Regulation of the c-met Proto-oncogene Promoter by p53*

(Received for publication, September 22, 1998, and in revised form, November 16, 1998)

Dai-Wu Seo†‡¶, Qiuyan Chen†‡¶, Martin L. Smith§¶, and Reza Zarnegar†‡¶

From the †Department of Pathology, Division of Cellular and Molecular Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261 and the §Indiana University Cancer Center, Indianapolis, Indiana 46202

In the present study, we have investigated the possible involvement of p53 in the transcriptional regulation of the c-met gene. Cotransfection of various c-met promoter reporter vectors with p53 expression plasmids demonstrated that only wild-type p53 but not tumor-derived mutant forms of p53 resulted in a significant enhancement of c-met promoter activity. Functional assays revealed that the p53 responsive element in the c-met promoter region is located at position –278 to –216 and confers p53 responsiveness not only in the context of the c-met promoter but also in the context of a heterologous promoter. Electrophoretic mobility shift assays using purified recombinant p53 protein showed that the p53 binding element identified within the c-met promoter specifically binds to p53 protein. Induction of p53 by UV irradiation in RKO cells that express wild-type p53 increased the level of the endogenous c-met gene product and p21WAF1/CIP1, a known target of p53 regulation. On the other hand, in RKO cells in which the function of p53 is impaired either by stable transfection of a dominant negative form of p53 or by HPV-E6 viral protein, no induction of the endogenous c-met gene or p21WAF1/CIP1 was noted by UV irradiation. These results suggest that the c-met gene is also a target of p53 gene regulation.

Hepatocyte growth factor (HGF) receptor (c-Met) is the product of the c-met proto-oncogene (1, 2), which was originally described as an activated oncogene in a chemically treated human osteosarcoma cell line (3, 4). c-Met is a transmembrane tyrosine kinase receptor, which is expressed in a wide variety of adult and embryonic tissues and transmits multiple biological responses such as mitogenesis (1), motogenesis (5), morphogenesis (6, 7), and anti-apoptotic activity (8) elicited by HGF (for a detailed review, see Refs. 9 and 10). Animal experiments demonstrated that HGF/c-Met are important in organ regeneration in adults, and studies using HGF or c-met gene knock-out mice have shown that this receptor-ligand system plays a pivotal role in embryonic development and normal growth (11–13).

Dysregulated c-met gene expression is observed in a variety of human carcinomas (14, 15) and sarcomas (16). It also mediates the movement and invasiveness of neoplastic cells and promotes metastasis (6). Transgenic mouse models that overexpress HGF or in which an autocrine loop between HGF and c-Met was established show accelerated tumor formation (17–19). The HGF/c-Met signaling system has also been shown to relay tumor suppressor signals, as activation of this signaling pathway results in growth inhibition of some tumor cells, and the expression of c-Met is reduced or lost in other tumor tissues (20–22). Moreover, in c-met/HGF double transgenic mice, HGF behaves as a tumor suppressor gene antagonizing the tumorigenic effect of c-myc (23). Thus, elucidation of the molecular mechanisms governing the transcriptional regulation of the c-met gene is crucial to understand the role of HGF/c-Met in normal and neoplastic growth.

The molecular mechanisms regulating c-met gene transcription are largely unknown. Previously, our laboratory reported the cloning and functional characterization of the mouse c-met gene promoter (24) and demonstrated that the region between –278 and –78 of the promoter contains positive regulatory elements, including two Sp1 binding sites that are essential for promoter function. In that study, we also identified a putative p53 binding site within the –278 to –78 region of the promoter by computer analysis. Therefore, we focused on the functional potential of the p53 binding site and its involvement in the transcriptional regulation of the c-met gene. Using various c-met gene promoter constructs and p53 expression vectors, our current study demonstrates that mouse c-met gene promoter activity is transactivated by wild-type p53 but not by several tumor-derived mutant forms of p53; the p53-mediated transactivation of the c-met gene promoter is dependent upon direct binding of p53 to the cognate binding site identified in the c-met gene promoter; induction of p53 by UV irradiation in RKO cells that express wild-type p53 increased the level of the endogenous c-met gene product and p21WAF1/CIP1, a known target of p53 regulation, and in RKO cells in which the function of p53 is impaired either by stable transfection of a dominant negative form of p53 or by HPV-E6 viral protein, no induction of the endogenous c-met gene or p21WAF1/CIP1 is observed. These findings shed new light on the regulation of c-met gene expression.

EXPERIMENTAL PROCEDURES

Plasmid Vector Construction—1.6-, 0.5-, and 0.4mc-met-CAT constructs were made as described previously (24). Briefly, the No1-EcoRI genomic DNA fragment containing the mouse c-met gene promoter region was subcloned into the pBluescript II SK+ EBI Data Bank with accession number(s) AF030200 and AC002080. § These authors contributed equally to this work.

The abbreviations used are: HGF, hepatocyte growth factor; CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; bp, base pair(s); TBP, TATA box-binding protein; EMSA, electrophoretic mobility shift assay; EGFR, epidermal growth factor receptor; TGF-α, transforming growth factor-α.

* This work was supported by American Cancer Society Grant CNE-97736 (to R. Z.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) AF030200 and AC002080.

† To whom all correspondence should be addressed: Dept. of Pathology, Div. of Cellular and Molecular Pathology, BST S 419, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261. Tel: 412-648-8657; Fax: 412-648-1916; E-mail: rezazar@pitt.edu.

‡ The abbreviations used are: HGF, hepatocyte growth factor; CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; bp, base pair(s); TBP, TATA box-binding protein; EMSA, electrophoretic mobility shift assay; EGFR, epidermal growth factor receptor; TGF-α, transforming growth factor-α.
constuct with Apal and KpnI, followed by self-ligation. RGC-W-3X-CAT and RGC-M-3X-CAT vectors, which are the positive and negative control vectors, respectively, for p53-mediated stimulation, have been described previously (25). To construct the p53-E1BCAT plasmid, a 63-bp pair synthetic DNA fragment (278 to –216) with an XhoI site of E1BCAT vector which contains only a minimal promoter element. The internal deletion mutant vector 1.6DP53mc-met-CAT was prepared by digestion of the 1.6mc-met-CAT construct with Apal and KpnI, followed by self-ligation. Wild-type and mutant p53 expression vectors have been described previously (26). They contain a full-length human cDNA sequence for wild-type or various mutant p53 proteins inserted downstream of the cytomegalovirus (CMV) promoter/enhancer in the pCMV-Neo-Bam vector. The p53 expression vectors and the RGC-W-3X-CAT, RGC-M-3X-CAT plasmids were kindly provided by Dr. Paul Robbins, Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA.

**Cell Lines**—The p53 mutant cell line C-33A obtained from the American Type Culture Collection (Rockville, MD) was maintained at 37 °C in Dulbecco’s modified Eagle's medium supplemented with 10% calf serum and gentamycin (100 μg/ml). The RKO cell line is a colon carcinoma cell line that contains the wild-type p53 gene. The RKOmp53 cell line is an RKO derivative stably transfected with a mutant p53 encoding gene. The RKO-E6 cell line is a clone isolated after RKO cells were transfected with human papillomavirus E6 oncoprotein gene driven by the RGC-W-3X-CAT plasmid containing three copies of mutant p53 binding site and the pCAT-Basic plasmid (which is promoterless) were not affected by forced expression of wild-type p53. Stimulation of the c-met gene promoter by wild-type p53 was dependent upon the input dose of wild-type p53 (Fig. 2F).

**Transfection and CAT Assay**—Transient transfection using the calcium phosphate precipitation method was carried out as described previously (28, 29). Cells were harvested 24 h after transfection and analyzed for CAT activity. Normalization for CAT activity was performed based on the protein concentration of each cell lysate.

**Oligonucleotides**—A 63-base pair DNA fragment containing the c-met p53 binding site (5′-CCAGGGAGACCTTGAGCAAACTCAGACCGACAGACCGACAGCTGCTGRTGGGGC-3′, from –278 to –216; p53 binding half-sites are underlined) and a consensus p53 binding site (5′-AGGCACTGCTAGCAAGCTG-3′) (28) were chemically synthesized and used as probes and competitors.

**Electrophoretic Mobility Shift Assay (EMSA)**—A purified p53 core domain protein (amino acids 102–292) was a generous gift of Dr. Nikola P. Pavletich (Memorial Sloan-Kettering Cancer Center, New York, NY) (30). The oligonucleotides described above were end-labeled with [γ-32P]dATP and used as probes. Protein-DNA binding analysis was carried out in a buffer described previously (29) consisting of 10 mM HEPES (pH 7.9), 100 mM KCl, 0.65 mM EDTA, 2.5 mM MgCl2, 6% glycerol, 2% Ficoll, and 50 ng of nonspecific DNA (poly[d(dC-dC)]) for competition experiments. 100- or 200-fold molar excess of specific or nonspecific oligonucleotide competitors were added to the reaction mixture. After incubation for 10 min at room temperature, reaction mixtures were loaded onto 4% polyacrylamide gels, and run in 0.5× TBE at room temperature. The gels were dried and exposed to x-ray film.

**Western Blot Assay**—Total cell lysate was separated by SDS-polyacrylamide gel electrophoresis under reducing conditions as described previously (31). The proteins were transferred to polyvinylidene difluoride membrane (Amersham Pharmacia Biotech) and Ponceau S (Sigma) staining was performed to confirm the proper loading and transfer of proteins and to normalize the signals. Nonspecific binding to the membrane was blocked by 5% nonfat milk in Tris-buffered saline/Tween buffer, and then specific antibodies were added. c-Met protein was detected by addition of a polyclonal anti-c-Met antibody (Santa Cruz Biotechnology, Inc.), respectively, followed by addition of horseradish peroxidase conjugated goat anti-mouse antibody (Sigma). The signals were visualized by enhanced chemiluminescence system (ECL) solution (NEP Life Science Products).

**RESULTS**

**Transcriptional Activation of the c-met Gene Promoter by Wild-type p53 in a Dose-dependent Manner**—Previously, our laboratory cloned and functionally characterized the mouse c-met 5′-flanking region (24). Nucleotide sequence analysis of the mouse c-met gene promoter region identified a potential p53 binding site at –278 to –216 (Fig. 1A), which indicates the possible involvement of the p53 tumor suppressor gene product in the transcriptional regulation of the c-met gene. To determine if p53 regulates c-met gene promoter activity, C-33A carcinoma cells, which harbor a mutated p53, were cotransfected with a reporter vector containing the c-met gene promoter region (–1390 to +184, relative to the transcription start site) (24) fused to the CAT reporter gene, and expression vectors for wild-type or tumor-derived mutant forms of p53. Fig. 1B is a representative CAT assay result. c-met gene promoter activity was enhanced significantly by coexpression of wild-type p53 as compared with the c-met promoter construct co-transfected with an expression vector lacking p53 encoding sequence (labeled vector in Fig. 1). However, cotransfection of expression plasmids encoding three different mutant forms of p53 did not affect c-met gene promoter activity in parallel experiments. The reporter construct RGC-W-3X-CAT containing three wild-type p53 binding sites was also stimulated by cotransfection with expression plasmid encoding wild-type p53, and, as expected, promoter activities of the RGC-M-3X-CAT plasmid containing three copies of mutant p53 binding site and the pCAT-Basic plasmid (which is promoterless) were not affected by forced expression of wild-type p53. Stimulation of the c-met gene promoter by wild-type p53 was dependent upon the input dose of wild-type p53 (Fig. 2F).

**Identification of the c-met Promoter Region Responsible for Conferring p53-mediated Stimulation**—To functionally identify the region in the c-met gene promoter that is responsible for p53-mediated stimulation, a series of 5′ deletion mutants were constructed and cotransfected with wild-type p53 expression plasmid (Fig. 2A). Fig. 2B shows the results of CAT assays. Deletion from the 5′ end to –279 did not alter the stimulating effect of wild-type p53 on the c-met gene promoter. However, deletion of 70 base pairs from –278 to –209 completely eliminated responsiveness to p53. To assess functionality of the identified c-met p53 response element in the context of the c-met gene promoter, an internal deletion mutant of the c-met promoter (1.6DP53mc-met-CAT) was constructed in which the identified c-met p53 response element (–278 to –216) was deleted (Fig. 3A). Cotransfection of the wild-type c-met construct (1.6mc-met-CAT) with the wild-type p53 expression plasmid resulted in stimulation of c-met gene promoter activity. In contrast, p53-mediated stimulation of c-met promoter activity was completely abolished in the mutant construct when cotransfected with wild-type p53 expression plasmid (Fig. 3B). These results indicate that the nucleotide sequence from –278 to –216 in the c-met promoter region contains an element(s) responsible for p53-mediated transactivation.

**Ability of a Nucleotide Sequence Containing the c-met p53 Response Element to Confer Stimulation by Wild-type p53 to a Heterologous Minimal Promoter**—The consensus p53 binding site is the 10-bp element of 5′-PuPuPuPuCAT/AT/GPyPyPy-3′ (32). For high affinity binding, two 10-bp sites are required. Nucleotide sequence analysis indicated that the c-met p53 response element within –278 to –216 region contains two 10-bp p53 binding sites, 5′-GGCACAACCT-3′ from –261 to –252 and 5′-GACACGTGC-3′ from –233 to –224 as shown in Fig. 4A. These two sites are separated by an 18-nucleotide spacer (Fig. 4A). Each site has only one and two mismatched nucleotides, respectively, compared with the published consensus sequence. To confirm whether this p53 binding element is responsible for conferring stimulation to the c-met gene promoter, a copy of the 63 base pair DNA fragment (–278 to –216) containing the p53 binding motif was inserted upstream of the minimal promoter element of the E1BCAT plasmid (Fig. 4A) and cotransfected with wild-type p53 expression plasmid. Cotransfection of the wild-type p53 expression vector dramatically stimulated promoter activity of two independently prepared plasmid con-
structs by more than 20-fold (p53-E1BCAT clones 1 and 2) (Fig. 4B). The extent of stimulation of the heterologous promoter by the 63-bp DNA fragment containing the c-met p53 binding site was comparable to that of the RGC-W-3X sequence containing three copies of a p53 consensus binding site (positive control) (Fig. 4B). The promoter activity of the E1BCAT control vector lacking the p53 binding site (negative control) was not affected by expression of wild-type p53 (Fig. 4B). Taken together, these results demonstrate that the DNA fragment (–278 to –216) containing the putative c-met p53 binding element is responsi-
FIG. 2. Mapping of the p53 regulatory region of the c-met gene promoter. A, schematic representation of the 5' end deletion of the c-met gene promoter. B, representative CAT assay results. Two µg of various 5' end deletion mutants of the c-met gene promoter were cotransfected with 2 µg of wild-type p53 expression plasmid using the calcium phosphate precipitation method. Cells were harvested 24 h after transfection and analyzed for CAT activity. The relative CAT activity in the presence or absence of wild-type p53 expression plasmid is plotted in the bar graph. Values are means ± S.D. of three separate experiments performed in duplicate.

FIG. 3. Dependence of p53-mediated stimulation of the c-met gene promoter on the c-met p53 response element. The 1.6Dp53mc-met-CAT construct in which the identified p53 response element (−278 to −216) was internally deleted from the 1.6mc-met-CAT construct was prepared as shown in A. 2 µg of 1.6mc-met-CAT or 1.6Dp53mc-met-CAT construct were cotransfected with 2 µg of wild-type p53 expression plasmid using the calcium phosphate precipitation method. Cells were harvested 24 h after transfection and analyzed for CAT activity. Transfection experiments and CAT assays were performed three times in duplicate, and the results are depicted as relative CAT activity (fold increase over the corresponding met-CAT construct that did not receive p53 expression plasmid) (B). The bar indicates the standard deviation.
ble for p53-mediated stimulation of mouse c-met gene promoter activity, and the identified p53 binding element is functional. Alignment of the mouse c-met promoter nucleotide sequence with that of the human indicates that the p53 binding site is well conserved between the two species (Fig. 5).

p53 Protein Binds to the c-met Promoter p53 Response Element in Vitro—To directly show the interaction of p53 protein and the c-met p53 response element, EMSAs were performed. The 63-bp DNA fragment (–278 to –216) containing the c-met p53 binding element was used as a probe, and the purified recombinant p53 core domain protein was used in the binding reaction. It is known that p53 contains 393 amino acids and is divided into three functional domains: amino acids 1–101 for transactivation by interacting with the basal transcriptional machinery, amino acids 102–292 for sequence-specific DNA binding, and amino acids 293–393 for oligomerization. The DNA binding specificity of the core domain protein is comparable to the full-length wild-type p53 protein (33). As we expected, the mobility of the labeled probe DNA was shifted by p53 core domain protein (Fig. 6, lane 2) and formed a binding complex. The shifted complex was diminished by increasing amounts of self-competitor as well as a wild-type consensus p53 binding site (lanes 3, 4, and 7) but not by nonspecific competitor (lanes 5 and 6). When the wild-type consensus p53 binding site was used as a probe, it specifically bound to the p53 core domain protein (lanes 9 and 10 as positive control). These results demonstrate that p53 protein directly binds to the identified c-met p53 response elements to exert p53-mediated stimulation of the c-met gene promoter.

Induction of Endogenous c-met Gene Expression by p53—We were interested to know whether p53 plays a role in regulating the expression of the endogenous c-met gene. We used RKO cells that express wild-type p53 and RKO cells that have been stably transfected either with a dominant negative mutant form of p53 or with viral E6 protein to impair p53 function. In this system, it is well documented that p53 expression is up-regulated after UV irradiation, resulting in the induction of its downstream target genes such as p21WAF1/CIP1 only in the parental RKO cell line, which has a functional wild-type p53 (27). As shown in Fig. 7, c-Met expression is up-regulated in RKO cells having a functional wild-type p53 protein within 3 h after UV light exposure. Similarly, the well known target of p53, p21WAF1/CIP1 protein, is also induced. In contrast, RKO cells transfected with mutant p53 or the viral E6 gene, which inactivates wild-type p53 protein, fail to show induction of c-Met and p21WAF1/CIP1 proteins. These results suggest that wild-type p53 is important in the up-regulation of c-Met under these experimental conditions.

DISCUSSION

p53 has been demonstrated to function as an important regulator of cell proliferation in response to certain stimuli such as DNA damage (34, 35). Despite the progress achieved toward understanding p53 functions, the molecular mechanisms by which p53 acts as a key regulator of cell growth and tumorigenesis are still unclear. Studies have demonstrated that p53 functions as a transcription factor and regulates a number of target genes at the transcriptional level. The central
region of the p53 protein interacts with the promoter of target genes in a sequence-specific manner, binding to two copies of a consensus element (5'-PuPuPuCA/T(A/T)GPyPyPy-3') (32). While wild-type p53 is a transactivator of the promoters containing a p53 binding motif, various tumor-derived mutant forms of p53 protein are defective in sequence-specific transactivation. Among the genes that are positively influenced by p53, c-Met, the protein tyrosine kinase cell surface receptor for HGF and transmits its multiple signals such as induction of cell growth, differentiation, and the apoptotic/antiapoptotic functions in normal and abnormal tissue growth. Previously, we have cloned and characterized the c-met promoter (24). Nucleotide sequence analysis identified a potential p53 binding element located within the p53-responsive promoter region (24), the c-met gene promoter (278 to 216) contains two 10-bp p53 binding sites (5'-PuPuPuC(A/T)(A/T)GPyPyPy-3'). These sites contain only one consensus element and 18 base pairs (Fig. 1). Unlike some p53 target gene promoters, which have been shown to be transactivated by tumor-derived mutant forms of p53 (32), c-met gene promoter activity was not affected by various p53 mutants (Fig. 1). In addition, the stimulatory effect of p53 on the c-met gene promoter was dependent upon the input dose of p53 expression plasmid (Fig. 1C). Doses ranging from 0.25 to 1.5 μg of p53 expression plasmid led to a continuous increase of p53-mediated stimulation. Maximal stimulation was reached at a dose of 1.5 μg of p53 expression plasmid and was slightly repressed at higher doses. It has been reported that p53 interacts with TATA box-binding protein (TBP) and interferes with the binding of TBP to the TATA box (43). Thus, it seems likely that, at a higher dose, p53 may sequester TBP and prevent its interaction with TFIID, which is required for the initiation of RNA polymerase II-dependent transcription.

p53-mediated stimulatory activity in the mouse c-met promoter was mapped to the region −278 to −216 by functional analysis (Figs. 2 and 3). These functional analysis results are in agreement with results of nucleotide sequence analysis of the c-met gene promoter. The potential p53 binding element identified within −278 to −216 contains two 10-bp p53 binding sites (5'-GGACAAACCT-3' and 5'-AGACACGTGC-3') separated by 18 base pairs (Fig. 1A). These sites contain only one and two nucleotide mismatches, respectively, compared with the published consensus p53 binding site, 5'-PuPuPuCA/T(A/T)GPyPyPy-3' (32). Previous studies demonstrated that at least two copies of the 10-bp base pair p53 binding site, separated by 0–13 base pairs, are required for high affinity p53 binding (32), and the number of intervening nucleotides is not absolutely crucial. It is of interest to note that the p53 binding element is well conserved between the mouse and human c-met promoters (Fig. 5). The extent of stimulation of the heterologous promoter by one copy of the DNA fragment (−278 to −216) containing the c-met p53 binding element was highly comparable to that produced by the RGC-W-3X-CAT containing three copies of the consensus p53 binding element (Fig. 4). Moreover, the purified DNA binding domain of p53 protein strongly and specifically bound to the c-met p53 response element in EMSA (Fig. 6).

p53 has been shown to interact with other transcription factors such as Sp1 and MDM-2 (44, 45). As shown by nucleotide sequence analysis (24), the c-met gene promoter is highly

---

**Fig. 5. Nucleotide sequence comparison of the mouse and human c-met 5′-flanking region.** Nucleotide sequence alignment of the mouse and human c-met promoter regions (GenBank accession numbers AF030200 and AC002080, respectively) were carried out using the DNASTAR alignment software. The 10-bp p53 binding sites are boxed and marked as I and II, respectively. The asterisks indicate identical nucleotides shared between species (m, mouse; h, human). The arrow indicates the transcription start site.
GC-rich. We identified two Sp1 binding sites (5′-GGCGG-3′) in the c-met gene promoter and demonstrated that Sp1 transcription factor is critically involved in transcriptional regulation of the c-met gene promoter (24). Thus, we cannot rule out the possibility that p53 also regulates the c-met gene promoter activity by cooperatively interacting with Sp1.

As the “guardian of the genome,” p53 is an important component of the DNA damage-inducible response. It is activated by genotoxic agents such as UV irradiation and then transactivates other genes to induce DNA repair and cell survival (27). In our experiment, we observed that the endogenous c-met gene product is induced after UV exposure, correlating with the expression of p53 and its target p21WAF1/CIP1. However, when wild-type p53 function was impaired by a mutant form of p53 or by viral E6 protein, induction of the p53 target gene p21WAF1/CIP1 as well as c-Met was abolished. These results demonstrate for the first time that c-Met is induced by UV light and that p53 plays a role in this activation process.

Although p53 is a transcription factor that serves a dual role in the regulation of cell proliferation, it is most recognized for its cell cycle arrest and apoptotic activity. As stated previously, HGF/c-Met is a multifunctional system that is involved in growth control and differentiation. HGF/c-Met inhibits the growth of some normal and tumor cells (20–22), and the level of expression of HGF and c-Met correlates with the degree of cell differentiation (31). c-Met is underexpressed in human breast carcinomas that harbor a mutant form of p53 (46). These results suggest that c-Met may cooperate with wild-type p53 to negatively regulate cell growth and induce differentiation. Experiments have demonstrated that wild-type p53 transactivates the genes encoding EGFR and TGF-α, which are known to positively modulate cell proliferation (37, 39). A number of studies have reported overexpression of EGFR (47, 48) and TGF-α (49, 50) in a wide variety of human cancers. It is also interesting to note that the HGF promoter was recently reported to be transcriptionally activated by p53 (51). These findings suggest that wild-type p53 plays a role in controlling the expression of both c-Met and its ligand, HGF, ultimately leading to regulation of cell growth and differentiation.

Acknowledgments—We thank Dr. Paul Robbins for RGC-W-3X-CAT, RGC-M-3X-CAT, and p53 expression plasmids, and Dr. Nikola P. Pavletich for purified p53 core domain protein. We also thank Dr. Marie C. DeFrances and Aaron Bell for critical reading of the manuscript.

REFERENCES
1. Bottaro, D. P., Rubin, J. S., Faletto, D. L., Kniecik, T. E., Vande Woude, G. F., andAaronson, S. A. (1991) Science 251, 802–804
