Physical Interaction and Functional Antagonism between the RNA Polymerase II Elongation Factor ELL and p53*

(Received for publication, February 8, 1999, and in revised form, March 25, 1999)

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ELL was originally identified as a gene that undergoes translocation with the trithorax-like MLL gene in acute myeloid leukemia. Recent studies have shown that the gene product, ELL, functions as an RNA polymerase II elongation factor that increases the rate of transcription by RNA polymerase II by suppressing transient pausing. Using yeast two-hybrid screening with ELL as bait, we isolated the p53 tumor suppressor protein as a specific interactor of ELL. The interaction involves respectively the transcription elongation activation domain of ELL and the C-terminal tail of p53. Through this interaction, ELL inhibits both sequence-specific transcription activation and sequence-independent transrepression by p53. Thus, ELL acts as a negative regulator of p53 in transcription. Conversely, p53 inhibits the transcription elongation activity of ELL, suggesting that p53 is capable of regulating general transcription by RNA polymerase II through controlling the ELL activity. Elevated levels of ELL in cells resulted in the inhibition of p53-dependent induction of endogenous p21 and substantially protected cells from p53-mediated apoptosis that is induced by genotoxic stress. Our observations indicate the existence of a mutually inhibitory interaction between p53 and a general transcription elongation factor ELL and raise the possibility that an aberrant interaction between p53 and ELL may play a role in the genesis of leukemias carrying MLL-ELL gene translocations.

The ELL gene (also known as MEN) was molecularly identified as a gene that was fused to the Drosophila trithorax-like MLL gene (also called ALL-1 or Hrx) in acute myeloid leukemia carrying the t(11;19)(q23;p13.1) chromosomal translocation (1, 2). The gene product, ELL, was recently demonstrated to function as an RNA polymerase II elongation factor that increases the overall rate of transcription elongation by RNA polymerase II via suppression of transient pausing by the polymerase at many sites along DNA (3).

The connection between transcription elongation factors and oncogenesis was first provided by the finding that the product of VHL tumor suppressor gene, VHL, associates with the complex of the B and C regulatory subunits of another transcription elongation factor, elongin (4, 5). Binding of VHL to the elongin BC complex inhibits its ability to activate elongin A subunit in transcription elongation. The observation that many of the naturally occurring VHL mutants exhibit substantially reduced binding to the elongin BC complexes led to the suggestion that tumor suppression by VHL may involve negative regulation of elongin as a transcription elongation factor.

ELL is the second transcription elongation factor found associated with human malignancy. The chimeric protein generated by MLL-ELL gene translocation contains an N-terminal AT-hook DNA binding domain and the methyltransferase-like domain of MLL that is fused to almost the entire ELL sequences (1, 2). Expression of the MLL portion of the fusion protein appears to be insufficient for inducing the leukemic phenotype in mice (6), indicating a role for the fusion partner of MLL in cellular transformation.

p53 is a tumor suppressor protein that is mutated in more than 50% of human cancers (7). Wild-type p53 inhibits cell growth and suppresses cellular transformation when ectopically expressed (8–11). p53 is also known to play a crucial role in apoptosis (12–15) and DNA repair (16–18). Evidence accumulates that the transcriptional activity of p53 is a major component of its biological effects. p53 has sequence-specific DNA binding properties and transcriptionally activates p53 response genes that contain p53-binding/responsive elements (19–22). Such genes include those for p21 cyclin-dependent kinase inhibitor (also known as WAF1 or Cip1) (23–25), MDM2 (26–28), GADD45 (29, 30), cyclin G (31), Bax (32, 33), and IGF-BP3 (34). Transcriptional regulation of these genes by p53 explains at least in part the pleiotropic functions of p53 in cell growth control. This transcriptional activity of p53 is negatively regulated by the MDM2 protein (35, 36) and by the adenovirus E1B protein (37). These proteins bind to the N-terminal domain of p53 and inhibit its transcriptional activity. Recent studies further demonstrated that MDM2-p53 interaction accelerates the ubiquitin-mediated degradation of p53 (38, 39). Common p53 mutants in tumors lack the sequence-specific DNA binding activity because of the point mutations in the DNA-binding core domain and, hence, cannot transactivate the p53-target genes (40).

p53 is also known to repress transcription from a wide variety of promoters that do not possess p53 binding/responsive elements (41–46). The potency of p53 as a transcriptional repressor is also thought to play a role in its function as a tumor suppressor. Although detailed mechanisms involved in this transrepression are not well understood, it appears to be mediated at least in part by the physical interaction between p53 and TATA-binding protein (TBP)1 (47–50). However, the observation that additional molecules other than TBP are also required.
required for the transpression by p53 (51) indicates that p53 may sequester factors that are necessary for efficient initiation and/or elongation of RNA polymerase II-dependent transcription in addition to TBP.

In order to understand the mechanisms through which the ELL transcription elongation factor affects human malignancy, we have investigated molecules that physically interact with ELL. In this report, we show that p53 specifically binds ELL. Through the interaction, ELL reduces the transcriptional capacity of p53, as well as its action as a general transcriptional repressor. Conversely, p53 binding blocks the transcription elongation activity of ELL. Furthermore, elevated levels of ELL in cells inhibit p53-dependent induction of p21 and protect cells from p53-mediated apoptosis. Our results indicate the existence of mutually inhibitory interaction between p53 and ELL and raise the possibility that aberrant interaction between p53 and ELL may be involved in leukemogenesis associated with MLL-ELL gene translocation.

MATERIALS AND METHODS

Yeast Two-hybrid Screening—ELL cDNA was amplified from human cDNA library by standard polymerase chain reaction, and the sequence of entire coding region was confirmed. Bait plasmid was constructed in pAS2C, a CEN-ARS version of pAS2, using full-length ELL cDNA. Yeast two-hybrid screening of a human B cell cDNA library was performed in the yeast strain Y190.

Plasmid Construction—A cDNA encoding the N-terminal Myc or hemagglutinin (HA) epitope-tagged human ELL (Myc-ELL or HA-ELL) was constructed by using polymerase chain reaction technique and was cloned into pcdNA3 or pSP6B/SB2 vector. Mammalian expression vectors pcdNA3-p53, pcdNA3-ELL, pcdNA3-mdm2, pcdNA3-hdm, and pcdNA3-β-galactosidase were constructed by inserting cDNAs encoding human p53, human ELL, mouse MDM2, human homologue of MDM2 (HDM2), and β-galactosidase, respectively, into pcdNA3. pOPTET vector is an inducible cDNA expression vector containing TcIP promoter, a modified tetracycline-regulatable promoter consisting of entire coding region was confirmed. Bait plasmid was constructed in E. coli. Protein concentration was determined using the Bradford method. The 6-1 cell is a BaF3-derived mouse pro-B cell line stably introducing expression plasmids for tTA and LacI by calcium-phosphate method. Two days after the transfection, cells were split into two 60-mm dishes (5 ml each). To each plate, either Tc (final concentration, 1 μg/ml) or IPTG (final concentration, 5 μM) was added. Luciferase assays were performed 48 h after the transfection. The luciferase activities of equal amounts of the extracted proteins were measured by Lumat 9507 luminometer (Berthold).

The Oligo(dC)-tailed Template Assay—The oligo(dC)-tailed template assay was performed with the use of pCpGR20A/PX plasmid (100 ng) and RNA polymerase (0.01unit) according to the method described (3). After 25 min of incubation, 100 μM cold CTP, 2 μM UTP and GST-ELL (100 ng), or His-ELL (600 ng) was added with or without 800 ng of GST-p53, and the reactions were further incubated. RNA transcripts were analyzed by electrophoresis through 6% polyacrylamide/7 M urea gels.

RESULTS

Physical Interaction between ELL and p53 in Cells—To isolate molecules that interact with ELL, we employed a yeast two-hybrid screen using ELL as a bait. By screening 6.5 × 106 independent cDNA clones prepared from an Epstein-Barr virus transformed human B lymphocyte library, we isolated cDNA encoding nearly full-length p53 that lacked the N-terminal 3 and the C-terminal 2 amino acids. This two-hybrid interaction was abolished by deleting 47 amino acids from the C terminus of p53.

The association of ELL and p53 in mammalian cells was then addressed by assessing the interaction of transiently expressed Myc-ELL with endogenous p53. In COS-7 cells expressing Myc-ELL, co-immunoprecipitation of endogenous p53 with ELL was readily detectable. Reciprocally, co-immunoprecipitation of ELL with endogenous p53 was detectable when the same lysates were immunoprecipitated with anti-p53 (Fig. 1A). Because COS-7 cells express SV40 large T antigen, which forms a complex with p53, we also examined the p53-ELL interaction in SAOS-2 human osteosarcoma cell or L929 mouse fibroblast cell, both of which do not possess SV40 large T antigen. Upon transient expression of p53 and Myc-ELL, reciprocal co-immunoprecipitation of ELL and p53 was again demonstrated in these cells (Fig. 1, B and C). In the absence of antibodies that specifically recognize ELL, it was impossible to examine the

N. Shinohu, T. Maeda, and M. Hatakeyama, unpublished observations.
interaction between endogenous ELL and p53.

**Nuclear Localization of ELL and p53**—The intracellular localization of p53 and ELL was next examined by co-expressing p53 and HA-ELL in SAOS-2 cells, which lack endogenous p53 (Fig. 2). Transfected SAOS-2 cells were doubly stained with anti-p53 (green) and anti-HA (red). As described previously, ELL was expressed diffusely in the nucleus but excluded from the nucleoli (55). Anti-p53 staining showed nuclear staining that was almost indistinguishable from that of ELL. Merging the two images produced yellow, suggesting co-localization of the two proteins in the nucleus.

**Localization of Interaction Sites in p53 and ELL**—In order to further characterize the ELL–p53 interaction, in vitro binding assays were performed using p53 fused with glutathione S-transferase (GST-p53) and in vitro-translated, 35S-labeled ELL protein. As demonstrated in Fig. 3A, ELL was specifically bound to immobilized GST-p53. 35S-Labeled p53 protein that forms oligomers with GST-p53 was used as a positive control. Reciprocally, in vitro-translated p53 was specifically bound to immobilized GST-ELL (Fig. 3B). Addition of RNase A to the binding reaction did not affect the formation of ELL–p53 complexes, excluding the possible involvement of RNA in this interaction. A direct interaction between ELL and p53 was also demonstrated by far Western blotting analysis using histidine-tagged ELL (His-ELL) as a protein probe (Fig. 3C).

Various deletion derivatives of p53 were used to map the part of p53 that is required for ELL binding. In accordance with the observed yeast two-hybrid interaction, GST-ELL did not bind p53 that lacks the C-terminal 47 amino acids (Fig. 4A, lane 2). Likewise, GST-p53 lacking the C-terminal 47 amino acids of p53 failed to bind ELL (Fig. 4B, lane 4). The results indicate that the C-terminal 47 amino acids of p53 are required for ELL binding.

Analysis using deletion derivatives of ELL revealed that a C-terminally truncated mutant, ELL(1–363), retained the ability to bind p53 (Fig. 4C, lane 3). However, further C-terminal deletion to residue 250 decreased binding significantly. Internal deletion of ELL between residues 35 and 249 strongly impaired p53 binding and further deletion completely abolished the interaction (Fig. 4C, lanes 9 and 11). The results indicate that the N-terminal half of ELL, a region where the transcription elongation factor activity resides (56), is required for its binding with p53.

**Effect of ELL on p53-dependent Transcriptional Activation**—Because the C terminus of p53, to which ELL binds, is known to be important for its transcriptional activity (19–22), we next examined whether ELL is capable of inhibiting p53-mediated transactivation of promoters containing p53-responsive elements. To this end, a transient luciferase reporter assay was conducted in SAOS-2 cells using RGC-, cyclin G-, or MDM2-promoter luciferase construct (28, 31, 57). As demonstrated in Fig. 5A, the p53-dependent transactivation of the RGC promoter was strongly repressed by co-expressing ELL. The inhibition was comparable to that induced by MDM2 or HDM2 (the human homologue of MDM2) under the experimental conditions employed. The effects of ELL on p53-responsive promoters were also examined by using an inducible cDNA expression system (53) in SAOS-2 cells. Again, induced expression of ELL inhibited the p53-dependent transactivation, whereas an ELL mutant that cannot interact with p53 failed to do so (Fig. 5B).
and C). However, in contrast to MDM2 (38, 39), expression of ELL did not reduce the expression levels of p53 as examined by anti-p53 immunoblotting. Hence, the results indicate that ELL inhibits the sequence-specific transactivation by p53 through the complex formation with p53.

**Effect of ELL on p53-dependent Transcriptional Repression**—In addition to acting as a transactivator, p53 represses the activity of a variety of promoters lacking p53-binding sites (41–50). Accordingly, the potential involvement of ELL in p53-dependent transrepression was next addressed by transient luciferase reporter assays in SAOS-2 cells. Induced expression of ELL in the SAOS-2 cells stimulated the activity of the p53-repressible CMV promoter, which lacks a p53-binding motif, in a dose-dependent manner (Fig. 6A). The
result was consistent with the fact that ELL potentiates general transcription by functioning as the transcription elongation factor. In contrast, p53 strongly suppressed CMV promoter activity (Fig. 6B). To address the effect of ELL on p53-mediated promoter suppression, subsequent experiments were performed under conditions in which induced expression of ELL alone did not significantly affect the CMV promoter activity (Fig. 6A, 3 μg of pOPTET-ELL). As shown in Fig. 6C, the suppression of the CMV promoter by p53 was efficiently reversed by the induced expression of wild-type ELL but not by a mutant ELL that fails to bind p53. The same observation was reproduced when SV40 promoter, which also lacks the p53-binding motif, was analyzed (Fig. 6D). Furthermore, elevated levels of ELL in SAOS-2 cells abolished p53-mediated transcriptional repression of the CMV promoter (Fig. 6E). Hence, these data indicate that ELL counteracts p53-dependent transcriptional repression.

Effect of p53 on Transcription Elongation Activity of ELL—Given that the N-terminal half of ELL contains the elongation activity (56) and also binds p53, it is possible that the association of p53 with ELL may modify the elongation activity of ELL. To address this effect, the effect of p53 on the activation of transcription elongation by ELL was examined in an in vitro RNA polymerase II transcription assay using an oligo(dC)-tailed template (3, 58). This promoter-independent assay has the advantage over the runoff assay because it permits a direct measurement of the activity of the elongation factor in the absence of basal transcription factors that might otherwise interact functionally with p53. Addition of purified GST-p53 itself to the assay did not have any effect on the RNA polymerase II-dependent RNA elongation (Fig. 7, A and B), indicating that p53 does not directly enhance or block transcription elongation. In contrast, addition of GST-ELL (Fig. 7, A and B) or His-ELL (Fig. 7C) in the assay substantially stimulated the rate of elongation of RNA transcript as reported previously (3). This ELL-dependent stimulation of RNA transcription was strongly suppressed by full-length p53 but not by a p53 mutant that cannot bind ELL (Fig. 7, B and C). The result indicates that p53 is capable of regulating general transcription by RNA polymerase II through its ability to bind ELL.

Effect of ELL upon p53-dependent Induction of p21 and Apoptosis—To further pursue functional relationship between ELL and p53 in vivo, we addressed whether ectopic expression of ELL can compromise the transcriptional activation of a p53-responsive p21 gene within its native chromosomal context. To this end, we generated stable transfectant clones that inducibly express Myc-ELL from 6-1 cell. (Fig. 8A). This 6-1 cell is a subline of interleukin 3-dependent mouse pro-B lymphoid cell line, BaF3, and stably co-expresses tTA and lacI (53, 54). The BaF3 cell is reported to possess functional p53 and undergo p53-dependent apoptosis by DNA-damaging agents, such as X-irradiation and cisplatin (59, 60). Upon transient trans-
fection of p53-responsive luciferase reporter plasmids into the 6-1 cells or the Myc-ELL-transfectants in an uninduced condition (i.e., expression of Myc-ELL was repressed in culture medium containing 1 μg/ml Tc), the reporter gene was inducibly expressed in response to cisplatin or X-irradiation. This indicated that, like BaF3 cells, the 6-1 cell and its Myc-ELL-transfectants possess functional p53.

Myc-ELL was induced to express in one of representative transfectants, 13-1, by treating the cells with IPTG in the absence of tetracycline for 27 h. The parental 6-1 and the Myc-ELL transfectant cells were then treated with cisplatin. After 6 h of incubation, cells were lysed, and expression of endogenous p53 or the p53-responsive p21 proteins was examined by anti-p53 or anti-p21 immunoblotting. As demonstrated in Fig. 8B, both cells inducibly expressed comparable amounts of endogenous p53 in response to cisplatin treatment. In addition, induction of endogenous p21 was observed in the parental 6-1 cells following cisplatin treatment. In contrast, the 13-1 cells expressing exogenous ELL failed to induce p21 despite the continued presence of functional p53 (Fig. 8C).

Finally, the effect of ELL on p53-mediated apoptosis was addressed. As shown in Fig. 8D, elevated expression of ELL resulted in greater protection from DNA damage-induced apoptosis. Hence, the results indicate that the transcriptional activity of p53 is blocked and that, as a result, p53-dependent apoptosis is inhibited by ELL.

DISCUSSION

We demonstrate in this work that the RNA polymerase II transcription elongation factor, ELL, physically interacts with the p53 tumor suppressor protein. The interaction involves the C-terminal 47 amino acids of p53 and the transcriptional elongation activation domain of ELL. The complex formation does not require DNA or RNA because it is directly demonstrated by far Western blotting with the use of recombinant proteins and is totally insensitive to RNase treatment.

The transcriptional activity of p53 is thought to play a crucial role in its function as a tumor suppressor. We show here that by forming a complex with p53, ELL inhibits p53-dependent transactivation of promoters containing p53-responsive elements. Furthermore, elevated expression of ELL in cells inhibits the function of endogenous p53 to transcriptionally induce apoptosis.

3 T. Kondo, T. Ito, and M. Hatakeyama, unpublished observations.
p21 in response to DNA damage. The C-terminal p53 region, to which ELL binds, is known to be required for the p53 oligomerization (61–63) and is capable of binding DNA or RNA nonspecifically (64–71). These C-terminal activities are suggested to be indispensable for transcriptional activation of certain p53-dependent promoters (21, 72, 73). Thus, through complex formation, ELL might directly inhibit the transcriptional activity associated with the C-terminal region of p53. Alternatively, ELL binding might sterically alter the central core region of p53 and impair the sequence-specific DNA binding activity of p53. This possibility is supported by the observation that double-strand DNA nonspecifically binds the C-terminal p53 and inhibits the sequence-specific DNA binding of p53 that is mediated by the central domain (74).

We also demonstrate here that ELL reverses p53-dependent transcriptional repression of promoters lacking the p53-binding motifs. The p53-dependent transrepression also requires the C-terminal p53 (41–46), to which ELL binds. Although precise mechanisms underlying the transrepression by p53 are yet to be understood, interaction of p53 with TBP is suspected to be involved (47–50). Because the TBP-p53 interaction also requires the C-terminal region of p53, it is possible that ELL competitively blocks the p53-TBP complex formation and, as a result, inhibits p53-dependent transrepression through TBP.

MDM2, a well-characterized p53 interactor, specifically inhibits p53-dependent transcription by binding to the N-terminal region of p53 (35, 36, 75). In contrast, ELL suppresses both p53-dependent transcription and transrepression through its binding with the C-terminal tail of p53. This indicates that ELL is a novel negative regulator of p53. Furthermore, in contrast to MDM2, which promotes the rapid degradation of p53 (38, 39), ELL does not appear to reduce the levels of p53. In normal cells, in which p53 levels are low, ELL may be involved in the down-regulation of the residual transcriptional activity of p53. Induction of p53 in response to genotoxic stress results in the accumulation of p53 in relative excess to ELL and provokes p53-dependent transactivation as well as transrepression. Alternatively, modifications such as phosphorylation of ELL and/or p53 in response to DNA damage may induce dissociation of the complex and convert p53 from a latent form to an active form, as has been shown in retinoblastoma protein–E2F interaction (76). Because the p53-mediated transcriptional activities have been implicated in playing crucial roles in the tumor suppressor function of p53, ELL is expected to act as an oncoprotein when it is inappropriately overexpressed, as is the case of MDM2 (35, 36, 77). Consistently, ectopic overexpression of ELL is capable of transforming fibroblast cells (78). The MLL-ELL fusion protein in leukemic cells contains almost the entire ELL protein sequence. Such a fusion protein might aberrantly inhibit p53 via quantitative and/or qualitative differences from the wild-type ELL. Consistent with this idea, leukemic cells with the MLL-ELL gene translocations examined to date contain wild-type p53 genes (79).

We demonstrate in this work that p53 interacts with the N-terminal half of ELL and inhibits the elongation activity of ELL in vitro. Our results thus indicate that p53 may have a role in controlling RNA polymerase by regulating ELL activity as a transcription elongation factor. In addition to the elongation activation factor, the N-terminal ELL is reported to possess a novel RNA polymerase II interaction domain that can inhibit RNA polymerase II activity in promoter-specific transcription initiation (56). More recently, a large ELL-containing complex that contains multiple proteins in addition to ELL was identified (80). This ELL complex possesses the transcription elongation activity but cannot inhibit RNA polymerase II activity in promoter-specific transcription, suggesting the presence of the ELL-associated proteins that suppress the transcriptional inhibitory activity of ELL. Our work presented here raises a possibility that p53 is also involved in this ELL-mediated polymerase regulation. As discussed, p53 has been suggested to inhibit initiation of transcription by directly binding TBP. Taken together, our finding provides a model in which ELL is capable of inhibiting general transcription in both initiation and elongation processes by targeting TBP and ELL, respectively. If this is the case, then elevated ELL activity as a result of p53 loss, ELL overexpression, or gain-of-function mutations of ELL may also contribute to cellular transformation.

The p53-ELL interaction presented here parallels recent findings on the VHL tumor suppressor gene, mutations of which are involved in the development of sporadic as well as hereditary forms of renal carcinoma. Wild-type VHL associates with the two regulatory subunits, B and C, of another general transcription elongation factor, elongin, and inhibits the elongation activity of the elongin A subunit, whereas mutant VHL molecules fail to do so (4, 5). In the case of p53-ELL interaction, common p53 mutants in tumors lose their transcriptional activities and hence do not receive regulation by ELL anymore. Furthermore, ELL mutants, such as the MLL-ELL fusion protein, may aberrantly inhibit p53 activity. Together with the VHL-elongin interaction, our results indicate the existence of functional connections between transcription elongation factors and tumor suppressors in the control of normal cell growth and in the prevention of cellular transformation.

Finally, functional antagonism between p53 and ELL indicates that the p53-mediated transcriptional control and the ELL-mediated transcriptional control should be executed in a mutually exclusive manner so as to exert coordinated expression of genes involved in cell growth and differentiation.

Acknowledgments—We thank L. Yamazaki, D. Cribinrik, and R. A. Weinberg for valuable comments and discussions; B. Vogelstein for p53 cDNAs; M. Oren for p53-responsive promoters; and B. Elenbaas for cDNAs encoding MDM2 and HD2M.

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