[CANCER RESEARCH 58, 2091–2094, May 15, 1998]

Advances in Brief

Wild-Type and Mutant Forms of p53 Activate Human Topoisomerase I: A Possible Mechanism for Gain of Function in Mutants[1]

Amador Albor, Shinsuke Kaku, and Molly Kulesz-Martin[2]

Department of Experimental Therapeutics, Roswell Park Cancer Institute, Buffalo, New York 14263

Abstract

p53-interacting proteins from mouse epidermal cells and human myelogenous leukemia cells were isolated by affinity chromatography using glutathione *S*-transferase (GST)-p53 fusion proteins. One of these proteins was topoisomerase I, whose interaction with p53 was recently reported. A carboxyl-terminal fragment containing the last 92 amino acids of p53 (GST-299–390) was sufficient for binding to topoisomerase I. Nanomolar concentrations of either GST-p53 or GST-299–390 enhanced the catalytic activity of purified human topoisomerase I. Purified wild-type human p53 and point mutants Ser-239, Ser-245, and His-273 were equivalent in their enhancement of human topoisomerase I activity. Because topoisomerase I is thought to promote genetic recombination, competence to enhance topoisomerase I catalytic activity coupled with a deficiency in transcriptional activity may be a mechanism for gain of function in mutant p53 proteins.

Introduction

Topoisomerase I belongs to a group of enzymes involved in the release of topological stress along the DNA double helix. Topoisomerases are necessary for nuclear metabolism, participating in a variety of processes involving the melting and reannealing of DNA including replication, transcription, and DNA repair (1). During its catalytic cycle, topoisomerase I sequentially breaks and religates one strand of DNA, allowing relaxation of the supercoiled DNA substrate. After the strand break, the enzyme remains covalently linked to the 3′ hydroxyl end through a phosphodiester bond via a tyrosine residue in its reaction center and then releases itself by transesterification onto the free 5′ hydroxyl end during the subsequent ligation. If a free 5′ hydroxyl group from an exogenous DNA molecule is available, topoisomerase I can catalyze the ligation of the two DNA strands (2–4), a property that allows topoisomerase I to mediate nonhomologous recombination in somatic cells. Strand exchange sites for nonhomologous recombination in mammalian cells contain sequences that are preferentially cleaved by topoisomerase I (5). Under certain conditions, topoisomerase I can be the cause of genomic instability; for instance, treatments or mutations that increase the stability of the covalent DNA-topoisomerase I reaction complexes increase the frequency of DNA recombination (6).

Recently, wild-type p53 has been described as a topoisomerase I-binding protein capable of enhancing the cleavage reaction step of the topoisomerase I catalytic cycle *in vitro* (7). The wild-type *p53* gene is a tumor suppressor with a role in maintaining genetic stability (8, 9). The p53 protein is a transcription factor that activates promoters of certain growth-regulatory genes through binding to a specific DNA sequence. DNA damage induces p53 protein which can lead to a G_1 arrest through transcriptional activation of WAF1, apoptosis through a mechanism partly dependent on p53 transcriptional activity, or a G_2 arrest (9, 10).

Wild-type and mutant p53 proteins differ in their biochemical properties. Typically, p53 mutants have a prolonged half-life, are defective in DNA binding, have lost the ability to suppress transformation, and can form heterotetramers with wild-type p53, resulting in a dominant negative effect on wild-type function (11). Often both *p53* gene copies are defective in human cancers, and the types of *p53* mutation differ from those of tumor suppressor genes *Rb* and *APC*. The most common alterations in these genes are deletions and nonsense mutations that eliminate protein expression. In contrast, most *p53* alterations involve missense mutations of one allele with loss or rearrangement of the other allele. This suggests that the presence, *i.e.*, a gain of function, of mutated p53 protein, rather than the loss of wild-type p53 protein, confers a selective growth advantage and promotes clonal expansion (8).

To examine further the interaction between wild-type p53 and topoisomerase I, we used wild-type p53 protein in an affinity chromatography assay, followed by *in vitro* studies with several recombinant p53 protein forms expressed in bacterial or insect cells. These studies confirm the interaction between topoisomerase I and p53 and localize the interaction to the carboxyl terminus of p53. In addition, several mutant p53 proteins deficient for transcription and growth inhibition retained the ability to enhance topoisomerase I catalytic function. Because mutant p53 protein is commonly overexpressed in cancer cells, this suggests a mechanism for gain of function by *p53* mutant genes.

Materials and Methods

Cell Lines and Cell Culture. Clone 291 is a mouse keratinocyte strain with a normal growth and differentiation phenotype (12). Clone 291.03RAT-C119 was derived by the transfection of 291.03RAT (a carcinoma derivative of 291 treated with 7,12-dimethylbenz(*a*)anthracene; Ref. 13) with a plasmid driving the expression of the temperature-sensitive p53 mutant Val-135. ML-1 is a human myeloblastic leukemia cell line with a wild-type *p53* gene (14).

Preparation of Nuclear Extracts. Nuclear extracts were prepared following a modification of the NaCl extraction method of Dignam *et al.* (15). The final preparation containing the salt-extracted soluble nuclear proteins was dialyzed against STA[3] buffer [10 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10 mM MgCl, 1 mM EDTA, and 10% glycerol.

Generation and Purification of p53 Recombinant Proteins. The fusion proteins GST-p53 and GST-299–390 (containing either the full-length or the last 92 amino acids of murine wild-type p53 protein fused to the carboxyl terminus of *Schistosoma japonicum* GST) were expressed in *Escherichia coli* DH5α cells (Life Technologies, Inc., Gaithersburg, MD) from plasmid pGEX-2T (Pharmacia Biotech, Uppsala, Sweden). GST protein itself was produced from bacteria carrying an empty pGEX-2T vector. Recombinant proteins were purified using essentially the protocol described by Frangioni

Received 3/2/98; accepted 3/31/98.

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.

[1] Supported by a research grant from Taisho Pharmaceutical Co., Ltd., NIH Grant CA31101, and Roswell Park Cancer Institute Core Grant CA16056.

[2] To whom requests for reprints should be addressed, at Department of Experimental Therapeutics, Room 403, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263.

[3] The abbreviations used are: STA, Sodium-Tris-EDTA; GST, glutathione *S*-transferase; TF, transcription factor.

and Neel (16) for the purification of enzymatically active GST fusion proteins. The final protein preparation was dialyzed against STA buffer.

To generate polyhistidine-tagged human p53 proteins, wild-type full-length cDNA, single-point mutant cDNA (Ser-239, Ser-245, and His-273 generated by *in vitro* mutagenesis), or cDNA for a 30-amino acid carboxyl-terminally truncated p53 was ligated into the baculovirus vector pFastBac HTb (Life Technologies, Inc.) in frame with a 6× polyhistidine amino terminal tag. The vector was introduced into DH10Bac competent bacteria carrying a baculovirus shuttle vector (bacmid). Recombinant bacmids containing the p53 cDNA inserts were transfected into Sf-9 insect cells to produce and amplify a recombinant viral stock, and the resulting proteins were purified from infected Sf-9 cells using nickel-agarose affinity chromatography (Qiagen, Valencia, CA). The final protein preparation was dialyzed against buffer D [(20 mM HEPES (pH 7.9), 100 mM KCl, 0.2 mM EDTA, 0.2 mM EGTA, 2 mM DTT, and 20% glycerol)], aliquoted, and stored at −80°C.

Binding Assay to GST Fusion Proteins and Amino Acid Sequencing. Purified GST-p53 fusion proteins or GST protein control was bound to glutathione-Sepharose beads (Pharmacia Biotech) at a concentration of 0.5 μg/μl wet Sepharose matrix. The beads were incubated with two bed volumes of nuclear extract (protein concentration, 1.7 μg/μl) in STA buffer for 30 min at 4°C. After washing with 6 volumes of STA buffer, bound proteins were sequentially eluted with 1 volume of STA buffer supplemented with 200, 500, and 1000 mM NaCl. A 5-μl aliquot of the initial nuclear extract or the NaCl eluates was separated in 7.5% SDS-PAGE, and proteins were detected either by silver staining (Bio-Rad, Cambridge, MA) or by immunoblotting with Scl70 (Immunovision, Springdale, AK), a polyclonal serum specific for topoisomerase I, followed by chemiluminescence (Pierce, Rockford, IL). Proteins p100 and p80 from the 500-mM NaCl fraction were subjected to internal amino acid sequencing at the W. M. Keck Foundation (Yale University School of Medicine, Department of Molecular Biophysics and Biochemistry, New Haven, CT).

Assays for Topoisomerase I Activity. Purified human topoisomerase I enzyme and supercoiled plasmid pHOT1 substrate (which contains a copy of a preferred topoisomerase I site cloned into pUC12) were purchased from Topogen (Colombus, OH). pHOT1 was maintained in DH5α cells and isolated using standard plasmid preparation procedures. Reactions were done in a total volume of 10 μl. The topoisomerase I reaction buffer contained 10 mM Tris-HCl (pH 7.9), 150 mM NaCl, 1 mM EDTA, 100 nM spermidine, 0.1% BSA, 5% glycerol, and the indicated amounts of p53 protein. All reactions contained an equal volume of the p53 protein solvent buffer to control for effects on enzyme activity. Reactions were started by the addition of 0.2 μg of supercoiled pHOT1 plasmid and incubated for 20 min at 37°C. Reactions were stopped by the addition of 2 μl of stop buffer (5% sarkosyl, 0.125% bromphenol blue, and 25% glycerol). Reaction products were separated by electrophoresis in 1% agarose at 2 V/cm for 18 h. After electrophoresis, gels were stained in 0.5 μg/ml ethidium bromide and photographed under UV light.

Results

Binding of Topoisomerase I to the Carboxyl Terminus of p53. Nuclear extracts from the nontransformed epidermal keratinocyte cell line 291 contained proteins that bound to GST-p53 fusion proteins immobilized on a glutathione-Sepharose matrix. A 100-kDa doublet and an 80-kDa protein eluted preferentially at 500 mM NaCl and were detected by silver staining after SDS-PAGE (Fig. 1*A*). These protein bands were identified as two different forms of topoisomerase I by both direct amino acid sequencing and western blotting with a topoisomerase I-specific serum Scl70 (Fig. 1*B*). The antiserum recognized three protein bands of 100, 82, and 80 kDa in the unfractionated nuclear extract. However, only the 100- and 80-kDa forms were retained by GST-p53. Topoisomerase I is known to undergo proteolysis during experimental handling, generating products of faster migration, whereas the 100-kDa form is the expected size of the full-length non-degraded form (17). The 82- and 80-kDa forms likely represent proteolytic products, which, in the case of the 82-kDa form, may be missing a domain necessary for interaction with p53. In similar experiments, the 100-kDa form

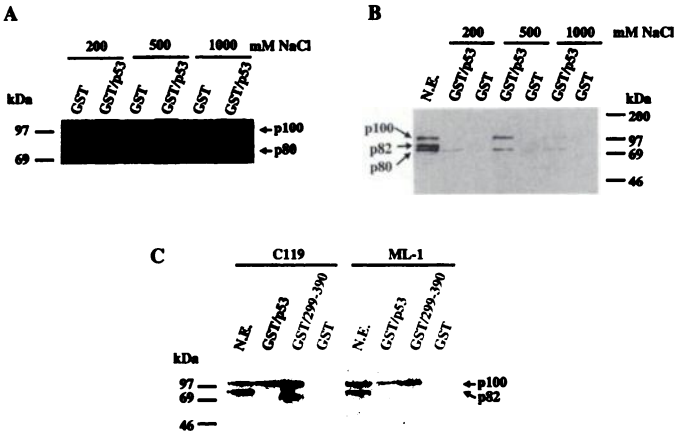

Fig. 1. Binding of topoisomerase I to the carboxyl terminus of p53. Nuclear extracts were prepared from mouse (291, *A* and *B*; 291.03RAT-C119, *C*) or human cells (ML-1, *C*). Nuclear extracts were incubated with full-length mouse p53 GST fusion protein (GST-p53) or GST fusion protein to the mouse p53 carboxyl terminus (GST-299–390) immobilized on glutathione-Sepharose beads. GST protein alone was used as a nonspecific binding control. Bound proteins were eluted with increasing NaCl concentrations as indicated and separated by 7.5% SDS-PAGE. Proteins were detected by silver staining (*A*) or by immunoblotting with topoisomerase I antiserum Scl70 (*B* and *C*). *N.E.*, unfractionated nuclear extract.

was retained from nuclear extracts of both mouse (291.03RAT-C119, a derivative of 291) and human (ML-1 leukemia cells) origin (Fig. 1*C*). Moreover, the GST-299–390 fusion protein retained topoisomerase I as efficiently as full-length GST-p53, indicating that the topoisomerase I interacting region was contained in the p53 carboxyl terminus.

The Effect of p53 Carboxyl Terminus on Topoisomerase I Catalytic Activity. Because the last 92 amino acids in the carboxyl terminus of p53 were sufficient for binding to topoisomerase I, we tested whether this region was also sufficient for effects on topoisomerase I activity. As shown in Fig. 2, the carboxyl terminus of p53 was sufficient for the activation of purified topoisomerase I activity. Fig. 2*A* shows the effect of 100 nM GST-299–390 on increasing amounts of topoisomerase I enzyme in a supercoiled plasmid relaxation assay. Topoisomerase I enzyme at a 1:1,000 dilution (Fig. 2*A*) was only minimally active in DNA relaxation in the absence p53 protein (−); however, at this dilution, enzyme activity was maximally stimulated in the presence of GST-299–390 (+). p53 protein enhanced topoisomerase I catalytic activity by at least 5-fold (for example, in Fig. 2*A*, the generation of relaxed forms at a 1:250 dilution of topoisomerase I in the presence of GST-299–390 is equivalent to a dilution of 1:50 in its absence; 1:16,000 + p53 is approximately equivalent to 1:1,000 − p53). The 1:1,000 concentration of enzyme was used to compare GST-p53, GST-299–390, and GST control recombinant proteins in the range of 4–400 nM for the capacity to stimulate topoisomerase I catalytic activity. Both full-length and carboxyl terminal p53 fusion proteins enhanced DNA relaxation by topoisomerase I (Fig. 2*B*). The effect was dose dependent up to 80 nM p53 proteins, with no further increase (GST-299–390) or an inhibitory effect (GST-p53) at 200 or 400 nM. The GST protein alone was inactive (4–20 nM) or minimally active (80–400 nM) in plasmid DNA relaxation, being well below the activities observed with GST-p53 or GST-299–300 at the same concentrations.

The Effect of Human p53 Mutant Proteins on Topoisomerase I Catalytic Activity. The capacity of mutant forms of p53 to activate topoisomerase I catalytic activity was tested using human wild-type and mutant p53 proteins produced in insect cells (Fig. 3). The wild-type protein and single-point mutant p53 proteins Ser-239, Ser-245, and His-273 were equivalent in capacity to increase topoisomerase I

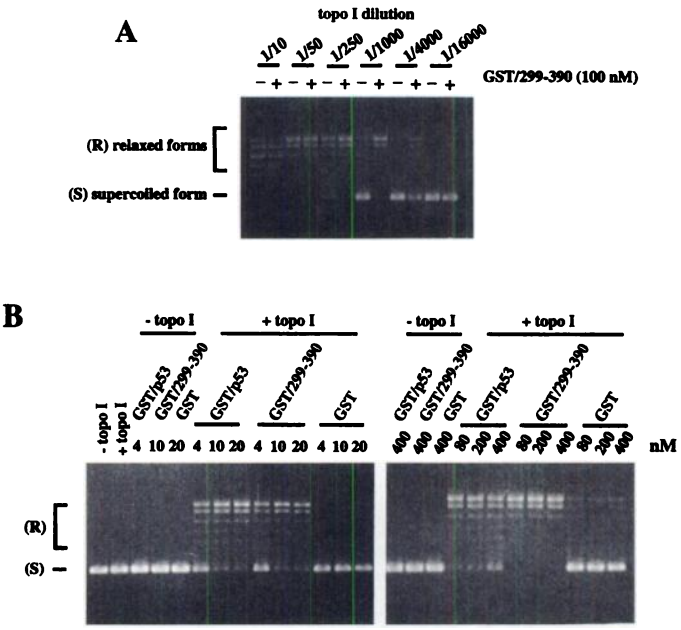

Fig. 2. Effect of GST, GST-p53, and GST-299–390 recombinant proteins on the DNA relaxation activity of purified human topoisomerase I. The indicated concentrations of recombinant proteins and topoisomerase I were included in reactions with a total volume of 10 μl, as described in "Materials and Methods." After the reaction, plasmid topological isomers were separated on a 1% agarose gel and detected by ethidium bromide staining. *A*, titration of topoisomerase I against a fixed concentration of GST-299–390. Topoisomerase I stock was subjected to serial dilution as indicated and incubated with 100 nM GST-299–390. *B*, dose dependence of recombinant p53 fusion protein activation of human topoisomerase I. Concentrations of 4–400 nM GST-p53, GST-299–390, or GST proteins were incubated with (+ *topo I*) or without (− *topo I*) a 1:1000 dilution (0.01 unit) of topoisomerase I.

catalytic activity. None of the p53 proteins relaxed plasmid DNA in the absence of topoisomerase I, indicating that the observed effects were not due to insect topoisomerases copurifying with the p53 proteins. The p53 protein concentrations needed for maximum topoisomerase I activation were higher than those in the previous experiments; for example, 1 μM human wild-type p53 protein compared to 80 nM GST fusion proteins. Because the GST fusion proteins were produced in bacteria, whereas the human recombinant p53 proteins were produced in insect cells, the differences between the two may be due to alternative posttranslational modifications in bacterial and insect cells.

Discussion

We have confirmed the observations of Gobert *et al.* (7) that wild-type p53 interacts with topoisomerase I and enhances its DNA relaxation activity and extended these observations to include a wild-type p53 carboxyl-terminal polypeptide and human p53 mutant proteins. Interactions are preserved between rodents and humans, because mouse p53 bound to topoisomerase I from both human and mouse nuclear extracts, and both mouse p53 produced as a GST fusion protein and human p53 produced in insect cells were able to enhance purified human topoisomerase I catalytic activity. We have demonstrated that the carboxyl terminus of p53 (residues 299–390) is sufficient for binding to topoisomerase I and enhancing its catalytic activity. The carboxyl terminus contains the p53 oligomerization domain and a terminal DNA-binding negative regulatory sequence (9). In addition, it has been reported to bind to the basal TFIIH components XPB (ERCC3) and XPD (ERCC2), inhibiting their helicase activity (18) and contributing to p53-mediated apoptosis (19).

Potential physiological functions for the interaction of wild-type p53 with topoisomerase I include roles in transcription and repair. Topoisomerase I is a transcriptional coactivator in *in vitro* transcription assays with a stimulatory effect on activator-mediated transcription (20, 21). Topoisomerase I can increase basal as well as activator-induced transcription in a defined reconstituted system, facilitating TFIID-TFIIA complex formation (22). The observed binding of p53 and topoisomerase I could be necessary for enhancement of the transcriptional activity of p53 in response to DNA damage and might even be expected to occur with other transcriptional transactivators. With regard to repair function, there is evidence that wild-type p53 is necessary for efficient DNA repair (23), and both topoisomerase I and II have been linked to DNA repair by the use of specific inhibitors (24).

We have shown that human mutant p53 forms commonly found in cancers retain the ability to stimulate catalytic activity of topoisomerase I. Topoisomerase I was activated to a similar extent by wild-type p53, His-273, and two other mutants, Ser-239 and Ser-245. Most human cancers with p53 alterations carry a missense p53 point mutant, which is frequently overexpressed. The high levels of mutant p53 can inactivate the wild-type form through the formation of heterotetramers, displaying a negative dominant effect. However, certain p53 missense mutants, like the His-273 used in this study, exhibit gain of function phenotypes. These include the promotion of tumorigenicity in nude and syngenic mice by cells lacking endogenous p53, the enhancement of plating efficiency in soft agar (25–27), and the enhancement of carcinogenesis in transgenic mice (28).

Mechanisms proposed to explain the positive dominant effect include the following possibilities: (*a*) anomalous transcriptional activity of mutant p53; or (*b*) genomic instability induced by mutant p53. Relative to the first, anomalous activation of the promoters of the multidrug resistance gene and the proliferating cell nuclear antigen gene has been observed in transient transfection experiments (27, 29). The current results are consistent with the proposal that the observed transcriptional activity of mutant p53 is due to the stimulation of topoisomerase I as a transcriptional coactivator (20–22), combined with a failure to inhibit the TFIIH-associated transcription/repair factors XPD and XPB (18, 19). Relative to the second possibility, the presence of mutant p53 has been reported to enhance genetic instability (30). Based on the results of the current study, mutant p53 could contribute to genetic

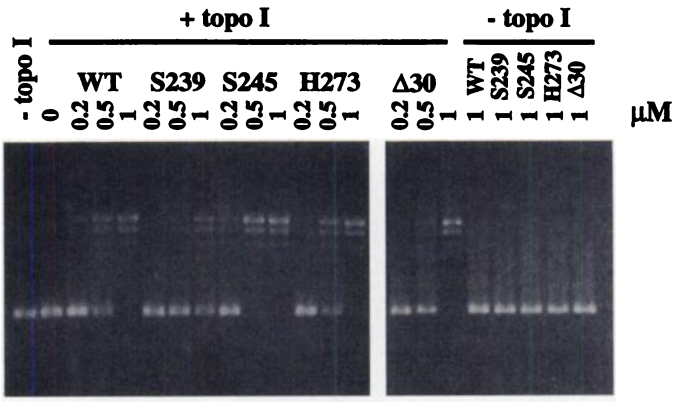

Fig. 3. Effect of mutant p53 recombinant proteins on topoisomerase I activity. His-tagged wild-type and mutant forms of human p53 were produced in insect cells, and their effect on topoisomerase I was assayed under the same conditions as described in the Fig. 2 legend. The p53 proteins at the indicated concentrations were incubated with 0.01 unit of purified human topoisomerase I (+ *topo I*) or without topoisomerase I (control; − *topo I*). Human p53 proteins: *WT*, wild type; *S239*, Asn-239 to Ser-239 mutant; *S245*, Gly 245 to Ser-245 mutant; *H273*, Arg-273 to His-273 mutant; Δ*30*, wild-type p53 sequence lacking the last 30 amino acid residues in the carboxyl terminus.

instability by stimulating the activity of topoisomerase I in nonhomologous recombination (2–4). This is supported by the fact that agents that increase the stability of the topoisomerase I cleavage complex, such as camptothecin, can induce genetic alterations, including sister chromatid exchange, deletions, and chromosomal aberrations (6). Mutant p53 proteins overexpressed in transformed and tumor cells could exhibit gain of function due to the retention of topoisomerase I catalytic enhancing activity coupled with a loss of tumor suppression activities (such as sequence-specific DNA binding, transcriptional activation of growth-inhibitory genes, DNA repair, and apoptosis), leading to increases in nonhomologous recombination and genomic instability. Future experiments will be required to directly assess these possibilities.

Acknowledgments

We are grateful to Dr. Stephen Friend for plasmid PC53SN3 used to generate wild-type human p53 cDNAs, to Dr. Moshe Oren for plasmids pLSVN51 used to generate mouse p53 cDNAs and pmMTp53val135-23 used to derive transfectant clone C119, and to Dr. Alexander Bloch and Rosemary Hromchak for ML-1 cells. We thank Laura Lee for sequencing of the plasmid constructs, Dr. Zoe Miner for guidance with the baculovirus system, Dr. Yu Wu for the construction of plasmids for the production of recombinant human p53 mutant proteins, and Dr. Terry Beerman for critical review of the manuscript.

References

1. Andersen, A. H., Bendixen, C., and Westergaard, O. DNA topoisomerases. *In:* M. L. DePanphilis (ed.), DNA Replication in Eukaryotic Cells, pp. 587–617. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1996.
2. Been, M. D., and Champoux, J. J. DNA strand breakage and closure by rat liver type I topoisomerase: separation of the half-reactions by using a single-stranded substrate. Proc. Natl. Acad. Sci. USA, *78:* 2883–2887, 1981.
3. Bullock, P., Champoux, J. J., and Botchman, M. Association of crossover points with topoisomerase I cleavage sites: a model for non-homologous recombination. Science (Washington DC), *230:* 954–958, 1985.
4. Christiansen, K., and Westergaard, O. Characterization of intra- and intermolecular DNA ligation mediated by eukaryotic topoisomerase I. J. Biol. Chem., *269:* 721–729, 1994.
5. Konopka, K. A. Compilation of DNA strand exchange sites for non-homologous recombination in somatic cells. Nucleic Acids Res., *16:* 1739–1758, 1988.
6. Anderson, R. D., and Berger, N. A. Mutagenicity and carcinogenicity of topoisomerase-interactive agents. Mutat., Res., *309:* 109–142, 1994.
7. Gobert, C., Bracco, L., Rossi, F., Olivier, M., Tazi, J., Lavelle, F., Larsen, A., and Riou, J. F. Modulation of DNA topoisomerase I activity by p53. Biochemistry, *35:* 5778–5786, 1996.
8. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res., *54:* 4855–4878, 1994.
9. Ko, L., and Prives, C. p53: puzzle and paradigm. Genes Dev., *10:* 1054–1072, 1996.
10. Hermeking, H., Lengauer, C., Polyak, K., He, T., Zhang, L., Thiagalingam, S., Kinzler, K. W., and Vogelstein, B. 14-3-3σ is a p53-regulated inhibitor of G_2/M progression. Mol. Cell, *1:* 3–11, 1997.
11. Levine, A. J., and Zambetti, G. P. A comparison of the biological activities of wild-type and mutant p53. FASEB J., *7:* 855–865, 1993.
12. Kulesz-Martin, M. F., Yoshida, M., Prestine, L., Yuspa, S. H., and Bertram, J. S. Mouse cell clones for improved quantitation of carcinogen-induced altered differentiation. Carcinogenesis (Lond.), *6:* 1245–1254, 1985.
13. Kulesz-Martin, M. F., Penetrante, R., and East, C. J. Benign and malignant tumor stages in a mouse keratinocyte cell line treated with 7,12-dimethylbenz[*a*]anthracene *in vitro*. Carcinogenesis (Lond.), *9:* 171–174, 1988.
14. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. Cancer Res., *51:* 6304–6311, 1991.
15. Dignam, D. J., Lebovitiz, P. L., and Roeder, R. G. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res., *11:* 1475–1485, 1983.
16. Frangioni, J., and Neel, B. Solubilization and purification of enzymatically active glutathione *S*-transferase (pGEX) fusion proteins. Anal. Biochem., *210:* 179–187, 1993.
17. Stewart, L., Irenton, G. C., Parker, L. H., Madden, K. R., and Champoux, J. J. Biochemical and biophysical analyses of recombinant forms of human topoisomerase I. J. Biol. Chem., *271:* 7593–7601, 1996.
18. Wang, X. W., Yeh, H., Schaffer, L., Roy, R., Moncollin, V., Egly, J., Wang, Z., Friedberg, E. C., Evans, M. K., Taffe, B. G., Bohr, V., Weeda, G., Hoeijmakers, J. H. J., Forrester, K., and Harris, C. C. p53 modulation of TFIIH associated nucleotide excision repair activity. Nat. Genet., *10:* 188–195, 1995.
19. Wang, X. W., Vermeulen, W., Coursen, J. D., Gibson, M., Lupold, S. E., Forrester, K., Xu, G., Elmore, L., Yeh, H., Hoeijmakers, J. H. J., and Harris, C. C. The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway. Genes Dev., *10:* 1219–1232, 1996.
20. Kretzschmar, M., Meisterernst, M., and Roeder, R. G. Identification of human DNA topoisomerase I as a cofactor for activator-dependent transcription by RNA polymerase II. Proc. Natl. Acad. Sci. USA, *90:* 11580–11512, 1993.
21. Merino, A., Madden, K. R., Lane, W. S., Champoux, J. J., and Reinberg, D. DNA topoisomerase I is involved in both repression and activation of transcription. Nature (Lond.), *365:* 227–232, 1993.
22. Shykind, B. M., Kim, J., Stewart, L., Champoux, J. J., and Sharp, P. Topoisomerase I enhances TFIID-TFIIA complex assembly during activation of transcription. Genes Dev., *11:* 397–407, 1997.
23. Ford, J. M., and Hanawalt, P. C. Li-Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV resistance. Proc. Natl. Acad. Sci. USA, *92:* 8876–8880, 1995.
24. Stevnsner, T., and Bohr, V. Studies of the role of topoisomerases in general, gene and strand specific DNA repair. Carcinogenesis (Lond.), *14:* 1841–1850, 1993.
25. Wolf, D., Harris, N., and Rotter, V. Reconstitution of p53 expression in a nonproduce Ab-MuLV-transformed cell line by transfection of a functional p53 gene. Cell, *38:* 119–126, 1984.
26. Shaulsky, G., Goldfinger, N., and Rotter, V. Alterations in tumor development *in vivo* mediated by expression of wild-type or mutant p53 proteins. Cancer Res., *51:* 5232–5237, 1991.
27. Dittmer, D., Pati, S., Zambetti, G. P., Chu, S., Teresky, A. K., Moore, M., Finlay, C., and Levine, A. J. Gain of function mutation in p53. Nat. Genet., *4:* 42–46, 1993.
28. Wang, X., Greenhalgh, D. A., Jiang, A., He, D., Zhong, L., Medina, D., Brinkley, R. B., and Roop, D. R. Expression of a p53 mutant in the epidermis of transgenic mice accelerates chemical carcinogenesis. Oncogene, in press, 1998.
29. Deb, S., Jackson, C. T., Subler, M. A., and Martin, D. W. Modulation of cellular and viral promoters by mutant human p53 proteins found in tumor cells. J. Virol., *66:* 6164–6170, 1992.
30. Liu, P. K., Kraus, E., Wu, T. A., Strong, L. C., and Tainsky, M. A. Analysis of genomic instability in Li-Fraumeni fibroblasts with germline p53 mutations. Oncogene, *12:* 2267–2278, 1996.

Wild-Type and Mutant Forms of p53 Activate Human Topoisomerase I: A Possible Mechanism for Gain of Function in Mutants

Amador Albor, Shinsuke Kaku and Molly Kulesz-Martin

Cancer Res 1998;58:2091-2094.