Radiation-induced Assembly of Rad51 and Rad52 Recombination Complex Requires ATM and c-Abl*

(Received for publication, February 24, 1999)

Gang Chen‡‡, Shyng-Shiou F. Yuan‡‡, Wei Liu‡‡, Yang Xu‡‡, Kelly Trujillo‡‡, Binwei Song‡‡, Feng Cong*, Stephen P. Goff**, Yun Wu‡‡, Ralph Arlinghaus‡‡, David Baltimore‡‡, Paul J. Gasser‡‡, Min S. Park§§, Patrick Sung§§, and Eva Y.-H. P. Lee‡‡‡

From the §Department of Molecular Medicine/Institute of Biotechnology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78245; the ¶Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, the ¶¶Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University, New York, New York 10032, the §§Department of Molecular Pathology, MD Anderson Cancer Center, Houston, Texas 77030, and the ¶¶¶Life Sciences Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Cells from individuals with the recessive cancer-prone disorder ataxia telangiectasia (A-T) are hypersensitive to ionizing radiation (I-R). ATM (mutated in A-T) is a protein kinase whose activity is stimulated by I-R. c-Abl, a nonreceptor tyrosine kinase, interacts with ATM and is activated by ATM following I-R. Rad51 is a homologue of bacterial RecA protein required for DNA recombination and repair. Here we demonstrate that there is an I-R-induced Rad51 tyrosine phosphorylation, and this induction is dependent on both ATM and c-Abl. ATM, c-Abl, and Rad51 can be co-immunoprecipitated from cell extracts. Consistent with the physical interaction, c-Abl phosphorylates Rad51 in vitro and in vivo. In assays using purified components, phosphorylation of Rad51 by c-Abl enhances complex formation between Rad51 and Rad52, which cooperates with Rad51 in recombination and repair. After I-R, an increase in association between Rad51 and Rad52 occurs in wild-type cells but not in cells with mutations that compromise ATM or c-Abl. Our data suggest signaling mediated through ATM, and c-Abl is required for the correct post-translational modification of Rad51, which is critical for the assembly of Rad51 repair protein complex following I-R.

Ataxia telangiectasia (A-T) is an autosomal recessive genetic disease characterized by diverse clinical symptoms that include neuronal degeneration, immune deficiency, gonadal abnormalities, cancer predisposition, premature aging, and oculocutaneous telangiectasias (1, 2). Cells from individuals with A-T are hypersensitive to ionizing radiation (I-R) and chemicals that cause DNA double-strand breaks (DSB). Upon I-R, A-T cells exhibit defects in multiple cell cycle checkpoint functions, including an aberrant p53 response and DNA DSB repair (1, 3). The DSB repair defect in A-T cells is subtle, but nevertheless contributes significantly to the radiosensitivity and chromosomal instability phenotype of these cells (4–6). At a time when rejoining of I-R-induced DSB is completed in normal mitotic cells, A-T cells still contain a significant number of DSBs, which may be converted into chromosomal breaks (7, 8). Additionally, the DSB repair process in A-T cells is error-prone (9, 10). A-T cells also possess an elevated level of spontaneous intrachromosomal recombination (11). In meiosis of A-T cells, the synaptonemal complexes fail to form at the pachytene stage, thus aborting gametogenesis (12–14). Little is known about the molecular basis for the meiotic and DNA DSB repair defects in A-T cells.

In eukaryotes, DNA DSB can be repaired by either homologous recombination or nonhomologous end-joining pathways (15). Homologous recombination is achieved through multiple enzyme-catalyzed steps that include processing and resection of the DSB, searching for and pairing of DNA homologs, strand invasion, DNA synthesis, and finally, resolution of recombination intermediates (16, 17). Rad51, a eukaryotic homolog of the bacterial recombinase RecA, is required for recombinational repair of DSB. In in vivo recombination assays using purified components, Rad51 catalyzes strand exchange in an ATP-dependent reaction (16, 17). Interactions between yeast Rad51 and other recombination factors including RPA, Rad52, Rad54, Rad55, and Rad57 have been described (18, 19). Human Rad51 also binds Rad52 (20, 21). Recent biochemical studies have demonstrated that the strand exchange activity of Rad51 is stimulated by Rad52, Rad54, and the Rad55-Rad57 complex, indicating that the protein-protein interactions are functionally significant (22–26). The results of studies with human Rad51 and Rad52 suggest that the mechanisms of DSB repair by homologous recombination are conserved in higher eukaryotes (27).

ATM, the gene mutated in A-T, encodes a 370 kDa protein that is predominantly nuclear (28–31). It contains a phosphatidylinositol kinase domain that is also present in several other proteins known to function in cellular responses to DNA damage, cell cycle control, and telomere maintenance (32, 33). Upon activation by DNA damage, the ATM kinase phosphorylates p53 on serine 15, which results in stabilization of p53 (34, 35). In addition to modifying p53, ATM is also required for the activation of the nonreceptor tyrosine kinase c-Abl (36, 37). ATM binds c-Abl constitutively and may phosphorylate c-Abl on serine 465, leading to enhanced kinase activity of c-Abl (37). Whether defective c-Abl activation affects DSB repair and thus...
contributes to the phenotypes of A-T cells remains to be addressed.

In this study, we tested the hypothesis that the aberrant DNA repair phenotype of A-T cells is due at least in part to defective modulation of protein components of the DNA DSB repair machinery by ATM kinase-mediated signals. We provide evidence that ATM and c-Abl kinase are required for correct post-translational modification and assembly of Rad51 protein complexes.

MATERIALS AND METHODS

Antibodies—The monoclonal anti-ATM antibody 3E8 was made using GST-ATM-LZP fusion protein as the immunogen. Polyclonal and monoclonal antibodies against human Rad51 and Rad52 were produced using HisRad51 and HisRad52 purified from bacteria. The anti-phosphotyrosine antibodies 4G10 and RC20, anti-β-actin antibody, and anti-Myc antibody 9E10 were from Upstate Biotechnologies, Transduction Laboratories, Sigma, and Santa Cruz Biotechnologies, respectively. SE9 and F6D are monoclonal antibodies that recognize human c-Abl.

Purification of Rad51, Rad52, and c-Abl—Human Rad51 and Rad52 were overexpressed in Escherichia coli. Consecutive chromatographic steps involving Q-Sepharose, Ni-NTA-agarose (for histidine-tagged Rad51 only), Affil-blue, hydroxypapitate, and Mono-Q were performed to purify Rad51, and chromatography on Ni-NTA and Mono-S columns was used to purify histidine-tagged Rad52. Myc-tagged c-Abl, overexpressed in COS-7 cells by transient transfection, was purified by affinity chromatography with the 9E10 antibody immobilized on agarose. The Myc epitope peptide was used to compete off the c-Abl bound to the column.

Analysis of Protein Interactions—Cells were lysed in EBC buffer (50 mM Tris, pH 7.6, 120 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA, 1 mM β-mercaptoethanol, 50 mM NaF, and 1 mM Na3VO4) plus protease inhibitors. Immunoprecipitation was performed as described earlier (28), using secondary antibodies cross-linked to magnetic beads (Dynal Inc. Oslo, Norway). To study whether protein complex formation requires DNA, immunoprecipitation was performed in the presence of ethidium bromide (20 μg/ml), and the immunoprecipitates were treated with 100 units of DNase I at 37 °C for 1 h. To examine Rad51 tyrosine phosphorylation, immunoblots containing Rad51 were incubated with anti-phosphotyrosine antibody and then horseradish peroxidase-conjugated secondary antibody. After detection by enhanced chemiluminescence (Amersham Pharmacia Biotech), bound antibodies in the blot were stripped off by incubation in 0.1 M glycine, pH 3.0, for 30 min. Total Rad51 amounts in the blot were determined with anti-Rad51 antibody. In GST pull-down assays, 1 μg of GST or GST fusion proteins that had been absorbed on glutathione beads was incubated with target proteins in EBC buffer for 2 h at 4 °C.

To examine the effects of Rad51 phosphorylation by c-Abl on interaction between Rad51 and Rad52, 1 μg of Rad51 was incubated with affinity purified wild-type or kinase inactive (K290R) c-Abl in kinase buffer (20 mM Tris, pH 7.5, 10 mM MgCl₂, 1 mM MnCl₂, and 1 mM dithiothreitol, 100 μM ATP) at 30 °C for 20 min. After addition of genistein to inactivate the c-Abl kinase, Rad51 in the kinase reactions was subjected to immunoprecipitation with a rabbit polyclonal anti-Rad51 antibody and the anti-phosphotyrosine antibody 4G10. Secondary antibodies conjugated with magnetic beads were used to capture the immune complex. 0.5 μg of Rad52 was then added to the mixture containing free and antibody-bound Rad51 and incubated at 4 °C for 45 min. Rad51 and Rad52 levels in antibody-bound and -unbound fractions were determined by immunoblotting with their respective antibodies.

Mapping the Residues of Rad51 Phosphorylated by c-Abl—HisRad51 cDNA was cloned into pcMV vector for expression in mammalian cells. Specific tyrosine residues of Rad51 were mutated to phenylalanine using Quick-change (Stratagene). 8 μg of pSV40-Myc-c-Abl or K290R-c-Abl, together with 2 μg of pcMV-His Rad51, were used to transfect COS-7 cells in one 10-cm plate. Two days later, the transfected cells were lysed in 1% Triton X-100 in EBC buffer containing 1 mM Na3VO₄ and protease inhibitors. HisRad51 in the cell lysates was absorbed to Ni-NTA resin. Proteins bound to the Ni-NTA resin were eluted with 250 mM imidazole in EBC and then subjected to immunoprecipitation with rabbit anti-Rad51 antibody. Tyrosine phosphorylation of the immunoprecipitated HisRad51 was detected by immunoblotting with the anti-phosphotyrosine antibody RC20.

FIG. 1. a, Rad51 and c-Abl bind the LZP domain of ATM. Left panel, Coomassie Blue staining of purified GST, GST-ATM-LZP, and GST-ATM-N after separation by SDS-PAGE. Molecular weight markers are shown on the left. Right panel, immunoblotting of T24 total cell extracts (lane 1), T24 cellular proteins pulled down by GST (lane 2), GST-ATM-LZP (lane 3), and GST-ATM-N (lane 4) with anti-c-Abl and anti-Rad51 antibodies. b, Co-immunoprecipitation of ATM, c-Abl, and Rad51. Raji cells (5 × 10⁶) were lysed in EBC buffer and subjected to immunoprecipitation (IP) using anti-ATM, anti-c-Abl, or anti-Rad51 antibodies. Proteins in the immunoprecipitates were detected by immunoblotting (IB) using the indicated antibodies.

RESULTS

I-R-induced Rad51 Tyrosine Phosphorylation Is Dependent on ATM and c-Abl—We used glutathione beads with glutathione S-transferase (GST)-ATM fusion proteins as the ligand to bind cellular proteins that may associate with ATM. Rad51 and c-Abl bound to a GST-ATM fusion protein encompassing the leucine zipper and proline-rich region (LZP) of ATM (residues 980–1,437, Fig. 1a, lane 3) but not to GST or a GST-ATM-N (a fusion protein containing residues 249–523 of ATM) (Fig. 1a, lanes 2 and 4). Immunoprecipitation was then carried out to further assess the association between ATM, c-Abl, and Rad51. Each of these three proteins was coimmunoprecipitated in immunoprecipitations with anti-ATM, anti-c-Abl or anti-Rad51 antibodies but not with preimmune IgG (Fig. 1b). Treatment of the immunoprecipitates with DNase I had no effect on the association between ATM, c-Abl, and Rad51, indicating that complex formation among these proteins is probably not mediated by DNA (Fig. 1b). The amount of co-immunoprecipitating proteins did not change significantly after I-R treatment (data not shown).

Previous studies have demonstrated an ATM-dependent ac-
vation of c-Abl tyrosine kinase following I-R (36, 37). Prompted by the physical interaction between Rad51 and c-Abl, we examined if Rad51 is tyrosine phosphorylated before and after I-R. Extracts prepared from T24 cells before and after I-R treatment were immunoprecipitated with anti-Rad51 antibody followed by sequential immunoblotting with anti-phosphotyrosine and anti-Rad51 antibodies. Although the levels of Rad51 did not change, tyrosine phosphorylation of Rad51 increased more than 3-fold following I-R treatment (Fig. 2a). Tyrosine phosphorylation of Rad51 was also examined in embryonic fibroblasts established from wild-type mice, with nullizygous c-Abl mutation (38), and also from ATM knockout mice (14). Induction of Rad51 tyrosine phosphorylation after I-R was seen in wild-type mouse embryonic fibroblasts (MEF), but not in c-Abl−/− or ATM−/− MEF (Fig. 2b). Thus, the induced tyrosine phosphorylation of Rad51 is dependent upon both ATM and c-Abl. An elevated basal level of Rad51 tyrosine phosphorylation was detected in c-Abl−/− MEFs (Fig. 2b). The reason for this result is unknown, but it could be because of the presence of a compensatory mechanism that results in an elevated basal level of c-Abl-like activity in c-Abl−/− MEF, which also leads to increased tyrosine phosphorylation of other c-Abl substrates (37).

Whether Rad51 protein can serve as a direct substrate for c-Abl was tested using purified Rad51, Myc-tagged c-Abl and kinase-inactive c-Abl (39). Rad51 (Fig. 3a) was phosphorylated by active c-Abl but not by the kinase-inactive c-Abl. Immunoblotting with an anti-phosphotyrosine antibody indicates that phosphorylation of Rad51 by c-Abl occurred on tyrosine residues (Fig. 3a). To investigate possible Rad51 phosphorylation by c-Abl in vivo, Rad51 was expressed together with kinase inactive or active c-Abl in COS-7 cells. Co-expression of Rad51 with wild-type c-Abl, but not kinase inactive c-Abl, stimulated Rad51 tyrosine phosphorylation (Fig. 3b). Mutation of Rad51 Tyr315, but not Tyr205, Tyr191, or Tyr54 to phenylalanine abolished Rad51 tyrosine phosphorylation by c-Abl (Fig. 3b). These results strongly suggest that c-Abl phosphorylates Rad51 Tyr315 in vivo. Interestingly, Rad51 Tyr191 is present in a YXXP context, a substrate sequence preferred by c-Abl kinase activity (40), and is a highly conserved residue in Rad51 in all known species of the animal kingdom. To determine whether the elevated tyrosine phosphorylation of Rad51 in c-Abl−/− MEFs is because of phosphorylation on Tyr315, wild-type and Y315F Rad51 was expressed in these cells by transient transfection. The levels of tyrosine phosphorylation of wild-type and Y315F Rad51 were similar (Fig. 3c), suggesting that the majority of Rad51 tyrosine phosphorylation in c-Abl−/− MEF does not occur on Tyr315.

Tyrosine Phosphorylation of Rad51 by c-Abl Enhances the Interaction between Rad51 and Rad52—Genetic and biochemical studies indicate that Rad51 cooperates with Rad52 in recombinational DNA repair (18, 22–24, 27). Whether phosphorylation of Rad51 by c-Abl modulates its interaction with human Rad52 was investigated in binding assays employing purified Rad51 and Rad52 (Fig. 4a). To avoid interference from possible un-phosphorylated Rad51, anti-phosphotyrosine antibody was used to isolate tyrosine phosphorylated Rad51 after kinase reaction with wild-type c-Abl (Fig. 4b). Approximately 4-fold more Rad52 was associated with tyrosine phosphorylated Rad51 than with Rad51 treated with K290R c-Abl (Fig. 4b), indicating that tyrosine phosphorylation of Rad51 by c-Abl enhances its interaction with Rad52 in vitro.

Because I-R induces Rad51 tyrosine phosphorylation in wild-type cells, but not in ATM−/− or c-Abl−/− MEF, we examined the association between Rad51 and Rad52 in cells with these distinct genetic backgrounds before and after I-R. Extracts prepared from mock-treated and irradiated T24, wild-type MEF, as well as c-Abl−/− and ATM−/− MEF were subjected to immunoprecipitation using anti-Rad51 antibody. The level of Rad52 in the Rad51 immunoprecipitates was quantitated by immunoblotting with anti-Rad52 antibody. Significantly more (3-fold) Rad52 was co-precipitated after I-R treatment of T24 than with Rad51 treated with K290R c-Abl (Fig. 4b). These results strongly suggest that c-Abl phosphorylates Rad51 Tyr315 in vivo.
Rad51 Phosphorylation and Complex Assembly

A-A

B

![Figure 4. Effect of Rad51 phosphorylation on its interaction with Rad52.](a) purified Rad52, 3 μg, was stained with Coomassie Blue after SDS-PAGE. A, binding of Rad52 to immobilized Rad51. Rad51, treated with K290R or wild-type c-Abl, was subject to immunoprecipitation by anti-Rad51 and anti-phosphotyrosine antibodies, respectively. Rad52 was then added to the mixture containing free and immunoprecipitated Rad51. The relative levels of Rad51 and Rad52 in the inputs, free, and antibody-immobilized (Bound) fractions were determined by immunoblotting. P, phosphate. Duplicate experiments (Exps. 1 and 2) are shown. The anti-Rad51 antibody did not interfere with the binding of Rad51 to His-Rad52 (data not shown).

![Figure 5. Increased association between Rad51 and Rad52 in vivo following I-R is dependent on ATM and c-Abl.](a) cell lysates prepared from T24, wild-type MEF, ATM−/− MEF, and c-Abl−/− MEF before and 60 min after I-R were subjected to immunoprecipitation (IP) with anti-Rad51 antibody. The immunoprecipitates were then analyzed by immunoblotting (IB) with anti-Rad51 and anti-Rad52 antibodies. The levels of Rad51 and Rad52 in the cell lysates (100 μg) were quantitated by immunoblotting with the indicated antibodies.

induced Rad51 tyrosine phosphorylation. Interestingly, there was an elevated association of Rad51 and Rad52 in c-Abl−/− MEF before I-R treatment (Fig. 5a), perhaps because of the increased basal level of Rad51 tyrosine phosphorylation in these cells.

**DISCUSSION**

Because A-T cells arrested in the G1 phase of the cell cycle are radiosensitive, it appears that defective checkpoint control is not the only cause of radiosensitivity of A-T cells (4–6). Moreover, the aberrant mitotic recombination and elevated chromosomal breaks in A-T cells after I-R suggest that ATM plays a direct role in the repair of DNA DSB (1, 4, 7, 9–11). In this study, we report that ATM and ATM-mediated signaling are required for both Rad51 phosphorylation and enhanced assembly of recombination complexes. Our data support the hypothesis that defective modulation of repair proteins may underlie the DNA repair deficiency in A-T cells.

Genetic and biochemical studies have assigned a central role for Rad51 in homologous recombination. The other recombination factors, Rad52, Rad54, and Rad55-Rad57 stimulate the strand exchange activity of Rad51 (22–27). This stimulation likely requires direct interaction between the recombination proteins because mutant Rad52 lacking the Rad51 binding site failed to stimulate Rad51-mediated strand exchange (23). Our study has identified an I-R-induced phosphorylation of Rad51 on tyrosine residues and a concomitant increase in association between Rad51 and Rad52, which may lead to increased DNA repair proficiency. The enhanced association of Rad51 and Rad52 after I-R is likely because of Rad51 tyrosine phosphorylation mediated by ATM and c-Abl since the enhanced binding was also observed after phosphorylation of Rad51 by c-Abl in vitro. It is not yet known whether phosphorylation of Rad51 affects its binding with other members of the recombination complex.

While this study was in progress, Yuan et al. (41) reported that phosphorylation of yeast and human Rad51 by c-Abl inhibited the binding of Rad51 to DNA. Furthermore, the strand exchange activity of yeast Rad51 also decreased upon treatment with c-Abl (41). The authors concluded that c-Abl might have inhibitory effects on Rad51 activities in vivo. In contrast, our studies of Rad51-Rad52 complex formation in vitro and in vivo suggest that the ATM and c-Abl-mediated signaling is likely to promote repair given the biochemical evidence that Rad51 acts in concert with Rad52 in homologous recombination (22–24, 27). Future biochemical studies are needed to address whether in the presence of Rad52, Rad51 would indeed exhibit higher recombination activity upon phosphorylation by c-Abl.

**Acknowledgments**—Anti-Rad51 antibody used in initial studies was kindly provided by Dr. T. Ogawa at the Osaka University, and the Rad51 expression plasmid was supplied by Drs. W.-H. Lee and P.-L. Chen. We thank Drs. Gopal K. Dasika and Hao-Chi Hsu for helping with site-specific mutagenesis and providing GST-c-Abl protein, respectively. Critical reading by Drs. Alan Tomkinson, Steve Skapek, McGregor Crowley, and John Leppard is greatly appreciated.

**REFERENCES**

1. Lavin, M. F., and Shiloh, Y. (1997) Annu. Rev. Immunol. 15, 177–202
2. Shiloh Y. (1997) Annu. Rev. Genet. 31, 655–662
3. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J., Jr. (1992) Cell 71, 587–597
4. Thacker, J. (1994) Int. J. Radiat. Biol. 66, suppl. 87–96
5. Meyn, M. S. (1993) Cancer Res. 55, 5991–6001
6. Cox, R. (1992) in Atlas Telengiectasias-A Cellular and Molecular Link Between Cancer, Neurofibromatosis, and Immune Deficiency: Bridges (B. A., and Harnden, D., eds.) Wiley Press, Chichester
7. Foray, N., Badic, C., Arlett, C. F., and Malaise, E. P. (1997) Int. J. Radiat. Biol. 71, 449–450
8. Blocher, D., Sigut, D., and Hannan, M. A. (1991) Int. J. Radiat. Biol. 60, 791–802
9. Luo, C. M., Tang, W., Mekael, K. L., DeFrank, J. S., Anne, P. R., and Powell, N. (1996) J. Biol. Chem. 271, 4497–4503
10. Dar, M. E., Winters, T. A., and Jorgensen, T. J. (1997) Mutat. Res. 384, 169–179
11. Meyn, M. S. (1993) Science 260, 1327–1330
12. Barlow C., Liyanage, M., Moens P. B., Tarsoumas N., Nagashima K., Brown K., Rottinghaus, S., Jackson, S. P., Tagle, D., Ried, T., and Wynshaw-Boris, A. (1998) Development 125, 4007–4017
13. Barlow, C., Hirotsune, S., Paylor, R., Liyanage, M., Eckhaus, M., Collins, F., Shiloh, Y., Crawley, J. N., Ried, T., and Wynshaw-Boris, A. (1996) Cell 86, 159–171
14. Xu, X., Ashley, T., Brairned, E. E., Bronson, R. T., Meyn, M. S., and Baltimore, D. (1996) Genes Dev. 10, 2411–2422
15. Kanaar, R., and Hoeijmakers, J. H. (1997) Genes Funct. 1, 165–174
16. Shinozuka, A., and Ogawa, T. (1995) Trends Biochem. Sci. 20, 387–391
17. Baumann, P., and West, S. C. (1998) Trends Biochem. Sci. 23, 247–251
18. Milne, G. T., and Weaver, D. T. (1993) Genes Dev. 7, 1755–1765
19. Hoyne, S. L., Fimnicz, A. B., and Berg, P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6925–6929
20. Shen, Z., Cloud, K. G., Chen, D. J., and Park, M. S. (1996) J. Biol. Chem. 271, 148–152
21. Van Dyke, E., Hajibagheri, N. M. A., Stasiak, A., and West, S. C. (1998) J. Mol. Biol. 284, 1027–1038
22. Sung, P. (1997) J. Biol. Chem. 272, 28194–28197
23. Shinozuka, A., and Ogawa, T. (1998) Nature 391, 404–407
24. New, J. H., Sugiyama, T., Zaitseva, E., and Kowalczykowski, S. C. (1998) Nature 391, 407–410
25. Petukhova, G., Straton, S., and Sung, P. (1998) Nature 393, 91–94
26. Sung, P. (1997) Genetics 111, 1111–1121
27. Benson, F. E., Baumann, P., and West, S. C. (1998) Nature 391, 401–404
28. Chen, G., and Lee, E. Y. (1996) J. Biol. Chem. 271, 33693–33697
29. Lakin, N. D., Weber, P., Stankovic, T., Rottinghaus, S. T., Taylor, A. M., and
Jackson, S. P. (1996) *Oncogene* 13, 2707–2716

30. Brown, K. D., Ziv, Y., Sadanandan, S. N., Chessa, L., Collins, F. S., Shiloh, Y., and Tagle, D. A. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 1840–1845

31. Watters, D., Khanna, K. K., Beamish, H., Birrell, G., Spring, K., Kedar, P., Gatei, M., Stenzel, D., Hobson, K., Kozlov, S., et al. (1997) *Oncogene* 14, 1911–1921

32. Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., Tagle, D. A., Smith, S., Uziel, T., Sfez, S., et al. (1995) *Science* 268, 1749–1753

33. Savitsky, K., Sfez, S., Tagle, D. A., Ziv, Y., Sartiel, A., Collins, F. S., Shiloh, Y., and Rotman, G. (1995) *Hum. Mol. Genet.* 4, 2025–2032

34. Banin, S., Moyal, L., Shieh, S., Taya, Y., Anderson, C. W., Chessa, L., Smerodinsky, N. I., Prives, C., Reiss, Y., Shiloh, Y., and Ziv, Y. (1998) *Science* 281, 1674–1677

35. Canman, C. E., Lim, D. S., Cimprich, K. A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M. B., and Siliciano, J. D. (1998) *Science* 281, 1677–1679

36. Shafman, T., Khanna, K. K., Kedar, P., Spring, K., Kozlov, S., Yen, T., Hobson, K., Gatei, M., Zhang, N., Watters, D., et al. (1997) *Nature* 387, 520–523

37. Baskaran, R., Wood, L. D., Whitaker, L. I., Canman, C. E., Morgan, S. E., Xu, Y., Barlow, C., Baltimore, D., Wynshaw-Boris, A., Kastan, M. B., and Wang, J. Y. (1997) *Nature* 387, 516–519

38. Tybulewicz, V. L., Crawford, C. E., Jackson, P. K., Bronson, R. T., and Mulligan, R. C. (1991) *Cell* 65, 1153–1163

39. Yuan, Z. M., Huang, Y., Whang, Y., Sawyers, C., Weinshelbaum, R., Kharbanda, S., and Kufe, D. (1996) *Science* 273, 3799–3802

40. Andoniou, C. E., Thien, C. B., and Langdon, W. Y. (1996) *Oncogene* 12, 1981–1989

41. Yuan, Z. M., Huang, Y., Ishiko, T., Nakada, S., Utsugisawa, T., Kharbanda, S., Wang, R., Sung, P., Shinohara, A., Weinshelbaum, R., and Kufe, D. (1998) *J. Biol. Chem.* 273, 3799–3802