Benzo[a]pyrene Activates the Human p53 Gene through Induction of Nuclear Factor κB Activity*

(Received for publication, June 14, 1999, and in revised form, September 20, 1999)

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p53 is known to be recruited in response to DNA-damaging genotoxic stress and plays an important role in maintaining the integrity of the genome. In the present study, the effect of a potent lung cancer carcinogen, benzo[a]pyrene (B[a]P) on p53 expression was investigated. We showed that exposure of A549 and NIH 3T3 cells to B[a]P resulted in an increase in p53 mRNA levels and in p53 promoter activation, indicating that B[a]P-induced p53 expression is partly regulated at the transcriptional level. The p53 promoter region which extends from −58 to −43, overlapping the κB motif, is essential for both the p53 basal promoter activity and p53 promoter activation induced by B[a]P. Nuclear factor κB (NF-κB) proteins have been revealed to be activated in B[a]P-induced p53 expression. Activated NF-κB complexes were shown to contain predominantly p50 and p65 subunit components in A549 cells and p65 subunit in NIH 3T3 cells. In addition, the overexpression of IκBα completely inhibited NF-κB activation, p53 promoter transactivation and the stimulatory effect on p53 transcription induced by B[a]P. We therefore conclude that B[a]P transcriptionally activates the human p53 gene through the induction of NF-κB activity.

Lung cancer is one of the most prevalent cancers in the world, and its mortality is expected to remain very high for many years to come (1). Concurrently, the environmental air quality is deteriorating and the number of smokers has increased. Epidemiological studies over the past several decades have provided a considerable body of evidence linking lung cancer to a number of mutagens and carcinogens detected both in the environment and cigarette smoke.

Polycyclic aromatic hydrocarbon (PAH)1 carcinogens, such as benzo[a]pyrene (B[a]P), are products of incomplete combustion of organic matter and are widespread in the environment. Carcinogenic and mutagenic effects of B[a]P have been well documented in human, animals, and mammalian cell systems (2, 3). Most PAH require metabolic activation to vicinal bay-

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1 The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; B[a]P, benzo[a]pyrene; m.o.i., multiplicity of infection; CDDP, cisplatin; NF-κB, nuclear factor-κB; β-gal, β-galactosidase; CAT, chloramphenicol acetyltransferase; DTT, dithiothreitol; bp, base pair(s); PCR, polymerase chain reaction; PBS, phosphate-buffered saline.

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and what cellular factors are involved. The results presented herein suggest the transcriptional activation of human p53 promoter by B[a]P-induced NF-κB complexes.

**EXPERIMENTAL PROCEDURES**

**Carcinogen and Antibodies—**B[a]P, obtained from Sigma-Aldrich, was dissolved in dimethyl sulfoxide immediately prior to use and then was added directly to the cell culture medium as 1000 x stocks to obtain the desired final concentration. Polyclonal antibodies against IκBα and the subunits of NF-κB (p50, p65, p52, and c-Rel) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal anti-p53 antibodies (Ab-6 and Ab-1) were obtained from Oncogene Science (Cambridge, MA).

**Plasmids—**The recombinant chloramphenicol acetyltransferase (CAT) vector, p35CAT3D255, which contains the human p53 gene promoter region −325 to +12 (the 3′ end of the fragment is an XbaI site and is about 12 bp upstream of the major transcription initiation site, the most 3′ end of which is defined as +1 in the present study) (22, 26) was constructed using PCR. Human genomic DNA, derived from normal human lymphocytes, was used as a template, and PCR was performed with the following primers: sense, 5′-GGTATGGCTGAGATATACG-3′; antisense, 5′-CAATCCAGGAAAGGTGTC-3′. The PCR product was digested with HindIII and XbaI and then ligated into pCAT Basic vector (Promega), which contains the CAT reporter gene but lacks a eukaryotic promoter region. The orientation and sequence of the inserted fragment were confirmed by sequencing.

With p35CAT3D255 as a template, p53 promoter fragments from −135 to +12 (d135), from −80 to +12 (d80), and from −50 to +12 (d50) with 5′ HindIII and 3′ XbaI ends were prepared by PCR and then were cloned into the pCAT Basic vector digested with HindIII and XbaI. Various linker-scanning mutations (mt 1 to mt 5) shown in Fig. 2 were introduced to p35CAT3D255 by an improved site-directed mutagenesis method using PCR (27).

The pSV-β-galactosidase (β-gal) control vector, a β-gal-expressing plasmid, was obtained from Promega.

**Cell Culture, DNA Transfection, and Exposure to B[a]P—**A human lung adenocarcinoma cell line, A549 (distal respiratory epithelium-like), was maintained in RPMI 1640 medium containing 10% fetal bovine serum (CC Laboratories, Cleveland, OH). A mouse fibroblast cell line, NIH 3T3 was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. For DNA transfection, cells were plated at a density of 5 x 10^4 cells/80-mm diameter culture dish 24 h before transfection. The cells were transfected with 5 μg of various CAT reporter plasmids and 1 μg of a pSV-β-gal vector, which contains the CAT reporter gene but lacks a eukaryotic promoter region. The orientation and sequence of the inserted fragment were confirmed by sequencing.

With p35CAT3D255 as a template, p53 promoter fragments from −135 to +12 (d135), from −80 to +12 (d80), and from −50 to +12 (d50) with 5′ HindIII and 3′ XbaI ends were prepared by PCR and then were cloned into the pCAT Basic vector digested with HindIII and XbaI. Various linker-scanning mutations (mt 1 to mt 5) shown in Fig. 2 were introduced to p35CAT3D255 by an improved site-directed mutagenesis method using PCR (27).

The pSV-β-galactosidase (β-gal) control vector, a β-gal-expressing plasmid, was obtained from Promega.

**Northern Blot Analysis—**The cells were grown for 24 h, then treated for 4, 8, or 24 h with B[a]P (10 μM). At the end of the treatments, the total RNA was extracted by using Isogen (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. The total RNAs (20 μg) from each treatment were denatured with formaldehyde-formamide and separated in a 1% agarose-formaldehyde gel. RNAs were then transferred to a nylon membrane (Amersham Pharmacia Biotech). The membrane was hybridized with the full-length human p53 cDNA or a 0.83-kilobase PstI fragment of mouse p53 cDNA (30). The probe was labeled with [α-32P]dCTP by random priming using the Megaprime DNA labeling system (Amersham Pharmacia Biotech), and purified through Nick spin columns (Amersham Pharmacia Biotech). A β-actin cDNA probe was later used to rehybridize the same membrane. The levels of p53 mRNA were quantified with the Fuji BAS 2000 phosphorimager and normalized to β-actin values.

**RESULTS**

Enhanced p53 Expression in A549 and NIH 3T3 Cells Treated by B[a]P—Although the results of previous studies showed that the cellular p53 protein levels increase upon exposure to B[a]P and its metabolites, and suggested that this increase is due mainly to an increase in p53 protein stability (24, 31, 32), it has not been studied as to whether transcriptional activation is also involved in p53 up-regulation in response to carcinogen treatment. To investigate the mechanisms leading to p53 protein accumulation in human lung adenocarcinoma cells after carcinogen treatment, the A549 cells that retain a wild-type p53 gene were exposed to B[a]P for increasing periods of time under serum-free conditions. Western blotting showed an increased p53 expression following stimulation...
B[a]P Activates p53 Transcriptionally

**FIG. 1.** B[a]P induces p53 mRNA as well as p53 proteins in A549 (A and B) and NIH 3T3 (C and D) cells. A and C, Western blot analysis. A549 (A) or NIH 3T3 (C) cells were exposed to B[a]P (10 μM) for various times as indicated under serum-free conditions, and nuclear extracts were analyzed by Western blotting using anti-p53 monoclonal antibodies (Ab-6 or Ab-1). The results are representative of four separate experiments. B and D, Northern blot analysis. After treatment with B[a]P as described above, total cellular RNA extracted from A549 (B) or NIH 3T3 (D) cells were hybridized to human or mouse p53 cDNA; the same blots were rehybridized to a β-actin probe. Ethidium bromide staining of the agarose gel is shown as an additional control for the amounts of RNA loaded in each lane (20 μg/ lane). The positions of 28S and 18S ribosomal RNAs was indicated. The results are representative of four separate experiments. Quantitative analysis of relative p53 mRNA levels is shown in the lower panel.

The discrepancy between p53 protein and mRNA levels after the treatment for 4 h may be the result of an increased stability of the protein, consistent with the previous findings (23, 24). CDDP, an anti-cancer agent that was reported to result in an increase in p53 mRNA (22), was used as a positive control. A similar stimulatory effect with B[a]P on p53 mRNA increase was observed in the A549 cells treated with the CDDP (data not shown). The p53 mRNA levels in NIH 3T3 cells also increased in response to B[a]P treatment (Fig. 1D), although the extent was less than in A549 cells.

**Activation of p53 Promoter by B[a]P—**The above described results prompted us to examine whether B[a]P activates the p53 gene promoter. A previous study identified that an 85-bp fragment spanning the p53 promoter region, the 3′ end of which is about 10 bp downstream of the major transcriptional initiation sites, is all that is required for full promoter activity (26), and this region is also required for genotoxic stress-responsive elements required for basal and B[a]P stress-responsive activities reside within this 80-bp region of the p53 promoter and further suggest that the p53 promoter region from −235 to −135 contains a region that represses the p53 proximal promoter activity.

As shown in Fig. 2, the 80-bp p53 core promoter region contains potential binding motifs for NF-κB (34) and basic helix-loop-helix transcription factors (35). We introduced three linker-scanning mutations in this 53-kb core promoter region (Fig. 2), and p53 promoter-CAT reporters containing these mutated 325-bp p53 promoter regions were examined for their basal promoter activities and responsiveness to B[a]P treatment. The results shown in Fig. 4 indicate that the mutations (mt 1 and mt 2) in the p53 promoter region from −58 to −43, overlapping the κB motif, result in 4.4- and 3.2-fold reduction in the basal promoter activity, respectively, and severely impair the p53 promoter activation induced by B[a]P. A mutation (mt 3) affecting a potential helix-loop-helix transcription factor binding motif did not have any significant deteriorative effect on the basal as well as on p53 B[a]P-stress-responsive promoter activity. These results thus indicate that the p53 promoter region extending from −58 to −43, overlapping the κB motif, is essential for both the p53 basal promoter activity and p53 promoter activation induced by B[a]P.

**B[a]P Activates NF-κB in A549 and NIH 3T3 Cells—**The results from a deletion analysis of p53 gene promoter region in the present and previous study (34) prompted us to determine whether NF-κB proteins are involved in B[a]P-induced p53 expression. We first examined the induction of NF-κB complexes in B[a]P-treated A549 and NIH 3T3 cells. Gel shift assays performed with nuclear extracts from A549 cells showed that stimulation with B[a]P at 10 μM induced a strong NF-κB DNA binding activity within 4 h and maintained until 24 h after B[a]P treatment. This induction was observed when the nuclear extracts were incubated with a canonical NF-κB consensus probe (data not shown) or with a NF-κB p53 probe derived from −39 to −60 of the p53 promoter (wt NF-κB p53 probe) (Fig. 5A). A similar stimulating effect of B[a]P on NF-κB DNA binding activity was also observed in NIH 3T3 cells (Fig. 5B).

Competition studies were performed with a 50-fold excess of the unlabeled wt NF-κB p53 probe, an oligonucleotide probe with mutations in the p53 NF-κB site (mt NF-κB p53 probe) and the canonical NF-κB consensus probe. As shown in Fig. 6, the protein binds NF-κB consensus site specifically, as the binding activity was fully inhibited by the unlabeled wt NF-κB p53 probe and by the canonical NF-κB consensus probe but not by the mt NF-κB p53 probe. A further characterization of the nuclear proteins binding to the NF-κB motif was obtained by preincubating the nuclear extracts with antibodies against the
p50, p65, p52, and c-Rel NF-kB subunits. Both p50 and p65 antibodies partially shifted the B[a]P-induced complex in A549 cells (Fig. 6A), whereas p52 and c-Rel antibodies did not. These data indicate that B[a]P activates NF-kB p50 and p65 proteins in A549 cells. Analysis of specific subunit components in the complex revealed the presence of p65 protein in NIH 3T3 cells (Fig. 6B).

**FIG. 2.** The structure of 5′ deletion and linker-scanning mutants of p53 promoter-CAT reporter genes. The human p53 promoter sequence from −30 to −60 and potential transcription factor binding motifs, kB (35) and helix-loop-helix (36), were shown. The most 3′ end of major transcription initiation sites for the human p53 gene as determined by Tuck and Crawford (28) is tentatively defined as +1 in the present study. Underlined sequences in p53CATmt 1, p53CATmt 2, and p53CATmt 3 represent the mutated positions.

**FIG. 3.** The activation of p53 promoter by B[a]P in A549 and NIH 3T3 cells. The cells were transfected with the p53CATd325 reporter plasmid and were exposed to various concentrations of B[a]P. CDDP (10 μg/ml) was used as a positive control (24). The CAT activity is expressed as the -fold increase in induction relative to the control (CTL) treated with vehicle only. The results represent the average of four independent experiments.

**FIG. 4.** Responsiveness to B[a]P treatment of 5′ deletion and linker-scanning mutants of the p53 promoter. A549 cells were transfected with the wild-type p53CATd325 or various 5′ deletion and linker-scanning mutants, and then exposed to B[a]P (10 μM). The CAT activity of each of the mutations have been assigned relative to the CAT activity of the wild-type p53CATd325, treated with vehicle only, taken as 1. The results represent the average of four independent experiments.

**FIG. 5.** B[a]P activates NF-kB in A549 and NIH 3T3 cells. A549 (A) and NIH 3T3 (B) cells were treated with either B[a]P (10 μM) or an equivalent volume of vehicle (control) for various times. The 32P-labeled double-stranded oligonucleotide containing the kB site from the human p53 promoter was used as a probe. Nuclear extracts were prepared following stimulation and analyzed for NF-κB binding activity as described under “Experimental Procedures.” The arrow corresponds to binding of NF-κB to the probe. The results are representative of four separate experiments.

**FIG. 6.** B[a]P—A recombinant adenosivirus expressing IxBa (rAd.IxBa) was used to infect A549 cells. After 3 days of infection with the recombinant adenovirus, the A549 cells expressed high levels of IxBa in a m.o.i.-dependent manner, based on the Western blotting findings (Fig. 7A). The amounts expressed...
were estimated to be about 60–70 times higher as compared with the endogenous protein. The stimulation of the infected cells at day 3 with B[a]P for 6 h did not detectably decrease the IκBα levels. In transient transfection assays, B[a]P stimulation in A549 cells infected with the rAd.IκBα did not induce any significant p53 promoter activation as compared with the level of activation in the control cells (Fig. 7B; compare with Fig. 3). An electrophoretic mobility-shift assay analysis demonstrated that the overexpression of the IκBα led to a complete suppression of B[a]P-induced NF-κB activation (Fig. 7C). No stimulatory effect of B[a]P on p53 mRNA levels was observed in the A549 cells infected with the rAd.IκBα (data not shown).

DISCUSSION

p53 recruitment in response to various genotoxic stresses is an important cellular response to maintain the integrity of the genome (14). After metabolic activation, carcinogens, such as PAHs, bind to DNA and form predominantly covalent carci-

Fig. 6. Specificity of p53wt probe containing the κB site from the human p53 promoter and antibody supershift assays. Binding reactions with nuclear extracts from A549 (A) and NIH3T3 (B) cells treated with B[a]P for 6 h were incubated with the 32P-labeled p53wt oligonucleotide (lane 8). Specificity of the oligonucleotide was confirmed by competition experiments with a 50-fold excess of unlabeled p53wt oligonucleotide (lane 5), unlabeled p53mt oligonucleotide (lane 6), or unlabeled canonical NF-κB consensus sequence (lane 7). Supershift assays performed using polyclonal antibodies against p50 (lane 1), p65 (lane 2), p52 (lane 3), or c-Rel (lane 4) identified p50 and/or p65 as constituents of the NF-κB complexes. The position of supershifted complexes is indicated. The results are representative of three separate experiments.

Fig. 7. A, adenovirus-mediated IκBα expression and responsiveness of the exogenous IκBα to B[a]P treatment. A549 cells were infected with the rAd.IκBα at a various of m.o.i. as indicated and, after 3 days of incubation, analyzed by Western blotting using the anti-IκBα antibody. In the last two lanes, cells were infected with rAd.IκBα at m.o.i. 10 and, after 3 days of incubation, treated with B[a]P (10 μM) for 4 and 8 h, respectively. The results are representative of three separate experiments. B, activation of p53 promoter by B[a]P in A549 cells infected with the rAd.IκBα. A549 cells were infected with the rAd.IκBα at m.o.i. 10 for 3 days, then transfected with the p53CAT325 reporter plasmid and exposed to various concentrations of B[a]P and CDDP (10 μg/ml). CAT activity is expressed in -fold induction relative to the control (CTL) treated with vehicle only. The results represent the average of three independent experiments. C, inhibition of NF-κB activation in cells infected with rAd.IκBα. A549 cells infected with rAd.IκBα at m.o.i. 10 were treated with B[a]P (10 μM) for the indicated times (lanes 2–4). The nuclear extracts were analyzed by electrophoretic mobility-shift assay using the labeled p53wt oligonucleotide. The black arrow corresponds to the binding of NF-κB to the probe. Lane 1 refers to control A549 cells treated with B[a]P for 6 h. A549 cells infected without (lane 5) or with rAd.IκBα 10 m.o.i. (lane 6) were shown. The results are representative of three separate experiments.
p53 promoter transactivation, and the stimulatory effect on p53 transcription induced by B[a]P.

The effect of PAHs, a ubiquitous environmental pollutant, on p53 expression was recently investigated (23, 24, 31, 32). p53 protein expression was first reported to be closely correlated with B[a]P-DNA adducts in carcinoma cell lines by Ramet et al. (31). They found the activation of B[a]P in both A549 lung cancer and MCF-7 breast adenocarcinoma cell lines containing wild-type p53 and formation of BPDE-DNA adduct were followed by an increase in p53 protein expression. The possible reasons for the increase in p53 expression are, like other DNA-damaging agents, the stabilization of p53 protein in the cells (24, 31, 32, 36) and the stimulation of p53 transcription (22, 23).

Since the p53 genotoxic stress response is a complex cellular process and may be regulated at the transcriptional level (22, 23), PAH-induced p53 expression may also be regulated in the same manner. Indeed, our results revealed that B[a]P stimulated the p53 expression transcriptionally through the p53 promoter activation, thus suggesting the existence of another pathway regulating p53 expression. Our results are supported by the conclusion of Sun et al. and Hellin et al. (22, 23), although both groups used anti-cancer drugs as genotoxic agents.

The previous study by Tuck and Crawford identified the human p53 promoter region (from −78 to +10) required for full basal p53 promoter activity (26). The results presented in our study indicate that this p53 promoter region is sufficient for p53 B[a]P stress-responsive promoter activity. Similar results have been reported by Sun et al. (22), in which they also found this region to be sufficient for p53 genotoxic stress-responsive promoter activity. This region contains potential binding motifs for several important transcription factors, including NF-κB and helix-loop-helix. The present study identified that a novel p53 promoter element involved in basal and B[a]P-induced promoter activation resides in the region from −58 to −43, overlapping the kB motif. B[a]P induced the p53 promoter through the binding of NF-κB proteins to the κB site.

NF-κB is a rapidly inducible transcription factor involved in the response of various stimuli, including cytokines, activators of protein kinase C, viral infections, and oxidants. Following its intracellular activation, NF-κB regulates the expression of many genes that code for cytokines, growth factors, acute phase response proteins, and cellular receptors and thus modulates the various cellular responses to the stimuli. NF-κB complexes bind DNA as dimers constituted from a family of proteins, Rel/NF-κB family. The family contains the proteins p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), RelB, and c-Rel (Rel) (37). The Rel/NF-κB family of proteins share an amino-terminal 300-amino acid domain (Rel homology domain), including DNA binding and dimerization domains and the nuclear translocation signal, which is most likely the binding site for IkB. In unstimulated cells, NF-κB is found in the cytoplasm and is bound to IkB (IkBo and IkBα), which prevents it from translocating to the nuclei. When these cells are stimulated, specific kinases phosphorylate IkB, causing its rapid degradation by proteasomes. The release of NF-κB from IkB results in the translocation of NF-κB into the nucleus, where it binds to specific sequences in the promoter regions of target genes.

Previous studies have identified the p53 responsive element that overlaps with an NF-κB binding site. This κB site is responsible for the induction of p53 transcription by transfection with p65, or treatment with either tumor necrosis factor-α or daunomycin, an anti-cancer agent (23, 34, 38). We herein demonstrated that, in human lung epithelial cells and mouse fibroblast cells, human p53 promoter was activated by NF-κB after exposure to B[a]P, thus supporting the above conclusion. Nevertheless, these results are in conflict with those reported by Sun et al. (22), who showed that NF-κB, while inducible by various anticancer drugs, does not play a major role in human p53 promoter activation induced by genotoxic agents. This discrepancy could result from using different cell types that may cause a lack of essential cellular factors (11, 22, 23). Indeed, it has previously been reported that daunomycin failed to trigger p53 gene expression in myeloid leukemia cells, indicating that anthracycline drugs might have distinct p53 induction activities in different cell types (11).

It is established that the dimer composition of the NF-κB complex determines its fine DNA binding specificity, giving rise to selective transcriptional activation or attenuation. Transcriptional activation of specific sets of genes will primarily depend on various dimer combinations being activated distinctly, or whether their relative amounts in cell types and tissues are subject to regulation (37, 39). Analysis of subunits present in the activated complexes from B[a]P-treated A549 cells indicated the presence of both p65 and p50 components, which together constitute the predominant transcriptionally active NF-κB dimer combination. We failed to find the presence of other subunits except p65 in the activated complexes from B[a]P-treated NIH3T3 cells. There are several possible explanations for the failure of identification of p50 subunit in NIH 3T3 cells. First, it is reported that p50 is barely detectable in quiescent NIH 3T3 cells that are derived by incubation of cells in serum-free medium (40). The p50 subunit in NIH 3T3 cells binding to NF-κB sequence may be too small to be detected by supershift assay in the present study. Second, binding ability of Rel/NF-κB dimers can be modulated by the κB sites, nearby DNA sequences, other DNA-binding proteins, and other unclear factors (37). Even using same cells' nuclear extract and identical canonical NF-κB consensus probe discrepant results were also observed (41–44). Third, another reason might be dependent on the cell line tested. There is a report suggesting at least two NIH 3T3 cell lines of different origins circulating in the world (45). Indeed, some researchers identified p50 in NIH 3T3 cells binding to NF-κB sequence (46), while others did not (47). Nevertheless, since p50 lacks a potent transcriptional activation domain, it does not generally activate transcription as homodimers in vitro (37). Transfection of p50 subunit did not activate transcription of p53 promoter (34). p65 subunit contains a more potent transcription activation domain than p50 subunit, and expression of p65 alone, or of p65, in combination with other subunits, result in more significant increase in NF-κB-dependent transcription than other subunits (37, 39). Therefore, the p65 subunit in the activated complexes is responsible mainly for transcriptional activity of NF-κB.

NF-κB activation in response to various stimuli is generally considered to occur rapidly within minutes (37). In the present study we found that the NF-κB activation by B[a]P was induced in 2–4 h and then was sustained for up to 24 h. The time course of NF-κB activation by B[a]P is concordant with the results when NF-κB was activated by daunorubicin and UV irradiation (38, 48). Bender et al. (48) recently found two totally different sequentially occurring mechanisms of UV-induced NF-κB activation and IkB degradation. They reported that the early IkBo degradation at 30 min to 6 h is not initiated by UV-induced DNA damage. It does not involve the phosphorylation of IkBo by IkBo kinase. On the other hand, IkB degradation and NF-κB activation at late time points, 15–20 h after UV irradiation, requires the phosphorylatable IkBo. This late mechanism thus clearly supports our results.

When IkBo was overexpressed in A549 cells, the B[a]P-induced NF-κB activation was suppressed and thus inhibited the p53 promoter activation in transient transfection assays.
and the stimulatory effect on p53 mRNA levels. These data provide direct evidence that the functional inhibition of NF-κB alters the B[a]P-induced p53 promoter activation and support the view that B[a]P-mediated p53 transcription is NF-κB-dependent.

In summary, the present study revealed that a potent lung carcinogen, B[a]P, increased the p53 expression at both the protein and mRNA levels. It activated the p53 promoter in two different cell lines, thus suggesting that the induced p53 was, at least partly, regulated transcriptionally. We identified that the p53 promoter region extending from −58 to −43, overlapping the κB motif, is essentially required for both p53 basal promoter activity and p53 promoter activation induced by B[a]P. Furthermore, NF-κB proteins are also identified to be involved in the B[a]P-induced p53 expression.

Acknowledgments—We thank Dr. Hikaru Ueno (Molecular Cardiology Unit, Research Institute of Angiocardiology and Cardiovascular Clinic, Kyushu University) for kindly providing recombinant adenovirus encoding IκBα and Dr. Yi-Ming Mu (Third Department of Internal Medicine, Kyushu University) for technical help. We are also most thankful to Dr. Kimitori Kohn (Department of Molecular Biology, University of Occupational and Environmental Health) for helpful and stimulating discussions.

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J. Biol. Chem. 1999, 274:35240-35246.
doi: 10.1074/jbc.274.49.35240

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