prepared from Saos-2 and U2OS cells infected with Ad-p53 and Ad-E2FBP1, respectively. Equal amounts (3 μg) of extracts were incubated with 32P-labeled double-stranded oligonucleotides in 10 μl of a reaction mixture containing 20 mM HEPEs (pH 7.9), 40 mM KCl, 6 mM MgCl2, 1 mM EGTA, 0.1% NP-40, 0.2 mM DTT, 10% glycerol, and 30 μg of BSA, and resolved by electrophoresis as described (30). Sonicated salmon sperm DNA (500 ng) and poly(di-dC) (1 μg) were added as non-specific competitors to the assays for p53 and E2FBP1/DRIL1, respectively. The following double-stranded oligonucleotides were used as probes and unlabeled competitors: E2FBP1p53BSWT, E2FBP1p53BSMUT, and E2FBP1 site 6–7 (5′-CTAGCGTTTTAAAATT-TATGAATTGTTATTA-3′ and 5′-GGACATTAATCTAGTTAAATTATTATATAAATCCTAAGTGTTCCG-3′). Pab421, Pab1801, and E2FBP1 (N-20) were added to the indicated samples.
Luciferase Assays
Saos-2 and U2OS cells were plated at 50–70% confluence in 12-well plates (Iwaki, Tokyo, Japan) 20–24 h before transient transfection with the indicated reporter plasmids together with pCMV-p53. The Renilla-luciferase expression vector (pRL/CMV) also was co-transfected as an internal control. The total amount of DNA was adjusted with a control CMV plasmid. Luciferase and Renilla luciferase activities were measured using the dual-luciferase reporter assay system (Promega). Luciferase activity was normalized to Renilla-luciferase activity.

RT-PCR
Total RNA was prepared from Ad-53-infected cells using the TRIZol Reagent (Life Technologies, Inc.). Complementary DNAs were synthesized from 5 μg of total RNA using the SuperScript Preamplification System (Life Technologies, Inc.). The RT-PCR exponential phase was determined to allow quantitative comparisons among cDNAs developed from identical reactions. Amplification by PCR involved a 94°C, 4-min initial denaturation step followed by 30 cycles at 94°C for 10 s and at 72°C for 2.5 min. The primer sequences were as follows: E2FBP1/DRIL1, 5’-TACGAGTGTGA-GAAGCCGGGCC and 5’-TTCGGAGGTGGCTGT-GATCCG; GAPDH, 5’-ACACAGTGGCATTGCATCAC and 5’-TCCACACCGCTGCTGTGTA. PCR products were resolved on 1% agarose gels and visualized by ethidium bromide and UV transillumination.

Western Blotting and Immunoprecipitation
Equal amounts of whole cell extracts were separated on polyacrylamide-SDS gels and transferred to polyvinylidene difluoride membranes (Pall, East Hills, NY). The blots were probed with primary antibodies and bands were detected using horseradish peroxide-conjugated secondary antibodies (Amer sham Biosciences, Buckinghamshire, UK) and the enhanced chemiluminescence (ECL) detection system (Amersham). To detect E2FBP1/DRIL1, lysates were immunoprecipitated with anti-E2FBP1/DRIL1 (N-20), and then Western blotted with the same or anti-E2FBP1/DRIL1 (243–593) antibodies to eliminate an overlapping non-specific band that appeared in standard Western blots. Both of these antibodies yielded the same results (data not shown).

DNA Damage
To examine the expression of E2FBP1/DRIL1 in response to DNA damage, cells were exposed to doxorubicin (0.2 μg/ml) (Sigma) or UV at 30 J/m² using an UV cross-linker (Stratagene, UV at 30 J/m²) or UV cross-linker (Stratagene, DNA damage, cells were exposed to doxorubicin (0.2 μg/ml). DNA damage was assessed through the detection of γH2AX foci using an overlapping non-specific band that appeared in standard Western blots. Both of these antibodies yielded the same results (data not shown).

Cell Cycle Analysis
To examine the subcellular localization of cyclin D1, cells plated on glass coverslips were fixed in 70% ethanol, double stained with anti-cyclin D1 and anti-sarcosmeric actin antibodies, and then immunostains were visualized using fluorescent tyramide reagent according to the manufacturer’s protocols (TSA-direct NEL-701, NEN, Boston, MA). To analyze the cell cycle profile, fixed cells were stained with anti-sarcosmeric actin and FITC-conjugated anti-mouse IgG. BrdUrd incorporation was scored in transfected cells (β-gal-positive) and the data represent the average of three independent experiments. At least 200 β-gal-positive cells were counted in each experiment.

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References


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