The Radioresistance to Killing of A1–5 Cells Derives from Activation of the Chk1 Pathway*

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Checkpoints respond to DNA damage by arresting the cell cycle to provide time for facilitating repair. In mammalian cells, the G2 checkpoint prevents the Cdc25C phosphatase from removing inhibitory phosphate groups from the mitosis-promoting kinase Cdc2. Both Chk1 and Chk2, the checkpoint kinases, can phosphorylate Cdc25C and inactivate its in vitro phosphatase activity. Therefore, both Chk1 and Chk2 are thought to regulate the activation of the G2 checkpoint. Here we report that A1–5, a transformed rat embryo fibroblast cell line, shows much more radioresistance associated with a much stronger G2 arrest response when compared with its counterpart, B4, although A1–5 and B4 cells have a similar capacity for nonhomologous end-joining DNA repair. These phenotypes of A1–5 cells are accompanied by a higher Chk1 expression and a higher phosphorylation of Cdc2. On the other hand, Chk2 expression increases slightly following radiation; however, it has no difference between A1–5 and B4 cells. Caffeine or UCN-01 abolishes the extreme radioresistance with the strong G2 arrest and at the same time reduces the phosphorylation of Cdc2 in A1–5 cells. In addition, Chk1 but not Chk2 antisense oligonucleotide sensitizes A1–5 cells to radiation-induced killing and reduces the G2 arrest of the cells. Taken together these results suggest that the Chk1/Cdc25C/Cdc2 pathway is the major player for the radioresistance with G2 arrest in A1–5 cells.

A1–5 and B4 are two independently isolated cell lines that were derived in the same laboratory from primary rat embryo fibroblasts transformed with activated Ras (T24) and mutant p53val-135 (1). Both A1–5 and B4 cells show the typical phenotypes of the temperature-sensitive p53val-135 (1), but as reported here, only A1–5 cells show extreme radioresistance to killing accompanied by a strong G2 checkpoint response.

G2 checkpoint activation plays an important role in promoting cell survival following DNA damage (2). The mechanism that regulates G2 arrest after DNA damage is conserved among species from yeast to human. The DNA damage checkpoint activated in G2 is believed to be mediated, at least in part, by an inhibition of the Cdc25C phosphatase that activates the Cdc2 kinase by removing inhibitory phosphates, thus allowing entry into mitosis (3, 4). Cdc25C could be phosphorylated in vitro at serine 216 by either Chk1 or Chk2 (5–7). This phosphorylation creates a binding site for the small acidic proteins 14-3-3 (3) that cause the transport of Cdc25C to the cytoplasm and prevent Cdc2 activation. The Chk1 and Chk2 (Chk2 is the homologue of Rad53 in Saccharomyces cerevisiae and Cds1 in Schizosaccharomyces pombe) kinases, two important checkpoint regulators (3, 5, 6, 8–13), were initially cloned in yeast, but homologues were subsequently identified in mammalian cells. Following DNA damage, both Chk1 and Chk2 are activated in human cells; however, it is not clear which pathway, Chk1/Cdc25C/Cdc2 or Chk2/Cdc25C/Cdc2, has a dominant role in G2 arrest. In this study we examine whether Chk1 or Chk2 plays the major role in the strong G2 arrest and the radioresistance to killing of A1–5 cells.

UCN-01 and caffeine are two efficient inhibitors of G2 checkpoint activation (14–20) that act by targeting different proteins. UCN-01, a protein kinase inhibitor, potentiates the cytotoxicity of a variety of anticancer agents, including cisplatin, camptothecin, and ionizing radiation (18–21). Therefore, it is currently undergoing testing in clinical trials for the treatment of human cancer. It is believed that UCN-01 sensitizes cells to DNA damage by abrogating the G2 checkpoint. Although UCN-01 inhibits multiple protein kinases (22), the way UCN-01 abrogates the G2 checkpoint is mainly by inhibiting Chk1 and affecting the Chk1-Cdc25C but not the Chk2-Cdc25C regulatory pathway (23, 24). Caffeine, which sensitizes cells to ionizing radiation and other genotoxic agents by abrogating DNA damage checkpoints, has been shown to be an effective inhibitor of ATM1 and ATR (25). ATM and ATR, both members of the phosphatidylinositol 3-kinase family, are upstream activators of Chk1 and Chk2 after DNA damage (8, 12, 13, 26). Recently it has been reported that in mammalian cells, caffeine abolishes the G2 checkpoint by inhibiting the ATM-Chk2 pathway (27). To determine the effects of Chk1 and Chk2 on G2 checkpoint response and radioresistance to the killing of A1–5 cells, we used these drugs (caffeine and UCN-01) and specific Chk1 or Chk2 antisense oligonucleotides in our experiments. Our results point to the Chk1 pathway as the major player for the extreme radioresistance and the strong G2 checkpoint response in A1–5 cells.

EXPERIMENTAL PROCEDURES

Cell Lines, Chemicals, and Irradiation—The two cell lines, A1–5 and B4 (obtained from Dr. A. Levine) (1), were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% iron-supplemented calf serum (Sigma). All incubations were at 37 °C in an atmosphere of 5% CO2 and 95% air. Caffeine (Sigma) was dissolved in water, and UCN-01 (NSC

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1 The abbreviations used are: ATM, ataxia telangiectasia-mutated; ATR, ATM- and Rad3-related; DSB, double strand breaks; HR, homologous recombination.

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For this purpose, cell pellets were suspended in lysis buffer (20 mM HEPES, pH 8.0, 150 mM NaCl, 1.5 mM EDTA, 0.5 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride in phosphate-buffered saline buffer) and then exposed to 6 Gy of x-rays. Cells were incubated for various times at 37 °C as noted and then collected to prepare whole cell lysates.

Flow Cytometry—For this purpose, either caffeine or UCN-01 for 30 min and then exposed to 6 Gy and returned to 37 °C. At different times thereafter, cells were trypsinized and fixed in 70% ethanol. Cells were stained in the solution (62 μg/ml RNase A, 40 μg/ml propidium iodide, and 0.1% Triton X-100 in phosphate-buffered saline buffer) at room temperature for 1 h. The distribution of cells in the cell cycle was measured in a flow cytometer (Coulter Epics Elite).

Induction and Repair of DNA Double Strand Breaks (DSB)—As described earlier (28), cells were labeled with 0.01 μCi/ml [32P]ATP. Samples were analyzed by 10% SDS-polyacrylamide gel electrophoresis, and the kinase activities were determined by the incorporation of [32P] into the Cdc25C protein using the PhosphorImager. The kinase immunoprecipitate was incubated at 30 °C for 30 min with 10 μg/ml Chk1 or Chk2 antibody in the presence of 10 μM cold thymidine. The kinase immunocomplexes were washed twice with 0.5% Tween 20, 1% Nonidet P-40, 0.5 mM EDTA, 2% N-lauryl sarcosyl, and 0.1 mg/ml proteinase E) for 16–18 h. Then the complexes were washed in a buffer containing 10 mM Tris, pH 8.0, and 0.1 mM EDTA and treated for 1 h with 0.1 mg/ml RNase A in the same buffer. A similar protocol was also employed to determine the induction of DNA DSB expression from checkpoint response.

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Colonies were collected 10–14 h later for the colony-forming assay, the flow cytometry assay (as described above), and Western blot by directly lysing the cell pellet in 1X SDS-polyacrylamide gel loading buffer.

RESULTS

The Radioresistance of A1–5 Cells Can Be Diminished by Caffeine or UCN-01—A1–5 and B4 cells have a similar genetic background (1). Indeed, in the absence of DNA damage, no differences in their phenotypes are apparent. However, after exposure to x-rays, A1–5 cells are extremely radioresistant to killing as compared with B4 cells (Fig. 1). The difference in radioresensitivity is greater than that observed in other transformed rat embryo fibroblast cell lines (29–31).

The radioresistance of A1–5 could be diminished by either 2 mM caffeine or 100 nM UCN-01. After caffeine or UCN-01 treatment, the survival of irradiated A1–5 cells decreased to levels similar to that of B4 cells. The observation that inhibitors of checkpoint activation sensitize A1–5 cells to radiation-induced killing suggests that a strong checkpoint response underlies the radioresistance of these cells.

A Strong G2 Delay in A1–5 Cells Can Be Abolished by Caffeine or UCN-01—The above results prompted experiments investigating the relationship between checkpoint response and cell radiosensitivity to killing in A1–5 cells. As shown in Fig. 2A, there is a large difference in the percentage of G2/M cells between irradiated A1–5 and B4 cells. B4 cells show a modest accumulation in G2 after exposure to 6 Gy, but cells are unable to complete the S phase and divide after ~12 h. However, after exposure to the same dose, A1–5 cells experience a high accumulation in the G2 phase and an unusually long G2 delay. The delay is not completely overcome during the 24-h follow-up period. This response is the strongest we have observed in a repair-proficient cell line. When the cells were treated with either caffeine or UCN-01, the enhanced G2 delay response in cells treated with either caffeine or UCN-01, the enhanced G2 delay response in

FIG. 1. Effects of caffeine or UCN-01 on the survival of A1–5 and B4 cells. 100 A1–5 (filled symbols) or B4 (open symbols) were grown for 2 days in 60-mm dishes and treated for 30 min with 2 mM caffeine (triangles) or with 100 nM UCN-01 (squares) before exposure to the indicated doses of radiation. Circles depict untreated cells. The survival fraction was determined using the clonogenic assay as described under “Experimental Procedures.” Data shown are the averages from four independent experiments.
irradiated A1–5 cells is abolished (Fig. 2B). The percentage of A1–5 cells in the G2/M phase is similar to that of B4 cells, and the cells overcome the arrest and divided after ~12 h.

The Radioresistance of A1–5 Cells Is Not Attributable to Reduced Induction or Increased Rejoining of DNA DSB—DNA DSB are thought to be severe lesions that if unrepaired or misrepaired will lead to cell death (32–34). Therefore, we investigated whether the altered induction or repair of DNA DSB underlies the radioresistance of A1–5 cells. There is no difference in the induction of DNA DSB between A1–5 and B4 cells (Fig. 3A). In addition, the rates of rejoining of DNA DSB are similar in the two cell lines, suggesting a similar capacity for nonhomologous end-joining repair (Fig. 3B).

Ha-Ras or p53 Does Not Directly Mediate the Increased Radioresistance and the Strong G2 Checkpoint Response of A1–5 Cells—We inquired whether the differences in the radiosensitivity between A1–5 and B4 cells derive from differences in the levels of expression of either the human Ha-Ras or the mouse mutant p53val-135. Therefore, we measured the kinetics of expression of these proteins after irradiation. There is no difference in the levels of Ha-Ras or p53 expression between A1–5 and B4 cells. In addition, the radiosensitivity and G2 response of A1–5 cells is not affected by a shift in temperature from 37 °C to 39 °C or to 32 °C (data not shown). Furthermore, neither caffeine nor UCN-01 affects the expression of these proteins (data not shown). Finally, several transformed cell lines generated in our laboratories by transfection with the same genes have a similar radiosensitivity to that of B4 cells (data not shown). These results in aggregate suggest that Ha-Ras and p53 expression are not directly related to the extreme radioresistance with the strong G2 checkpoint response of A1–5 cells. Because Chk1 and Chk2 are considered important regulators of the G2 checkpoint, we investigated next whether they are involved in the strong G2 checkpoint response in A1–5 cells.

High Expression of Chk1 Correlates with High Phosphorylation of Cdc2 in A1–5 Cells—Fig. 4A shows that the constitutive levels of Chk1 were higher in A1–5 than in B4 cells. The difference is much larger after a 6-Gy radiation. G2 arrest after DNA damage is achieved by maintaining inhibitory phosphorylations on Cdc2 through the inactivation of Cdc25C. To further determine whether the higher expression of Chk1 is related to the Cdc25C/Cdc2 pathway, we compared Cdc2 expression as well as its phosphorylation status in A1–5 and B4 cells. Although there is no difference in Cdc2 expression between A1–5 and B4 cells, and there is no change in Cdc2 expression after irradiation (data not shown), there is a difference in the levels of Cdc2 phosphorylation between A1–5 and B4 cells (Fig. 4B). The level of phosphorylated Cdc2 is higher in nonirradiated A1–5 cells (0 h) than in B4 cells, which is consistent with the higher Chk1 expression level and the percentage of cells in G2 (Fig. 2B and Fig. 4, A and B, at 0 h for controls). Also similar to the kinetics of Chk1 expression, the levels of phosphorylated Cdc2 increased in irradiated A1–5 and B4 cells with a stronger effect observed in A1–5 cells (Fig. 4B). Caffeine or UCN-01 clearly reduces Chk1 expression in A1–5

Fig. 2. Effects of caffeine or UCN-01 on the G2 delay of A1–5 and B4 cells. A, as described under “Experimental Procedures,” at various times after a 6-Gy radiation cells were trypsinized, fixed with 70% ethanol, and stained with propidium iodide. The distribution of cells through the cell cycle was measured by flow cytometry, and the fraction of cells in G1, S, and G2/M was determined. The fraction of cells in the G2/M phase is plotted as a function of time after irradiation (A1–5, filled circle; B4, open circle). B, A1–5 (filled symbols) and B4 (open symbols) were treated with 2 mM caffeine (circles) or 100 nM UCN-01 (triangles) for 30 min before and continuously after irradiation until samples were collected for analysis.

Fig. 3. Induction and rejoining of DNA DSB in A1–5 and B4 cells. A, dose-response curves for the induction of DNA DSB. Cells were trypsinized, embedded in agarose blocks, and exposed to various doses of x-rays while kept on ice. The amount of DNA DSB was measured by asymmetric field inversion gel electrophoresis and is expressed as the fraction of activity released (FAR). The results shown are obtained by quantitating gels from three experiments. The mean (A1–5, filled circles; B4, open circles) and S.E. are plotted as a function of time. B, rejoining of DNA DSB as a function of time. Cells were exposed to 30-Gy x-rays and returned to 37 °C. At various times thereafter, cells were trypsinized and prepared for asymmetric field inversion gel electrophoresis. The value of fraction of activity released measured in nonirradiated cells has been subtracted from all data points (A1–5, filled circles; B4, open circles) (28).
cells starting at 6 h after irradiation (Fig. 4A) and correlates with the reduction of Cdc2 phosphorylation, which leads to the similar levels in A1–5 and B4 cells (Fig. 4, A and B). Similar results are also obtained when the kinase activity of Chk1 is evaluated, but quantitation is hampered by the parallel changes in the levels of the protein (data not shown). Thus, a relationship is indicated between Chk1 activation and G2 arrest in A1–5 cells.

**Chk2 Expression and Kinase Activity Are Similar in A1–5 and B4 Cells**—Because Chk2 could also affect the phosphorylations of Cdc2 through the inactivation of Cdc25C, we then examined whether Chk2 also contributes to the phosphorylation of Cdc2 in irradiated A1–5 cells. Fig. 5A shows that there is no apparent difference in the levels of Chk2 expression between A1–5 and B4 cells, although Chk2 expression increased somewhat in both cell lines after irradiation. In addition, neither caffeine nor UCN-01 affects Chk2 expression in A1–5 or B4 cells (Fig. 5A). To determine whether this observation also holds at the level of kinase activity, we measured the ability of Chk2 to phosphorylate Cdc25C in vitro. The results obtained are consistent with those obtained at the level of protein expression and suggest that there is no difference in Chk2 activity between irradiated A1–5 and B4 cells (Fig. 5B). In addition, neither caffeine nor UCN-01 apparently affects the Chk2 kinase activity (Fig. 5B). These data suggest that Chk2 is not directly related to the phosphorylation of Cdc25C in irradiated A1–5 cells.

To confirm that the higher expression of Chk1 is the major reason for the extreme radioresistance with the stronger G2 arrest response in A1–5 cells, we then examined the effects of Chk1 or Chk2 antisense oligonucleotides on the survival and G2 arrest of irradiated A1–5 cells.

**Chk1 but Not Chk2 Antisense Oligonucleotides Can Reduce the G2 Delay and Radiosensitize A1–5 Cells to Killing**—Without irradiation, the Chk1 and Chk2 antisense oligonucleotides show a different toxicity to A1–5 cells. Compared with the nontreated cells, the cells treated with the Chk1 antisense show a remarkable reduction of their number and reach about one-third of the control at 24 h after the treatment (Fig. 6A). On the other hand, Chk2 shows little effect on the cell number (Fig. 6A). We checked whether the antisense oligonucleotides could specifically inhibit Chk1 or Chk2 expression. The Western blot data are shown in Fig. 6B; the Chk1 or Chk2 antisense oligonucleotide specifically reduces the protein expression. Next, as we expected, the anti-Chk1 oligonucleotide radiosensitizes the survival of A1–5 cells down to the levels similar to that observed after UCN-01 treatment (Figs. 1 and 6C). Also, anti-Chk1 oligonucleotide reduces the strong G2 delay response in A1–5 cells (Fig. 6D). The percentage of the Chk1 antisense-treated A1–5 cells in the G2/M phase is much less than that of non-antisense-treated A1–5 cells, and cells overcome the arrest and divided ~4 h after radiation (Fig. 6D). At the same time, the Chk2 antisense oligonucleotide has little effect on A1–5 cell survival and G2 arrest (Fig. 6, B and C). These observations provide the direct evidence that the Chk1 but not the Chk2 pathway plays the major role in the special phenotypes of A1–5 cells.

ATM and ATR are considered upstream modifiers of Chk1. To determine whether the activation of these upstream modifiers causes the activation of the Chk1/Cdc2 pathway, we examined their expression in A1–5 cells. Although the expression of ATM and ATR increases after irradiation, there is no difference in the expression of these proteins between A1–5 and B4 cells (data not shown). This result suggests that the higher expression of Chk1 in A1–5 cells is not directly related to the expression of ATM or ATR.

**DISCUSSION**

The activation of DNA damage checkpoints arrests the normal progression through the cycle and facilitates repair, which in turn increases the survival probability. Although the underlying mechanism of checkpoint response remains unclear in its details, the essential aspects have been elucidated. A1–5 cells with their extreme radioresistance and strong G2 checkpoint response provide a useful model for studying the relationship between radiosensitivity to killing and checkpoint activation.

The results presented here indicate that the radioresistance to the killing of A1–5 cells can be abolished by drugs preventing the activation of the G2 checkpoint and suggest that G2 check-
point activation promotes cell survival. Phosphorylated Cdc2 is in an inactive state but can be activated by Cdc25C-mediated dephosphorylation. Therefore, the inactivation of Cdc25C could lead to an arrest of cells in G2 (7). Chk1 or Chk2 could phosphorylate Cdc25C after DNA damage and induce such inactivation. It is important to establish whether both of them are involved in the strong G2 arrest in A1–5 cells. The use of this information will allow the mechanistic characterization of the A1–5 phenotypes to DNA damage and also further our understanding of the G2 checkpoint. Our results suggest that A1–5 cells with higher levels of Cdc2 phosphorylation are accompanied by a stronger arrest in G2. Drugs that abolish this arrest, such as caffeine and UCN-01, also reduce the levels of Cdc2 phosphorylation. Thus, as expected, the strong G2 arrest in irradiated A1–5 cells is associated with an increase in Cdc2 phosphorylation.

Interesting also is the observation that the increased phosphorylation of Cdc2 correlates with the higher expression of Chk1 in A1–5 cells and that caffeine or UCN-01 not only reduces the levels of Cdc2 phosphorylation but also affects the expression levels of Chk1. It has been reported that Chk1 is phosphorylated and activated by upstream modifiers following DNA damage (5). Our results don’t show any phosphorylation of Chk1 in irradiated A1–5 cells, which might be attributable to the fact that the regular one-dimensional gel is not sensitive enough to observe the phosphorylation of Chk1 (26). On the other hand, the expression levels of Chk2 are not correlated with the changes of Cdc2 phosphorylation. UCN-01 at 100 nM could inhibit Chk1 but has no effect on Chk2 (23, 24). In our experiments, this concentration of UCN-01 abolishes G2 arrest with radioresistance in A1–5 cells, which correlates with the reduction of Cdc2 phosphorylation. These results suggest that Chk1 but not Chk2 is responsible for maintaining Cdc2 phosphorylation in irradiated A1–5 cells.

Caffeine is an inhibitor of checkpoint activation known to sensitize cells to radiation (14, 35–39). However, the mechanism by which caffeine abolishes the G2 checkpoint and causes cell radiosensitization to killing remained unknown until recently. The discovery that caffeine inhibits the kinase activities of ATM and ATR (25) suggested a mechanism for caffeine radiosensitization. Following DNA damage, the activation of Chk2 requires ATM (8, 12, 13), whereas the activation of Chk1 requires ATR (26). Caffeine down-regulates both Chk1 expression and Cdc2 phosphorylation but has only a small effect on Chk2. Also the Chk1 but not the Chk2 antisense oligonucleotide reduces the G2 delay and sensitizes A1–5 cells to killing by irradiation. These results suggest by mainly affecting the Chk1 pathway, caffeine abolishes the strong G2 arrest in irradiated A1–5 cells. Our results differ from those of an earlier study (27), which suggested that caffeine abolishes the G2 checkpoint by inhibiting the ATM/Chk2 pathway. The contrasting conclusions may be attributable to the different cell lines used and the different time frame in which Chk2 activity was measured. However, our results do not exclude the possibility that the

FIG. 6. Effects of Chk1 or Chk2 antisense oligonucleotide on A1–5 cells. A, the effects of the oligonucleotides on the number of A1–5 cells. After the Chk1 antisense (black bars) or the Chk2 antisense (gray bars) treatment, the cells were counted at different times and plotted as the percentages of nontreated control. Data shown are the averages from three independent experiments. B, Western blot data. As described under “Experimental Procedures,” 20 h after antisense oligonucleotide treatment, cells were collected and directly lysed in 1× protein gel loading buffer. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was used as the internal standard. C, survival data. Twelve h after antisense oligonucleotide treatment, the cells were irradiated (6 Gy) and incubated for another 12 h. Then the cells were collected for the clonogenic assay as described under “Experimental Procedures.” Data shown are the averages from three independent experiments. D, G2 delay data. Twelve h after antisense oligonucleotide treatment, the cells were irradiated (6 Gy) and incubated for another 14 h. The histograms represent the distribution of cells through the cell cycle measured by flow cytometry.

FIG. 7. A model for the DNA damage response pathway in mammalian cells. The ATR/Chk1 pathway has a predominant role of G2 arrest, whereas ATM/Chk2 plays a supporting role. The ATR/Chk1 pathway can be interrupted either by caffeine (inhibiting ATR and ATM) or by UCN-01 (inhibiting Chk1).
Chk2 pathway plays a role in maintaining the strong G2 arrest (40) in A1–5 cells. In fact, although Chk1 expression in A1–5 cells is decreased to the level similar to that in B4 cells at 24 h after irradiation (Fig. 4A), at the same time, the percentage of G2 phase in A1–5 cells is still higher than that in B4 cells (Fig. 2A), which might depend upon Chk2 activation.

Because there is no difference in the expression of ATR between A1–5 and B4 cells (data not shown), the higher Chk1 expression may not be directly related to ATR, the upstream regulator of Chk1. It remains unclear why A1–5 and B4 cells, despite their similar genetic background, display such a different DNA damage response. One possibility is that during the process of transformation, Chk1 is modified because it is directly or indirectly targeted by the specific recombination in A1–5 cells. This hypothesis is under investigation in our laboratory now.

As mentioned above, it is thought that checkpoint activation is exploited by the cell to perform DNA repair and thus to reduce cell killing. Although DNA DSB are repaired most efficiently through homologous recombination (HR) in yeast and through nonhomologous end-joining in mammalian cells, some experiments show that both repair pathways are conserved from yeast to humans (41). The results presented here indicate that A1–5 cells show a stronger G2 checkpoint response but no difference in cell death (26) compared with B4 cells (Fig. 2), whereas a supporting role for the ATR/Chk1 functions in addition to the ATM/Chk2 pathway is essential for cell survival. Checkpoint activation and HR repair may be two parallel functions controlled by the specific recombination in A1–5 cells. This hypothesis is under investigation in our laboratory now.

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REFERENCES
1. Martinez, J., Geiger, O., Martinez, J., and Levine, A. J. (1991) Genes Dev. 5, 151–159
2. Elledge, S. J. (1996) Science 274, 1664–1672
3. Peng, C.-Y., Graves, P. R., Thoma, R. S., Wu, Z., Shaw, A. S., and Piwnica-Worms, H. (1997) Science 277, 1501–1506
4. Dalal, S. N., Schweitzer, C. M., Gan, J., and DeCaprio, J. A. (1999) Mol. Cell. Biol. 19, 4465–4479
5. Sanchez, Y., Wang, C., Thoma, R. S., Richman, R. Wu, Z., Piwnica-Worms, H., and Elledge, S. J. (1997) Science 277, 1497–1501
6. Nurse, P. (1997) Cell 91, 865–867
7. Matsuzaki, S., Huang, M., and Elledge, S. J. (1998) Science 282, 1893–1897
8. Behrens, P., Furnari, B., and Russell, P. (1998) Science 277, 1495–1497
9. Zeng, Y., Forbes, K. C., Wu, Z., Moreno, S., Piwnica-Worms, H., and Enoch, T. (1998) Nature 395, 507–510
10. Brondello, J. M., Boddy, M. N., Furnari, B., and Russell, P. (1999) Mol. Cell. Biol. 19, 4262–4269
11. Brown, A. L., Lee, C. H., Schwartz, J. K., Mitiku, N., Piwnica-Worms, H., and Chung, J. H. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3745–3750
12. Chaturvedi, P., Eng, W. K., Zhu, Y., Mattern, M. R., Mishra, R., Hurle, M. R., Zhang, X., Annan, R. S., Lu, Q., Fauceette, L. F., Scott, G. F., Li, X., Carr, S. A., Johnson, R. K., Winkler, J. D., and Zhou, B. B. S. (1999) Oncogene 18, 4047–4054
13. Lau, C. C., and Pardee, A. B. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2942–2946
14. Schlegel, R., and Pardee, A. B. (1986) Science 232, 1264–1266
15. Powell, S. N., DeFrank, J. S., Connell, P., Eogan, M., Preffer, F., Dombovski, D., Tang, W., and Friend, S. (1995) Cancer Res. 55, 1643–1648
16. Yao, S.-L., Akhtar, A. J., McKenna, K. A., Bedi, G. C., Sidransky, D., Mabry, M., Ravi, R., Collector, M. J., Fuchs, S. J., Fuchs, E. J., and Bedi, A. (1996) Nat. Med. 2, 1140–1143
17. Wang, Q., Fan, S., Eastman, A., Wolland, P. J., Saussville, E. A., and O’Connor, P. M. (1986) J. Natl. Cancer Inst. 88, 856–865
18. Bunch, R. T., and Eastman, A. (1996) Clin. Cancer Res. 2, 791–797
19. Bunch, R. T., and Eastman, A. (1997) Cell Growth Differ. 8, 779–788
20. Sha, R.-G., Cao, C.-X., Shimizu, T., O’Connor, P. M., Kohn, K. W., and Rotmier, Y. (1997) Cancer Res. 57, 112–116
21. Kawakami, K., Futami, H., Takahara, J., and Yamaguchi, K. (1996) Biochem. Biophys. Res. Commun. 219, 778–783
22. Ghesquière, P., Yu, L., Luy, J., Gales, J., Sauriau, E. V., O’Connor, P. M., and Piwnica-Worms, H. (2000) J. Biol. Chem. 275, 5600–5605
23. Busby, E. C., Leistritz, D. F., Abraham, R. T., Karnitz, L. M., and Sarkaria, J. N. (2000) Cancer Res. 60, 2108–2112
24. Sarkaria, J. N., Busby, E. C., Tibbetts, R. S., Roos, P., Tata, Y., Karnitz, L. M., and Abraham, R. T. (1999) Cancer Res. 59, 4375–4382
25. Liu, Q., Guntuku, S., Cui, X. S., Matsuzaki, S., Cortez, D., Yamai, K., Luo, G., Carrattini-Rivera, M. L., Losson, K. J., Kruk, J., Donehower, L. A., and Elledge, S. J. (2000) Genes Dev. 14, 1448–1459
26. Zhou, B. B. S., Chaturvedi, P., Springer, K., Scott, S. P., Johansen, R. A., Mishra, R., Matern, M. R., Winkler, J. D., and K汉, K. (2000) J. Biol. Chem. 275, 10342–10348
27. DiBlasi, S., Jeng, Z.-C., Chen, R., Hyslop, T., Curran, W. J., Jr., and Iliakis, G. (2000) Cancer Res. 60, 1245–1253
28. Iliakis, G. (1991) Radiat. Res. 120, 267–279
29. McKenna, W. G., Iliakis, G., Weiss, M. C., Bernhard, E. J., and Muschel, R. J. (1991) Radiat. Res. 125, 283–287
30. Wang, Y., and Iliakis, G. (1991) Cancer Res. 52, 508–514
31. Iliakis, G. (1991) Bioessays 13, 641–648
32. Frankenberger-Schwager, M. (1990) Radiat. Environ. Biophys. 29, 273–292
33. Jeggo, P. A. (1990) Mutat. Res. 239, 1–16
34. Tomaszovics, S. P., and Dewey, W. C. (1978) Mutat. Res. 42, 1–16
35. Kimler, B. F., Leeper, D. B., Snyder, M. H., Rowley, R., and Schneiderman, M. H. (1982) Int. J. Radiat. Biol. 41, 47–58
36. Lucke-Hulhe, C. (1982) Radiat. Res. 89, 298–308
37. Jung, T., and Streffer, C. (1992) Int. J. Radiat. Biol. 62, 161–168
38. Rowley, R. (1992) Radiat. Res. 132, 144–152
39. Hirao, K., Kong, Y. Y., Matsuoka, S., Wakeham, A., Buland, J., Yoshida, H., Liu, D., Elledge, S. J., and Mak, T. W. (2000) Proc. Natl. Acad. Sci. U. S. A. 287, 1824–1827
40. Kanaar, R., and Hoeijmakers, J. H. (1997) Genet. Funct. 1, 165–174
41. Morrison, C., Sonoda, E., Takegawa, K., Aihara, H., and Lee, W.-H. (2000) EMBO J. 19, 463–471
42. Li, S., Ting, N. S. Y., Zheng, L., Chen, P.-L., Xu, Y., Shih, L., Lee, Y.-H. P., and Lee, W.-H. (2000) Nature 406, 210–215
43. Asiedu, N. A., Zeng, Z.-C., Ouan, J., Thacher, J., and Iliakis, G. (2000) Oncogene 19, 5788–5800
44. Zhou, B.-S. B., and Elledge, S. J. (2000) Nature 408, 433–439