Insights into Selective Activation of p53 DNA Binding by c-Abl*

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As a transcription factor, p53 recognizes a specific consensus DNA sequence and activates the expression of the target genes involved in either growth arrest or apoptosis. Despite our wealth of knowledge on the genes that are targeted by p53 in growth arrest and apoptosis, relatively little is known about the promoter specificity triggered by p53 in these processes. Here we show that interaction with c-Abl stabilized p53 tetrameric conformation, and as a consequence c-Abl stimulated p53 DNA binding only when all quarter binding sites (a perfect binding sequence) on p53-responsive promoters were present. This result suggests that in response to DNA damage, c-Abl binding may specifically stimulate p53 DNA binding on the promoters with perfect binding sequences. A sequence comparison of several known p53-responsive elements illustrates the presence of the perfect binding sequences on the p21 but not the Bax promoter. Significantly, we show that c-Abl indeed enhanced p53 DNA binding and transcription from p21 but not Bax. These results suggest that the promoter specificity plays an important role in selective activation of p53 DNA binding by c-Abl. The implications of this with relation to selective activation of p53 target genes involved in either growth arrest or apoptosis are discussed.

p53 exerts its tumor suppression function by inducing cell cycle G1 arrest and apoptosis in response to DNA damage (1, 2). p53 is a transcription factor that recognizes a specific consensus DNA sequence and activates the expression of the target genes involved in either growth arrest (3) or apoptosis (4, 5). Despite our wealth of knowledge about the spectrum of genes that p53 targets in growth arrest and apoptosis, relatively little is known about the promoter specificity it triggers in these processes.

The c-Abl tyrosine kinase and its transforming variants have been implicated in tumorigenesis and many important cellular processes including cell growth arrest and apoptosis (6). The effects of c-Abl are mediated by multiple protein-protein interactions and by its tyrosine kinase activity. The underlying mechanisms of G1 arrest induced by c-Abl are largely unknown, and several models have been proposed (7). Studies by several laboratories including our own suggest a role of p53 in this process (8–13). c-Abl can regulate the p53 protein levels possibly by phosphorylating Mdm2 (13) and inhibiting Mdm2-mediated degradation of p53 (12). In addition, c-Abl binds directly to p53 (8, 9) and stabilizes the p53-DNA complex (11). Interestingly, c-Abl stimulation of p53 DNA binding does not require its tyrosine kinase activity (9, 11) and the alteration of the p53 protein levels (11). These findings suggest that c-Abl stimulates p53-dependent transcription through multiple mechanisms, which may provide selectivity for the regulation of p53 function.

The underlying mechanisms of apoptosis induced by c-Abl are also largely unknown; however, phosphorylation of p73 by c-Abl clearly plays a role (14–16). p73 is a member of the p53 family and has similar transcriptional activation, DNA binding, and oligomerization domains (17). p73 can activate transcription from promoters containing the p53-responsive element and induce apoptosis. c-Abl phosphorylates p73 in response to ionizing radiation, and mutation at the c-Abl phosphorylation site (Tyr-99) blocks the apoptotic response to ionizing radiation. In addition to p73, the pro-apoptotic function of c-Abl to a lesser extent is mediated through p53 (18). c-Abl has been shown to phosphorylate Mdm2 at Tyr-394, and this phosphorylation may lead to a maximal accumulation of p53 in response to DNA damage (13). Nevertheless, these results suggest that c-Abl exerts its apoptotic function via its tyrosine kinase activity, and an accumulation of p53 or p73 seems to be required for c-Abl-mediated apoptosis.

We have shown that c-Abl stimulates p53 DNA binding independent of its kinase activity and in the absence of the accumulation of p53 (11). These results prompted us to test whether the interaction of c-Abl with p53 may selectively activate p53 target genes involved in growth arrest but not apoptosis. Previously, we reported that c-Abl interacts with the C terminus of p53 and stimulates p53 DNA binding by decreasing the dissociation rate of the p53-DNA complex. We speculated that the C-terminal domain might interfere with the tetramerization of p53, resulting in a less stable p53-DNA complex. The interaction with c-Abl may disrupt the inhibition and enhance tetramerization of the protein (11). As each p53 subunit binds to one quarter-site in its consensus sequence, resulting in all four quarter-sites being bound stably by one p53 tetramer (19–23), if our model is correct the activation should preferably occur on a promoter containing the full consensus sequence (i.e. all four quarter-sites). To support this model, we show in this study that indeed c-Abl stimulated p53 DNA binding only when all four quarters of the binding site on p53-responsive promoters were present. This result raises the possibility that, in response to DNA damage, c-Abl binding may specifically stimulate p53 DNA binding on the promoters that contain perfect binding sites (full consensus sequences) and therefore provide the selectivity. A sequence comparison of several known p53-responsive elements indeed illustrates the presence of the full consensus sequences on p53-responsive promoters that were involved in G1 arrest (such as p21) but not on those involved in apoptosis (such as Bax). Significantly, we showed that, in vitro and in vivo, c-Abl indeed enhanced p53 DNA binding on p21 but not on Bax. Finally, we showed that interaction with c-Abl indeed stabilized p53 tetrameric conformation. Together, our results reveal that c-Abl binding stabilizes the tetrameric form of p53 and selectively stimulates p53 DNA binding on promot-

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Experimental Procedures

Plasmid Constructs—The luciferase reporter plasmids were constructed by cloning either wild-type or mutant p53 binding elements (Fig. 1B) into the BamH I site of pE4Luc (11). Similarly, the p21 or Bax luciferase reporter plasmids were constructed by cloning the p53-responsive elements from either the p21 promoter (5’-gagcccagacttcaac-gcatgtgggctaggc-3’) or the Bax promoter (5’-gactcttcaagtcgaga-cagctggtgctaggc-3’) into pE4Luc. All constructions were confirmed by sequence analyses.

Transcriptional Activation Assay—The transcriptional activity of p53 was measured using luciferase reporter plasmids containing one copy of the p53 binding sites. Various combinations of plasmid DNAs were transfected into endogenous p53-negative Saos-2 cells by the calcium phosphate method. The amounts of plasmids transfected for 60-mm dishes were as follows: 1 µg of reporter plasmid, 0.2 µg of pcDNA-p53, and 1 µg of pRSaMVtkNeo-Abl (8). All samples for luciferase assays were normalized for ß-galactosidase activity from a ß-galactosidase plasmid transfected control expression vector as described previously (11). The protein levels were determined by Western blot analysis with the anti-p53 antibody DO1 and anti-actin antibody (Santa Cruz Biotechnology).

Purification of the c-Abl and p53 Proteins—The baculovirus-expressed GST–c-Abl fusion protein was purified as described previously (11). To purify p53, sf21 cells were either infected with recombinant baculovirus expressing a hemagglutinin epitope-tagged p53 (HA-p53) or co-infected with recombinant baculoviruses expressing GST or GST-c-Abl. p53 was purified from the nuclear extract of infected insect cell lysates by binding to a matrix of monoclonal antibody (12CA5) specific for the epitope tag, followed by elution with the epitope peptide (24).

The purified proteins were analyzed by SDS-polyacrylamide gel electrophoresis. Native PAGE—Purified proteins were analyzed on native gels containing 7.5% polyacrylamide, 1.5 M Tris, pH 8.8 (resolving gel), and 5% polyacrylamide, 0.5 M Tris, pH 6.8 (stacking gel). Samples in sample application buffer (312.5 mM Tris, pH 6.8, 20% glycerol, and 0.05% bromphenol blue) were prepared prior to loading onto gels. Gel electrophoresis was performed overnight at constant current of 1 mA in the presence of 0.5 M Tris, pH 6.8, and 192 mM glycine. Protein bands were visualized by silver staining.

Electrophoretic Mobility Shift Assay—Electrophoretic mobility shift assay was carried out as described previously (11). Briefly, the p53-binding site probes were labeled with the Klenow fragment of Escherichia coli DNA polymerase. All probes had the same number of base pairs, and the p53 recognition sequences were located in the centers of the probe. Binding reaction contained 60 mM KCl, 12% glycerol, 5 µM MgCl₂, 1 mM EDTA, 0.2 µg of bovine serum albumin, 0.1 µg of poly(dC-dG), 100 cpm of labeled probe, and 40 ng of purified p53 as 1X. Reactions were incubated at 30 °C for 30 min and analyzed on a 5% polyacrylamide gel containing 0.5X TBE (0.045 M tris, 0.045 M sodium borate, 0.001 M EDTA, pH 8.0). DNA-protein complexes were visualized with PhosphorImager and Adobe Photoshop software.

Chromatin-Immunoprecipitation (ChIP) Assay—ChIP was carried out as described previously (25). After reversing the formaldehyde cross-linking by heating at 65 °C more than 5 h, DNA was extracted with phenol and chloroform, precipitated with ethanol, and dissolved in 50 µl of TE (10 mM Tris, 1 mM EDTA, pH 8.0) buffer. PCR amplifications were performed with 4 µl of DNA, at 30 cycles, unless indicated for U2OS and Saos-2 cells. PCR products were resolved by agarose-gel electrophoresis and visualized by ethidium bromide staining. The p21 primers were: 5’-GTGGCTCTGTGTCCTTCTG-3’ (forward) and 5’-GTGAAAAACGAGGGCCCAAG-3’ (reverse). The RGC primers were: 5’-AGGCTACCCACATTGCTGAT-3’ (forward) and 5’-AGTCACAGTGTAAAGGGGCAC-3’ (reverse). The Bax primers were: 5’-TACAGGATTGTGAGGTGAGG-3’ (forward) and 5’-TCTGCAGACCTGAAAGGTG-3’ (reverse). The TATA box primers were: 5’-GTGGTTGGTCCAACTCTCATG-3’ (forward) and 5’-AGTCACAGTGTAAAGGGGCAC-3’ (reverse).

1 The abbreviations used are: GST, glutathione S-transferase; ChIP, chromatin immunoprecipitation; RGC, ribosomal gene cluster.
Selective Activation of p53 DNA Binding by c-Abl

To directly test the requirement of all quarter binding sites in c-Abl activation of p53 DNA binding in vivo, we performed a chromatin immunoprecipitation assay (ChIP) using the promoters either with full consensus sequence (wild-type) or lacking one quarter-site (M4) as targets. As illustrated in Fig. 2B, c-Abl increased the binding activity of p53 to the wild-type promoter by 2.8-fold but had no effect on p53 binding with the M4 promoter. We also tested the binding of p53 to the M4 promoter at 40 PCR cycles, and no c-Abl stimulation was detected (data not shown). To ensure that both wild-type and M4 reporter plasmids could be detected by ChIP, we also performed the assay with anti-TATA box-binding protein antibody (Fig. 2B). These data argue strongly that the stimulation of p53 DNA binding by c-Abl requires all four quartets of the binding site. The most likely biochemical explanation is that c-Abl binding stabilizes the tetrameric conformation of p53. These data also imply that the promoter specificity may define a layer of selectivity for activation of p53 target genes by c-Abl binding.

**c-Abl Selectively Stimulates p53 DNA Binding on the p21 Promoter, Suggesting A Possible Role in the Specific Activation of p53 Target Genes Involved in G1 Arrest**—A number of studies have shown that c-Abl exerts its apoptotic function via its kinase activity and an accumulation of p53 or p73 is required for c-Abl-mediated apoptosis (14, 16). Because c-Abl stimulates p53 DNA binding independent of its kinase activity and in the absence of accumulated p53 (11), we hypothesized that the interaction of c-Abl with p53 might selectively activate the growth-arrest function of p53. Our results that the full consensus sequence is essential for the activation of p53 DNA binding by c-Abl prompted us to analyze several well characterized p53-responsive promoters for which protein products play a role in either G1 arrest or apoptosis. Fig. 3A summarizes the sequence comparison results of the p53-responsive elements in these promoters and shows that two p53-responsive elements from G1-arrest promoters, p21 and Gadd45, contained full consensus sequences, whereas none of the six p53-responsive elements from apoptosis promoters did. This was of particular interest in light of our results suggesting that all four quarts of the DNA-binding site in the p53 consensus sequence are required for stimulation of p53 DNA binding by c-Abl. Because positions 1 and 10 of the 10-bp palindromic half-site appear to be less critical for the high affinity DNA binding (20, 26), we consider all base pairs at these two positions equally acceptable in our sequencing analysis.

To test our hypothesis, we carried out transient transfection experiments from reporter constructs containing p53-responsive elements from p21 or Bax promoter (Fig. 3B, upper panel). The addition of c-Abl to the reporter with p53-responsive elements from the p21 promoter resulted in an almost 5-fold enhancement of p53-dependent transcription, whereas the addition of c-Abl to the elements from the Bax promoter lacking the full consensus sequence had no detectable effect on the transcription. To ensure that the lack of c-Abl stimulation on the Bax promoter was not due to the low transcription activity of p53, we tested p53-dependent transcription over a range of concentrations and found no c-Abl stimulation (Fig. 3B, inset). Western blot analysis was performed to ensure that the proteins were expressed at equal levels (Fig. 3B, bottom panel). These findings implied that the presence of all quarter binding sites in the p21 promoter would be critical for c-Abl stimulation.

To test the effect of c-Abl on the occupancy of p53 on p21 and Bax promoters in vivo, a ChIP assay testing the binding of endogenous p53 on the endogenous p21 or Bax promoter was carried out. As shown in Fig. 3C, c-Abl increases the binding activity of p53 to the 5′ binding site (that contains full consensus sequence, Fig. 3A) on the p21 promoter by 3.2-fold.
were transfected with 1 μg of each reporter construct and 0.2 μg of p53 expression plasmid with or without 1 μg of c-Abl expression plasmid. The transfected cells were lysed, and the luciferase activity was measured after normalization to β-galactosidase activity. The p53 proteins levels were determined by Western blot analysis with DO1 anti-p53 antibody. Inset, Saos-2 cells were transfected with 1 μg of M4 promoter (M4-luc (luciferase)) and increased concentrations of p53 expression plasmid as indicated in the presence or absence of c-Abl expression plasmid. WT, wild type. B, in vivo p53 DNA binding activity was analyzed in Saos-2 cells by ChIP assay under the same transfection conditions as in A. PCR amplifications were performed with 4 μl of DNA, using 30 cycles for p53 immunoprecipitate (IP) and 35 cycles for TATA box-binding protein immunoprecipitate. PCR products were resolved by agarose-gel electrophoresis and visualized by ethidium bromide staining. Fold of Act., -fold of activation.

contrast, c-Abl shows no effect on the binding activity of p53 on the 3′ binding site and on the binding site on Bax promoter, both of which lack full consensus sequences (Fig. 3A). p53 bound to the 3′ binding site (36 PCR cycles rather than 30 as for the 3′ binding site) and Bax (40 PCR cycles rather than 30) to a lesser extent. These data further support the model that the c-Abl interaction regulates p53 DNA binding, at least in part, in a promoter-dependent manner and therefore may selectively activate the growth arrest function of p53.

To directly examine the requirement of the full consensus sequence for c-Abl activation, we replaced “C” at the second position of the 10-bp palindromic half-site with “A” and generated a full consensus sequence on the Bax p53-responsive element (Fig. 4B, Bax-4Q) and examined the effect of c-Abl on p53 DNA binding in an electrophoretic mobility shift assay. The addition of c-Abl (Fig. 4A; 1:1 molar ratio to p53, IX) resulted in a significant stimulation of p53 DNA binding on the p53-responsive elements from the p21 promoter (25–30-fold activation; Fig. 4A), whereas the addition of c-Abl to the elements from the Bax promoter at the same molar ratio had no detectable effect. p53 bound to the Bax probes to a less extent (four times more p53 is required for the binding to the Bax sequence; 4X), which agrees with the previous finding that higher levels of p53 are required to induce cell apoptosis than to G1 arrest (27) and with our results shown in Fig. 3. Remarkably, although c-Abl did not activate p53 DNA binding on the wild-type Bax promoter, the single base pair mutation restored the c-Abl activation of p53 DNA binding. In addition, this mutation also restored the efficient p53 binding (50 ng of p53; Fig. 4A, IX).

To test whether this mutant can rescue the ability of c-Abl to activate p53 transcription in vivo, we performed transient transfection experiments from the Bax-4Q promoter in Saos-2 cells. As shown in Fig. 4C, the addition of c-Abl to the Bax-4Q promoter indeed resulted in more than 2-fold activation of p53-dependent transcription. In contrast, the addition of c-Abl to the wild-type promoters had no detectable effect on the transcription. Together, these data show that c-Abl binding stimulates p53 DNA binding on promoters containing all quarter DNA-binding sites.

c-Abl Binding Stabilizes the Tetrameric Conformation of p53—To directly test our hypothesis that the interaction of c-Abl with p53 may stabilize its tetrameric conformation, we compared the migration profiles of the p53 proteins purified from insect cell lysates co-infected with either p53 and c-Abl or p53 and GST using a native polyacrylamide gel approach. As shown in Fig. 5A, approximately half of the p53 protein co-infected with GST migrated at a position similar to a 440-kDa marker, a well-established position where the tetrameric form of p53 is migrated (28). We also detected approximately half-motifs of a larger form of p53 at positions similar to a 660-kDa marker, which indicates the existence of other oligomeric forms of the protein. The density of the 440-kDa band was enhanced when p53 was purified from the c-Abl co-infected cell lysates, which suggests that the interaction of c-Abl with p53 stabilized its tetrameric conformation. To exclude the possibility that our results may be peculiar to the co-infection, further native gel analysis with purified p53 in the presence of purified GST or c-Abl proteins revealed similar results (Fig. 5B), which demonstrated that the purified c-Abl protein stabilized p53 tetrameric conformation in vitro. To confirm these results, we also compared the elution profiles using fast protein liquid chromatography-based gel filtration on a Superose 6 column.
As shown in Fig. 5C, most of the p53 protein again eluted at a position similar to a 440-kDa protein marker. We also detected small amounts (15–20% of the total protein) of a larger form at positions similar to a 660-kDa protein marker. Importantly, p53 purified from the c-Abl co-infected lysates shifted the protein from the larger oligomer to the tetramer (Fig. 5C), indicating that the interaction of c-Abl stabilized the tetrameric conformation of p53.

To further confirm these results, we tested the effect of c-Abl on the C-terminal truncated form of p53 (ΔC363). The p53 proteins levels were determined by Western blot analysis with DO1 anti-p53 antibody. Inset, Saos-2 cells were transfected with 1 μg of Bax-luc and increased concentrations of p53 expression plasmid as indicated in the presence or absence of c-Abl expression plasmid. c-Abl was transfected into U2OS cells, and the binding of endogenous p53 to the p21 or Bax promoter was analyzed by ChIP assay. PCR amplifications were performed with 4 μl of DNA, using 30 cycles for the 5′ binding site, 36 cycles for the 3′ binding site on the p21 promoter, and 40 cycles for the binding site on the Bax promoter. IP, immunoprecipitate; Fold of Act., fold of activation.

DISCUSSION

We reported previously that c-Abl interacts with the C terminus of p53 and stimulates p53 DNA binding by decreasing the dissociation rate of the p53-DNA complex. The C-terminal regulatory domain has been proposed by others to interact with a motif in the core DNA binding domain of the p53 tetramer, thereby forming a conformational inactive complex (29).
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Despite compelling evidence for such a model, the increased association rate of p53 and DNA after disrupting the C-terminal inhibition was not observed (11). In addition, full-length and C-terminally deleted forms of p53 were found to be structurally indistinctive (30). An alternative explanation, therefore, is that the C-terminal domain may interfere with the tetramerization of p53, resulting in a less stable p53-DNA complex. Interaction with the C-terminal domain by c-Abl therefore may disrupt the inhibition and enhance tetramerization of the protein (Fig. 6).

In this study, we show that interaction with c-Abl indeed stabilizes the p53 tetrameric conformation (Fig. 5).

Consistent with this notion, we also show that the full consensus sequence is required for activation of p53 DNA binding by c-Abl. We note that, although approximately half of the purified p53 protein exists as tetramer (Fig. 5), the DNA binding activities of p53 on M2, M3, M4 probes were reduced only slightly compared with that of the wild-type probe (Fig. 1). Additionally, c-Abl showed no effect on M2, M3, and M4 sequences in the reporter assay (Fig. 2) but displayed a weak stimulation on the binding of p53 to, at least, M2 and M3 sequences (Fig. 1). This observation is likely due to the difference between in vitro and in vivo analysis. In fact, we observed that, in the absence of c-Abl, transfected p53 binds to the wild-type promoter and stimulates transcription better than the M4 promoter in vivo (Fig. 2) and that the addition of c-Abl did not show any detectable effect on the binding of p53 to M4 sequences (Fig. 2B, data not shown). Nevertheless, these results imply that, in response to DNA damage, the binding of the C-terminal domain by c-Abl may specifically stimulate p53 DNA binding on the promoters with the required sequences and therefore provide the selectivity.

Several lines of evidence support this view. First, c-Abl has been shown to regulate p53 through multiple mechanisms. Second, an accumulation of p53 is associated with c-Abl-mediated apoptosis, whereas the c-Abl stimulation of p53 DNA binding does not affect the p53 protein levels (11). Third, c-Abl exerts its apoptotic function via its tyrosine kinase activity, whereas c-Abl-p53 complexes are detected in cells expressing either wild-type or the kinase-inactive c-Abl mutant in response to ionizing radiation (9). Fourth, p53 binds to its responsive elements in G1 arrest promoters at higher affinity than to those in apoptosis ones, which, in theory, could lead to differential regulation in DNA binding. Finally, the fact that c-Abl enhances p53 DNA binding on p21, the full consensus sequence-containing G1 arrest promoter, but not Bax, a partial consensus sequence-containing apoptosis promoter, provides direct evidence that c-Abl binding selectively regulates p53 function at the promoter level.

The model that c-Abl may specifically activate p53 DNA binding on G1 promoters relies on the fact that p53 must adopt a more stable tetramer in response to DNA damage. The p53 protein is active when it is tetrameric, and in this conformation it binds with high affinity to DNA (31). Interestingly, the dissociation constant ($2.3 \times 10^{-6} \text{ M}$) for tetramer formation has been determined for the protein fragment 303–393 (32). Our data that most of the purified p53 protein at an approximate concentration of $4 \times 10^{-6} \text{ M}$ exists as a tetramer support this analysis (Fig. 5). Nevertheless, based on the $K_d$ analysis, Sakkaguchi et al. (33) suggest that because the cellular concentration of p53 is very low in human cells with undamaged DNA (1–10 nM), p53 may exist in these cells predominantly as monomers. Therefore, in response to DNA damage, increased p53 protein levels should favor for tetramer formation. Furthermore, tetramerization of p53 is stimulated by post-translational modifications, such as the phosphorylation of Ser-392 (34) induced in response to DNA damage (34). Thus it is likely that, in response to DNA damage, p53 forms a more stable tetramer. Taken together, our study provides evidence that the interaction of the C-terminal domain by c-Abl binding en-

![Fig. 4. A full consensus sequence is required for c-Abl activation.](image-url)

- **A**: radiolabeled p21, Bax, and mutated Bax (containing the full consensus sequence BAX-4Q) probes (shown in B) were incubated with 40 ng of purified p53 in the presence of GST-Abl (c-Abl) or GST at a molar ratio of 1:1 (1X) or 1:4 (4X). C, Saos-2 cells were transfected with 1 μg of each reporter construct and 0.2 μg of p53 expression plasmid in the presence or absence of 1 μg of c-Abl expression plasmid. The luciferase activity was measured after normalization to β-galactosidase activity. The p53 proteins levels were determined by Western blot analysis with DO1 anti-p53 antibody.

- **B**: TCAAGTTAGAGACAGCCTTGCGCTGGGC

- **C**: Bax Luc

- **p53**

- **c-Abl**

- **Bax-Luc**

- **Bax-4Q-Luc**

- **Actin**
hances p53 DNA binding by stabilizing the p53 tetramer.

These results are in agreement with our previous observation that c-Abl stimulates p53 DNA binding by decreasing the dissociation rate of the p53-DNA complex as the breaking of the contacts between four DNA binding domains of a p53 tetramer and DNA at the same time should be an energetically unfavorable step. Similar studies were also done with nuclear receptors and showed that dimerization of nuclear receptors stabilizes the binding of the receptors to DNA (35). In the absence of the dimerization domain, DNA binding domain monomers dissociate from the DNA very rapidly. In contrast, a dimer of the full-length receptor was found to dissociate from the DNA very slowly. In the case of p53, the stabilization of DNA binding should be more effective as p53 exists as a tetramer.

Although broadening our analysis will be required to firmly determine whether c-Abl indeed stimulates p53 DNA binding on all G1 but not apoptosis promoters, our study implies that the promoter specificity defines a layer of selectivity for activation of p53 target genes involved in either growth arrest or apoptosis. It is well established that higher levels of p53 are required to induce cell apoptosis than G1 arrest (27) (Fig. 3); however, the molecular basis of this difference is relatively unknown. The data presented in this study may provide an explanation. Promoters involved in G1 arrest contain full consensus sequences. Upon DNA damage, post-translational modification promotes tetramerization of p53, resulting in activations of p53-mediated transcription on the promoters involved in G1 arrest. In contrast, promoters involved in apoptosis may lack full consensus sequences. Upon DNA damage, again post-translational modification promotes tetramerization of p53; however, the tetramerization, by itself, would not be sufficient to...
to activate p53-mediated transcription on the promoters involved in apoptosis. Accumulation of p53 would also be required for full activation. Therefore, higher levels of p53 are required to induce cell apoptosis than G1 arrest. Nevertheless, required for full activation. Therefore, higher levels of p53 are involved in apoptosis. Accumulation of p53 would also be re-

Discussion

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REFERENCES

1. Vogelstein, B., Lane, D., and Levine, A. J. (2000) Nature 16, 307–310
2. Sharpless, N. E., and DePinho, R. A. (2002) Cell 9, 9–12
3. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) Cell 10, 817–825
4. Miyashita, T., and Reed, J. C. (1995) Cell 29, 293–299
5. Polyak, K., Xia, Y., Zweier, J. L., Kinzler, K. W., and Vogelstein, B. (1997) Nature 389, 300–305
6. Wang, J. Y. J. (2000) Oncogene 19, 5643–5650
7. Shaul, Y. (2000) Cell Death Differ. 7, 10–16
8. Goga, A., Liu, X., Hambuch, T. M., Senechel, K., Major, E., Berk, A. J., Witte, O. N., and Sawyer, C. L. (1995) Oncogene 17, 791–799
9. Yuan, Z. M., Huang, Y., Whang, Y., Sawyer, C., Weichselbaum, R., Kharbanda, S., and Kufe, D. (1994) Science 265, 809–812
10. Sionov, R. V., Moallem, E., Berger, M., Kazaaz, A., Gerlitz, O., Ben-Neriah, Y., Oren, M., and Haput, Y. (1999) J. Biol. Chem. 274, 8371–8374
11. Nie, Y., Li, H.-H., Bula, C. M., and Liu, X. (2000) Mol. Cell. Biol. 20, 741–748
12. Sionov, R. V., Cen, S., Goldberg, Z., Berger, M., Bercovich, B., Ben-Neriah, Y., Ciechanover, A., and Haput, Y. (2001) Mol. Cell. Biol. 21, 5869–5878
13. Goldberg, Z., Sionov, R. V., Berger, M., Zawar, Z., Perets, R., Van Etten, R. A., Oren, M., Taya, Y., and Haput, Y. (2002) EMBO J. 21, 3715–3727
14. Agami, R., Blandino, G., Oren, M., and Shaul, Y. (1999) Nature 22, 809–813
15. Gong, J. G., Costanzo, A., Yang, H. Q., Melino, G., Kaelin, W. G., Jr., Revrero, M., and Wang, Y. F. (1999) Nature 24, 806–809
16. Yuan, Z. M., Shiyou, H., Ishiko, T., Sun, X., Gu, J., Huang, Y. U., Lu, H., Kharbanda, S., Weichselbaum, R., and Kufe, D. (1999) Nature 24, 814–817
17. Klaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J. C., Valant, A., Minty, A., Chalin, P., Leitais, J. M., Dumont, X., Ferrara, P., McKeon, F., and Caput, D. (1997) Cell 90, 806–818
18. W. T., Jackson, P. K., and Van Etten, R. A. (1996) EMBO J. 15, 1583–1595
19. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C., and Vogelstein, B. (1991) Science 252, 1708–1711
20. El-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W., and Vogelstein, B. (1992) Nat. Genet. 1, 45–49
21. Funk, W. D., Pak, D. T., Karas, R. H., Wright, W. E., and Shay, J. W. (1992) Mol. Cell. Biol. 12, 2866–2875
22. Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. (1994) Science 265, 334–335
23. McLaren, K. G., and Lee, P. W. (1999) EMBO J. 18, 763–770
24. Liu, X., and Berk, A. J. (1995) Mol. Cell. Biol. 5196–5202
25. Freeman, D. J., Li, A. G., Wei, G., Li, H.-H., Lesche, R., Chief, A. K., Martinez-Diaz, H., Rosengurt, N., Liu, X., and Wu, H. (2003) Cancer Cell 3, 117–130
26. Thukral, S. K., Lu, Y., Blain, G. C., Harvey, T. S., and Jacobsen, V. L. (1995) Mol. Cell. Biol. 15, 5196–5202
27. Chen, X., Ko, L. J., Jayaraman, L., and Prives, C. (1996) Genes Dev. 10, 2438–2451
28. Friedman, P. N., Chen, X., Bargnotti, J., and Prives, C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3319–3323
29. Hupp, T. R., Sparks, A., and Lane, D. P. (1995) Cell 83, 237–245
30. Ayed, A., Mulder, F. A. A., Yi, G., Lu, Y., Ray, L. E., and Arrowsmith, C. H. (2003) Nat. Struct. Biol. 10, 756–760
31. Chene, P. (2001) Oncogene 29, 2611–2617
32. Sakamoto, H., Lewis, M. S., Kodama, H., Appella, E., and Sakaguchi, K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8974–8978
33. Sakaguchi, K., Sakamoto, H., Lewis, M. S., Anderson, C. W., Erickson, J. W., Appella, E., and Xie, D. (1997) Biochemistry 36, 10117–10124
34. Keller, D., Zeng, X., Wang, Y., Zhang, Q. H., Kuo, M., Shu, H., Goodman, K., Lozano, G., Zhao, Y., and Lu, H. (2001) Mol. Cell 7, 283–292
35. Glass, C. K. (1994) Endocr. Rev. 15, 391–407
36. Chao, C., Hergenhahn, M., Kasner, M. D., Wu, Z., Saito, S., Iggo, R., Holstein, M., Appella, E., and Xu, Y. (2003) J. Biol. Chem. 278, 41028–41032
37. McKinney, K., Mattia, M., Gottfried, V., and Prives, C. (2004) Mol. Cell 16, 413–424