A Role for the Fanconi Anemia C Protein in Maintaining the DNA Damage-induced G₂ Checkpoint*

Received for publication, June 25, 2004, and in revised form, September 9, 2004
Published, JBC Papers in Press, September 17, 2004, DOI 10.1074/jbc.M407160200

Brian W. Freie‡§, Samantha L. M. Ciccone‡§, Xiaxin Li‡, P. Artur Plett‡, Christie M. Orschell§§, Edward F. Srour‡§, Helmut Hanenberg**, Detlev Schindler‡‡, Suk-Hee Lee§§, and D. Wade Clapp‡§¶¶

From the ‡Herman B. Wells Center for Pediatric Research and §Departments of Microbiology and Immunology, ¶¶Pediatrics, ¶¶¶Medicine, and §§Department of Biochemistry, Indiana University School of Medicine, Indianapolis, Indiana 46202, **Department of Pediatrics, University of Dusseldorf, Dusseldorf, Germany D-40225, and the ¶¶¶Biozentrum der Universität Würzburg, Institut für Humangenetik, Würzburg 97074 Germany

Fanconi anemia (FA) is a complex, heterogeneous genetic disorder composed of at least 11 complementation groups. The FA proteins have recently been found to functionally interact with the cell cycle regulatory proteins ATM and BRCA1; however, the function of the FA proteins in cell cycle control remains incompletely understood. Here we show that the Fanconi anemia complementation group C protein (Fancc) is necessary for proper function of the DNA damage-induced G₂/M checkpoint in vitro and in vivo. Despite apparently normal induction of the G₂/M checkpoint after ionizing radiation, murine and human cells lacking functional Fancc did not maintain the G₂ checkpoint as compared with wild-type cells. The increased rate of mitotic entry seen in Fancc−/− mouse embryo fibroblasts correlated with decreased inhibitory phosphorylation of cdc2 kinase on tyrosine 15. An increased inability to maintain the DNA damage-induced G₂ checkpoint was observed in Fancc−/− /Trp53−/− cells compared with Fancc−/− cells, indicating that Fancc and p53 cooperated to maintain the G₂ checkpoint. In contrast, genetic disruption of both Fancc and Atm did not cooperate in the G₂ checkpoint. These data indicate that Fancc and p53 in separate pathways converge to regulate the G₂ checkpoint. Finally, fibroblasts lacking FANCd2 were found to have a G₂ checkpoint phenotype similar to Fancc-deficient cells, indicating that FANCd2, which is activated by the FA complex, was also required to maintain the G₂ checkpoint. Because a proper checkpoint function is critical for the maintenance of genomic stability and is intricately related to the function and integrity of the DNA repair process, these data have implications in understanding both the function of FA proteins and the mechanism of genomic instability in FA.

Fanconi anemia (FA) is an autosomal recessive disorder characterized by a range of congenital abnormalities, progressive bone marrow failure, and a propensity to develop myeloid leukemia as well as epithelial and other cancers (1, 2). FA cells show a characteristic pattern of hypersensitivity to bifunctional alkylating agents such as mitomycin C or diepoxybutane (3). This hypersensitivity was utilized in cell fusion studies to identify 11 complementation groups (FAA-L). Eight of these gene products (for review, see Refs. 4 and 5) have been cloned. The FA proteins have recently been found to function in G₂ checkpoint regulation, we measured the DNA repair process, these data have implications in understanding both the function of FA proteins and the mechanism of genomic instability in FA.
We report that the G2 checkpoint was induced normally but not maintained in cells lacking functional FANCC (Fancc) after treatment with IR. Immortalized cells lacking FANCD2 exhibited a similar phenotype. The inability of cells to maintain the G2 checkpoint correlated with an inability to maintain tyrosine 15 phosphorylation on cdc2 despite apparently normal induction of the Atm/Chk2 pathway. Utilizing genetic intercrosses, we found that the G2 checkpoint defect in Atm+/−; Fancc−/− cells was similar to Atm−/− cells, whereas Fancc−/−; Trp53−/− cells had a more severe inability to maintain the G2 checkpoint compared with cells that were mutant at Fancc or Trp53 only. These data are consistent with Fancc functioning via a p53-independent mechanism to maintain the G2 checkpoint. These data have implications in understanding both the function of FA proteins and the mechanism of genomic instability in FA.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Primary murine embryo fibroblasts (MEFs) were obtained from day 12–14 mouse embryos derived from breeding of Fancc−/− and Trp53−/− mice that were less than passage 6. Primary human skin fibroblasts were isolated from two FA complementation group C patients and cultured in Dulbecco’s modified Eagle’s/F-12 media containing 10% fetal calf serum supplemented with 2 mM l-glutamine, 100 units/ml penicillin and streptomycin (Invitrogen). MEFs were maintained based on a 3T3 protocol (32). All experiments using MEFs were performed with cells that were less than passage 6. Primary human skin fibroblasts were isolated from two FA complementation group C patients and cultured in Dulbecco’s modified Eagle’s containing 10% fetal calf serum and supplemented with 2 mM l-glutamine, 100 units/ml penicillin and streptomycin. These cells were transduced with a retrovirus expressing FANCC (33) and selected in G418. Both control and FANC2-corrupted immortalized fibroblasts from a FANC2 patient (P2D2 and P2D2/Trp53−/−) were gifts of the Fanconi anemia foundation and were cultured the same as primary skin fibroblasts.

**Mouse Models**—Mice harboring a disruption of the Fancc gene were generously provided by Dr. Manuel Buchwald (34). These mice were backcrossed for 10 generations into the C57BL/6J strain. Mice were genotyped using PCR to detect wild-type and mutant Fancc alleles as described (35). Mice with the disrupted Atm allele have been previously described (36) and were obtained from The Jackson Laboratory (Bar Harbor, ME) in the 129 genetic background. Genotyping for Atm was carried using PCR primers as suggested by the vendor. These mice were crossed with Fancc mutant mice in the C57BL/6J strain to yield mice with a mixed genetic background. F2 embryos were generated by crossing Atm and Fancc double heterozygous animals, and MEFs were isolated from embryos. Experiments comparing the Fancc and Atm genotypes to one another were carried out using MEF lines isolated from a common pregnant female. Mice harboring a disruption of the Trp53 gene have been previously described (37) and were obtained in the C57BL/6J strain from The Jackson Laboratory. Trp53 genotype was determined using a PCR method and primer sequences as provided by The Jackson Laboratory. Fancc and Trp53 compound heterozygous mice were generated by mating Fancc+/− mice with Trp53+/− mice. The F2 generation was produced by crossing the compound heterozygotes derived from the F1 mice.

**Treatment of Cells with IR and Mitotic Index Determination**—For analyzing G2 progression of a defined S-phase population, cells were pulse-labeled with 10 μM bromodeoxyuridine (BrDU). Cells were then treated with ionizing radiation using a GammaCell-40 exactor (Nordion) containing a 137Cs source and cultured for the indicated time period followed by analysis of Brdu incorporation. BrDU incorporation was determined using anti-BrDU FITC antibody (PharMingen) according to the manufacturer’s specifications. Briefly, cells were fixed in 70% ethanol, dehydrated (2 M HCl), and neutralized (0.1 M sodium borate). Cells were stained with anti-BrDU FITC antibody, then resuspended in 50 μg/ml propidium iodide (Sigma) and analyzed on a FACScan flow cytometer (BD Biosciences). For mitotic index determination, cells were cultured on tissue culture slides (Nunc), treated with IR, and incubated for the indicated time. Slides were fixed in 100% methanol containing 2 μg/ml 4,6-diamidino-2-phenylindole (Sigma) and condensed, and mitotic nuclei were visualized and scored by fluorescent microscopy. For analysis of phosphorylated histone H3, MEFs of the appropriate genotypes were treated with 10 Gy of IR, cultured for the indicated time period, then trypsinized and fixed in 70% ethanol. After fixation cells were permeabilized (0.25% Triton X-100 on ice for 15 min), washed, and incubated for 1 h in phosphate-buffered saline containing 0.1% bovine serum albumin and 0.75 μg/ml of phospho-histone H3 antibody (Upstate Biotechnology). After washing, cells were then incubated in secondary antibody (goat anti-mouse FITC, Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min at room temperature. Cells were then incubated in 50 μg/ml propidium iodide and 100 μg/ml RNase A for 30 min and analyzed on a flow cytometer for FITC (FL-1) and propidium iodide (FL-2). For all cells analyzed, the G1 peak was standardized at a fluorescence intensity of 200 in FL-2.

**G2 Checkpoint Analysis in Keratinocytes**—The analysis of the G2 checkpoint was carried out in vivo as previously described (38) by measuring the percent of mitotic cells in keratinocytes in syngeneic, 3–6-month-old age and sex-matched mice. Mice were irradiated with 5 Gy of IR and then allowed to recover for the given time period. Mice were then sacrificed, and skin was fixed in 10% formalin. Processing was performed for phosphohistone H3 antibody (Upstate Biotechnology), used at a 1:500 dilution. Approximately one mitotic event was scored in untreated keratinocytes per high power field.

**S-phase Checkpoint Analysis**—Analysis of the IR-induced S phase checkpoint was carried out according to previously established methods. Fibroblasts were first labeled with [3H]thymidine (0.5 nCi/ml) for 36 h and washed, and cells were then cultured overnight. Cells were irradiated (20 Gy) and pulse-labeled for 15 min with [3H]thymidine at varying time periods after IR. Labeling was terminated (10% 2.3 M citric acid), cells were fixed (10% 2.3M citric acid), and the solution was counted on a scintillation counter for both 14C and H.

**Immunoblotting**—Whole cell protein extracts were obtained from cells in 100 mM Tris pH 7.4, 0.1% SDS, 50 mM NaCl, 1 mM dithiothreitol, and equivalent amounts of protein were electroeluted on 10% SDS-PAGE gels, transferred to polyvinylidene difluoride membranes (Amershamsciences), and detected by Western blotting using the ECL system (Amershamsciences). Antibodies used were cdc2 (C-19) polyclonal (Santa Cruz Biotechnology), Chk2 (clone 7) monoclonal (Upstate Biotechnology), and tyrosine 15 phosphorylated cdc2 (New England Biolabs).

**RESULTS**

**Murine (Fancc) and Human (FANCC) Fibroblasts Have an Impaired IR-induced G2 Checkpoint**—To test the hypothesis that the murine homologue (Fancc) of Fanconi anemia type C (FANCC) functions in G2 checkpoint control, the cell cycle kinetics of a defined population of primary Fancc−/− and Fancc+/+ MEFs were examined after treatment with ionizing radiation. We monitored the progression of a BrdUrd-labeled (S phase) population of cells through G2/M phases of the cell cycle after IR treatment, similar to the methodology used previously (39, 40). Cells were cultured for the indicated times and analyzed for simultaneous detection of BrdUrd and DNA content using flow cytometry (Fig. 1a). The pulsed cells were arrested with primarily 4N DNA content by 8 h after IR treatment in both genotypes. Although Fancc+/+ cells remained arrested in G2/M, the Fancc−/− cells began to exit G2/M, and a significant proportion of cells (30%) escaped from 4N arrest 12–16 h post-IR. The data are summarized in a graph depicting the results from three independently isolated MEF lines (Fig. 1b).

To determine whether the checkpoint abnormality observed in Fancc null MEFs also occurred in human cells, FANCC mutant cutaneous fibroblasts were examined for G2 arrest after IR treatment. Fibroblasts from two FA patients with FANCC mutations were transduced with a retrovirus encoding the FANCC cDNA and neo or a retrovirus containing neo only. FANCC fibroblasts (control and FANC2-corrected) were treated with BrDUrd for 1 h, exposed to 5 Gy of IR, and cultured for up to 48 h (Fig. 1, c–d). In both patients the BrdUrd-labeled cells (both Fancc gene-corrected and FANCC mutant) were essentially arrested with 4N DNA content 8 h after IR. However, although most of the cells expressing recombinant FANCC maintained the arrest at 24 and 48 h post-IR, a much higher percentage of the patient fibroblasts...
expressing only neo exited G₂ and mitosis into the subsequent cell cycle. These data demonstrate that a significant portion of cells lacking functional murine Fancc and human FANCC did not maintain cell cycle arrest at 4n compared with controls expressing functional FANCC.

To further analyze the DNA damage-induced G₂ checkpoint in Fancc+/− MEFs, we determined the mitotic index of murine Fancc+/−/H11001 and Fancc+/−/H11002 cells after treatment with IR. Compared with Fancc+/−/H11001 cells, a significantly increased percentage of Fancc+/−/H11002 cells were observed in mitosis after IR treatment (Fig. 2a). In human fibroblasts a similar reduction in mitotic index was observed after IR in the FANCC mutant cells expressing recombinant FANCC protein, whereas uncorrected FANCC mutant cells demonstrated a significantly higher rate of mitosis after IR treatment (Fig. 2b).

Since biochemical pathways exist for both the induction and maintenance of the G₂ checkpoint (41, 42), we tested whether Fancc is important for initiating or maintaining the G₂ checkpoint. Mitotic index was assessed as a function of time after treatment of Fancc+/−/H11001 and Fancc+/−/H11002 cells with IR by microscopy (Fig. 2c, d–e), which specifically occurs during mitosis (43, 44). Consistent with previous observations by others (42, 45, 46), we observed in Fancc+/−/H11002 cells a marked reduction in mitosis by 1 h after IR treatment that was essentially maintained for a prolonged time course (up to 12 h). Although Fancc+/−/H11001 cells demonstrated normal induction of the G₂ checkpoint, they began to exit G₂ and enter mitosis between 4 and 6 h after IR.
To test whether the G₂ checkpoint was functional in Fancc−/− cells in vivo, we compared the induction and maintenance of G₂ in keratinocytes from irradiated Fancc+/+ and Fancc−/− mice. Histologic sections of skin from the irradiated mice were processed and analyzed by immunohistochemistry for phosphohistone H3 (38). Consistent with previous studies (38), a reduction in the percentage of phosphohistone H3 positive cells was observed in wild-type cells at 6 and 24 h after IR treatment (Fig. 3). In contrast, although a reduction in mitotic cells was observed in Fancc−/− cells 6 h after IR treatment, the percentage of mitotic cells had increased to untreated levels in Fancc−/− skin sections by 24 h after treatment. Overall these data indicate that in primary cells Fancc is dispensable for initiation but required for proper maintenance of the G₂ checkpoint.

Evaluation of cdc2 Phosphorylation in Fancc−/− Cells after IR—A critical regulator of the G₂/M transition is the phosphoprotein cdc2, which is highly regulated during G₂ and M phases (47). Sustained phosphorylation of tyrosine 15, which occurs as a result of DNA damage, prevents entry of cells into mitosis (47, 48). We, therefore, determined whether the inability of Fancc−/− cells to maintain the IR-induced G₂ checkpoint was associated with altered cdc2 phosphorylation (Fig. 4a, top panel). Fancc+/+ and Fancc−/− cells were treated with IR, and phosphorylation of cdc2 was assessed using an antiserum specific to tyrosine 15-phosphorylated cdc2 (Fig. 4a). Induction of tyrosine 15 phosphorylation was observed in both Fancc+/+ and Fancc−/− cells after IR treatment. Interestingly, although tyrosine 15-phosphorylated cdc2 was evident at all time points after IR in Fancc+/+ cells, we observed that cdc2 phosphorylation was decreased significantly by 8 h post-IR in Fancc−/− cells. A good correlation between increased mitotic index and decreased tyrosine 15-phosphorylated cdc2 was observed in both Fancc+/+ and Fancc−/− cells (Fig. 4b). Taken together, these data indicate that the inability of Fancc−/− cells to maintain the IR-induced G₂ checkpoint correlated with decreased tyrosine 15-phosphorylated cdc2.

ATM is an important regulator of the G₂ checkpoint that activates a signaling cascade, resulting in accumulation of tyrosine 15-phosphorylated cdc2 after IR (42, 49, 50). Fancc could function downstream of ATM or in a separate pathway to maintain the G₂ checkpoint. To differentiate these possibilities, we crossed Fancc−/− deficient mice with Atm−/− deficient mice to determine whether null mutations in Fancc and Atm cooperate in loss of G₂ checkpoint control. We measured the mitotic index by phosphohistone H3 analysis in primary MEFs from the F2 progeny of this intercross (Fig. 4c). Compared with Fancc+/+, Atm+/+ MEFs, Fancc+/+, Atm−/− MEFs failed to induce a normal G₂ checkpoint, consistent with previous literature (42, 45), whereas Fancc−/−, Atm+/+ cells induced but did not maintain the G₂ check point. Cells that were Fancc−/−, Atm−/− exhibited a checkpoint defect that was very similar to similar results is shown. Statistical significance was assessed by Student’s t test, d, a representative analysis of cells analyzed for phosphohistone H3 staining. Cells were cultured after treatment with IR, fixed, and stained with antibody and propidium iodide (PI). The stained cells were then subjected to bivariate analysis for phosphohistone H3 in FITC (FL-1) and PI (FL-2) on a flow cytometer. e, P-H3 analysis of cells cultured for various times after IR treatment. Cells were prepared and analyzed as in d and are represented graphically as the mean percent of control phosphohistone H3 ± S.E. of three independently performed experiments. Percent of control untreated cells is represented on the y axis, and time following IR treatment is shown on the x axis. Statistical analysis was carried out using a paired t test. NT, not treated; IR, treated with ionizing radiation.
Fancc and Atm null alleles do not cooperate in the G2 checkpoint. One important target of ATM is the checkpoint kinase CHK2 (49). To test whether Fancc is required for Atm-dependent activation of Chk2, we examined Chk2 activation in Fancc\(+/-\) and Fancc\(-/-\) cells after IR (Fig. 4b). In both Fancc\(-/-\) and Fancc\(+/-\) cells, Chk2 protein was converted to the phosphorylated, slower migrating form after IR treatment (49), indicating normal phosphorylation of Chk2. Taken together, these data suggest that Fancc may be in an Atm-dependent pathway subsequent to Atm and Chk2 activation.

Fancc and Trp53 Cooperate in G2 Checkpoint Control—Previous studies have demonstrated a critical function in G2 checkpoint control for the tumor suppressor p53. Fig. 3. Analysis of mitotic index by phosphohistone H3 determination in Fancc\(+/-\) and Fancc\(-/-\) mice in vivo in keratinocytes. Mice were treated with 5 Gy of IR, then allowed to recover for 6 or 24 h. Mice were then euthanized, and skin sections were prepared for analysis by immunohistochemistry for phosphohistone H3. The data shown are the mean percent of control phosphohistone H3 (±S.E.) of five mice for each experimental group. Statistics were performed using Students' t test.
Fancc measured the G2 checkpoint by mitotic index determination after Fancc and p53 cooperate in maintaining the G2 checkpoint. The G2 checkpoint was induced by ionizing radiation both in fibroblasts and keratinocytes. Collectively, these data indicate that the FA complex substrate FANCD2 is also required for maintaining the G2 checkpoint. Because mouse cells doubly mutant for Fancc and Atm exhibit cooperative defects in maintaining the G2 checkpoint, cooperation was not observed between Fancc and Atm. ATM is an important mediator of Cdc2 activity via the CHK1/CHK2 kinases and Cdc25 phosphatases. Our genetic data indicating that Atm and Fancc do not cooperate would be consistent with Fancc being downstream of Atm in a linear pathway of G2 checkpoint regulation. However, further characterization of the interaction between effectors in the ATM and FA pathway using biochemical means will be required to investigate this possibility. ATM was shown previously to phosphorylate FANC2, suggesting a potential interaction between FANCC and ATM (25). Other studies indicate that ATR interacts with the FA pathway in the response to DNA cross-linking agents (28); hence, ATR could also mediate the FA protein function in the G2 checkpoint. Because mouse cells doubly mutant for Atm and Atr exhibit cooperative defects in maintaining the G2 checkpoint (42), one would expect that an ATR effector would also cooperate with ATM. Because Fancc did not cooperate with Atm, the data are not consistent with a role for Fancc in an ATR-dependent pathway after IR.

Emerging evidence increasingly supports a role for the FA proteins in mediating the dsDNA break response (12, 16, 24, 60). Our data support a model in which, after FA complex activation and FANC2 localization to foci (12), FANC2

**FIG. 5.** Fancc and p53 cooperate in G2 checkpoint function after IR treatment. Mitotic index (y axis) was blindly assessed by chromatin condensation and plotted at a function of time (x axis) for cells of the four genotypes shown. The mean percent of control mitosis (+SE) of a representative experiment is shown. Statistical comparison was performed using Student’s t test.

*Fig. 6. The G2 checkpoint is not maintained following IR in an immortalized cell line lacking functional FANC2. The G2 checkpoint was measured as a function of time after IR treatment by assessing the mitotic index using phosphohistone H3 analysis by flow cytometry as described under “Experimental Procedures.” PD20 cells derived from an FA-D2 patient and PD20 cells corrected with a retrovirus expressing FANC2 were treated with 5 Gy of IR, then fixed after culture for the indicated time period. The data are shown as percent mitotic phosphohistone H3 (y axis) as a function of hours after IR treatment (x axis). The data shown are a mean of three independent experiments. *, p < 0.01.

Role of Fanconi Anemia C in Maintaining the G2 Checkpoint 50991

**FIG. 6.** The G2 checkpoint is not maintained following IR in an immortalized cell line lacking functional FANC2. The G2 checkpoint was measured as a function of time after IR treatment by assessing the mitotic index using phosphohistone H3 analysis by flow cytometry as described under “Experimental Procedures.” PD20 cells derived from an FA-D2 patient and PD20 cells corrected with a retrovirus expressing FANC2 were treated with 5 Gy of IR, then fixed after culture for the indicated time period. The data are shown as percent mitotic phosphohistone H3 (y axis) as a function of hours after IR treatment (x axis). The data shown are a mean of three independent experiments. *, p < 0.01.
Role of Fanconi Anemia C in Maintaining the G2 Checkpoint

3 Similar to the defect shown here in observed a defect in the S phase, but not in the G2 phase.

10 The methodology employed in the current studies allows a more detailed measurement of the cellular capacity to maintain the G2 checkpoint.

15 Although FA cells are hypersensitive to cross-linking agents (3), they exhibit only a modest hypersensitivity to IR (25, 61, 62). DNA cross-linking agents produce bulky adducts that interfere with DNA replication in S phase, and a failure to promptly resolve cross-links (or partially processed cross-links) in FA cells during S phase may account for the characteristic hypersensitivity to these agents (63). This would be consistent with homologous recombination repair-deficient cells such as the Rad51 paralogs, which have a profound hypersensitivity to cross-linking agents (64, 65) and lesser hypersensitivity to IR (64). FA cells exhibited an apparently normal capacity to initiate the G2 checkpoint, as opposed to ATM cells that do not initiate a normal G2 checkpoint and have a profound hypersensitivity to IR (42, 66, 67). Furthermore, IR-induced dsDNA breaks are rapidly rejoined within 1 h in normal cells (for review, see Ref. 68), and there is no detectable defect in rejoicing of breaks in FA cells. We also found that Fanc−/− cells exhibited a normal IR-induced S phase checkpoint (data not shown), consistent with previous findings in FANCC fibroblasts (25) and similar to that observed in the murine model for Fancd−/− (69). Therefore, an S phase checkpoint defect is unlikely to contribute to the G2 phenotype observed in the current study. However, FA proteins are activated by IR (12, 24, 25), and FA-deficient cells exhibit genomic instability as a result of IR (16), indicating an important role in this response.

20 Further studies are required to determine whether Fanc−/− deficient cells and cells from other FA complementation groups have altered fidelity of repair in response to IR, as might be predicted based on an inability to maintain the G2 checkpoint. Importantly, FA cells can survive low dose IR; hence, genomic instability induced by IR could persist in these cells. This could have important clinical implications since IR is utilized in conditioning regimens before bone marrow transplantation of FA patients. In conclusion, the role of FA proteins in maintaining the G2 checkpoint has significant implications for genomic instability and in understanding the function of the FA proteins.

Acknowledgments—We thank Daisy D. Zeng and Jin Yuan for excellent technical assistance. We also thank Janice Walls for secretarial support in preparing the manuscript. The Herman B. Wells Center for Pediatric Research is a Core Center of Excellence in Molecular Hematology (NIDDK, National Institutes of Health Grant P50 DK49218).

REFERENCES

1. Rosenberg, P. S., Greene, M. H., and Alter, B. P. (2003) Blood 101, 822–826
2. Auerbach, A. D., and Allen, H. G. (1991) Cancer Genet. Cytogenet. 51, 1–12
3. Cicone, B., Freie, D. W., and Clapp, unpublished results.
4. Cicone and S.-H. Lee, unpublished results.
Role of Fanconi Anemia C in Maintaining the G2 Checkpoint

50993

T. D., Galloway, D. A., Donehower, L. A., Tainsky, M. A., and Kaufmann, W. K. (1995) Cancer Res. 55, 1763–1773
46. Bunz, F., Dutriaux, A., Lengauer, C., Waldman, T., Zhou, S., Brown, J. P., Sedivy, J. M., Kinzler, K. W., and Vogelstein, B. (1998) Science 282, 1497–1501
47. Merta, A. O., Draetta, G., Beach, D., and Wang, J. Y. (1989) Cell 58, 193–203
48. Norbury, C., Blow, J., and Nurse, P. (1991) EMBO J. 10, 3321–3329
49. Matsuoka, S., Huang, M., and Elledge, S. J. (1998) Science 282, 1893–1897
50. Melchionna, R., Chen, X. B., Blasina, A., and McGowan, C. H. (2000) Nat. Cell Biol. 2, 762–765
51. Flatt, P. M., Tang, L. J., Scatena, C. D., Szak, S. T., and Pietenpol, J. A. (2000) Mol. Cell. Biol. 20, 4210–4223
52. Timmers, C., Taniguchi, T., Hejna, J., Reifsteck, C., Lucas, L., Bruun, D., Thayer, M., Cox, B., Olson, S., D’Andrea, A. D., Moses, R., and Grompe, M. (2001) Mol. Cell 7, 241–248
53. Ando, T., Kawabe, T., Ohara, H., Ducommun, B., Itoh, M., and Okamoto, T. (2001) J. Biol. Chem. 276, 42971–42977
54. Taylor, W. R., Schonthal, A. H., Galante, J., and Stark, G. R. (2001) J. Biol. Chem. 276, 1998–2006
55. Jin, S., Tong, T., Fan, W., Fan, F., Antinore, M. J., Zhu, X., Mazzacurati, L., Li, X., Petrak, K. L., Rajasekaran, B., Wu, M., and Zhan, Q. (2002) Oncogene 21, 8696–8704
56. Winters, Z. E., Ongkeko, W. M., Harris, A. L., and Norbury, C. J. (1998) Oncogene 17, 673–684
57. Lock, R. B., and Ross, W. E. (1990) Cancer Res. 50, 3761–3766
58. Kupper, G. M., Yamashita, T., Naf, D., Suliman, A., Asano, S., and D’Andrea, A. D. (1997) Blood 90, 1047–1054
59. Heinrich, M. C., Silvey, K. V., Stone, S., Zigler, A. J., Griffith, D. J., Mentallo, M., Chai, L., Zhi, Y., and Hoatlin, M. E. (2000) Blood 95, 3970–3977
60. Smith, J., Andrau, J. C., Kallenbach, S., Laquerbe, A., Boyen, N., and Papadopoulo, D. (1998) J. Mol. Biol. 281, 815–825
61. Duckworth-Rysiecki, G., and Taylor, A. M. (1985) Cancer Res. 45, 416–420
62. Yang, Y., Huang, X., De Oca, R. M., Hays, T., Moreau, L., Lu, N., Seed, B., and D’Andrea, A. D. (2001) Blood 98, 3435–3440
63. Rothfuss, A., and Grompe, M. (2004) Mol. Cell. Biol. 24, 123–134
64. Takata, M., Suei, S., Tachiiri, S., Fukushita, T., Sonoda, E., Schild, D., Thompson, L. H., and Carrano, A. V. (1991) Mutat. Res. 254, 143–152
65. Taylor, A. M., Harnden, D. G., Arlett, C. F., Harcourt, S. A., Stevens, S., and Bridges, B. A. (1975) Nature 258, 427–429
66. Beamish, H., Williams, R., Chen, P., and Lavin, M. F. (1996) J. Biol. Chem. 271, 20486–20493
67. Iliakis, G., Wang, H., Perrault, A. R., Boecker, W., Rosidi, B., Windhofer, F., Wu, W., Guan, J., Terzoudi, G., and Pantelias, G. (2004) Cytogenet. Genome Res. 104, 14–20
68. Houghtaling, S., Timmers, C., Noll, M., Finegold, M. J., Jones, S. N., Meyn, M. S., and Grompe, M. (2003) Genes Dev. 17, 2021–2035