Identification and Characterization of the IKKα Promoter

POSITIVE AND NEGATIVE REGULATION BY ETS-1 AND p53, RESPECTIVELY*

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IKKα, a subunit of IkB kinase (IKK) complex, has an important role in the activation of nuclear factor-kB (NF-kB), a key regulator of normal and tumor cell proliferation, apoptosis, and response to chemotherapy. However, little is known about the transcriptional regulation of the IKKα gene itself. The present study revealed that the transcriptional induction of the IKKα gene is positively regulated by binding ETS-1, the protein product of the ETS-1 proto-oncogene. Furthermore, ETS-1 mediated activation of IKKα is negatively regulated by p53 binding to ETS-1. By analyzing the genomic DNA sequence, we identified the putative IKKα promoter sequence in the 5′-flanking untranslated region of the IKKα gene. Transfection of EU-4, an acute lymphoblastic leukemia (ALL) cell line, with plasmids containing the IKKα 5′-untranslated region sequence upstream of the luciferase reporter showed that this region possessed major promoter activity. Induction or enforced overexpression of p53 represses IKKα mRNA and protein expression as well as IKKα promoter activity. Deletion and mutation analyses as well as chromatin immunoprecipitation and electrophoretic mobility shift assay indicated that ETS-1 binds to the core IKKα promoter and strongly induces its activity. Although p53 does not directly bind to the IKKα promoter, it physically interacts with ETS-1 and specifically inhibits ETS-1-induced IKKα promoter activity. These results suggest that the proximal 5′-flanking region of the IKKα gene contains a functional promoter reciprocally regulated by p53 and ETS-1. Furthermore, loss of p53-mediated control over ETS-1-dependent transactivation of IKKα may represent a novel pathway for the constitutive activation of NF-kB-mediated gene expression and therapy resistance in cancer.

NF-kB is a transcription factor that plays an essential role in regulating the balance between cell proliferation and apoptosis, including the response of tumor cells to chemotherapy (1–3). Activation of the IkB kinase complex (IKKa/β/γ) is a critical step in the activation of the NF-kB pathway (4, 5). Recent studies have identified an important role for IKKα distinct from that of IKKβ in regulating NF-kB-mediated gene expression (6–8). Unlike IKKβ, which is localized predominantly in the cytoplasm, IKKα can shuttle between the cytoplasm and nucleus (9, 10). In the nucleus, IKKα is recruited to the promoter region of the NFkB-regulated genes, and activates their expression by interacting with cAMP-response element-binding protein as well as by phosphorylating histone H3, which is critical for the activation of NF-kB-directed gene expression (10, 11).

Previous studies have shown that NF-kB in tumor cells is activated by treatment with tumor necrosis factor α (12, 13) and certain chemotherapeutic agents (13–15). Similarly, constitutively activated NF-kB (i.e. activation of NF-kB in cells without stimulation) has been associated with increased cell proliferation and survival in cancer cells, and may be linked to drug resistance in these cells (16–18). Although the mechanisms by which NF-kB regulates resistance to apoptosis are not completely understood, it is believed that activated NF-kB in the nucleus protects cells against apoptosis through directly activating transcription of NF-kB-dependent genes. These downstream genes include members of the Inhibitor-of-Apoptosis Protein (IAP) family such as cIAP1, cIAP2, XIAP (19–22), and Bcl-2 family members Bcl-XL and Bfl-1/A1 (23, 24).

In contrast to the role of NF-kB in protecting cells from apoptosis, the p53 tumor suppressor gene plays an important role in inducing apoptosis in response to various types of stress including chemotherapeutic drug treatment. p53 functions as a transcription factor that can either positively or negatively regulate transcription of a particular gene promoter (25). Previous studies have shown that p53 induces apoptosis by activating apoptosis-promoting genes such as Bax, DR5, and Fas (26–28), or by repressing apoptosis-inhibiting genes such as MDR, Bcl-2, and survivin (29–32).

A recent study using microarrays representing over 33,000 individual human genes aimed at identifying differentially expressed genes in response to p53-induced apoptosis has found that a total of 1501 genes (4.4%) responded to p53, and ~80% of these were repressed by p53 (33). In activating gene expression, p53 functions via DNA sequence-specific binding (34). The specific p53 consensus sequence consists of two copies of a decamer motif separated by 0 to 13 bp of random nucleotide (35). Transcriptional repression mediated by p53 is complex and may occur via multiple mechanisms. A few genes are suppressed by p53 through direct DNA binding (36–39), whereas most genes repressed by p53 lack p53 binding (40). Repression of the latter genes by p53 involves interactions of p53 with other transcriptional factors such as TBP, the TATA box-binding protein (41), Sp-1 (42), and ETS-1 (43, 44).

Both NF-kB activation and loss of wild-type (wt) p53 function are involved in progressive development of many cancers.
and resistance to chemotherapy; thus these two events may be associated. Previous studies have shown that WT p53 and the p65 (RelA) subunit of NF-kB mutually repress each other’s ability to activate transcription through competitive binding to the cAMP-response element-binding protein (45, 46). Furthermore, we found that WT p53 can directly repress p65 promoter activity (47).

In the present study, we demonstrate an additional mechanism for the interaction of p53 and NF-kB. Our studies showed that p53 represses the expression of IKKα, and p53-repression of IKKα occurs at the transcriptional level. Because IKKα is important in regulating a novel NF-kB activation pathway, loss of p53 function by point mutation or overexpression of MDM2 may contribute to uncontrolled transactivation of IKKα in cancer cells resulting in increased survival and resistance to apoptosis because of enhanced NF-kB activation.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—Three cell lines, EU-1, EU-4, and EU-8, established from children with B-cell precursor ALL were used in this study. The EU-1 line was provided by Dr. D. Golbik (University of Southern California, Los Angeles) and was derived from the umbilical cord of an 8-year-old child with B-cell precursor ALL. The EU-4 line was derived from an 8-year-old child with B-cell precursor ALL (48). The cell lines were grown in standard culture medium (RPMI 1640 containing 10% fetal bovine serum, 2 mmol/liter τ-glutamine, 50 units of penicillin, and 50 μg/ml streptomycin) at 37 °C in 5% CO2 in air. 

**Identification and Construction of Full-length IKKα Promoter Plasmid**—To date, there have been no studies of the IKKα gene promoter reported. We have searched the 5′-flanking untranslated DNA sequence of the IKKα gene by navigating the Human Genome web site (ncbi.nlm.nih.gov/Genes/index.html). A 1010-bp fragment from −940 to +70 in this region was generated by PCR using primers 5′-ATGAACTACTGGCTGCG-3′ and 5′-GCTCCATGCGGCGCG-3′, and cloned into the promoterless luciferase vector pGL3 basic (Promega) at XhoI and HindIII sites to produce the pLuc-1010 construct.

**Generation of IKKα Promoter Deletion and Mutation Constructs**—In an attempt to identify IKKα promoter sequences, we constructed a series of deletion constructs and site-directed mutants were made. For construction of the putative IKKα promoter, deletion constructs pLuc-508, pLuc-168, pLuc-120, pLuc-110, pLuc-100, and pLuc-890 and PCR primer pairs were determined from the corresponding sites in the sequence of the 1010-bp fragment, and then used to make different constructs. Mutant pLuc-1680-mm construct was made using mutated nucleotides at positions 5′-GGCGCATCTTCTGCAGTTACTCATCCCGT-3′ and 5′-GCGCAAGGTTTGCATACCTGTCGCACTGCA-3′, and cloned into the promoterless luciferase vector pGL3 basic (Promega) at XhoI and HindIII sites to produce the pluc-Luc-1010 construct.

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Fig. 1. Nucleotide sequence of the 5′-flanking UTR of the IKKα gene. Three putative p53-binding sites are underlined that are highly associated with a canonical p53 consensus sequence, i.e., the three regions contain 4-, 1-, and 10-nucleotide (italics) spacers, respectively, between the two decamer “half-sites” of the p53 consensus elements (bold). The potential core promoter region contains a consensus for binding transcription factors ETS-1 (bold and lowercase). A vertical arrow marks the cDNA start site and a bent arrow indicates the first codon.

DNA was recovered by phenol/chloroform extraction and ethanol precipitation. Pellets were resuspended in TE buffer and subjected to PCR amplification using forward and reverse primers (5′-GTGTTTCCGGTTTACGCCC-3′ and 5′-TGCTCCGGGTCTTG-3′) to the pLuc-508 fragment. The resulting product was 188 bp and contained the last two putative p53 consensus sequences as well as the ETS-1-binding motif in the IKKα promoter. The PCR product was sequenced by agarose gel electrophoresis.

EMSA—Sequence-specific DNA binding activity of ETS-1 to IKKα promoter was assessed by EMSA. Nuclear protein extraction was prepared using a kit (NE-PERTM from Pierce). Nuclear protein (5 μg) from EU-1 and EU-4 cells was incubated for 15 min in a binding buffer (10 mM Tris-HCl, 50 mM NaCl, 0.5 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 7.5 mM MgCl₂) plus 0.1 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml pepstatin. After centrifugation, 30 μg of the clarified cell lysate was incubated with 15 μl of protein G plus/protein A-agarose and 1 μg of p53, MDM2, or ETS-1 antibodies, respectively. After 24 h incubation, the agarose was centrifuged, washed four times with ice-cold lysis buffer, suspended in electrophoresis sample buffer, and boiled for 5 min. The immunoprecipitated protein was further analyzed by Western blotting as described above.

RESULTS

Identification of the Proximal 5′-Flanking Region of the IKKα Gene—By navigating the Human Genome, we found the nucleotide sequence of the proximal 5′-flanking UTR of the IKKα gene. The DNA sequence 5′ to the start site that represents a known transcription start site of the IKKα coding sequence is shown in Fig. 1. The DNA sequence of the 5′-flanking UTR of the IKKα gene contains three potential p53 response consensus...
sites located between nucleotides −345 to −322, −98 to −78, and −29 to +1. These putative p53 response elements are associated with a canonical p53 consensus sequence that consists of two copies of a decamer motif 5′-RRRCWWGYYY-3′ separated by 0 to 13 bp of random nucleotides, in the motif, R or A, W or A, Y or C or T (34). Moreover, an ETS-1-binding site (−46 to −39) was found in the most likely core promoter region of the IKKα gene.

Repression of IKKα Promoter Activity by WT p53—To determine the promoter activity of the sequence located 5′ to the IKKα gene, a fragment of DNA spanning nucleotides −940 to +70 was placed 5′ to the promoterless luciferase vector pGL3 basic. The level of luciferase activity was examined upon transient transfection into EU-4 cells. Substantial levels of luciferase activity were detected if the transfected reporter construct contained the −940 to +70 sequence in the sense but not in the antisense orientation (data not shown). To investigate whether the IKKα promoter is regulated by p53, the IKKα promoter-luciferase construct and plasmids containing WT p53 and various forms of mutant p53 were co-transfected into EU-4 cells, respectively. From the data presented in Fig. 2A, it can be seen that WT p53 produced a 70–80% decrease in IKKα promoter activity (relative to the control plasmid CMV-neo), whereas p53 mutations in codon 143, 175, 248, and 273 increased (2.5–4.5-fold) the IKKα promoter activity. Repression of IKKα promoter activity by WT p53 was dose-dependent, and this inhibitory effect was reversed by co-transfection with MDM2 (Fig. 2B). Titrations of transfected p53 and MDM2 in cellular extracts were determined by Western blot assay (Fig. 2B, inset).

Inhibition of IKKα Expression by WT p53—To further evaluate whether p53 regulates IKKα expression, the p53-null ALL cell line EU-4 was utilized for either stable transfection with a temperature-sensitive p53 allele encoding alanine at codon 143 or transient transfection with a p21 promoter-luciferase construct. The p53 protein in p53–143 transfected EU-4 (EU-4/p53–143) cells selected from a single clone exists in a mutant conformation at 37.5 °C, and temperature shift to 32.5 °C induces a WT conformation of p53. As shown in Fig. 3A, the promoter activity of the p53 target gene p21 was induced in a time-dependent manner in EU-4/p53–143 cells cultured at 32.5 °C. Moreover, Western blot assay showed that expression of endogenous p21 and MDM2, also p53 targets, was increased in EU-4/p53–143 cells at 32.5 °C (Fig. 3B), indicating that a functional WT p53 was induced in EU-4 transfected with p53–143 mutant at 32.5 °C. Under the same experimental conditions, however, expression of IKKα was down-regulated in EU-4/p53–143 cells, whereas the levels of IKKβ, IKKγ, and the housekeeping gene actin remained unchanged (Fig. 3C). To further characterize whether IKKα mRNA is repressed by p53, we performed RT-PCR analysis in EU-4/p53–143 cells. As shown in Fig. 3D, the expression of IKKα mRNA was rapidly and strongly decreased in EU-4/p53–143 cells cultured at 32.5 °C.

FIG. 3. Inhibition of IKKα expression by WT p53 in a p53-inducible cellular model. Functional WT p53 was induced in the p53-null EU-4 ALL line transfected with temperature-sensitive p53 mutant plasmid p53–143 after shifting to the p53-permissive temperature (32.5 °C), as examined by gene reporter assay to test p21 promoter-luciferase activity (A) and by Western blot analysis to detect the expression of MDM2 as well as p21 (B). Kinetic analysis of IKKα protein and mRNA expression was performed by Western blotting (C) and RT-PCR (D), respectively, in EU-4/p53–143 cells cultured at 32.5 °C. Cells grown at 37.5 °C (non-permissive temperature) were used as control. Controls also included transfection of CMV-neo plasmid in A and D, expression of IKKβ and IKKγ in C and expression of actin in B–D.
The expression of IKKα mRNA remained unchanged in EU-4 cells transfected with a control plasmid (CMV-neo) at 32.5 °C. IKKα Promoter Activity Is Induced Primarily by ETS-1—To determine the core promoter region of the IKKα gene, we generated a series of deleted constructs of the IKKα promoter and performed transfection and luciferase activity assays. Our results demonstrated that the core promoter region resides between −50 and −1 as shown in Fig. 4. Construct pLuc-120 (−50 to +70) expressed maximum luciferase activity similar to that of the full-length promoter pLuc-1010, and both 3′−5′ deleted constructs pLuc-890 (−940 to −51) and 5′−3′ deleted construct pLuc-70 (+1 to +70) showed no promoter activities as compared with control (transfection of pGL3 basic vector only). Interestingly, a narrow region (−50 to −30, 20 bp) containing the ETS-1 binding site expressed the majority of promoter activity, because activity of construct pLuc-100 (−30 to +70) was significantly lower than that of pLuc-120 (−50 to +70).
was approximately one-seventh of that of pLuc-120. Furthermore, there were approximately two-thirds reductions in the activity of deleted promoter pLuc-110 (H11002 to H11001) with a partial deletion (truncation) of the ETS-1 binding site compared with the activity of pLuc-120 with an intact ETS-1 binding site, and the full-length promoter with a mutated ETS-1 binding site (pLuc-1010m) showed significant reduction of promoter activity, suggesting that the transcription factor ETS-1 is an important regulator for IKK/H9251 expression.

To further confirm that ETS-1 regulates the IKK/H9251 promoter, we evaluated whether co-transfection of the ETS-1 expression plasmid would increase the activity of the IKK/H9251 promoter in cell line EU-8 with no ETS-1 expression. Furthermore, we examined whether blockage of endogenous ETS-1 by siRNA would decrease IKK/H9251 promoter activity in EU-8 cells with high levels of ETS-1 expression. We have generated a pSUPER/ETS-1 plasmid containing a 19-nucleotide siRNA sequence specific for targeting ETS-1 (Fig. 5A). Transfection of this plasmid into EU-4 cells significantly suppressed expression of the endogenous ETS-1 protein (Fig. 5B). Co-transfection of ETS-1 siRNA plasmid remarkably reduced IKK/H9251 promoter activity in a dose-dependent manner (Fig. 5C). As we expected, co-transfection of ETS-1 expression plasmid into EU-8 cells significantly increased IKK/H9251 promoter activity (Fig. 5D), although the transfection efficiency in EU-8 cells was lower than in EU-4 cells as tested for activity of transfected β-galactosidase (data not shown).
CHIP assay. The transcriptional factors. Our results showed that p53 does not bind most genes repressed by p53 lack DNA binding. Repression of genes are repressed by p53 via direct DNA binding, whereas p53 physically binds to ETS-1 (Fig. 7A), which is consistent with the result reported by Kim et al. (44). We additionally demonstrated that the presence of WT p53 reduced binding of ETS-1 to the IKKα promoter, whereas mutant p53 enhanced this binding (Fig. 7B). Furthermore, we compared the promoter activity between construct pLuc-120 with an intact ETS-1 response consensus and construct pLuc-110 with deletion of the ETS-1 core consensus GGAA in co-transfection with the WT p53 expression plasmid. As shown in Fig. 7C, the promoter activity of pLuc-120 is significantly inhibited by p53, whereas the promoter activity of pLuc-110 is not repressed by p53.

**DISCUSSION**

In this study we report the identification and analysis of the promoter region of the IKKα gene and the regulation of its expression, hoping to gain insight into the properties of IKKα in regulating NF-κB activation in tumor cells. By searching the Human Genome, we identified the nucleotide sequence of the IKKα gene promoter has several potential p53 response consensus sequences that are associated with classical p53-binding sites, our results showed that mutations of the putative p53-binding sites, our results showed that mutations of the putative p53-binding sites in the promoter region of the IKKα gene were found between −50 to +1, which was predominantly activated by ETS-1 and negatively regulated by p53. The negative regulation of IKKα expression by p53 was further proven by the evidence that induction of WT p53, using a temperature-sensitive p53 mutant transfected in EU-4 cells, repressed both mRNA and protein expression of IKKα.

It has been known that many genes that are negatively regulated by p53 lack p53 response elements in their promoter. Although the IKKα gene promoter has several potential p53 response consensus sequences that are associated with classical p53-binding sites, our results showed that mutations of the putative p53 response elements in the core promoter region did not interfere with p53 repression of IKKα promoter activity. Further-

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**Fig. 6.** Analyses for binding capacity of p53 and ETS-1 to IKKα promoter. A, agarose gel electrophoresis shows the PCR results from each CHIP assay. The IKKα promoter construct pLuc-508 and either WT p53 or ETS-1 expression plasmids were cotransfected into EU-4 cells and precipitated with anti-p53 or anti-ETS-1 antibodies (lanes 2 and 5, respectively). For negative control, immunoprecipitation was performed either using a normal mouse or rabbit IgG or in the absence of antibody (no) in each experiment (lanes 3, 6, and 4, 7, respectively). Lane 1 shows DNA size markers. The PCR product (188 bp) in lane 5 contains the ETS-1 binding site in the IKKα promoter. B, EMSA to examine the binding of ETS-1 to IKKα promoter. Nuclear extracts from EU-1 and EU-4 cells were incubated in binding reactions with 32P-labeled WT or mutant (mut) probes containing the CCGGAAGT sequence spanning −46 to −39 of the IKKα promoter. Samples were run on a nondenaturing 5% polyacrylamide gel and imaged by autoradiography. Lane 1, labeled probe with the EU-1 nuclear extract; lanes 2–5, labeled WT probe with nuclear extracts of EU-1; lanes 6–8, labeled WT probe with nuclear extracts of EU-4 cells. In reactions depicted in lane 2, 25-fold molar excess of non-labeled WT probe was added. In reactions depicted in lanes 4 and 7, cell extracts were preincubated with 2 μg of rabbit IgG for 1 h at 4 °C before probes were added. In reactions depicted in lanes 5 and 8, cell extracts were preincubated with 2 μg of rabbit anti-ETS-1 antibody. The specific protein-DNA complexes and supershift with antibodies are indicated.

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| Antibody | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 | Lane 7 | Lane 8 |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|
| WT probe |         |        |        |        |        |        |        |        |
| wt-competitor | + | + | + | + | + | + | | |
| mut-probe | + | + | + | + | + | + | | |
| rabbit IgG | - | - | - | - | - | - | - | - |
| anti-ets-1 | + | + | + | + | + | + | | |

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**Fig. 7.** A, analyses for binding capacity of p53 and ETS-1 to IKKα promoter. A, agarose gel electrophoresis shows the PCR results from each CHIP assay. The IKKα promoter construct pLuc-508 and either WT p53 or ETS-1 expression plasmids were cotransfected into EU-4 cells and precipitated with anti-p53 or anti-ETS-1 antibodies (lanes 2 and 5, respectively). For negative control, immunoprecipitation was performed either using a normal mouse or rabbit IgG or in the absence of antibody (no) in each experiment (lanes 3, 6, and 4, 7, respectively). Lane 1 shows DNA size markers. The PCR product (188 bp) in lane 5 contains the ETS-1 binding site in the IKKα promoter. B, EMSA to examine the binding of ETS-1 to IKKα promoter. Nuclear extracts from EU-1 and EU-4 cells were incubated in binding reactions with 32P-labeled WT or mutant (mut) probes containing the CCGGAAGT sequence spanning −46 to −39 of the IKKα promoter. Samples were run on a nondenaturing 5% polyacrylamide gel and imaged by autoradiography. Lane 1, labeled probe with the EU-1 nuclear extract; lanes 2–5, labeled WT probe with nuclear extracts of EU-1; lanes 6–8, labeled WT probe with nuclear extracts of EU-4 cells. In reactions depicted in lane 2, 25-fold molar excess of non-labeled WT probe was added. In reactions depicted in lanes 4 and 7, cell extracts were preincubated with 2 μg of rabbit IgG for 1 h at 4 °C before probes were added. In reactions depicted in lanes 5 and 8, cell extracts were preincubated with 2 μg of rabbit anti-ETS-1 antibody. The specific protein-DNA complexes and supershift with antibodies are indicated.

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| Antibody | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 | Lane 7 | Lane 8 |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|
| WT probe |         |        |        |        |        |        |        |        |
| wt-competitor | + | + | + | + | + | + | + | + |
| mut-probe | + | + | + | + | + | + | + | + |
| rabbit IgG | - | - | - | - | - | - | - | - |
| anti-ets-1 | + | + | + | + | + | + | + | + |
more, neither CHIP analysis nor EMSA detected binding of p53 to the \textit{IKKa} promoter. These results indicate that the putative p53 response consensus sequences are dispensable for p53-mediated repression of the \textit{IKKa} promoter activity, which is consistent with many previous observations that p53 negatively regulates a gene promoter in the absence of direct DNA binding (40). The mechanism is generally ascribed to sequestration of components of the basal transcription machinery by p53 through protein-protein interaction in the absence of DNA binding (42).

In the present study, we identified that the p53 repression of the \textit{IKKa} gene promoter is through regulation of ETS-1. ETS-1 is a DNA-binding protein that regulates transcription by specific binding to sequence containing a GGAA core usually found in the ETS-1-regulated promoter (50). A previous study found that the DNA sequence CCGGAAGT (ETS1–3) was the most efficient binding motif for purified ETS-1 protein (51). Intriguingly, the \textit{IKKa} core promoter region contains one such sequence (–46 to –39) as shown in Fig. 1. Our promoter studies and analysis of ETS-1 DNA binding \textit{in vitro} indicate that ETS-1 binds to the region containing the ETS1–3 sequence in the \textit{IKKa} promoter and strongly activates \textit{IKKa} promoter activity. Previous studies have demonstrated that ETS-1 and p53 are closely associated proteins. ETS-1 is required for the formation of a stable p53-DNA complex under physiological conditions in UV-induced apoptosis (52). Other studies have reported that p53 inhibits transcriptional activation of thromboxane synthase by binding to the gene promoter and physically interacting with ETS-1 (44), whereas p53 represses the human presenilin-1 gene by interacting with ETS-1 but without direct DNA binding (43). Consistent with the latter study, our results showed that p53 does not bind to \textit{IKKa} promoter and represses \textit{IKKa} promoter activity by physically interacting with ETS-1 and inhibiting ETS-1-mediated activation.

The critical interaction between ETS-1 and p53 in regulating promoter activity is well characterized in our study, in which p53 significantly inhibits the binding of ETS-1 to the \textit{IKKa} promoter and the activity of the \textit{IKKa} promoter construct containing intact ETS-1 binding site but not the construct with deletion of the core GGAA for ETS-1 binding. It has been demonstrated that the tumor suppressor p53 and the protooncogenic factor ETS-1 are important regulators in neoplastic...
transformation and cancer progression. The co-regulation of IKKα, an important activator of NF-kB, by p53 and ETS-1 may represent one of the mechanisms for p53- and ETS-1-mediated cancer formation and progression.

In our study, the inhibitory effect of p53 on IKKα promoter activity was abrogated by co-transfection of the p53-inhibitory oncogene MDM2. Furthermore, transfection of mutant p53 genes activated IKKα promoter activity. The molecular mechanism by which mutant p53 is able to directly up-regulate expression of a number of genes in contrast to the down-regulation of these genes by WT p53 has not been completely understood. Our results showed that enforced expression of mutant p53 increased ETS-1 DNA binding to the IKKα promoter, suggesting that mutant p53 proteins may gain a function to activate IKKα via interacting with ETS-1. These results further support the involvement of p53 regulation of IKKα expression in tumorigenesis and in development of resistance to chemotherapy-induced apoptosis. Constitutive NF-kB activation frequently occurs in cancer and leukemia cells, which is not always associated with increased degradation of IkB. We were prompted to study the p53 regulation of IKKα expression by our preliminary observation that several ALL cell lines with the p53 mutation expressed constitutive NF-kB, whereas ALL cell lines with WT p53 increased ETS-1 DNA binding to the IKKα promoter, thus supporting the involvement of p53 regulation of IKKα expression at the transcriptional level. Loss of p53 function either by mutation or by overexpression of MDM2, which frequently occurs in cancer and leukemia, will release the repression of IKKα, ultimately resulting in constitutive NF-kB-mediated gene activation. Thus, transcriptional induction of IKKα may represent a novel pathway through which loss of p53 may contribute to tumorigenesis.

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