E2F Mediates Sustained G2 Arrest and Down-regulation of Stathmin and AIM-1 Expression in Response to Genotoxic Stress*

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Exposure of cells to genotoxic agents results in activation of checkpoint pathways leading to cell cycle arrest. These arrest pathways allow repair of damaged DNA before its replication and segregation, thus preventing accumulation of mutations. The tumor suppressor retinoblastoma (RB) is required for the G1/S checkpoint function. In addition, regulation of the G2 checkpoint by the tumor suppressor p53 is RB-dependent. However, the molecular mechanism underlying the involvement of RB and its related proteins p107 and p130 in the G2 checkpoint is not fully understood. We show here that sustained G2/M arrest induced by the genotoxic agent doxorubicin is E2F-dependent and involves a decrease in expression of two mitotic regulators, Stathmin and AIM-1. Abrogation of E2F function by dominant negative E2F abolishes the doxorubicin-induced down-regulation of Stathmin and AIM-1 and leads to premature exit from G2. Expression of the E7 papilloma virus protein, which dissociates complexes containing E2F and RB family members, also prevents the down-regulation of these mitotic genes and leads to premature exit from G2 after genotoxic stress. Furthermore, genotoxic stress increases the levels of nuclear E2F-4 and p130 as well as their in vivo binding to the Stathmin promoter. Thus, functional complexes containing E2F and RB family members appear to be essential for repressing expression of critical mitotic regulators and maintaining the G2/M checkpoint.

Cell cycle arrest in response to DNA damage is an important mechanism for maintaining genomic integrity. This cell cycle arrest provides time for DNA repair to prevent replication or segregation of damaged DNA. Induction of growth arrest by DNA damage occurs mainly through the activation of checkpoint pathways that delay cell cycle progression at G1, S, and G2 (1, 2).

Growth arrest at both G1 and G2 is believed to occur in two steps, resulting in a rapid arrest that is followed by a more sustained arrest. Initial arrest at G1 involves phosphorylation and degradation of both the protein phosphatase cdc25A and cyclin D1, resulting in inhibition of G1 cyclin-dependent kinases (3–5). Initial arrest at G2 involves phosphorylation and inhibition of the protein phosphatase cdc25C, leading to inhibition of cdc2 activity (6, 7).

Through transactivation of p21, the tumor suppressor p53 is one of the essential mediators of sustained arrest at both G1 and G2 in response to DNA damage (8–11). p21 binds to cyclin-cyclin-dependent kinase complexes and inhibits their ability to phosphorylate the retinoblastoma tumor suppressor, RB, and its related proteins, p107 and p130 (12). Cells deficient of RB fail to arrest at G1 after DNA damage, indicating that RB plays an essential role in this arrest (8, 13). In addition, p53 regulation of DNA damage-induced G2 arrest was shown to be RB-dependent (14).

RB, p107, and p130 play a key role in negative regulation of cell cycle progression, and their growth inhibitory activity is largely attributed to their association with members of the E2F family of transcription factors (15, 16). The E2F family is composed of six members, E2F-1–E2F-6, which heterodimerize with the DP proteins, DP-1 or DP-2, to form the DNA-binding, active transcription factor (15, 16). E2F plays a crucial and well established role in the control of cell cycle progression mainly by up-regulating expression of genes required for the G1/S transition as well as for DNA replication (15, 16).

This transcriptional activity of E2F is inhibited by its interaction with RB, p107, and p130 (15, 16). In addition, the complex containing E2F and RB family members also actively represses transcription. Assembly of such repressive complexes, containing E2F and RB family members (referred to herein as E2F-RB), although they may contain p107 or p130, on promoters that have E2F-binding sites is critical for growth suppression by RB family members (17, 18). The combination of cessation of repression of some E2F-regulated genes by the E2F-RB complex and the activation of others by activated E2F constitutes a major step in promoting G1 exit.

E2F-1, -2, and -3 comprise a subgroup of the E2F family. These E2Fs are specifically regulated by RB and not by the RB-related proteins, p107 and p130. Their release from RB precedes the activation of E2F-responsive genes as well as S-phase entry (19), and their overexpression induces quiescent cells to enter S-phase (20–24). In addition, E2F-1, and possibly also E2F-2 and -3, induce apoptosis (21, 24, 25). E2F-4 and -5 constitute another subgroup of the E2F family. They interact also with p107 and p130 and are implicated mainly in repression of gene expression (26, 27). Unlike E2F-1, -2, and -3, which are constitutively nuclear, E2F-4 and -5 are found in the nucleus only in G1 and early G2, when many of the E2F-regulated genes are repressed (28–31). Furthermore, binding of E2F-4 to promoters is associated with gene repression (26, 32).

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Recent studies indicate that E2F regulates the expression of genes involved not only in cell cycle progression but also in other biological processes (33–36). Many of these novel E2F targets function in various cellular responses to DNA damage, including activation of checkpoints, DNA repair, and apoptosis, thus implicating E2F in the DNA damage response. Further support to this notion comes from recent reports demonstrating that the E2F-1 protein is stabilized and its levels are increased following DNA damage (37–40). This stabilization is due to phosphorylation of E2F-1 by the protein kinase ATM, one of the master controllers of the response to DNA damage (41).

We and others have shown that E2F up-regulates expression of a number of genes involved in entry to and progression through mitosis (33, 35). However, the mode of regulation of these mitotic genes by E2F and the biological consequences of this regulation are not fully understood. We show here that expression of two of these mitotic genes, AIM-1 and Stathmin, is also elevated by transcriptionally inactive E2F-1, indicating that they are subjected to E2F-dependent repression. Furthermore, we show that E2F-containing complexes are required for DNA damage-induced down-regulation of AIM-1 and Stathmin. This repression of gene expression is correlated with E2F-dependent maintenance of DNA damage-induced growth arrest at G2.

EXPERIMENTAL PROCEDURES

Cell Culture—NIH3T3 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% bovine calf serum. Rat-1a-MT-wtE2F-1 and Rat-1a-MT-E2F-1IdITA, which are the Rat 1 cell lines transfected with an inducible plasmid expressing either wild type of a dominant negative mutant of E2F-1, were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and G418 (500 μg/ml). For zinc induction, cells were maintained for 48 h in medium with 0.1% serum, and then 100 μM ZnCl2 were added to the medium.

293 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. All cells were maintained at 37 °C in a humidified 8% CO2-containing atmosphere.

Activation of the E2F-1 fused to estrogen receptor ligand-binding domain (ER-E2F-1) was induced by addition of 4-hydroxytamoxifen to a final concentration of 30 nM. Cycloheximide was added to a final concentration of 10 μg/ml. Doxorubicin was added to a final concentration of 0.2 μg/ml.

Plasmids—The pSV-α-EM-LV packaging plasmid and pBABE-ER-E2F-1 were described previously (24, 42). pBABE-E2F-1IdITA was generated by inserting the E2F-1IdITA XbaI-HindIII fragment from pRsCMV-E2F-1 (1–363) (43) into the pBABE-puro vector.

Retroviral Infection—Cells of the packaging cell line 293 were cotransfected with 10 μg of φ etropic packaging plasmid, pSV-α-EM-LV, and 10 μg of the relevant plasmid using the calcium phosphate method in the presence of chloroquin (55 μM final concentration, Sigma C6628). After 8 h, the transfection medium was replaced with fresh medium, and 5 ml of retroviral-containing cell supernatant was collected at 6-h intervals. Five collections were pooled together and frozen in aliquots. For infection, NIH3T3 cells were incubated for 5 h at 37 °C in 3 ml of retroviral supernatant, supplemented with 8 μg/ml polybrene (Sigma H8926). Then, 7 ml of medium was added, and after 24 h the medium was replaced with fresh medium containing 10% fetal calf serum and 2 μg/ml puromycin (Sigma P7130).

RT-PCR—Reverse transcription-PCR (RT-PCR) was performed on total RNA prepared by the Tri Reagent method. For this assay, 7.5 μg of RNA was used for cDNA synthesis using Moloney murine leukemia virus reverse transcriptase (Promega, 200 u) and oligo(dT) (Amberson Biosciences, 0.5 μg). Following are the number of cycles, annealing temperature, and the sequences of 5′ and 3′ primers used for each of the tested genes, respectively: for the gene encoding AIM-1, 28 cycles, 58 °C using 5′-AGATTGGGGCTCCCTCGGG and 5′-TCTACCTCCTCGGGACG for the gene encoding Stathmin, 25 cycles, 58 °C using 5′-GGTGAAGAACAATGTACCAAGG and 5′-TGTCCTTACCTTTCCCTCG; for the gene encoding ARF-PO, 19 cycles, 58 °C using 5′-TGGGTGGGACAGAATGTTG and 5′-CAGCTGACACAGGC for the gene encoding GAPDH, 20 cycles, 58 °C using 5′-ACCAAGATGCTCATGGCAATC and 5′-TCCACACCACCTCTTGTTG.

Fractionation and Western Blotting—For nuclear and cytoplasmic fractions, cell pellets were resuspended in four packed cell volumes of hypotonic buffer (10 mM HEPS, pH 7.9, 10 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, and 10 μg/ml leupeptin) and incubated for 15 min on ice. Cells were then lysed by adding Nonidet P-40 to a final concentration of 0.6% and vortexing. After a short centrifugation, the cytoplasmic supernatant was taken out, and the nuclear pellet was lysed in two packed cell volumes of lysis buffer (20 mM HEPS, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, and 10 μg/ml leupeptin). Protein concentrations were determined by Bradford assay. The ratio of loaded volumes of cytoplasmic and nuclear extracts from a given cell population was equivalent to the ratio of volumes of hypotonic and lysis buffers and therefore it is considered as the per cell ratio of cytoplasmic and nuclear proteins. For whole cell extracts, cells were lysed in lysis buffer (20 mM HEPS, pH 7.8, 450 mM NaCl, 25% glycerol, 0.2 mM EDTA, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, and 1 μg/ml leupeptin). Equal amounts of protein from each lysate, as determined by Bradford assay, were resolved by electrophoresis through an SDS 7.5–12.5% polyacrylamide gel and transferred to a filter (Protran BA 85, Schleicher & Schuell). Filters were incubated with a primary antibody for 2 h, after 1 h blocking in PBS with 0.05% Tween 20 and 5% dry milk. Primary antibodies used were as follows: anti-E2F-1 (sc-251, Santa Cruz), anti-RB (14001A, PharMingen), anti-p130 (sc-619, Santa Cruz), anti-AIM-1 (BD Biosciences), anti-Stathmin (ST/ATC, gift of Andre Sobel), anti-E2F-4 (sc-866 Santa Cruz), and anti-B23 (sc-6013, Santa Cruz). Binding of the primary antibody was detected using an enhanced chemiluminescence kit (ECL, Amersham Biosciences).

Cell Cycle Flow Cytometry Assays—Cells were trypsinized and fixed with methanol (−20 °C). After fixation, cells were centrifuged for 5 min at 200 g, resuspended in PBS and incubated for 30 min at 4 °C. After recentrifugation cells were resuspended in PBS containing 5 μg/ml propidium iodide and 50 μg/ml RNase A and incubated for 30 min at room temperature. Fluorescence intensity was analyzed using a BD Biosciences flow cytometer.

Chromatin Immunoprecipitation (ChIP)—Approximately 108 cells were cross-linked by addition of formaldehyde directly to the growth medium (final concentration 1%). Cross-linking was stopped after 10 min at room temperature by the addition of glycine (final concentration: 0.125 M). Cross-linked cells were washed with PBS, trypsinized, scraped, washed with PBS, and then resuspended in buffer I (10 mM HEPS, pH 6.5, 10 mM EDTA, 0.5 mM EGTA, and 0.25% Triton X-100). Cells were pelleted by microcentrifugation and then resuspended in buffer II (10 mM HEPS, pH 6.5, 1 mM EDTA, 0.5 mM EGTA, and 200 mM NaCl). After microcentrifugation, nuclei were resuspended in lysis buffer (50 mM Tris, pH 8.1, 10 mM EDTA, 1% SDS, and protease inhibitors). The resulting chromatin was sonicated to an average size of 1000 bp and then microcentrifuged. The supernatant (10 μl with 10 μl lysis buffer) was diluted with 80 μl of dilution buffer (50 mM Tris, pH 8.1, 150 mM NaCl, 2 mM EDTA, and 1% Triton X-100) and divided into aliquots. After preclearing with blocked protein A-Sepharose beads, 1 μg of antibody was added to each aliquot of chromatin and incubated on a rotating platform overnight at 4 °C. Immunocomplexes were recovered with blocked protein A-Sepharose beads. Following extensive washing, bound DNA fragments were eluted and analyzed by subsequent PCR. Antibodies used were as follows: anti-E2F-4 (sc-866, Santa Cruz) and anti-p130 (sc-317, Santa Cruz). Primers used for PCR were for Statlin promoter (forward) 5′-AAAGCTGCCTGTGCTGCAGGC-3′ and (reverse) 5′-CTGGGAGAGAGCATTCTGGG-3′ and for β-actin (forward) 5′-ACTCTTCCAGCCTTCTCTTTCC-3′ and (reverse) 5′-CTCTGTCAGTCTTACG-3′.

RESULTS

Our initial studies aimed at understanding the regulation of mitotic genes by E2F focused on one of these E2F-regulated mitotic genes, Statlin (also known as oncprotein 18), which encodes a protein involved in microtubule dynamics and spindle assembly (44). To determine whether Statlin is a direct target of E2F, we infected NIH3T3 cells with a retrovirus carrying E2F-1 fused to the estrogen receptor ligand-binding domain (ER-E2F-1). The ER-E2F-1 is expressed as an inactive fusion protein, which is activated upon addition of the ligand 4-hydroxymoxifin (24). As was previously reported by Ishida et al. (33) induction of E2F-1 led to an increase in Statlin mRNA levels (Fig. 1A). Interestingly, a similar E2F1-induced increase in Statlin mRNA levels was detected in the pres-
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The decrease in mRNA levels of the studied genes could be detected 24 h after treatment and was most evident at 48 h (Fig. 2C). Thus, the increase in hypophosphorylated RB and p130, which are the growth repressive forms, coincided with or preceded the down-regulation of Stathmin and AIM-1. This result raises the possibility that complexes containing either RB or p130 mediate the repression of Stathmin and AIM-1 in response to DNA damage.

Next we tested whether E2F mediates the decrease in expression of these mitotic genes in response to DNA damage. To this end, NIH3T3 cells were infected with retroviruses containing either E2F-1dITA or an empty vector and then treated with doxorubicin.

As shown earlier for uninfected cells (Fig. 2), in vector-infected NIH3T3 cells, the doxorubicin-induced growth arrest was accompanied by a significant increase in levels of hypophosphorylated RB and p130 (Fig. 3B). In addition, as shown earlier for uninfected cells, treatment of empty vector-infected NIH3T3 cells with doxorubicin led to a decrease in mRNA levels of the E2F-regulated genes, AIM-1 and Stathmin (Fig. 3A). A similar doxorubicin-induced decrease was detected in protein levels of AIM-1 and Stathmin (Fig. 3B). Expression of E2F-1dITA did not significantly affect the doxorubicin-induced accumulation of hypophosphorylated RB and p130; however, it abolished the decrease in mRNA and protein levels of AIM-1 and Stathmin (Fig. 3, A and B). Thus, in cells expressing E2F-1dITA, levels of AIM-1 and Stathmin remained unchanged throughout the experiment.

Following doxorubicin addition, cells infected with an empty vector, similarly to uninfected cells, arrested at the G1 and G2/M phases of the cell cycle. This arrest at G1 and G2/M persisted for at least 96 h (Fig. 3C). Expression of E2F-1dITA did not result in noticeable changes in cell cycle distribution of untreated cells; however, it had profound effects on cell cycle distribution after treatment with doxorubicin. Cells expressing E2F-1dITA failed to arrest at G1 and accumulated at G2/M. Interestingly, growth arrest at G2/M was not maintained, the percentage of cells at G2/M gradually decreased, and a concomitant increase in cells with <2 N and >4 N DNA content was detected (Fig. 3C). These data suggest that while E2F is not essential for the initiation of G2/M arrest, it is required for its maintenance.

To study more directly the role of E2F-RB complexes in the response to DNA damage, we tested the effect of their dissociation by the papilloma virus E7 protein. To this end, NIH3T3 cells were infected with a retrovirus containing either E2F-1 or E7 (data not shown). These findings suggest that endogenous E2F-RB complexes mediate repression of mitotic genes and sustained G2/M arrest in response to DNA damage.

To identify the distinct members of the E2F and RB families that mediate the repression of E2F-regulated genes in response
to DNA damage we performed a ChIP using antibodies directed against specific family members. The human Stathmin promoter contains three putative E2F-binding sites at positions −28, −577, and −701 upstream to the transcription start site (47), and our analysis of the murine Stathmin promoter indicates that it too contains three putative E2F-binding sites. Taken together with our observation that E2F-induced up-regulation of Stathmin does not require de novo protein synthesis (Fig. 1A), this sequence information suggests that E2Fs may interact with the Stathmin promoter. Therefore, we analyzed this promoter for occupancy by E2F and RB family members. We observed a significant enrichment of the Stathmin promoter when using E2F-4 or p130 antibodies (Fig. 5A). We did not detect any enrichment after amplification of an unrelated genomic DNA fragment (Fig. 5A). The enrichment of the Stathmin promoter when using p130 and E2F-4 antibodies was more prominent in cells treated with...
doxorubicin (Fig. 5B), indicating an increase in the occupancy of this promoter by E2F-4 and p130 after genotoxic stress. In agreement with this observation, we detected an increase in levels of E2F-4 and hypophosphorylated p130 in the nuclei of cells treated with doxorubicin (Fig. 5C). Taken together, our data indicate that endogenous E2F-RB complexes down-regulate expression of the mitotic genes AIM-1 and Stathmin in response to DNA damage. Furthermore, our findings strongly suggest that E2F-RB complexes play a role in the maintenance of DNA damage-induced G2/M arrest.

**DISCUSSION**

Recent screens aimed at identifying novel genes regulated by E2F suggest that E2F modulates the expression of a number of genes involved in entry to and progression through mitosis as well as genes that affect the G2/M checkpoint (33, 35, 36). We show here that two pivotal mitotic regulators, Stathmin and AIM-1, are subjected to E2F-mediated negative regulation, and their expression is elevated by a dominant negative mutant of E2F-1 that lacks the transactivation and RB-binding domains.
Stathmin/oncoprotein 18 is a conserved cytoplasmic phosphoprotein that physically interacts with tubulin dimers (reviewed in Ref. 44). It is a critical regulator of microtubule dynamics during cell cycle progression and governs preferential microtubule growth around chromosomes during spindle assembly (48). Stathmin is expressed at elevated levels in various human tumors (49, 50), and it is expressed at higher levels in proliferating cells as compared with non-proliferating cells (51). The Stathmin promoter contains three E2F-binding sites (47), and a recent study indicates that Stathmin levels are regulated by E2F (33). Importantly, antisense inhibition of Stathmin expression results in growth arrest and accumulation of cells in G2/M (52, 53). Conversely, overexpression of Stathmin abrogates irradiation-induced G2/M arrest. Thus, Stathmin appears to have an essential role in the G2/M arrest mechanism (54).

The second E2F-regulated gene studied here, AIM-1, is an Aurora/Ipl1-related serine/threonine kinase that is required for cytokinesis (reviewed in Refs. 45 and 46). Regulation of AIM-1 expression is not well characterized, and its promoter has not been studied extensively. Analysis of genomic sequences upstream to human AIM-1 coding sequence reveals an E2F-binding site at −61 upstream to the putative transcription start site, which is conserved in mouse AIM-1. Furthermore, E2F-4 was shown to interact with the human AIM-1 promoter (36).

AIM-1 expression is cell-cycle-regulated, and its mRNA and protein accumulate at G2/M (55–57). The kinase activity of AIM-1 is also cell-cycle-regulated with peak activity at the M phase (58). The intracellular localization of AIM-1 is consistent with a role in the later stages of mitosis as it is found in the central spindles of anaphase cells and at the midbody of telophase cells (56, 58). Both expression of a kinase-inactive form of AIM-1 and overexpression of wt AIM-1 block cytokinesis and lead to polyploidy, indicating that proper AIM-1 expression and activity is critical for cytokinesis (55, 56).

The results presented here demonstrate that expression of AIM-1 and Stathmin is repressed in response to DNA damage. We show that this repression is abrogated by dominant negative E2F-1 as well as by dissociation of E2F-RB complexes, indicating that such complexes play a critical role in the regulation of AIM-1 as well as by dissociation of E2F-RB complexes, resulting in a sustained arrest at G1 and G2/M. In sharp contrast, doxorubicin treatment of cells expressing either E7 or E2F-1dlTA, the premature exit from G2 after genotoxic stress (14, 59, 61), and with previous studies demonstrating that lack of RB is correlated with increased endoreduplication (60). The data presented here further support a role for endogenous E2F in preventing endoreduplication after DNA damage.

Overall, the data presented here demonstrate that E2F-RB complexes play an important role both in maintaining G2/M arrest and in repression of two mitotic genes after DNA damage. The complete panel of genes that are repressed by E2F-RB complexes and their relative contribution to sustained G2/M arrest remain to be determined. Cdc2 and cyclin B1 are two E2F-regulated genes (32, 33) whose promoter activity was shown to be down-regulated upon sustained G2/M arrest or p53 activation (59, 61, 62). Protein levels of cdc2 and cyclin B1 as well as cyclin B1/cdc2 kinase activity also decrease in association with sustained G2/M arrest after DNA damage (14, 59, 61), and this decrease is abrogated by E7, implicating the RB family in their negative regulation. These data strongly suggest that upon DNA damage, expression of both cyclin B1 and cdc2 is repressed by E2F-RB complexes. However, there is conflicting data regarding the dependence of sustained G2/M arrest on reduction of cyclin B1/cdc2 activity. While one study demonstrated that constitutive activation of cyclin B1/cdc2 kinase activity overrides p53-mediated G2/M arrest (63), others detected prolonged G2/M arrest after DNA damage even in cells with high cyclin B1/cdc2 activity (61). Thus, additional genes repressed by E2F-RB complexes probably play a pivotal role in sustaining G2/M arrest. A number of additional E2F-regulated genes, including cyclin A, thymidine kinase, topoisomerase I, and RAD51 are repressed upon DNA damage (64). We show here that the E2F-dependent decrease in expression of two genes involved in mitosis, AIM-1 and Stathmin, is associated with sustained G2/M arrest after DNA damage. It is tempting to speculate that sustaining G2 arrest after DNA damage involves the concerted, E2F-mediated, repression of a large panel of genes.

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REFERENCES
1. Zhou, B. B., and Elledge, S. J. (2000) Nature 405, 433–439
2. Khanna, K. K., and Jackson, S. P. (2001) Nature 27, 247–254
3. Agami, R., and Bernards, R. (2000) Cell 102, 55–66
4. Costanza, V., Robertson, K., Czurda, C., Y. Kim, R., Avededo, E., Gottesman, M., Grieco, D., and Gauthier, J. (2000) Mol. Cell 6, 649 –659
5. Mailand, N., Falck, J., Lukas, C., Syljuasen, R. G., Welcker, M., Bartek, J., and Lukas, J. (2000) Science 288, 1425–1429
6. Peng, C. Y., Graves, P. R., Thoma, R. S., Wu, Z., Shaw, A. S., and Piwnica-Worms, H. (1997) Science 277, 1501–1505
7. Sanchez, Y., Wong, C., Thoma, R. S., Richman, R., Wu, Z., Piwnica-Worms, H., and Elledge, S. J. (1997) Science 277, 1497–1501
8. Bruganillas, J., Moberg, K., Boyd, S. D., Taya, Y., Jacks, T., and Lees, J. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1002–1007
9. Deng, C., Zhang, P., Harper, J. W., Elledge, S. J., and Leder, P. (1995) Cell 82, 675–684
10. Waldman, T., Kinzler, K. W., and Vogelstein, B. (1995) Cancer Res. 55, 5187–5190
11. Bux, 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
12. Harper, J. W., Adam, G. E., Wu, N., Keyomarsi, K., and Elledge, S. J. (1993) Cell 75, 805–816
13. Harrington, E. A., Bruce, J. L., Harlow, E., and Dyson, N. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11945–11950
14. Platt, P. M., Tang, L. J., Scatena, C. D., Szak, S. T., and Pietenpol, J. A. (2000) Mol. Cell. Biol. 20, 4210–4220
15. Dyson, N. (1999) Genes Dev. 12, 2245–2262
16. Nevins, J. R. (1998) Cell Growth Differ. 9, 585–593
17. Harbour, J. W., and Dean, D. C. (2000) Genes Dev. 14, 2493–2499
18. Zhang, H. S., and Dean, D. C. (2001) Oncogene 20, 3134–3138
19. Moberg, K., Sturz, M. A., and Lees, J. A. (1996) Mol. Cell. Biol. 16, 1436–1449
20. Johnson, D. G., Schwarz, K. J., Cress, W. D., and Nevins, J. R. (1990) Nature 349, 342–349
21. Qin, X. Q., Livingston, D. M., Kaelin, W. G., Jr., and Adams, P. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10918–10922
22. Lukas, J., Petersen, B. O., Holm, R., Bartek, J., and Helin, K. (1996) Mol. Cell. Biol. 16, 1947–1957
23. DeGregori, J., Leone, G., Miron, A., Jakl, L., and Nevins, J. R. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7245–7250
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24. Vigo, E., Muller, H., Prosperini, E., Hateboer, G., Cartwright, P., Moroni, M. C., and Helin, K. (1999) *Mol. Cell. Biol.* 19, 6379–6395
25. Kowalk, T. F., DeGregori, J., Leone, G., Jakoi, L., and Nevins, J. R. (1998) *Cell Growth Diff.* 9, 113–118
26. Takahashi, Y., Rayman, J. B., and Dymalch, B. D. (2000) *Genes Dev.* 14, 804–816
27. Trimarchi, J. M., and Lees, J. A. (2002) *Nat. Rev. Mol. Cell. Biol.* 3, 11–20
28. Magie, J. W., C. L., Illenye, S., Harlow, E., and Reutz, N. H. (1996) *J. Cell Sci.* 109, 1717–1726
29. Lindeman, G. J., Gaubatz, S., Livingston, D. M., and Ginsberg, D. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 5085–5100
30. Muller, H., Moroni, M. C., Vigo, E., Petersen, B. O., Bartek, J., and Helin, K. (1997) *Mol. Cell. Biol.* 17, 5508–5520
31. Verona, R., Moherg, K., Estes, S., Starz, M., Vernon, J. P., and Lees, J. A. (1997) *Mol. Cell. Biol.* 17, 7288–7292
32. Tommasi, S., and Pfeifer, G. P. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 109, 816
33. Ishida, S., Huang, E., Zuzan, H., Spang, R., Leone, G., West, M., and Nevins, J. R. (2001) *Mol. Cell. Biol.* 21, 4684–4699
34. Muller, H., Bracken, A. P., Vernell, R., Moroni, M. C., Christians, F., Grassilli, E., Prosperini, E., Vigo, E., Olner, J. D., and Helin, K. (2001) *Genes Dev.* 15, 287–295
35. Polager, S., Kalma, Y., Berkovich, E., and Ginsberg, D. (2002) *Oncogene* 21, 437–446
36. Reo, B., Cam, H., Takahashi, Y., Volkert, T., Terragni, J., Young, R. A., and Dynalch, B. D. (2002) *Genes Dev.* 16, 245–256
37. Huang, Y., Ishiko, T., Nakada, S., Utsumi, T., Kato, T., and Yuan, Z. M. (1997) *Cancer Res.* 57, 3640–3643
38. Blattner, C., Sparks, A., and Lane, D. (1999) *Mol. Cell. Biol.* 19, 3704–3714
39. Hoferer, M., Wibelauer, C., Humar, B., and Krek, W. (1999) *Nucleic Acids Res.* 27, 491–495
40. O'Connor, D. J., and Lu, X. (1999) *Oncogene* 18, 2369–2376
41. Lin, W. C., Lin, F. T., and Nevins, J. R. (2001) *Genes Dev.* 15, 1833–1844
42. Muller, A. J., Young, J. C., Pendergast, A. M., Pondel, M., Landaz, N. R., Littman, D. B., and Witte, O. N. (1991) *Mol. Cell. Biol.* 11, 1785–1792
43. Hofmann, F., Martelli, F., Livingston, D. M., and Wang, Z. (1996) *Genes Dev.* 10, 2949–2959
44. Cassimieri, L. (2002) *Curr. Opin. Cell Biol.* 14, 23–34
45. Bischoff, J. R., and Plowman, G. D. (1999) *Trends Cell Biol.* 9, 454–459
46. Terasa, Y. (2001) *Cell Struct. Funct.* 26, 103–107
47. Melhem, R. F., Zhu, X. H., Hailat, N., Strahler, J. R., and Hanash, S. M. (1991) *J. Biol. Chem.* 266, 17747–17753
48. Andersen, S. S., Ashford, A. J., Tournebize, R., Gavet, O., Sobel, A., Hyman, A. A., and Karstens, E. (1997) *Nature* 389, 640–643
49. Hanash, S. M., Strahler, J. R., Kuick, R., Chui, E. H., and Nichols, D. (1988) *J. Biol. Chem.* 263, 12813–12815
50. Bischoff, J. R., Lachkar, S., Becette, V., Cifuentes-Diaz, C., Sobel, A., Lidereau, R., and Curmi, P. A. (1998) *Br. J. Cancer* 78, 701–709
51. Rowlands, D. C., Williams, A., Jones, N. A., Guest, S. S., Reynolds, G. M., Barber, P. C., and Brown, G. (1995) *Lab. Invest.* 72, 100–113
52. Luo, X. N., Mookerjee, B., Ferrari, A., Mistry, S., and Atwell, G. F. (1994) *J. Biol. Chem.* 269, 10312–10318
53. Marklund, U., Osterman, O., Melander, H., Bergh, A., and Gullberg, M. (1994) *J. Biol. Chem.* 269, 30626–30635
54. Johnson, J. I., Aurelio, O. N., Kwa, Z., Jergensen, G. E., Pellegata, N. S., Plattner, R., Stanbridge, E. J., and Cajot, J. F. (2000) *Int. J. Cancer* 88, 685–691
55. Tatsuka, M., Katayama, H., Otani, T., Tanaka, T., Odashima, S., Suzuki, F., and Terada, Y. (1998) *Cancer Res.* 58, 4811–4816
56. Terasa, Y., Tatsuka, M., Suzuki, F., Yano, Y., Fujita, S., and Otsu, M. (1998) *EMBO J.* 17, 667–676
57. Kawasaki, A., Tanizawa, M., Miyazawa, J., Enoe, S., Tanaka, H., Terasa, Y., Tatsuka, M., Machi, T., Miyazaki, H., Furukawa, Y., and Kanakura, Y. (2001) *J. Cell Biol.* 152, 275–287
58. Bischoff, J. R., Anderson, L., Zhu, Y., Massie, K., Ng, L., Souza, B., Schryver, B., Flanagan, P., Vassart, B., Ginther, C., Chan, C. S., Novotny, M., Slamon, D. J., and Plowman, G. D. (1998) *EMBO J.* 17, 3052–3065
59. Taylor, W. R., Schneithal, A. H., Galante, J., and Stark, G. R. (2001) *J. Biol. Chem.* 276, 1998–2006
60. Nicolasou, E. B., III, Chen, S., Smeets, M., Hengst, L., Prives, C., and Reed, S. I. (1998) *Mol. Cell. Biol.* 18, 629–643
61. Passalas, T. M., Benanti, J. A., Gewin, L., Kiyono, T., and Galloway, D. A. (1996) *Mol. Cell. Biol.* 16, 5672–5681
62. Manni, I., Mazzaro, G., Gurtner, A., Mantovani, R., Haugwitz, U., Krause, K., Engeland, K., Sasch, A., Soddu, S., and Piaggio, G. (2001) *J. Biol. Chem.* 276, 5570–5576
63. Park, M., Chae, H. D., Yun, J., Jung, M., Lim, Y. S., Kim, S. H., Han, M. H., and Shin, D. Y. (2000) *Cancer Res.* 60, 542–545
64. de Toledo, S. M., Arzam, E. I., Keng, P., Laffrenier, S., and Little, J. B. (1998) *Cell Growth Diff.* 9, 867–896