The extracellular matrix is a crucial component in determining cell fate. Fibrillar collagen in its native form inhibits cell proliferation, whereas in its monomeric form it stimulates proliferation. The observation of elevated levels of p27KIP1 in cells plated in the presence of fibrillar collagen has led to the assumption that this kinase inhibitor was responsible for cell cycle arrest on fibrillar collagen. Here we provide evidence that p15INK4b, rather than p27KIP1, is the cyclin-dependent kinase inhibitor responsible for G0/G1 arrest of human melanoma cells grown on fibrillar collagen. Additionally, we demonstrate that fibrillar collagen can also arrest cells at the G2 phase, which is mediated in part by p21CIP1. Our data, in addition to identifying cyclin-dependent kinase inhibitors important in cell cycle arrest mediated by fibrillar collagen, demonstrate the complexity of cell cycle regulation and indicate that modulating a single cyclin-dependent kinase inhibitor does not disrupt cell proliferation in the presence of fibrillar collagen.

The extracellular matrix (ECM) is a complex network of structural and functional proteins that in addition to providing cell anchorage regulates migration, differentiation, survival, and proliferation (1). During tumor progression significant changes occur in the interactions between tumor cells and the ECM. For example, during early stages of melanoma progression, characterized by a radial growth, melanoma cells are confined to the epidermis and have little interaction with the ECM (2, 3). However, as melanoma progresses toward a vertical growth phase, tumor cells invade the basement membrane and the adjacent dermis and become exposed to many ECM proteins, including type I collagen, the most abundant protein in the body (4, 5). Type I collagen can have either a stimulatory or inhibitory effect on cell proliferation, and this is determined as a function of its native structure. When present in a monomeric fibrillar or denatured form (gelatin), type I collagen acts as a growth stimulatory protein by promoting integrin clustering and activation of focal adhesion kinase (6, 7). However, when present in its native organized fibrillar form, type I collagen has been shown to inhibit the proliferation of a variety of cell types, including vascular and bladder smooth muscle cells (8, 9), endothelial cells (10), and tumor cells (7, 11). Accordingly, loss of the fibrillar structure of type I collagen by oxidation (12) or proteolytic degradation (13) switches its regulatory effect on proliferation from growth restrictive to growth promoting. It has also been shown that the growth inhibitory activity of fibrillar collagen (FC) on tumor cells involves an arrest at the G0/G1 to S phase transition (7, 8).

The mechanism by which fibrillar collagen exerts a growth restrictive activity has not been fully elucidated. Progression through the cell cycle is dependent upon the activity of cyclin-cyclin-dependent kinase complexes, which is regulated by the levels of cyclin-dependent kinase inhibitors (CKIs) (14–16). The observation that cells cultured on FC have elevated levels of p27KIP1 suggested that this elevation was responsible for FC-induced cell cycle arrest (7, 8). Here we have tested whether p27KIP1 was necessary for the inhibitory effect of FC by down-regulating p27KIP1 by small interfering RNA (siRNA) in melanoma cells cultured on FC. Our data revealed that p27KIP1 is not necessary for cell cycle arrest and point to an important role that p15INK4b and p21CIP1 play in the complex control of FC on cell proliferation.

EXPERIMENTAL PROCEDURES

**Antibodies**—Polyclonal antibodies against p19INK4d (M-167), p21CIP1 (C-19), p27KIP1 (C-19), and Skp2 (H-435) were purchased from Santa Cruz Biotechnology. Murine monoclonal antibodies against p15INK4b (Ab-6), p18INK4c (Ab-3), and p57KIP2 (Ab-5) were from Lab Vision Corp. (Fremont, CA), and the anti-β-tubulin antibody (Clone Tub 2.1) was from Sigma.

**Cell Lines**—M24met cells were cultured in RPMI 1640 (Cellgro) supplemented with 10% fetal bovine serum and 2 mM 1-glutamine. Cell images were captured using a CKX41 inverted microscope (Olympus) with a Microfire digital color camera, 4Mpixel (Optronics).

**Preparation of Collagen**—To prepare monomeric-fibrillar collagen (MFC), 0.01 M HCl was used to dilute a skin bovine type I collagen stock (Angiotech Biomaterials) to a final concentration of 1 mg/ml. FC was prepared by neutralizing the...
p15 and p21 Are Critical for FC-induced Cell Cycle Arrest

acidic collagen with 1 m NaOH according to the manufacturer’s instructions to a final concentration of 1.5 mg/ml. Collagen was incubated at 37°C for a minimum of 5 h and rinsed with phosphate-buffered saline (PBS: 137 mM NaCl, 2.6 mM KCl, 10 mM Na₂HPO₄, and 1.7 mM KH₂PO₄, pH 7.4) before cell addition. We confirmed this method generated FC by transmitted electron microscopy that showed the presence of collagen fibers with the characteristic periodic striation.

Cell Collection, Protein Isolation, and Fluorescence-activated Cell Sorting (FACS) Analysis—Subconfluent cells were harvested and plated on the specific collagen matrices and collected after the desired time. Cells on MFC were harvested by trypsin/EDTA dissociation, whereas cells on FC were collected using collagenase A (2.5 mg/ml) (Roche Applied Science). Cell number was determined with a hemacytometer. Following collection, cells were rinsed with PBS, pelleted, and lysed with modified radioimmunoprecipitation buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 10 mM sodium pyrophosphate containing 1.5 mM MgCl₂, 1 mM EGTA, 1% sodium deoxycholate, 0.25 mM Na₃VO₄, 100 mM NaF, 10 mg/ml each of leupeptin and aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Lysis was completed by vortexing three times for 10 s with a 10-min incubation on ice between each vortexing. Lysates were centrifuged (14,000 rpm) and the supernates transferred to a fresh tube. The protein concentration in the lysates was determined using the BCA Protein Assay kit (Pierce) following the manufacturer’s instructions. For FACS analysis, cells were collected, rinsed with PBS, and fixed in 70% ethanol (in PBS) overnight at 4°C. Following fixation, nuclei were incubated for 30 min at 37°C with 20 μg/ml RNase A in PBS, rinsed with PBS, and then resuspended at 3 × 10⁶ nuclei/ml in 40 μg/ml propidium iodide in PBS. Nuclei were then passed through a cell strainer and analyzed on an EPICS Elite ESP cell sorter (Beckman Coulter, Inc.) using Expo 32 (version 1.2) software. The data were expressed as a percentage of total cells excluding apoptotic/necrotic cells, which contributed to an average of 6.5 ± 0.8% of the total of untransfected cells and an average of 14.2 ± 1.3% of transfected cells.

Western Blotting—Proteins (20 μg of total cell lysate) processed by SDS-PAGE were transferred to nitrocellulose membranes. The membranes were blocked for 1 h at room temperature with 5% skim milk powder in washing solution (25 mM Tris-HCl, pH 7.4, 137 mM NaCl, 2.7 mM KCl, and 0.1% Tween 20) and the primary antibodies were incubated at 4°C overnight. A monoclonal anti-β-tubulin antibody was used at a 1:1,000 dilution, and all other primary antibodies were used at 1 μg/ml. Blots were developed using a horseradish peroxidase-coupled secondary antibody at a 1:10,000 dilution for 1 h at room temperature and Enhanced Chemiluminescence (Amersham Biosciences). Blots were quantified using Labworks Imaging and Analysis Software (UVP, Upland, CA).

siRNA—The following siRNA sequences were used: p15, 5’-AATCAGTGCAAGCGCTAGA-3’ (NM_004936, base pairs 1833–1853); p21, 5’-AACATACTGGCCTGGACTGTT-3’ (NM_000389, base pairs 1941–1961); p27, 5’-AATGATCTGAAACGCCTAGA-3’ (NM_000389, base pairs 1941–1961); p21, 5’-AACATACTGGCCTGGACTGTT-3’ (NM_000389, base pairs 1941–1961); p27, 5’-AATGATCTGAAACGCCTAGA-3’ (NM_000389, base pairs 1941–1961). Cells were cultured in T150 culture flasks (Corning) in RPMI 1640, containing 10% fetal calf serum, without antibiotics until 80% confluence was reached. The cells were rinsed twice with PBS, and siRNA was added for 5 h at 37°C. The medium was then replaced with fresh RPMI 1640 containing 10% fetal calf serum, and the cells were incubated for 72 h before being used in specific experiments. For siRNA transfection we prepared the following reagents: tube 1 containing 61 μl of siRNA (20 μM stock; Qiagen) and 2.2 ml of RPMI 1640, and tube 2 containing 90 μl of Lipofectamine 2000 (Invitrogen) and 2.16 ml of RPMI 1640. Dual siRNA reactions had equal amounts of siRNA. Tubes were incubated for 5 min at room temperature and then combined and incubated for a further 20 min. Next, 13.5 ml of RPMI 1640 was added and the siRNA mix was added to the cells. Final siRNA concentration was 68 nM (or 136 nM for dual). Transfection efficiency was assessed using fluorescein isothiocyanate-labeled siRNA and viewing cells after 5 h with a Leica MZ FL III fluorescence stereomicroscope and 75W Xenon arc lamp and triple pass filter set. In all experiments, the transfection efficiency was 90 ± 2.6%.

RNA Extraction and Reverse Transcription Polymerase Chain Reaction—RNA was isolated from cell pellets using Trizol (Invitrogen) following the manufacturer’s instructions. RNA concentration and purity were determined by measuring absorbance at 260 and 280 nm, and the integrity was checked by agarose gel electrophoresis and staining with ethidium bromide. For each 20-μl reaction, 500 ng of total RNA was added in 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, with 500 μM each dNTP, 10 mM dithiothreitol, 50 units of RNase inhibitor, 500 ng of N9 random oligonucleotide, and 200 units of M-MLV-RT (Invitrogen). Reaction was performed according to the manufacturer’s instructions. A total of 2.5 μl of the reverse transcriptase reaction mix was added to 25 μl of polymerase chain reaction (PCR) buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 200 μM each dNTP, 1.5 mM MgCl₂, 1.25 units of Taq polymerase, and 500 pm forward and reverse primers (Invitrogen). PCR cycle conditions for primers were 1 cycle at 94°C for 1 min, 35 cycles of 95°C for 30 s, 51°C for 30 s, 72°C for 30 s, and then 72°C for 10 min. Glyceraldehyde-3-phosphate dehydrogenase primers were added after 5 cycles at 500 pm/reaction. PCR products were analyzed by agarose gel electrophoresis and ethidium bromide staining. Primer sequences are available upon request.

Statistical Analysis—Data were considered to have a parametric distribution. Therefore, a Student’s t-test (two-tail) assuming equal variance was used, with p < 0.05 being considered significant.

RESULTS

Down-regulation of p27KIP1 Does Not Prevent FC-induced Cell Cycle Arrest—We first examined the levels of p27KIP1 in M24met cells plated on FC over 48 h. Consistent with our previous report (7) we observed an increase in p27KIP1 protein levels at 12 h that was maintained at least up to 48 h (Fig. 1A). To determine whether p27KIP1 was necessary for the growth inhibitory effect of FC, we used transient transfection with a p27KIP1 siRNA to specifically down-regulate p27KIP1. Transfection of M24met cells with a p27KIP1 siRNA completely suppressed
p27KIP1 expression for at least 72 h after transfection (Fig. 1B).

A cell cycle analysis performed 72 h after transfection, on cells plated for 24 h on FC, did not indicate a difference in the distribution of the phases of the cell cycle between cells transfected with the p27KIP1 siRNA, scrambled siRNA, or Lipofectamine alone (Fig. 1C). Additionally, no difference was detected in cell number between cells transfected with p27KIP1 siRNA or scrambled siRNA when cultured on FC (Fig. 1D). The data thus suggest that, although elevated in the presence of FC, p27KIP1 is not a necessary regulator of cell cycle progression in melanoma cells plated in contact with FC.

**Down-regulation of Skp2 Does Not Affect M24met Proliferation on MFC**—The levels of p27KIP1 in cells are controlled by proteasome degradation following p27KIP1 polyubiquitinylation via the Skp1-L-Cullin-F-box protein (SCF) complex (17, 18). The F-box protein that provides the SCF complex specificity for p27KIP1 is Skp2. Consistent with Skp2 being a regulator of p27KIP1 expression in M24met cells, we observed a corresponding down-regulation of Skp2 in M24met cells plated on FC as the levels of p27KIP1 increased (Fig. 2A). Down-regulation of Skp2 by siRNA in these cells (Fig. 2B), however, did not affect their cell cycle distribution in the presence of MFC (Fig. 2C) and did not inhibit the proliferation of M24met cells on MFC (Fig. 2D). This lack of effect occurred even though down-regulation of Skp2 resulted in elevated levels of p27KIP1 on MFC (Fig. 2E). These data provide a second line of evidence that p27KIP1 is not responsible for the growth regulatory effect of collagen.

**p15INK4b Levels Are Up-regulated in M24met Cells in the Presence of FC**—In the absence of evidence supporting a causal role for p27KIP1, we examined the levels of other CKI in M24met cells plated on FC. This analysis indicated an increase in p15INK4b mRNA (Fig. 3A) and protein (Fig. 3B) 24 h after contact with FC. There were, however, no changes in the expression of p18INK4b, p19INK4b, p21CIP1, and p57KIP2, and p16INK4a was not expressed. Thus the data suggested that p15INK4b could be responsible for the G0/G1-S growth arrest observed in the presence of FC.

**Loss of p15INK4b Allows Cells to Pass the G0/G1-S Checkpoint**—To test this possibility, we transfected M24met cells with p15INK4b-specific siRNA and demonstrated a complete inhibition of p15INK4b expression when compared with a scrambled siRNA sequence for at least 72 h after transfection (Fig. 4A). Interestingly, down-regulation of p15INK4b in these cells prevented the G0/G1 arrest on FC (55.8 ± 0.6% of cells transfected with p15INK4b siRNA were in G0/G1 compared with 84.1 ± 1.3% of cells transfected with scrambled siRNA, p = 0.0001) (Fig. 4B). However, down-regulation of p15INK4b increased the percentage of cells in G2/M from 3.1% to 38.2% when compared with cells transfected with a scrambled siRNA (p = 0.00002). Consistent with this arrest at G0, down-regulation of p15INK4b in M24met cells did not result in an increase in cell cycle arrest.

**Additional samples not required for this figure were loaded between the lanes of interest and therefore have been removed by cropping, as indicated by the spaces between lanes.**
proliferation (Fig. 4). The data thus suggest that p15INK4b, although necessary for the cell cycle arrest at G0/G1-S by FC, is not the sole factor inhibiting cell proliferation on FC. Because it has previously been suggested that the action of FC in inducing a G0/G1 arrest is linked to the ability of FC to prevent cell spreading (13, 19), we examined cell morphology in response to p15INK4b siRNA treatment and escape from G0/G1 arrest. Down-regulation of p15INK4b had no effect on cell morphology when cultured on FC (Fig. 3D), thus supporting our previous published work indicating that FC-induced cell cycle arrest is independent of cell morphology (11).

Role of p21CIP1—Cyclin-dependent kinase inhibitors of the CIP and KIP family, in particular p21CIP1 and p27KIP1, are known regulators of cell cycle progression beyond the G1 checkpoint (15, 20) and at the G2 arrest (21). To test whether these inhibitors could be involved in the G2 arrest upon down-regulation of p15INK4b in M24met cells, we examined their expression in M24met cells transfected with p15INK4b siRNA. Although the level of p27KIP1 remained unchanged, we observed an increase in the levels of p21CIP1 upon down-regulation of p15INK4b (Fig. 5A). To determine the significance of this increase in p21CIP1 expression we measured the effect of simultaneously down-regulating p15INK4b and p21CIP1 on cell cycle progression in M24met cells plated on FC, using transfection with siRNA (Fig. 5B). This experiment indicated that upon down-regulation of p15INK4b and p21CIP1, the percentage of cells in G2 decreased from 38.17 ± 0.66% (p15INK4b siRNA alone) to 23.1 ± 2.7% (Fig. 5C). At the same time, the percent of cells in G1 increased from 55.77 ± 1.1 (p15INK4b siRNA alone) to 67.6 ± 2.7% and in S phase from 6.07 ± 0.5 (p15INK4b siRNA alone) to 9.3 ± 2.5% (Fig. 5C). Although the S phase increase is small, it represents a 50% increase when compared with cells in which only p15 was down-regulated. Consistent with this increase in cells in S phase, we found that dual suppression of
p15INK4b and p21CIP1 expression in M24met cells plated on FC stimulated proliferation by 146 ± 10% at 24 h ($p = 0.006$) and by 177 ± 11% at 72 h ($p = 0.009$) compared with 97 ± 6 and 106 ± 7% with scrambled siRNAs, respectively (Fig. 5D). This level of stimulation was, however, lower than when cells were plated on MFC (404 ± 21% after 72 h; $p = 0.0003$, data not shown). Changes in cell cycle and proliferation in the presence of FC were not associated with changes in morphology, suggesting a mechanism independent of cell spreading (Fig. 5E). p21CIP1 siRNA alone had a minimal effect on cell cycle distribution and did not significantly increase cell proliferation (data not shown), indicating that it is the cooperation between p15INK4b and p21CIP1 that is responsible for the cell cycle arrest observed when M24met cells are plated in the presence of FC.

DISCUSSION

Although able to proliferate in the absence of exogenous signals, cancer cells nevertheless remain sensitive to external stimuli. Those include growth factors, cell-cell contact, and cell-ECM contact (22–24). Contact between malignant and non-malignant cells and proteins of the ECM has generally a proliferative effect on cells as they spread and integrins become activated (25, 26). Type I collagen is unique as it has been shown to stimulate or inhibit cell proliferation as a function of its structural state. When organized in a cross-linked multimeric structure, collagen inhibits the proliferation of normal cells as well as malignant cells (7–11). In contrast, in the absence of this multimeric organization or when denatured by heat or proteolytic degradation, type I collagen promotes cell spreading, integrin binding, and clustering, activation of focal adhesions, and cell proliferation (7, 13). It has been proposed that p27KIP1 plays a critical role in mediating the growth inhibitory effect of FC, because elevated levels of p27KIP1 have been typically associated with cell cycle arrest when cells are grown in the presence of FC (8). We had previously reported elevated levels of p27KIP1 associated with a decrease in cyclin E-cyclin-dependent kinase activity and G0/G1 cell cycle arrest in M24met and two other human cancer cell lines, A2058 melanoma and HT1080 fibrosarcoma cells plated on FC (7, 11). However, in the absence of examination of the effect of down-regulation of p27KIP1, it was impossible to determine the causal role of p27KIP1. Here we provide evidence indicating that p27KIP1 is not necessary for growth arrest when M24met cells are plated in contact with FC by demonstrating an absence of effect of p27KIP1 down-regulation on cell cycle distribution and proliferation of M24met cells in the presence of FC. We also demonstrate an absence of effect of Skp2 down-regulation on the proliferation of melanoma cells on MFC despite an anticipated up-regulation of p27KIP1, further supporting the concept that contact with collagen does not affect cell proliferation by altering the p27KIP1-Skp2 balance.

However, our data point to a role for p15INK4b. Changes in p15INK4b have been primarily involved in modulating the growth of cells in response to growth factors, in particular transforming growth factor-β (27, 28). Our data demonstrate for the first time that p15INK4b is also responsive to changes in cell-ECM contact and is necessary for the increase in G0/G1 observed in the presence of FC. However down-regulation of p15INK4b alone failed to increase cell proliferation on FC. We documented that this is due to an unanticipated additional arrest at $G_{S}$/M, mediated by p21CIP1. p21CIP1 has been shown to induce a $G_{S}$/M arrest in a variety of cancer cells, including gastric, osteosarcoma, and lung cancer, and to be responsible for the antiproliferative and pro-apoptotic effect of many anticancer agents, like histone deacetylase inhibitors, farnesyl transferase inhibitors, or cyclooxygenase inhibitors (29–33).

In contrast to p15INK4b, it has been previously shown that p21CIP1 is regulated by contact between cell and the ECM. Elevated levels of p21CIP1 are associated with the growth inhibition of hepatocytes cultured of Engelbreth-Holm-Swarm gels (34) and on hepatic stellate cells grown on a collagenase-resistant form of collagen I (35). p21CIP1 can also be down-regulated by the ECM via β-1 integrin-mediated activation of phosphatidylinositol 3-kinase, providing a mechanism for the ECM to override DNA damage-induced cell cycle arrest (36) or by cell contact with fibronectin (37). Here we provide for the first time evidence that, in conjunction with p15INK4b, p21CIP1 is actively responsible for the cell cycle arrest observed when tumor cells are grown in contact with FC. It should be noted that upon dual down-regulation of p15INK4b and p21CIP1, an increasing number of melanoma cells move from G0 to S phase but that this change in cell cycle distribution does not allow the cells to reach a proliferative rate similar to the one observed when cells are plated in the presence of MFC. They also fail to spread. This is consistent with our proposed model (11) in which contact between cells and FC provides a growth inhibitory signal to cells that is associated with an absence of cell spreading, whereas contact between cells and MFC not only removes the growth inhibitory signal but also provides a growth-stimulating signal associated with cell spreading (Fig. 6).

We have previously reported that the collagen receptor dis-coidin domain receptor 2 is involved in the control of FC on cell proliferation, as we demonstrated that down-regulation of DDR2 in tumor cells allows them to proliferate in the presence of FC.
of FC (11). These data presented here raise the question whether p15
INK4b and p21
CIP1 are downstream targets of DDR2 signaling. This aspect is currently being investigated in our laboratory.

In summary, our observations provide evidence that p27
KIP1 is not necessary for the G0/G1-S arrest observed when cells are in contact with FC and that rather p15
INK4b and p21
CIP1 are responsible for arresting cells at the G0/G1-S and G2-M transition, respectively.

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REFERENCES
1. Lukashev, M. E., and Werb, Z. (1998) Trends. Cell Biol. 8, 437–441
2. Elder, D. (1999) Acta Oncol. 38, 535–547
3. Satyamoorthy, K., and Herlyn, M. (2002) Exp. Mol. Pathol. 64, 71–81
4. Karsenty, G., and Park, R. W. (1995) Cell 87, 1069–1078
5. Xia, H., Nho, R. S., Kahm, J., Kleidon, J., and Henke, C. A. (2004) J. Biol. Chem. 279, 33024–33034
6. Henriet, P., Zhong, Z. D., Brooks, P. C., Weinberg, K. I., and DeClerck, Y. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10026–10031
7. Pilcher, B. K., Sudbeck, B. D., Dumin, J. A., Welgus, H. G., and Parks, W. C. (1998) Arch. Dermatol. Res. 290, (suppl.) S37–S46
8. Koyama, H., Raines, E. W., Bornfeldt, K. E., Roberts, J. M., and Ross, R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5629–5634
9. Herz, D. B., Aitken, K., and Bagli, D. J. (2003) J. Biol. Chem. 278, 39757–39765
10. Roberts, J. M., and Forrester, J. V. (1990) Cancer Metastasis Rev. 9, 317–329
11. Bacakova, L., Wilhelm, J., Herget, J., Novotna, J., and Eckhart, A. (1997) Exp. Mol. Pathol. 64, 185–194
12. Hotary, K. B., Allen, E. D., Brooks, P. C., Datta, N. S., Long, M. W., and Weiss, S. J. (2003) Cell 114, 33–45
13. Hengstschlager, M., Braun, K., Soucek, T., Miloloza, A., and Hengstschlager-Ottnad, E. (1999) Mutat. Res. 436, 1–9
14. Sherr, C. J., and Roberts, J. M. (1999) Genes Dev. 13, 1501–1512
15. Johnson, D. G., and Walker, C. L. (1999) Annu. Rev. Pharmacol. Toxicol. 39, 295–312
16. Sutterluty, H., Chatelain, E., Marti, A., Wirbelauer, C., Senften, M., Muller, U., and Krek, W. (1999) Nat. Cell Biol. 1, 207–214
17. Carrano, A. C., Eytan, E., Hershko, A., and Pagano, M. (1999) Nat. Cell Biol. 1, 193–199
18. Roberts, J. M., and Forrester, J. V. (1990) J. Biol. Chem. 265, 165–172
19. Kim, H. S., Kim, J. W., Gang, J., Wen, J., Koh, S. S., Koh, J. S., Chung, H. H., and Song, S. Y. (2006) Toxicol. Appl. Pharmacol. 215, 317–329
20. Dvory-Sobol, H., Cohen-Noymann, E., Kazanov, D., Figler, A., Birkenfeld, S., Madar-Shapiro, L., Benamouzig, R., and Arber, N. (2006) Eur. J. Cancer 42, 422–426
21. Sherr, C. J., and Roberts, J. M. (1999) Biochem. J. 339, Pt. 3, 481–488
22. Hannon, G. J., and Beach, D. (1994) Nature 371, 257–261
23. Johnson, D. G., and Walker, C. L. (1999) J. Urol. 170, 2072–2076
24. Carrano, A. C., Eytan, E., Hershko, A., and Pagano, M. (1999) Nature 371, 288–293
25. Boudreau, N. J., and Jones, P. L. (1999) Biochem. J. 339, Pt. 3, 481–488
26. Boudreau, N. J., and Jones, P. L. (1999) Cell 97, 10026–10031
27. Karsenty, G., and Park, R. W. (1995) Cell 87, 1069–1078
28. Dvory-Sobol, H., Cohen-Noymann, E., Kazanov, D., Figler, A., Birkenfeld, S., Madar-Shapiro, L., Benamouzig, R., and Arber, N. (2006) Eur. J. Cancer 42, 422–426
29. Sharma, N., Timmers, C., Trikha, P., Saavedra, H. I., Obery, A., and Leone, G. (2006) J. Biol. Chem. 281, 36124–36131
30. Sharma, N., Timmers, C., Trikha, P., Saavedra, H. I., Obery, A., and Leone, G. (2006) J. Biol. Chem. 281, 36124–36131
31. Koyama, H., Raines, E. W., Bornfeldt, K. E., Roberts, J. M., and Ross, R. (1996) Cell 87, 1069–1078
32. Koyama, H., Raines, E. W., Bornfeldt, K. E., Roberts, J. M., and Ross, R. (1996) Cell 87, 1069–1078
33. Hengstschlager, M., Braun, K., Soucek, T., Miloloza, A., and Hengstschlager-Ottnad, E. (1999) Mutat. Res. 436, 1–9
34. Sherr, C. J., and Roberts, J. M. (1999) Genes Dev. 13, 1501–1512
35. Johnson, D. G., and Walker, C. L. (1999) Annu. Rev. Pharmacol. Toxicol. 39, 295–312
36. Sutterluty, H., Chatelain, E., Marti, A., Wirbelauer, C., Senften, M., Muller, U., and Krek, W. (1999) Nat. Cell Biol. 1, 207–214
37. Carrano, A. C., Eytan, E., Hershko, A., and Pagano, M. (1999) Nat. Cell Biol. 1, 193–199
38. Roberts, J. M., and Forrester, J. V. (1990) J. Biol. Chem. 265, 165–172
39. Dvory-Sobol, H., Cohen-Noymann, E., Kazanov, D., Figler, A., Birkenfeld, S., Madar-Shapiro, L., Benamouzig, R., and Arber, N. (2006) Eur. J. Cancer 42, 422–426
40. Nagaki, M., Sugiyama, A., Naiki, T., Ohsawa, Y., and Moriwaki, H. (2000) J. Hepatol. 32, 488–496
41. Zhou, X., Jamil, A., Nima, N., Sekino, Y., Iwai, Y., and Benyon, R. C. (2006) J. Biol. Chem. 281, 39757–39765
42. Sherr, C. J., and Roberts, J. M. (1999) Cancer Biol. Ther. 2, 14–17
43. Hotary, K. B., Allen, E. D., Brooks, P. C., Datta, N. S., Long, M. W., and Weiss, S. J. (2003) Cell 114, 33–45
44. Sherr, C. J., and Roberts, J. M. (1999) Cell 97, 10026–10031
45. Hannon, G. J., and Beach, D. (1994) Nature 371, 257–261
46. Boudreau, N. J., and Jones, P. L. (1999) Biochem. J. 339, Pt. 3, 481–488