[CANCER RESEARCH 63, 2596–2605, May 15, 2003]

p53 Interacts with hRAD51 and hRAD54, and Directly Modulates Homologous Recombination[1,2]

Steven P. Linke, Sagar Sengupta, Nissim Khabie, Beth A. Jeffries, Sabine Buchhop, Stefan Miska, Wilhelm Henning, Remy Pedeux, Xin W. Wang, Lorne J. Hofseth, Qin Yang, Susan H. Garfield, Horst-Werner Stürzbecher, and Curtis C. Harris[3]

Laboratories of Human Carcinogenesis [S. P. L., S. S., N. K., B. A. J., R. P., X. W. W., L. J. H., Q. Y., C. C. H.] and Experimental Carcinogenesis [S. H. G.], National Cancer Institute, NIH, Bethesda, Maryland 20892, and Institute of Human Genetics, University of Luebeck, Lübeck, Germany D-23538 [S. B., S. M., W. H., H-W. S.]

ABSTRACT

p53 inhibits tumorigenesis through a variety of functions, including mediation of cell cycle arrest, premature senescence, and apoptosis. p53 also can associate with several DNA helicases and proteins involved in homologous recombination. In this study, we show that p53, hRAD51, and hRAD54 coimmunoprecipitated and colocalized with each other at endogenous levels in normal cells. Colocalization was observed with the phosphoserine-15 form of p53 at presumed DNA processing sites after the induction of DNA breaks. hRAD54 bound directly to the p53 COOH terminus *in vitro* without a nucleic acid intermediate. We then investigated the functional consequences of these protein interactions. A host cell reactivation assay revealed that the elevation in recombination observed after p53 inactivation is dependent on the hRAD51 pathway and that p53-dependent antirecombinogenic activity can be attributed to p53 binding to hRAD51 directly. These data support the hypothesis that p53 helps maintain genetic stability through transcription-independent modulation of homologous recombination factors.

INTRODUCTION

HR[4] is important for the generation of genetic diversity and for chromosome segregation during meiosis, as well as the repair of complex forms of DNA damage, such as double-strand breaks, interstrand cross-links, or DNA adducts in close proximity on opposite strands. Such damage can be induced by exogenous agents, like ionizing radiation, or it can arise from endogenous processes, like replication fork stalling during attempted replication over a single-strand break (1).

Maintenance of genetic stability likely relies heavily on the high fidelity of HR-mediated repair. Cells with mutations in HR genes (*e.g.*, *BRCA1*, *BRCA2*, *RAD51*, *RAD54*, *XRCC2*, or *XRCC*3) exhibit high levels of genetic instability and sensitivity to cross-linking agents and ionizing radiation (2). Conversely, inappropriate activity of the HR pathway also could contribute to genetic instability by causing improper recombination (*e.g.*, between repeat sequences or mutant alleles), resulting in translocations, deletions, duplications, or loss of heterozygosity (3–7). hRAD51 overexpression is observed in several tumor-derived cells (8–12) and correlates with elevated HR frequencies (8, 13, 14), which could account for some of these observed instabilities. In addition, some cancer-derived mutations have been reported for *RAD51*, *RAD54*, and *RAD54B* (a *Rad54* paralog), and there are reports indicating loss of heterozygosity at the chromosomal locations of *RAD51*, *RAD51B* (a *Rad51* paralog), *RAD52*, and *RAD54*, as well as *BRCA1* and *BRCA2* (reviewed in Ref. 2). Direct links between hRAD51 overexpression or HR gene modifications and the underlying genome instability in tumorigenesis remain to be established. However, these early reports are consistent with the idea that HR-mediated repair activity must be tightly regulated to maintain genetic stability (15).

The initial steps of HR involve recognition and preprocessing of the DNA lesions to form single-stranded DNA tails. Similar to RecA and yeast Rad51, hRAD51 can form polymers along single-stranded DNA, forming nucleoprotein filaments *in vitro* (16). hRAD51 also associates with hRAD54 (17). RAD54 belongs to the SWI2/SNF2 family of helicase-like proteins (18), which contain seven evolutionarily conserved motifs, including a DNA-dependent ATPase motif. Although hRAD54 does not possess classic helicase activity, it can alter DNA conformation (19–21). There is evidence that RAD54 can bind to RAD51 stoichiometrically and stabilize the protein-DNA complex, thereby stimulating strand invasion (22, 23). RAD51 also possesses DNA-dependent ATPase activity through a conserved Walker motif, which presumably catalyzes the pivotal homologous pairing and strand exchange reactions between the nucleoprotein filaments and the repair template DNA (24, 25).

Expression of mammalian RAD51 and RAD54 is induced in late G_1, S, and G_2 (18, 23, 26, 27). RAD51 forms nuclear foci in S phase cells (26, 28). Several other proteins implicated in HR, including BRCA1, BRCA2, RAD54, BLM, and RPA, bind to RAD51 and/or colocalize with RAD51 foci (17, 19, 29–34). These foci probably represent large complexes of proteins engaged in, or attempting, HR-mediated DNA repair, because they increase in number and intensity after DNA damage induced by agents such as ionizing radiation, cross-linkers, and UV radiation. HR may be favored in S phase cells because of the presence of sister chromatids as proximal repair templates.

Mutations in the *p53* tumor suppressor gene are common in a variety of human cancers (35). p53 can be activated by a variety of stress signals, including DNA damage (36), ribonucleotide depletion (37), hyperploidy (38, 39), hypoxia (40), activated oncogenes (41), and loss of cell adhesion (42). On stimulation, p53 can initiate cell cycle arrest, premature senescence, or apoptosis by activating transcription of several downstream genes (43–46). On the basis of data derived from a transactivation-deficient mouse model, it has been suggested that transcriptional transactivation is the determining tumor suppressor function of p53 (47).

However, there is also evidence that p53 can initiate apoptosis in a transcription-independent manner (48–50). We have shown previously that this can occur through physical interactions of p53 with members of the DExH-containing DNA helicase superfamily, includ-

Received 10/31/02; accepted 3/13/03.

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] N. K. was supported as an NIH-Howard Hughes Medical Institute scholar by the Howard Hughes Medical Institute. This research was supported, in part, by grants EUR94-858 and EUR98-1775 from the Dutch Cancer Society, Grant PGN 901-010097 from the Netherlands Organization for Scientific Research, Grant RG0063/1997 from the Human Frontiers Science Program Organization, Grant F14PCT950010 from the EURATOM/EEC, and Grant QLG7-CT-1999-00851 from the FP5 program of the EEC.

[2] Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org).

[3] To whom requests for reprints should be addressed, at Laboratory of Human Carcinogenesis, National Cancer Institute, NIH, 37 Convent Drive, Building 37, Room 2C05, Bethesda, MD 20892-4255. Phone: (301) 496-2048; Fax: (301) 496-0497; E-mail: Curtis_Harris@nih.gov.

[4] The abbreviations used are: HR, homologous recombination; hRAD51, human RAD51; hRAD54, human RAD54; NHF, normal human fibroblast; co-IP, coimmunoprecipitation; NCS, neocarzinostatin; GFP, green fluorescent protein; BFP, blue fluorescent protein; HU, hydroxyurea; CMV, cytomegalovirus; GST, glutathione *S*-transferase.

ing the repair factors BLM, XPB, XPD, and WRN (51–56). This superfamily also includes members of the SWI2/SNF2 family, such as CSB and RAD54 (18), with no demonstrated classic helicase activity. We showed previously that p53 can bind to CSB (52).

p53 also may help maintain genetic stability by modulating HR activity. p53-deficient cells often exhibit elevated frequencies of gene amplifications and karyotypic abnormalities, such as aneuploidy, deletions, inversions, and translocations (*e.g.*, 57–59). In addition, mammalian cells in which p53 has been inactivated exhibit elevated spontaneous and induced HR (60–70), as well as DNA damage-induced sister chromatid exchange (71–73), a process that can be mediated by HR. Furthermore, p53 can bind directly or indirectly to several proteins implicated in HR, including BLM, BRCA1, BRCA2, and RAD52 (54, 74–77). We showed recently that p53 can modulate the helicase activities of BLM and WRN (56), which are likely involved in recombinational repair (78). In addition, p53 can bind to and inhibit the HR factors hRAD51, RecA, and RPA (79–81). Interestingly, the primary binding site on hRAD51 is the putative homo-oligomerization domain, which may be necessary to form functional hRAD51 nucleoprotein filaments (16, 82). Several studies indicate that p53 modulation of HR activity is independent of its transcriptional activation function (65, 67, 70, 83).

In this report, we characterize p53 interactions with the HR factors hRAD51 and hRAD54. We demonstrate both physical interactions and colocalization of the proteins in nuclear foci. As a functional consequence of these interactions, we demonstrate p53-dependent modulation of HR frequencies *in vivo*. These findings indicate that p53 has a transcription-independent effect on HR via direct interaction with hRAD51 and hRAD54.

MATERIALS AND METHODS

Cells and Treatments. NHF strain GM07532 was obtained from Coriell Cell Repositories (Camden, NJ). LFS041 cells are postcrisis Li-Fraumeni fibroblasts that are p53 null. All of the cells were maintained in DMEM supplemented with glutamine, penicillin, streptomycin, and 10% fetal bovine serum. HU was obtained from Sigma, and NCS was obtained from the Drug Synthesis Branch of the National Cancer Institute. For HU treatments, cells were grown to confluency to attain synchronization in G_1, then subcultured at a lower density for 24 h so that most of the cells were in S phase. The cells were then either left untreated or treated with 1 mM HU for 16 h. For NCS treatments, asynchronous cells were treated at a concentration of 100 ng/ml for the indicated times.

Plasmids and Antibodies. cDNAs coding for wild-type hRAD51, the p53 nonbinding mutant of hRAD51 (186P), and the Walker motif dominant-negative mutant of hRAD54 (189R) were subcloned into and expressed from the pCLXSN retroviral vector (84). Subsequent sequencing revealed that hRAD51/186P also contains the following mutations, which did not substantially affect its ability to enhance HR in the host cell reactivation assay: 47R, 59G, and 122V. cDNAs with incompatible restriction sites were first subcloned into pJS-1, a shuttle vector produced by changing the *BssH*II sites flanking the polylinker of pBluescript to *Not*I sites, and then subcloned into a version of pCLXSN in which the *EcoR*I site had been converted to a *Not*I site. In this study, no retrovirus was produced; rather, the plasmids were transfected and the genes expressed under the control of the CMV promoter. Wild-type p53 and hRAD54 were expressed from the pcDNA3 expression plasmid (Invitrogen, Carlsbad, CA). The tumor-derived p53 mutants 175H and 248W were expressed from the pCMV-Neo-Bam expression plasmid. Dominant-negative RAD51 (dnRAD51 and SMRAD51) is a chimera of *Saccharomyces cerevisiae* and murine Rad51 (85), which was expressed from the pcDNA3.1 expression plasmid (Invitrogen). Transcriptionally inactive p53 (22/23) was expressed from pRCp53 (22, 23, 86).

GST-p53 NC encodes GST fused to full-length human wild-type p53. GST-p53 N5, NTD, 2C, 3C, 25, and 35, kindly provided by Bruce Stillman (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), encode GST fused to wild-type p53 amino acid residues 2–293, 2–124, 94–393, 155–393, 94–293, and 155–293, respectively.

The antibodies used in this study were: mouse monoclonal anti-p53 PAb421 (Ab-1), PAb1801 (Ab-2), and DO-1 (Ab-6; Oncogene Research Products); rabbit polyclonal anti-hRAD51 Ab-1 and mouse monoclonal anti-hRAD51 Ab-2 (Oncogene Research Products); rabbit polyclonal anti-phospho-p53 (Ser15; Cell Signaling Technology, Beverly, MA); and goat polyclonal anti-hRAD54 D-18 and L-17 (Santa Cruz Biotechnology). Horseradish peroxidase-linked secondary antibodies were donkey antirabbit IgG and donkey antimouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), and goat antimouse κ horseradish peroxidase, which does not recognize the heavy chain (SouthernBiotech, Birmingham, AL).

***In Vivo* Binding Assays.** Cells were lysed with immunoprecipitation buffer [0.5% NP40, 50 mM Tris (pH 8.0), and 120 mM NaCl, or 1% sodium deoxycholate, 0.1% SDS, 1 mM Tris (pH 7.4), and 150 mM NaCl] containing protease inhibitors. Protein concentrations were determined, and the samples were incubated for 1–2 h at 4°C with the indicated primary antibody or an equivalent amount of the appropriate control (mouse IgG or rabbit serum). Protein complexes were then precipitated with agarose- or Sepharose-conjugated protein A and/or G. Development was carried out by enhanced chemiluminescence according to the manufacturer instructions (Amersham), and densitometry analysis was performed on a Macintosh computer using the public domain NIH Image program (developed at the NIH and available on the internet).[5] Experiments were repeated at least twice, and representative blots are shown.

Immunostaining and Microscopy. The cells were seeded onto coverslips or glass chamber slides (Nalge Nunc International) and treated as indicated at least 24 h later. Just before fixation, the cells were washed with PBS (pH 7.4). In some cases, the live cells were fixed immediately with 4% paraformaldehyde in PBS or ice-cold absolute methanol. Paraformaldehyde-fixed cells were permeabilized with 0.2–0.5% Triton X-100 in PBS for 20 min, and methanol-fixed cells were washed briefly with ice-cold acetone. Cells were then washed several times with PBS and blocked for 20 min with 2.0% BSA/0.15% glycine in PBS. In other cases, the live cells were treated for 8 min with a hypotonic lysis solution [0.5% NP40, 10 mM Tris (pH 7.4) 2.5 mM $MgCl_2$, and 1 mM phenylmethylsulfonyl fluoride) on ice. The cells were washed once with ice-cold PBS and then fixed for 4 min in ice-cold 100% ethanol. They were then washed three times with PBS containing 0.2% Tween 20 and blocked for 30 min with 10% normal chicken serum in PBS.

All of the subsequent antibody incubations were carried out at room temperature in a humidified, light-proof chamber with the antibody diluted in a blocking solution. Incubations with the indicated primary antibodies were conducted for either 1 h at room temperature or overnight at 4°C, and all of the secondary antibody incubations were carried out for ~1 h at room temperature. Extensive washing with PBS or PBS containing 0.2% Tween 20 was carried out after both incubations. Secondary antibodies used were goat antimouse IgG-Alexa488, goat antirabbit IgG-Alexa568, and donkey antigoat-Alexa488 from Molecular Probes (Eugene, OR), as well as donkey antirabbit IgG-fluorescein-5-isothiocyanate and horse antigoat IgG-Texas Red (Jackson Immuno Laboratories). Coverslips were applied with Vectashield antifade mounting medium with or without 0.5 μg 4′,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA).

For conventional immunofluorescence microscopy, the cells were examined with a Zeiss Axioskop fluorescence microscope equipped with a Photometrics SenSys CCD camera. Images were captured, pseudocolored, and, in some cases, merged using IP Lab Spectrum v3.1 software (Scanalytics, Inc., Fairfax, VA). Alternatively, confocal fluorescent images were collected with a Bio-Rad MRC 1024 confocal scan head mounted on a Nikon Optiphot microscope with a 60× Planapochromat lens or a Zeiss LSM410. Z sections were collected at 0.5-μm intervals for each cell using LaserSharp software (Bio-Rad). Images were analyzed with Confocal Assistant software (Bio-Rad). For calculation of percentages of cells with foci, 50 cells were scored in three separate experiments. For quantitation of signal overlap, the Z sections of five random cells that were scored as containing foci were analyzed and averaged with the Bio-Rad LaserSharp software.

[5] Internet address: http://rsb.info.nih.gov/nih-image/.

***In Vitro* Binding Assays.** For the p53-hRAD54 assays, recombinant GST-p53 fusion proteins were produced in *Escherichia coli* and purified on glutathione-agarose beads according to the manufacturer (Sigma). Protein concentrations were determined by Coomassie blue staining of SDS-PAGE gels and comparison with molecular weight standards (Bio-Rad). hRAD54 was produced *in vitro* using the TNT-coupled rabbit reticulolysate system (Promega) and radiolabeled with [^{35}S]cysteine according to the manufacturer. Immediately after protein production, 5 μl of the hRAD54 lysate was incubated with 1 μg each of the recombinant GST-p53 proteins for the specified time period in immunoprecipitation buffer (120 mM NaCl, 50 mM Tris, and 0.5% NP40) on a rocker at room temperature. Then, the agarose beads were washed five times with at least 25 volumes of buffer. The precipitated proteins were denatured in loading buffer by heating at 95°C for 10 min. Samples were run on a 10% SDS-PAGE gel. The gel was then fixed in 50% methanol/10% acetic acid, enhanced in 1.5 M sodium salicylate, dried, and exposed overnight to autoradiographic film. Binding was measured as the amount of radiolabeled hRAD54 in the binding lanes compared with a control direct-translation product. Antibodies used in the competition studies were obtained from Oncogene Research Products (San Diego, CA).

Transfections. All of the transfections were carried out for 6 h in serum-free medium with Lipofectamine Plus or Lipofectamine 2000 according to manufacturer instructions (Invitrogen). Complete medium was added, and cells were incubated for a total of 36 h for the host cell reactivation assay or 72 h for analysis of hRAD51 structures.

HR Assay. The host cell reactivation assay to assess HR capacity was carried out as described (69). Different combinations of expression plasmids were cotransfected with the pBHRF plasmid, encoding a truncated GFP and a full-length BFP. In the absence of HR, only BFP is expressed. However, HR between the BFP and truncated GFP can lead to the creation of a functional GFP. Green and blue fluorescence were examined simultaneously with a Becton-Dickinson FACS Vantage flow cytometer with a 488-nm argon laser (GFP) and an UV (350–360 nm) laser (BFP). Each experiment was carried out at least three times.

RESULTS

Wild-Type p53 Associates with Wild-Type hRAD51 *in Vivo*. We showed previously an *in vitro* interaction between hRAD51 and p53 (80). We also showed an *in vivo* interaction between RAD51 and murine temperature-sensitive p53 in rat embryo fibroblasts, and between hRAD51 and mutant p53 (220C) in the hepatocarcinoma cell line, HuH7 (82). To investigate the association of hRAD51 with wild-type p53 at endogenous levels in normal cells, we conducted co-IP experiments in NHFs. hRAD51 co-IPed with p53 in untreated NHF (Fig. 1*A*). On the basis of densitometry, the amount of hRAD51 that co-IPed with p53 increased modestly within 2 h of treatment with the double-strand break-inducing agent NCS, but then decreased at later time points. Although the amount of co-IPed hRAD51 had decreased significantly after 18 h of treatment, the decrease was comparable with that observed for the overall hRAD51 level (Fig. 1*A*). A 16-h treatment with the ribonucleotide reductase inhibitor HU, which can collapse replication forks leading to DNA breaks in S phase, led to an increase in the amount of hRAD51 that co-IPed with p53 (87). Similar results were observed after γ-irradiation of the hepatoma cell line HepG2, which contains wild-type p53 (data not shown).

Because hRAD51 colocalizes in nuclear foci with other factors involved in HR-mediated DNA repair in S phase cells and/or DNA-damaged cells, we sought to determine whether p53 is also present in these foci. After standard paraformaldehyde fixation of untreated NHF, p53 staining with the NH_2-terminal antibody, DO-1, or a phosphoserine-15-specific antibody showed only faint staining with little evidence of nuclear foci (data not shown). However, within 2 h of NCS treatment, which induces ATM-dependent phosphorylation of p53 at serine 15, a focal staining pattern of endogenous p53 phosphoserine-15 was observed in 15% ± 3% (mean ± SD) of cells.

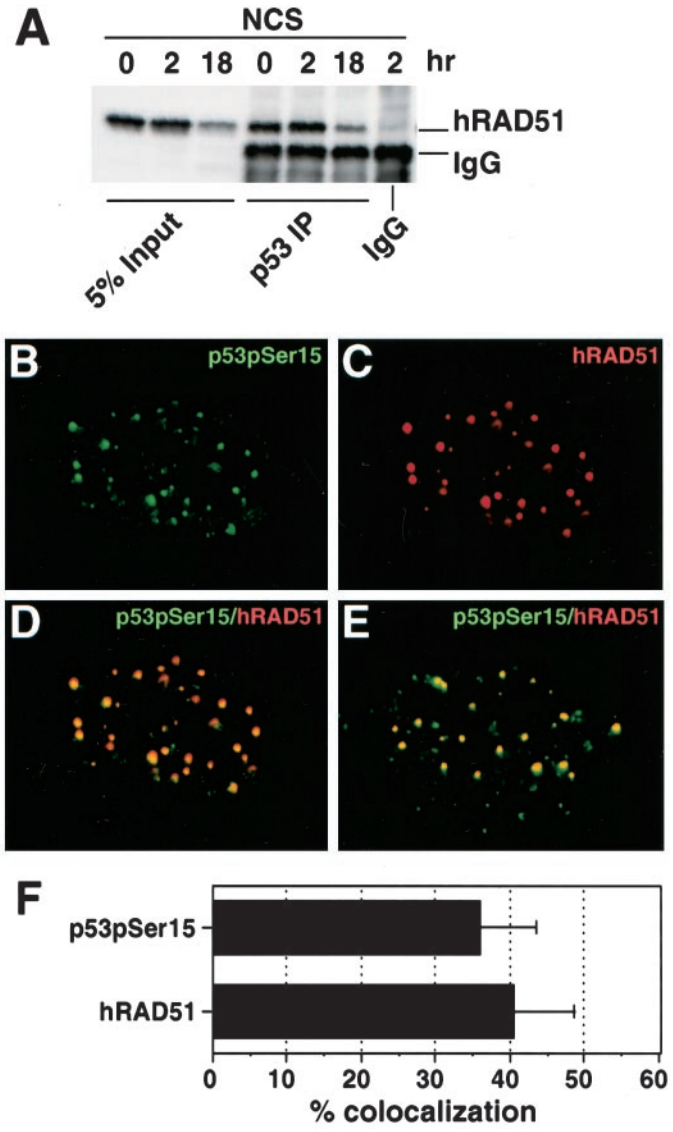

Fig. 1. p53 associates with hRAD51 *in vivo*. *A*, NHFs were treated with NCS for the indicated times. Lysates (500 μg) were immunoprecipitated with anti-p53 DO-1 or mouse IgG and then run on a gel along with 25 μg total lysate. The blot was probed with anti-RAD51 Ab-2. *B–E*, NHFs were treated with NCS for 2 h, and stained for p53 with anti-phospho-p53 (Ser15) and anti-hRAD51 Ab-2. *B* and *C* are the same cell with *D* being a merged image. *E*, merged image of another cell. *F*, percentage colocalization of p53-phosphoserine-15 and hRAD51 signals calculated by analysis of confocal images with LaserSharp software. Five cells were averaged; *bars*, ±SD.

These foci colocalized with hRAD51 (Fig. 1, *B–E*). The relatively low fraction of asynchronously growing cells with RAD51 foci is consistent with their formation specifically in S phase cells. Approximately 35–40% of the signal overlapped in these cells (Fig. 1*F*). The remainder of the cells contained less than five foci of either protein, so they were not scored. A much higher percentage of NHF exhibited focal p53-hRAD51 colocalization after HU treatment, possibly because of an accumulation of cells in S phase (87). The focal staining was specific, because no staining was apparent in NCS-treated p53-null cells (data not shown). Although no colocalization of endogenous p53 and hRAD51 was evident in untreated NHF, p53 overexpression led to colocalization in a subset of cells when stained with a mixture of p53 NH_2- and COOH-terminal antibodies (data not shown).

Wild-Type p53 Associates with Wild-Type hRAD54 *in Vivo*. p53 can physically interact with several DNA helicases, including BLM, XPB, XPD, and WRN (51–54). We investigated whether p53

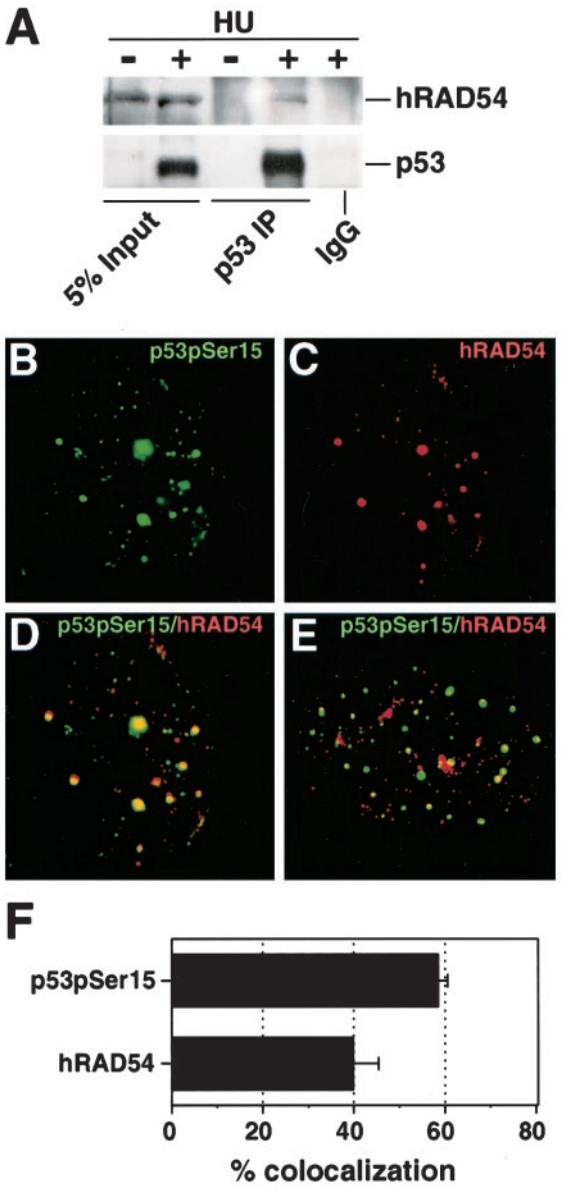

Fig. 2. p53 associates with hRAD54 *in vivo*. *A*, contact-inhibited NHFs were released into HU (+) or no treatment (−) for 16 h. The lysates (400 μg) were immunoprecipitated with anti-p53 DO-1 or mouse IgG and then run on a gel along with 20 μg of total lysates. The blot was probed with anti-hRAD54 D-18 and L-17 (Santa Cruz Biotechnology), then reprobed with anti-p53 DO-1. *B*--*E*, NHFs were treated as in *A*, and stained for p53 with anti-phospho-p53 (Ser15), and anti-hRAD54 D-18 and L-17. *B* and *D* are the same cell with *D* being a merged image. *E*, merged image of another cell. *F*, percentage colocalization of p53-phosphoserine-15 and hRAD54 signals calculated by analysis of confocal images with LaserSharp software. Five cells were averaged; *bars*, ±SD.

could also interact with hRAD54, a member of the same superfamily. HU treatment of NHF for 16 h also induced association, as evidenced by an increase in hRAD54 in p53 immunoprecipitates (Fig. 2*A*). Treatment of NHF or normal human lymphoblasts with γ radiation also induced the p53 signal in hRAD54 immunoprecipitates (data not shown). Consistent with these findings, p53 also colocalized with hRAD54 after HU treatment, with approximately 40–60% signal overlap in cells with foci (Fig. 2, *B*--*E*). In cells overexpressing hRAD54, the hRAD54 antibodies revealed well-defined bands of the same and predicted molecular weight as that seen in the p53 immunoprecipitates (Supplementary Fig. 1). In addition, the hRAD54 focal staining was not present when the hRAD54 antibodies were preincubated with control peptides (data not shown). These data support the specificity of the hRAD54 antibodies.

hRAD54 Binds Directly to p53 *in Vitro*. On the basis of the data described above, binding of p53 to hRAD54 could be indirect through mutual interaction with hRAD51. However, hRAD54 is the human homologue of a yeast DNA helicase that shares structural motifs with other members of a superfamily that includes DNA helicases to which p53 can bind (BLM, CSB, XPB, XPD, and WRN). Therefore, we sought to determine whether p53 could bind directly to hRAD54. Radiolabeled, *in vitro*-translated hRAD54 was incubated with *E. coli*-produced, GST-tagged, wild-type p53, which had been conjugated to reduced glutathione-linked agarose beads. hRAD54 precipitated specifically with GST-p53, but not with GST alone or unconjugated beads (Fig. 3*A*). These results show a clear, specific interaction between p53 and hRAD54 *in vitro*.

Given that p53 can bind directly to DNA and that hRAD54 is a homologue of a DNA helicase, it is possible that the interaction between these proteins could be through a nucleic acid intermediate (88). To test this possibility, binding of the *in vitro*-translated hRAD54 to GST-p53 was assayed after treatment of the lysate with DNase I, RNase A, or both. Nuclease treatment had no effect on the hRAD54-p53 interaction (Fig. 3*B*), indicating that the association is direct.

The COOH-Terminal Domain of p53 Binds Strongly to hRAD54. p53 can be divided into three topographical domains based on function. The NH_2-terminal domain is active in transactivational activities; the central region is a specific DNA-binding domain, where most of the cancer-associated p53 point mutations are found; and the multifunctional COOH-terminal domain possesses oligomerization, nuclear localization, and apoptosis-inducing activities (reviewed in Ref. 89). A panel of p53 deletion mutants was used to determine which functional domain(s) of p53 were required for hRAD54 binding. Equal amounts of GST-tagged, full-length p53 or p53 deletion mutants were incubated for equal time periods with radiolabeled hRAD54. The three mutants with an intact COOH terminus showed strong binding, whereas the other two mutants showed weak binding (Fig. 4*A*). A major reduction in binding was observed with the N5 p53 deletion mutant relative to full-length p53 (NC), showing that hRAD54 binds preferentially to the multifunctional COOH-terminal domain of p53 within residues 294–393 (gray boxes in Fig. 4*A*).

To additionally verify the hRAD54 interaction with the p53 COOH-terminal domain, competition experiments were conducted on the p53 deletion mutants with the p53 antibody PAb421, which binds to residues 371–380 (Ref. 90; Fig. 4*B*). Wild-type and deletion mutants of p53 were incubated with radiolabeled hRAD54 in either the presence or absence of saturating amounts of PAb421 (Fig. 4*C*).

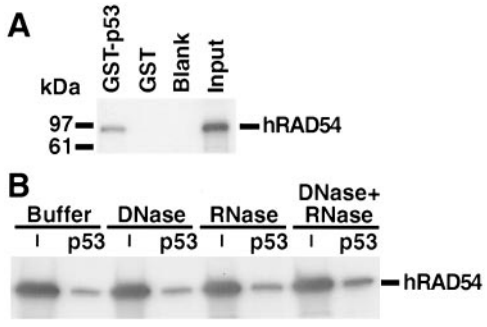

Fig. 3. hRAD54 binds to p53 *in vitro*. *A*, radiolabeled, *in vitro*-produced hRAD54 was incubated for 30 min with full-length GST-p53, GST only, or agarose beads only (Blank). The precipitated samples were run on an SDS-PAGE gel and visualized by autoradiography. Input, 20% of total hRAD54 per sample loaded directly onto the gel. *B*, radiolabeled, *in vitro*-produced hRAD54 was treated for 30 min with buffer, DNase I, RNase A, or both DNase I and RNase A. The hRAD54 was then incubated with GST-p53 for 2 h (p53). The precipitated samples were run on an SDS-PAGE gel and visualized by autoradiography. Input, 20% of total hRAD54 per treatment loaded directly onto the gel.

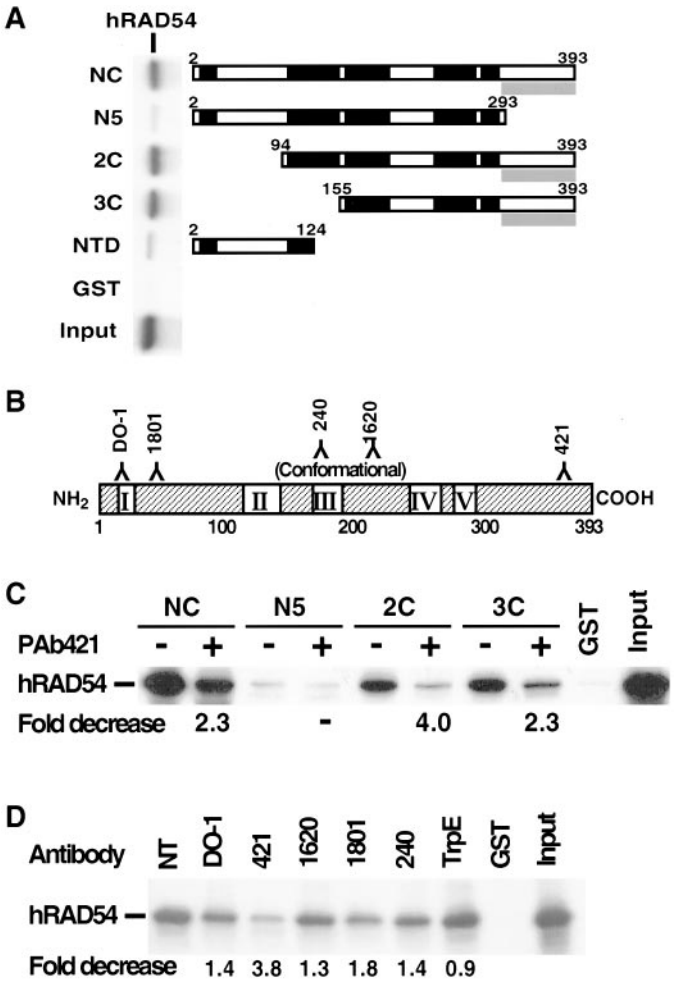

Fig. 4. hRAD54 binds to the extreme COOH terminus of p53 *in vitro*. Radiolabeled, *in vitro*-produced hRAD54 was incubated with equal amounts of the indicated form of GST-p53 or GST only for 2 h. *A*, hRAD54 binds to the extreme COOH terminus of p53 between amino acids 293–393 (▨). Schematic drawings to the *right of each lane* demonstrate which portions of the protein were present. The *numbers* denote amino acid residues. ■ indicate conserved regions, and ▨ represent the proposed binding site. *B*, schematic drawing indicating the epitopes for each anti-p53 antibody. The antibodies (and epitopes) used included DO-1 (residues 21–25), PAb1801 (residues 46–55), and PAb421 (residues 371–380). Antibodies 240 and 1620 are conformational only, and their epitopes depend on an intact tertiary structure of the p53 protein. *C*, radiolabeled, *in vitro*-produced hRAD54 was incubated with the indicated form of GST-p53 or GST only, along with either saturating amounts of PAb421 (+) or no antibody (−) for 2 h. "Fold decrease" is "+" relative to "−" for each GST-p53 form. *D*, radiolabeled, *in vitro*-produced hRAD54 was incubated for 2 h with GST-p53NC (full-length, wild-type) and either no antibody (*NT*), saturating levels of the indicated anti-p53 antibody, or antibacterial TrpE antibody. "Fold decrease" is relative to NT. The precipitated samples were run on an SDS-PAGE gel and visualized by autoradiography. *Input*, 20% of total hRAD54 per sample loaded directly onto the gel.

PAb421 diminished the binding of hRAD54 to the forms of p53 containing the COOH terminus. Little binding occurred with the form of p53 lacking the COOH terminus (N5), either with or without the antibody.

To show that the competitive disruption of hRAD54-p53 binding by PAb421 was specific to its binding to the COOH terminus of p53, a panel of p53 antibodies that bind to various regions were used (Fig. 4*B*). The antibodies were incubated with GST-tagged, full-length p53 and radiolabeled hRAD54 (Fig. 4*D*). Consistent with the above results, PAb421 strongly inhibited binding, whereas TrpE (an antibody to a bacterial protein used as a negative control) did not decrease binding at all. The other p53 antibodies caused only minor reductions in binding.

hRAD51 Associates with hRAD54 *in Vivo*. Previous studies have shown focal colocalization between murine RAD51 and RAD54 (19), and between overexpressed hRAD51 and hRAD54 (91). To demonstrate colocalization of hRAD51 and hRAD54 at endogenous levels, we used a mixture of two goat polyclonal Rad54 antibodies. Focal nuclear staining of hRAD54 was observed in 5% ± 1% of untreated cells, and these foci colocalized with hRAD51 foci (data not shown). The percentage of cells with these hRAD54 foci increased to 15% ± 4% within 4 h of NCS treatment (Fig. 5, *A–D*). Similar to the results with hRAD51, the relatively low fraction of asynchronously growing cells with hRAD54 foci is consistent with their formation specifically in S phase cells. In addition, hRAD54 was present in hRAD51 immunoprecipitates in both untreated and HU-treated NHF (Fig. 5*E*), which may reflect an HU-induced increase in the fraction of S phase cells.

p53 Inhibits hRAD51 Polymerization. When hRAD51 is overexpressed it can polymerize into high-order, filamentous nuclear structures (92). Although the physiological significance of these structures has not been established, they serve as a marker for RAD51 polymerization. Given that p53 can bind to the homo-oligomerization domain of hRAD51 (82), we determined whether p53 could affect hRAD51 structure formation by transfecting postcrisis LFS041 fibroblasts (p53 null) with combinations of wild-type or mutant p53, or hRAD51. About 60% of the cells expressing wild-type hRAD51 contained the structures (Fig. 6, *A*, *B*, and *G*), whereas the remainder exhibited diffuse nuclear staining. In contrast, only ~18% of cells coexpressing wild-type hRAD51 and wild-type p53 contained the structures (Fig. 6*G*), whereas the remainder exhibited diffuse (Fig. 6, *C* and *D*) or more focal (Fig. 6, *E* and *F*) nuclear staining. The p53/273H point mutant exhibits greatly reduced binding to hRAD51 (80). Consistent with this, coexpression of p53/273H with wild-type

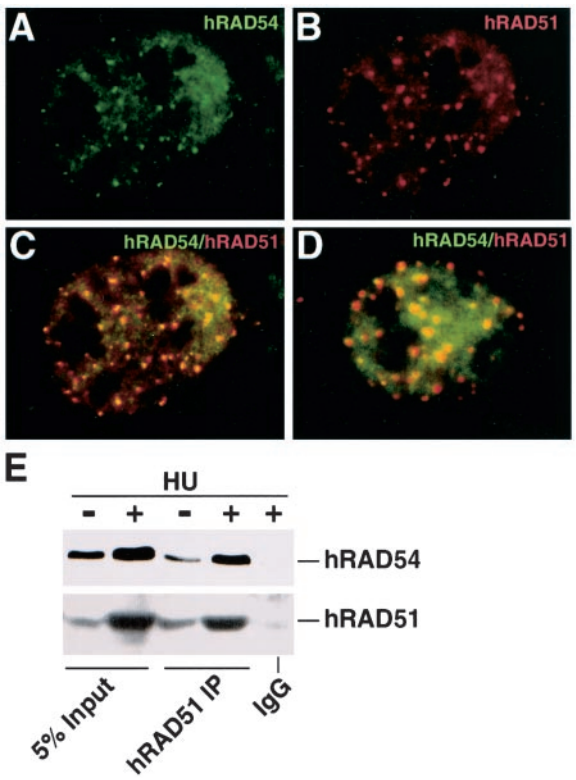

Fig. 5. hRAD51 associates with hRAD54 *in vivo*. *A–D*, NHFs were treated with NCS for 4 h, and stained with anti-hRAD51 Ab-1, and hRAD54 D-18 and L-17. *A–C* are the same cell with *C* being a merged image. *E*, contact-inhibited NHFs were released into HU (+) or no treatment (−) for 16 h. The lysates (400 μg) were immunoprecipitated with anti-hRAD51 Ab-1 or rabbit serum and then run on a gel along with 20 μg of total lysates. The blot was probed with anti-hRAD54 D-18 and L-17, and then reprobed with anti-hRAD51 Ab-2.

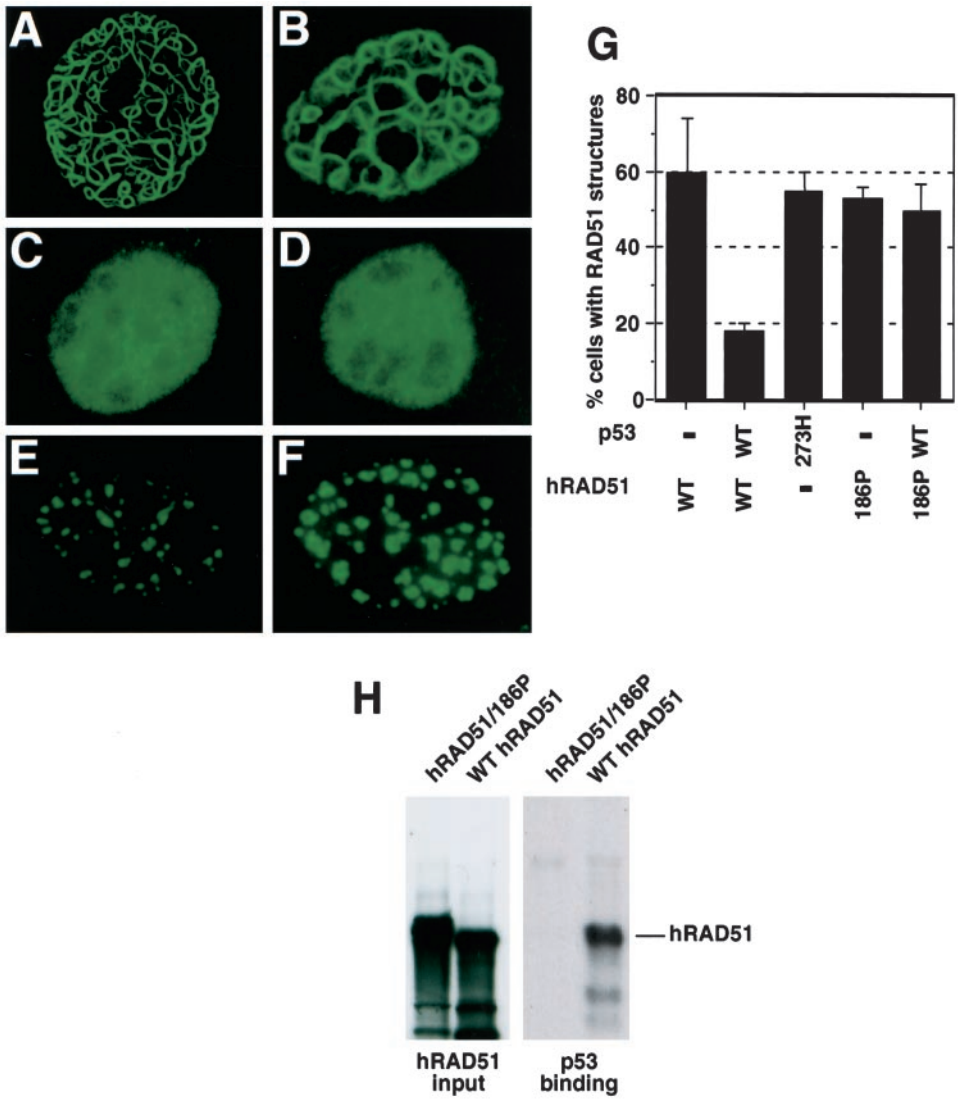

Fig. 6. p53 inhibits hRad51 polymerization through direct binding. LFS041 cells were transfected with pCLXSN-hRAD51 with or without pcDNA3-p53, fixed 72 h later, and then stained with anti-Rad51 Ab-1 (Oncogene Research Products). *A* and *B*, nuclear structures arising from hRAD51 expression alone. The transfected nuclei exhibited diffuse nuclear staining (*C* and *D*) or more focal nuclear staining (*E* and *F*) when wild-type p53 was coexpressed with hRAD51. *G*, LFS041 cells were transfected with the indicated plasmids, and the percentage of cells that contained RAD51 structures was calculated relative to the number that stained positively for RAD51. The number of RAD51-positive cells was dependent on both transfection and expression efficiencies, and was ~8% and 5%, respectively, for wild-type hRAD51 and 186P. Fifty cells were counted for each transfection in two separate experiments; *bars*, ±SD. *G*, binding of *in vitro*-translated, wild-type hRAD51 or the 186P mutant to *E. coli* produced His-tagged, wild-type p53.

hRAD51 failed to significantly inhibit structure formation (Fig. 6*G*). The hRAD51/186P mutant exhibits greatly reduced binding to p53 *in vitro* (Fig. 6*H*). Although this mutant still formed hRAD51 structures in over half of the RAD51-positive cells, coexpression of wild-type p53 failed to significantly inhibit structure formation (Fig. 6*G*). These data indicate that p53 can inhibit hRAD51 polymerization *in vivo* through direct binding to hRAD51.

Elevated Recombination Because of p53 Deficiency Is Dependent on hRAD51/hRAD54. Several studies have shown that recombination is elevated in p53-deficient cells (60–70). To help establish a mechanistic role for p53 in this process, a host cell reactivation assay (69) was conducted to assess HR. NHFs were transfected with p53, hRAD51, and/or hRAD54 mutants, along with a plasmid encoding a truncated GFP and a full-length BFP. In the absence of recombination, only BFP is expressed, but successful recombination between the BFP and truncated GFP can lead to the creation of functional GFP. Although the recombination occurs extrachromosomally in this assay, the intermediate DNA structures and mechanism are presumably the same as those that occur during chromosomal HR. Consistent with previous reports (62, 65, 70), expression of the tumor-derived p53/175H and p53/248W mutants led to an elevation in spontaneous HR (Fig. 7*A*). In contrast, expression of a dominant-negative RAD51, SMRAD51 (85), or a dominant-negative RAD54, hRAD54/189R (19), led to a reduction in spontaneous HR. Interestingly, when either of the p53 mutants was coexpressed with either of the RAD dominant-negatives, the HR levels did not increase over the levels seen with the dominant-negatives alone (Fig. 7*A*). These data validate the assay for the study of the RAD51 pathway and indicate that the elevation in HR observed after p53 inactivation is dependent on the RAD51/RAD54 pathway.

p53 Inhibits HR through a Direct Interaction with hRAD51. The RAD51 overexpression observed in many cancer cells may result in elevated HR. We next determined the effects of p53 on hRAD51-mediated HR using the host cell reactivation assay. Overexpression of wild-type hRAD51 or hRAD51/186P increased spontaneous HR approximately 2–3-fold over the basal level (Fig. 7*B*). For this study, the transcriptionally inactive 22/23 mutant of p53 (86) was used to minimize the potential contribution of secondary, transcription-dependent effects of p53 on HR. Consistent with a recent report (93), p53 did not significantly affect the basal level of spontaneous HR in the plasmid substrate. However, when coexpressed with hRAD51, p53 prevented any significant increase in HR by hRAD51. In contrast, p53 did not significantly inhibit the elevated HR induced by overexpression of the p53 nonbinding mutant of hRAD51 (Fig. 7*B*). Expression of the transfected proteins was verified by Western blot (Fig. 7, *C* and *D*). Although indirect effects of p53 on HR cannot be fully excluded,

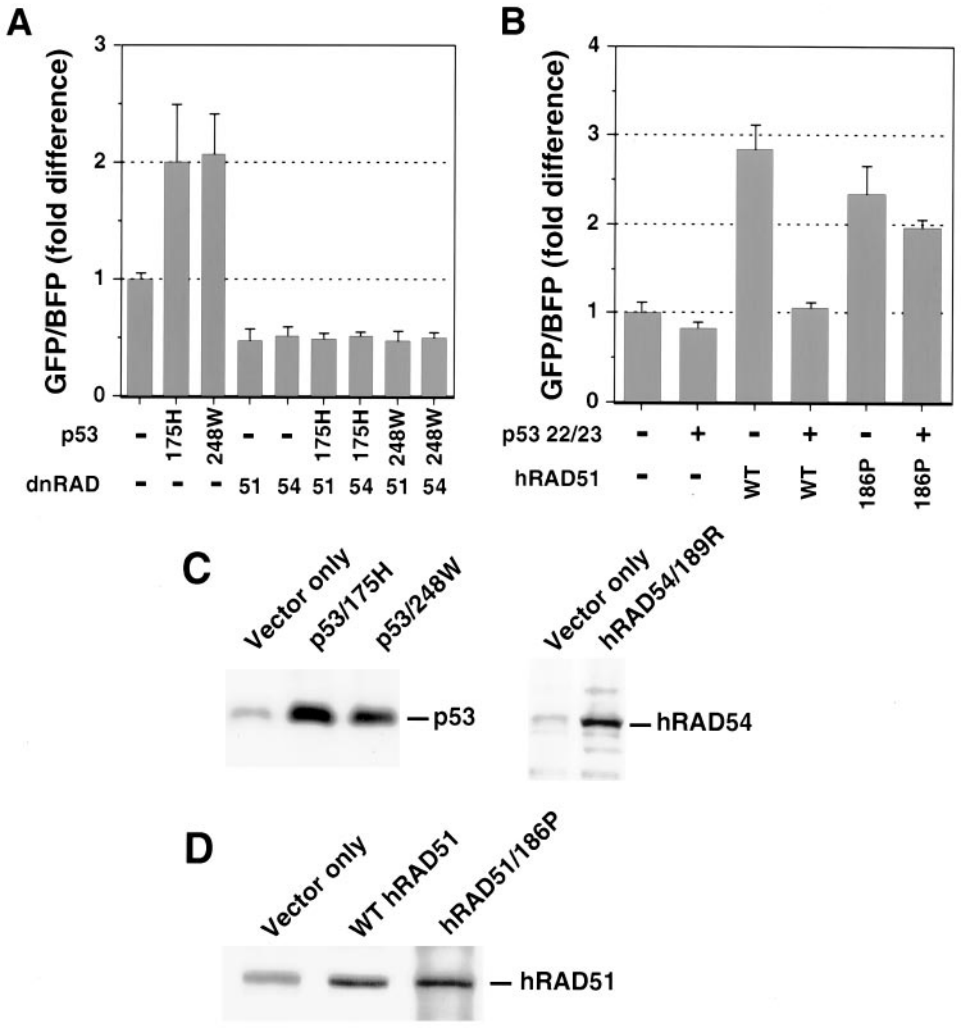

Fig. 7. p53 inhibits HR through a direct functional interaction with the hRAD51 pathway. NHFs were transfected with the HR substrate pBHRF and the indicated expression plasmids. Cells were harvested for protein or flow cytometry 36 h later. *A* and *B*, host cell reactivation assay for HR. Results are presented as the ratio of GFP to BFP cells, indicating the HR frequency level. Each sample was done twice in duplicate; *bars*, ±SD. *A*, effects of p53 inactivation and/or RAD51 pathway proteins. −, no plasmid; *175H*, pCMV-Neo-p53/175H; *248W*, pCMV-Neo-p53/248W; *dnRAD51*, pcDNA3.1-SMRAD51; *dnRAD54*, pCLXSN-hRAD54/189R. *B*, effects of p53 on RAD51 overexpressing cells. −, no plasmid; +, pRCp53 (22, 23); *WT*, wild-type hRAD51; *186P*, pCLXSN-hRAD51/186P. *C* and *D*, Western blots to verify expression of the transfected genes.

these data additionally support the hypothesis that p53 can repress HR through a direct interaction with hRAD51.

DISCUSSION

Previous studies have correlated p53 status with HR frequencies (60–70). Other studies have suggested a direct interaction between p53 and RAD51 (80–82), a central component of the HR pathway. In this study, co-IP experiments indicate that p53, hRAD51, and hRAD54 can form complexes at endogenous levels in normal cells. In addition, these proteins colocalize in nuclear foci, presumably during the S and/or G_2 phases of the cell cycle when the HR proteins are at their peak levels and sister chromatids are available as repair templates. The phosphoserine 15-specific p53 antibody used in this study resulted in focal staining, but similar results were achieved with antibody #588 (87), raised against the COOH terminus of p53 (94), so it is unclear whether any post-translational modifications to p53 are required for the interactions. The foci may form during the processing of replication-associated or exogenously induced DNA damage and, thus, represent sites of active repair. Another, more likely possibility is that the amount of protein at sites of active repair is too low to detect with conventional immunofluorescence, and that the visible foci represent accumulations of protein at persistent lesions that are irreparable or only slowly repaired (95). DNA breaks can stimulate recombination, and persistent breaks are prime candidates for misrepair. Thus, the accumulation of p53 in hRAD51 and hRAD54 foci may inhibit inappropriate recombination.

A host cell reactivation assay was used to demonstrate the direct role of p53 in modulating HR (Fig. 7*A*). This study confirms previous reports that expression of the tumor-derived p53 mutants 175H or 248W leads to elevated recombination (62, 65, 70). This elevation is independent of the transactivation activity of p53 (65). We showed previously that p53/248W exhibits reduced binding to Holliday junctions (56). We also demonstrate that dominant-negative forms of either RAD51 or RAD54 reduce recombination in the assay. The dominant-negative RAD51 used was a chimera of yeast and murine RAD51, SMRAD51, which reportedly eliminates spontaneous recombination in a Chinese hamster ovary reporter line. The dominant-negative RAD54 used, hRAD54/189R, contains a mutation in the conserved DNA-dependent ATPase domain that may be require for RAD54-mediated DNA unwinding (19). Coexpression of the p53 mutants with either of these dominant-negatives failed to elevate recombination over the reduced level observed with the dominant-negatives alone. This indicates that p53 modulation of HR is dependent on hRAD51 and hRAD54.

Elevated levels of hRAD51 are observed in a variety of tumor cell lines, suggesting that hyper-recombination may play a role in tumorigenesis (8–12). We demonstrate here that hRAD51 overexpression leads to elevated HR in the host cell reactivation assay and that

coexpression of transactivation-deficient p53 reduces the recombination to baseline levels. The binding site for p53 on hRAD51 between amino acids 125 and 220 is highly conserved from bacteria to human and includes the putative homo-oligomerization domain, which may be necessary to form functional hRAD51 nucleoprotein filaments (16, 80). Interestingly, expression of the p53 nonbinding mutant hRAD51/186P also elevated HR, but coexpression of p53 did not significantly inhibit this activity (Fig. 7*B*). Parallel results were achieved in assays of the ability of hRAD51 to form higher-order structures *in vivo* using these same mutants (Fig. 6). These data indicate that p53 modulates HR through direct interaction and inhibition of hRAD51.

This study also shows for the first time that p53 can interact directly with hRAD54 *in vitro* and *in vivo*. Although it has been demonstrated that hRAD51 and hRAD54 can bind directly to each other (17), the data presented here and elsewhere (80) indicate that p53 can bind to both separately. hRAD54 binds mainly to the extreme COOH terminus of p53, which is consistent with the binding of p53 to several other members of the same superfamily. It is also possible that *in vivo* p53 binds to either hRAD51 or hRAD54 only indirectly through association with the other protein.

Several studies indicate an even more direct role of p53 in DNA repair, including HR-related functions. For example, p53 reportedly binds to single- and double-stranded DNA ends through its COOH terminus, and can catalyze DNA renaturation and strand transfer reactions (88, 96–98). Other studies show that p53 binds to DNA base mismatches through its COOH terminus, resulting in HR inhibition (83, 99). In addition, there is evidence that p53 binds to Holliday junctions and may catalyze their resolution or inhibit HR (81, 100). It is also of interest that the hRAD51 paralog, RAD51B, can phosphorylate p53 (101), and that p53 reportedly associates directly with a number of other proteins implicated in HR, including BLM, BRCA1, BRCA2, RAD52, and RPA (54, 74–77, 79). The specific nature of these physical and functional interactions remains to be elucidated.

We also have shown that p53 colocalizes with BLM in nuclear foci that presumably form at sites of replication fork collapse, and that p53 localization in these foci is dependent on functional BLM (87). BLM is an antirecombinase that may catalyze reverse branch migration at these sites (102). In addition, we have shown that p53 can cooperate with BLM to inhibit RAD51 pathway-dependent sister chromatid exchanges (87). The data support a model in which BLM recruits p53 to certain sites at which recombination is favorable, but not necessarily desirable. Inhibitory interactions of p53 with hRAD51 and/or hRAD54 may assist in the down-regulation of inappropriate recombination.

Normally, HR serves to enhance genetic stability by mediating the accurate repair of double-strand breaks and other DNA lesions that cannot be handled by other repair processes. However, inappropriate activity of the HR machinery may lead to indiscriminate recombination, which could lead to genetic instability in the form of chromosomal aberrations. Although it has been suggested that transactivation is the determining function of p53 in tumor suppression (47), HU-induced replication fork stalling is known to induce transcriptionally inactive p53 in S phase (103). The relocalization of p53 to nuclear foci and formation of complexes with hRAD51 and hRAD54 are consistent with this finding. The results presented here, in combination with other studies showing that HR levels are elevated in the absence of functional p53 (60–70), support the hypothesis that p53 inhibits inappropriate recombination through inhibitory biochemical interactions with both hRAD51 and hRAD54. Thus, regulation of recombination may be another mechanism by which p53 maintains genetic stability.

ACKNOWLEDGMENTS

We thank John Kolman for pJS-1, Robert Naviaux for pCLXSN, and Robbert Slebos for pBHRF. We also thank Jan Hoeijmakers and Roland Kanaar for additional reagents and advice, Jill Coursen for technical support, Ana Robles for critical review of the manuscript, Dorothea Dudek for editorial assistance, and Karen MacPherson for bibliographic assistance.

REFERENCES

1. Haber, J. E. DNA recombination: the replication connection. Trends Biochem. Sci., *24:* 271–275, 1999.
2. Thompson, L. H., and Schild, D. Homologous recombinational repair of DNA ensures mammalian chromosome stability. Mutat. Res., *477:* 131–153, 2001.
3. Luo, G., Santoro, I. M., McDaniel, L. D., Nishijima, I., Mills, M., Youssoufian, H., Vogel, H., Schultz, R. A., and Bradley, A. Cancer predisposition caused by elevated mitotic recombination in Bloom mice. Nat. Genet., *26:* 424–429, 2000.
4. Zhou, Z. H., Akgun, E., and Jasin, M. Repeat expansion by homologous recombination in the mouse germ line at palindromic sequences. Proc. Natl. Acad. Sci. USA, *98:* 8326–8333, 2001.
5. Cheng, R. Z., Shammas, M. A., Li, J., and Shmookler Reis, R. J. Expression of SV40 large T antigen stimulates reversion of a chromosomal gene duplication in human cells. Exp. Cell Res., *234:* 300–312, 1997.
6. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instabilities in human cancers. Nature (Lond.), *396:* 643–649, 1998.
7. Windle, B., Draper, B. W., Yin, Y. X., O'Gorman, S., and Wahl, G. M. A central role for chromosome breakage in gene amplification, deletion formation, and amplicon integration. Genes Dev., *5:* 160–174, 1991.
8. Xia, S. J., Shammas, M. A., and Shmookler Reis, R. J. Elevated recombination in immortal human cells is mediated by HsRAD51 recombinase. Mol. Cell. Biol., *17:* 7151–7158, 1997.
9. Maacke, H., Opitz, S., Jost, K., Hamdorf, W., Henning, W., Kruger, S., Feller, A. C., Lopens, A., Diedrich, K., Schwinger, E., and Sturzbecher, H. W. Over-expression of wild-type Rad51 correlates with histological grading of invasive ductal breast cancer. Int. J. Cancer, *88:* 907–913, 2000.
10. Maacke, H., Jost, K., Opitz, S., Miska, S., Yuan, Y., Hasselbach, L., Luttges, J., Kalthoff, H., and Sturzbecher, H. W. DNA repair and recombination factor Rad51 is over-expressed in human pancreatic adenocarcinoma. Oncogene, *19:* 2791–2795, 2000.
11. Han, H., Bearss, D. J., Browne, L. W., Calaluce, R., Nagle, R. B., and Von Hoff, D. D. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. Cancer Res., *62:* 2890–2896, 2002.
12. Raderschall, E., Stout, K., Freier, S., Suckow, V., Schweiger, S., and Haaf, T. Elevated levels of Rad51 recombination protein in tumor cells. Cancer Res., *62:* 219–225, 2002.
13. Vispe, S., Cazaux, C., Lesca, C., and Defais, M. Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. Nucleic Acids Res., *26:* 2859–2864, 1998.
14. Yanez, R. J., and Porter, A. C. Gene targeting is enhanced in human cells overexpressing hRAD51. Gene Ther., *6:* 1282–1290, 1999.
15. Powell, S. N., Willers, H., and Xia, F. BRCA2 keeps Rad51 in line. High-fidelity homologous recombination prevents breast and ovarian cancer? Mol. Cell., *10:* 1262–1263, 2002.
16. Benson, F. E., Stasiak, A., and West, S. C. Purification and characterization of the human Rad51 protein, an analogue of E. coli RecA. EMBO J., *13:* 5764–5771, 1994.
17. Golub, E. I., Kovalenko, O. V., Gupta, R. C., Ward, D. C., and Radding, C. M. Interaction of human recombination proteins Rad51 and Rad54. Nucleic Acids Res., *25:* 4106–4110, 1997.
18. Kanaar, R., Troelstra, C., Swagemakers, S. M., Essers, J., Smit, B., Franssen, J. H., Pastink, A., Bezzubova, O. Y., Buerstedde, J. M., Clever, B., Heyer, W. D., and Hoeijmakers, J. H. Human and mouse homologs of the Saccharomyces cerevisiae RAD54 DNA repair gene: evidence for functional conservation. Curr. Biol., *6:* 828–838, 1996.
19. Tan, T. L., Essers, J., Citterio, E., Swagemakers, S. M., de Wit, J., Benson, F. E., Hoeijmakers, J. H., and Kanaar, R. Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation. Curr. Biol., *9:* 325–328, 1999.
20. Ristic, D., Wyman, C., Paulusma, C., and Kanaar, R. The architecture of the human Rad54-DNA complex provides evidence for protein translocation along DNA. Proc. Natl. Acad. Sci. USA, *98:* 8454–8460, 2001.
21. Alexeev, A., Mazin, A., and Kowalczykowski, S. C. Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51-ssDNA nucleoprotein filament. Nat. Struct. Biol., 2003.
22. Mazin, A. V., Alexeev, A. A., and Kowalczykowski, S. C. A novel function of RAD54 protein: stabilization of the RAD51 nucleoprotein filament. J. Biol. Chem., 2003.
23. Essers, J., Hendriks, R. W., Wesoly, J., Beerens, C. E., Smit, B., Hoeijmakers, J. H., Wyman, C., Dronkert, M. L., and Kanaar, R. Analysis of mouse Rad54 expression and its implications for homologous recombination. DNA Repair (Amst.), *1:* 779–793, 2002.
24. Baumann, P., Benson, F. E., and West, S. C. Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions *in vitro*. Cell, *87:* 757–766, 1996.

25. Gupta, R. C., Bazemore, L. R., Golub, E. I., and Radding, C. M. Activities of human recombination protein Rad51. Proc. Natl. Acad. Sci. USA, *94:* 463–468, 1997.
26. Flygare, J., Benson, F., and Hellgren, D. Expression of the human RAD51 gene during the cell cycle in primary human peripheral blood lymphocytes. Biochim. Biophys. Acta, *1312:* 231–236, 1996.
27. Yamamoto, A., Taki, T., Yagi, H., Habu, T., Yoshida, K., Yoshimura, Y., Yamamoto, K., Matsushiro, A., Nishimune, Y., and Morita, T. Cell cycle-dependent expression of the mouse Rad51 gene in proliferating cells. Mol. Gen. Genet., *251:* 1–12, 1996.
28. Haaf, T., Golub, E. I., Reddy, G., Radding, C. M., and Ward, D. C. Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes. Proc. Natl. Acad. Sci. USA, *92:* 2298–2302, 1995.
29. Golub, E. I., Gupta, R. C., Haaf, T., Wold, M. S., and Radding, C. M. Interaction of human rad51 recombination protein with single-stranded DNA binding protein, RPA. Nucleic Acids Res., *26:* 5388–5393, 1998.
30. Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D. M. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell, *88:* 265–275, 1997.
31. Sharan, S. K., Morimatsu, M., Albrecht, U., Lim, D. S., Regel, E., Dinh, C., Sands, A., Eichele, G., Hasty, P., and Bradley, A. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. Nature (Lond.), *386:* 804–810, 1997.
32. Chen, P. L., Chen, C. F., Chen, Y., Xiao, J., Sharp, Z. D., and Lee, W. H. The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. Proc. Natl. Acad. Sci. USA, *95:* 5287–5292, 1998.
33. Wu, L., Davies, S. L., Levitt, N. C., and Hickson, I. D. Potential role for the BLM helicase in recombinational repair via a conserved interaction with RAD51. J. Biol. Chem., *276:* 19375–19381, 2001.
34. Bischof, O., Kim, S. H., Irving, J., Beresten, S., Ellis, N. A., and Campisi, J. Regulation and localization of the Bloom syndrome protein in response to DNA damage. J. Cell Biol., *153:* 367–380, 2001.
35. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. p53 mutations in human cancers. Science (Wash. DC), *253:* 49–53, 1991.
36. 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.
37. Linke, S. P., Clarkin, K. C., Di Leonardo, A., Tsou, A., and Wahl, G. M. A reversible, p53-dependent G_0/G_1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Genes Dev., *10:* 934–947, 1996.
38. Cross, S. M., Sanchez, C. A., Morgan, C. A., Schimke, M. K., Ramel, S., Idzerda, R. L., Raskind, W. H., and Reid, B. J. A p53-dependent mouse spindle checkpoint. Science (Wash. DC), *267:* 1353–1356, 1995.
39. Di Leonardo, A., Khan, S. H., Linke, S. P., Greco, V., Seidita, G., and Wahl, G. M. DNA rereplication in the presence of mitotic spindle inhibitors in human and mouse fibroblasts lacking either p53 or pRb function. Cancer Res., *57:* 1013–1019, 1997.
40. Graeber, T. G., Peterson, J. F., Tsai, M., Monica, K., Fornace, A. J., Jr., and Giaccia. A. J. Hypoxia induces accumulation of p53 protein, but activation of a G_1- phase checkpoint by low-oxygen conditions is independent of p53 status. Mol. Cell. Biol., *14:* 6264–6277, 1994.
41. Kamijo, T., Weber, J. D., Zambetti, G., Zindy, F., Roussel, M. F., and Sherr, C. J. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc. Natl. Acad. Sci. USA, *95:* 8292–8297, 1998.
42. Nigro, J. M., Aldape, K. D., Hess, S. M., and Tlsty, T. D. Cellular adhesion regulates p53 protein levels in primary human keratinocytes. Cancer Res., *57:* 3635–3639, 1997.
43. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G_1 cyclin- dependent kinases. Cell, *75:* 805–816, 1993.
44. el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. WAF1, a potential mediator of p53 tumor suppression. Cell, *75:* 817–825, 1993.
45. Di Leonardo, A., Linke, S. P., Clarkin, K., and Wahl, G. M. DNA damage triggers a prolonged p53-dependent G_1 arrest and long-term induction of Cip1 in normal human fibroblasts. Genes Dev., *8:* 2540–2551, 1994.
46. Miyashita, T., and Reed, J. C. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell, *80:* 293–299, 1995.
47. Jimenez, G. S., Nister, M., Stommel, J. M., Beeche, M., Barcarse, E. A., Zhang, X. Q., O'Gorman, S., and Wahl, G. M. A transactivation-deficient mouse model provides insights into Trp53 regulation and function. Nat. Genet., *26:* 37–43, 2000.
48. Zhou, X., Wang, X. W., Xu, L., Hagiwara, K., Nagashima, M., Wolkowicz, R., Zurer, I., Rotter, V., and Harris, C. C. COOH-terminal domain of p53 modulates p53-mediated transcriptional transactivation, cell growth, and apoptosis. Cancer Res., *59:* 843–848, 1999.
49. Haupt, Y., Rowan, S., Shaulian, E., Vousden, K. H., and Oren, M. Induction of apoptosis in HeLa cells by *trans*-activation-deficient p53. Genes Dev., *9:* 2170–2183, 1995.
50. Caelles, C., Helmberg, A., and Karin, M. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature (Lond.), *370:* 220–223, 1994.
51. Wang, X. W., Yeh, H., Schaeffer, L., Roy, R., Moncollin, V., Egly, J. M., Wang, Z., Freidberg, E. C., Evans, M. K., Taffe, B. G., and *et al.* p53 modulation of TFIIH-associated nucleotide excision repair activity. Nat. Genet., *10:* 188–195, 1995.
52. Wang, X. W., Vermeulen, W., Coursen, J. D., Gibson, M., Lupold, S. E., Forrester, K., Xu, G., Elmore, L., Yeh, H., Hoeijmakers, J. H., and Harris, C. C. The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway. Genes Dev., *10:* 1219–1232, 1996.
53. Spillare, E. A., Robles, A. I., Wang, X. W., Shen, J. C., Yu, C. E., Schellenberg, G. D., and Harris, C. C. p53-mediated apoptosis is attenuated in Werner syndrome cells. Genes Dev., *13:* 1355–1360, 1999.
54. Wang, X. W., Tseng, A., Ellis, N. A., Spillare, E. A., Linke, S. P., Robles, A. I., Seker, H., Yang, Q., Hu, P., Beresten, S., Bemmels, N. A., Garfield, S., and Harris, C. C. Functional interaction of p53 and BLM DNA helicase in apoptosis. J. Biol. Chem., *276:* 32948–32955, 2001.
55. Robles, A. I., and Harris, C. C. p53-mediated apoptosis and genomic instability diseases. Acta Oncol., *40:* 696–701, 2001.
56. Yang, Q., Zhang, R., Wang, X. W., Spillare, E. A., Linke, S. P., Subramanian, D., Griffith, J. D., Li, J. L., Hickson, I. D., Shen, J. C., Loeb, L. A., Mazur, S. J., Appella, E., Brosh, R. M., Jr., Karmakar, P., Bohr, V. A., and Harris, C. C. The processing of Holliday junctions by BLM and WRN helicases is regulated by p53. J. Biol. Chem., *21:* 21, 2002.
57. White, A. E., Livanos, E. M., and Tlsty, T. D. Differential disruption of genomic integrity and cell cycle regulation in normal human fibroblasts by the HPV oncoproteins. Genes Dev., *8:* 666–677, 1994.
58. Yin, Y., Tainsky, M. A., Bischoff, F. Z., Strong, L. C., and Wahl, G. M. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell, *70:* 937–948, 1992.
59. Livingstone, L. R., White, A., Sprouse, J., Livanos, E., Jacks, T., and Tlsty, T. D. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell, *70:* 923–935, 1992.
60. Meyn, M. S., Strasfeld, L., and Allen, C. Testing the role of p53 in the expression of genetic instability and apoptosis in ataxia-telangiectasia. Int. J. Radiat. Biol., *66:* S141–149, 1994.
61. Wiesmuller, L., Cammenga, J., and Deppert, W. W. *In vivo* assay of p53 function in homologous recombination between simian virus 40 chromosomes. J. Virol., *70:* 737–744, 1996.
62. Bertrand, P., Rouillard, D., Boulet, A., Levalois, C., Soussi, T., and Lopez, B. S. Increase of spontaneous intrachromosomal homologous recombination in mammalian cells expressing a mutant p53 protein. Oncogene, *14:* 1117–1122, 1997.
63. Mekeel, K. L., Tang, W., Kachnic, L. A., Luo, C. M., DeFrank, J. S., and Powell, S. N. Inactivation of p53 results in high rates of homologous recombination. Oncogene, *14:* 1847–1857, 1997.
64. Honma, M., Zhang, L. S., Hayashi, M., Takeshita, K., Nakagawa, Y., Tanaka, N., and Sofuni, T. Illegitimate recombination leading to allelic loss and unbalanced translocation in p53-mutated human lymphoblastoid cells. Mol. Cell. Biol., *17:* 4774–4781, 1997.
65. Saintigny, Y., Rouillard, D., Chaput, B., Soussi, T., and Lopez, B. S. Mutant p53 proteins stimulate spontaneous and radiation-induced intrachromosomal homologous recombination independently of the alteration of the transactivation activity and of the G_1 checkpoint. Oncogene, *18:* 3553–3563, 1999.
66. Xia, F., Amundson, S. A., Nickoloff, J. A., and Liber, H. L. Different capacities for recombination in closely related human lymphoblastoid cell lines with different mutational responses to X- irradiation. Mol. Cell. Biol., *14:* 5850–5857, 1994.
67. Willers, H., McCarthy, E. E., Wu, B., Wunsch, H., Tang, W., Taghian, D. G., Xia, F., and Powell, S. N. Dissociation of p53-mediated suppression of homologous recombination from G_1/S cell cycle checkpoint control. Oncogene, *19:* 632–639., 2000.
68. Willers, H., McCarthy, E. E., Alberti, W., Dahm-Daphi, J., and Powell, S. N. Loss of wild-type p53 function is responsible for upregulated homologous recombination in immortal rodent fibroblasts. Int. J. Radiat. Biol., *76:* 1055–1062, 2000.
69. Slebos, R. J., and Taylor, J. A. A novel host cell reactivation assay to assess homologous recombination capacity in human cancer cell lines. Biochem. Biophys. Res. Commun., *281:* 212–219, 2001.
70. Saintigny, Y., and Lopez, B. S. Homologous recombination induced by replication inhibition, is stimulated by expression of mutant p53. Oncogene, *21:* 488–492, 2002.
71. Schwartz, J. L., and Russell, K. J. The effect of functional inactivation of TP53 by HPV-E6 transformation on the induction of chromosome aberrations by γ rays in human tumor cells. Radiat. Res., *151:* 385–390, 1999.
72. Ishizaki, K., Ejima, Y., Matsunaga, T., Hara, R., Sakamoto, A., Ikenaga, M., Ikawa, Y., and Aizawa, S. Increased UV-induced SCEs but normal repair of DNA damage in p53- deficient mouse cells. Int. J. Cancer, *58:* 254–257, 1994.
73. Cleaver, J. E., Afzal, V., Feeney, L., McDowell, M., Sadinski, W., Volpe, J. P., Busch, D. B., Coleman, D. M., Ziffer, D. W., Yu, Y., Nagasawa, H., and Little, J. B. Increased ultraviolet sensitivity and chromosomal instability related to P53 function in the xeroderma pigmentosum variant. Cancer Res., *59:* 1102–1108, 1999.
74. Marmorstein, L. Y., Ouchi, T., and Aaronson, S. A. The BRCA2 gene product functionally interacts with p53 and RAD51. Proc. Natl. Acad. Sci. USA, *95:* 13869–13874, 1998.
75. Ouchi, T., Monteiro, A. N., August, A., Aaronson, S. A., and Hanafusa, H. BRCA1 regulates p53-dependent gene expression. Proc. Natl. Acad. Sci. USA, *95:* 2302–2306, 1998.
76. Zhang, H., Somasundaram, K., Peng, Y., Tian, H., Bi, D., Weber, B. L., and El-Deiry, W. S. BRCA1 physically associates with p53 and stimulates its transcriptional activity. Oncogene, *16:* 1713–1721, 1998.
77. Shen, Z., Pardington-Purtymun, P. E., Comeaux, J. C., Moyzis, R. K., and Chen, D. J. Associations of UBE2I with RAD52, UBL1, p53, and RAD51 proteins in a yeast two-hybrid system. Genomics, *37:* 183–186, 1996.
78. Saintigny, Y., Makienko, K., Swanson, C., Emond, M. J., and Monnat, R. J., Jr. Homologous recombination resolution defect in werner syndrome. Mol. Cell. Biol., *22:* 6971–6978, 2002.

79. Dutta, A., Ruppert, J. M., Aster, J. C., and Winchester, E. Inhibition of DNA replication factor RPA by p53. Nature (Lond.), *365:* 79–82, 1993.
80. Buchhop, S., Gibson, M. K., Wang, X. W., Wagner, P., Sturzbecher, H. W., and Harris, C. C. Interaction of p53 with the human Rad51 protein. Nucleic Acids Res, *25:* 3868–3874, 1997.
81. Susse, S., Janz, C., Janus, F., Deppert, W., and Wiesmuller, L. Role of heteroduplex joints in the functional interactions between human Rad51 and wild-type p53. Oncogene, *19:* 4500–4512, 2000.
82. Sturzbecher, H. W., Donzelmann, B., Henning, W., Knippschild, U., and Buchhop, S. p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. EMBO J., *15:* 1992–2002, 1996.
83. Dudenhoffer, C., Kurth, M., Janus, F., Deppert, W., and Wiesmuller, L. Dissociation of the recombination control and the sequence-specific transactivation function of p53. Oncogene, *18:* 5773–5784., 1999.
84. Naviaux, R. K., Costanzi, E., Haas, M., and Verma, I. M. The pCL vector system: rapid production of helper-free, high-titer, recombinant retroviruses. J. Virol., *70:* 5701–5705, 1996.
85. Lambert, S., and Lopez, B. S. Role of RAD51 in sister-chromatid exchanges in mammalian cells. Oncogene, *20:* 6627–6631, 2001.
86. Lin, J., Chen, J., Elenbaas, B., and Levine, A. J. Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. Genes Dev., *8:* 1235–1246, 1994.
87. Sengupta, S., Linke, S. P., Pedeux, R., Yang, Q., Farnsworth, J., Garfield, S. H., Valerie, K., Shay, J. W., Ellis, N. A., Wasylyk, B., and Harris, C. C. BLM helicase-dependent transport of p53 to sites of stalled DNA replication forks modulates homologous recombination. EMBO J., *22:* 1210–1222, 2003.
88. Oberosler, P., Hloch, P., Ramsperger, U., and Stahl, H. p53-catalyzed annealing of complementary single-stranded nucleic acids. EMBO J., *12:* 2389–2396., 1993.
89. Robles, A. I., Linke, S. P., and Harris, C. C. The p53 network in lung carcinogenesis. Oncogene, *21:* 6898–6907, 2002.
90. Legros, Y., Lafon, C., and Soussi, T. Linear antigenic sites defined by the B-cell response to human p53 are localized predominantly in the amino and carboxy-termini of the protein. Oncogene, *9:* 2071–2076, 1994.
91. Essers, J., Houtsmuller, A. B., van Veelen, L., Paulusma, C., Nigg, A. L., Pastink, A., Vermeulen, W., Hoeijmakers, J. H., and Kanaar, R. Nuclear dynamics of RAD52 group homologous recombination proteins in response to DNA damage. EMBO J., *21:* 2030–2037, 2002.
92. Raderschall, E., Bazarov, A., Cao, J., Lurz, R., Smith, A., Mann, W., Ropers, H. H., Sedivy, J. M., Golub, E. I., Fritz, E., and Haaf, T. Formation of higher-order nuclear Rad51 structures is functionally linked to p21 expression and protection from DNA damage-induced apoptosis. J. Cell. Sci., *115:* 153–164, 2002.
93. Willers, H., McCarthy, E. E., Hubbe, P., Dahm-Daphi, J., and Powell, S. N. Homologous recombination in extrachromosomal plasmid substrates is not suppressed by p53. Carcinogenesis (Lond.), *22:* 1757–1763, 2001.
94. Sengupta, S., and Wasylyk, B. Ligand-dependent interaction of the glucocorticoid receptor with p53 enhances their degradation by Hdm2. Genes Dev., *15:* 2367–2380, 2001.
95. Haaf, T., Raderschall, E., Reddy, G., Ward, D. C., Radding, C. M., and Golub, E. I. Sequestration of mammalian Rad51-recombination protein into micronuclei. J. Cell Biol., *144:* 11–20, 1999.
96. Bakalkin, G., Yakovleva, T., Selivanova, G., Magnusson, K. P., Szekely, L., Kiseleva, E., Klein, G., Terenius, L., and Wiman, K. G. p53 binds single-stranded DNA ends and catalyzes DNA renaturation and strand transfer. Proc. Natl. Acad. Sci. USA, *91:* 413–417, 1994.
97. Bakalkin, G., Selivanova, G., Yakovleva, T., Kiseleva, E., Kashuba, E., Magnusson, K. P., Szekely, L., Klein, G., Terenius, L., and Wiman, K. G. p53 binds single-stranded DNA ends through the C-terminal domain and internal DNA segments via the middle domain. Nucleic Acids Res., *23:* 362–369, 1995.
98. Reed, M., Woelker, B., Wang, P., Wang, Y., Anderson, M. E., and Tegtmeyer, P. The C-terminal domain of p53 recognizes DNA damaged by ionizing radiation. Proc. Natl. Acad. Sci. USA, *92:* 9455–9459, 1995.
99. Lee, S., Elenbaas, B., Levine, A., and Griffith, J. p53 and its 14 kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. Cell, *81:* 1013–1020, 1995.
100. Lee, S., Cavallo, L., and Griffith, J. Human p53 binds Holliday junctions strongly and facilitates their cleavage. J. Biol. Chem., *272:* 7532–7539, 1997.
101. Havre, P. A., Rice, M., Ramos, R., and Kmiec, E. B. HsRec2/Rad51L1, a protein influencing cell cycle progression, has protein kinase activity. Exp. Cell Res., *254:* 33–44, 2000.
102. Haber, J. E., and Heyer, W. D. The fuss about Mus81. Cell, *107:* 551–554, 2001.
103. Gottifredi, V., Shieh, S., Taya, Y., and Prives, C. p53 accumulates but is functionally impaired when DNA synthesis is blocked. Proc. Natl. Acad. Sci. USA, *98:* 1036–1041, 2001.