Up-regulation of Bcl-2 Homology 3 (BH3)-only Proteins by E2F1 Mediates Apoptosis*

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The E2F1 transcription factor is a critical downstream target of the tumor suppressor pRB. The retinoblastoma (RB) pathway is often inactivated in human tumors, resulting in deregulated E2F activity that can induce both proliferation and apoptosis. Bcl-2 homology 3 (BH3)-only proteins are pro-apoptotic members of the Bcl-2 protein family that trigger apoptosis in response to diverse stimuli. We show here that E2F1 up-regulates the expression of the pro-apoptotic BH3-only proteins PUMA, Noxa, Bim, and Hrk/DP5 through a direct transcriptional mechanism. Expression of the E7 protein of HPV16, which disrupts RB/E2F complexes, also up-regulates the expression of these four BH3-only proteins, implicating endogenous E2F in this phenomenon. Indeed, endogenous E2F1 binds the promoters of these four genes. Furthermore, inhibition of E2F1-induced expression of either Noxa or PUMA results in a significant reduction in E2F1-induced apoptosis, indicating that increased Noxa and PUMA levels mediate this E2F1-induced apoptosis. Importantly, inhibition of E2F activity abolishes DNA damage-induced elevation of PUMA levels, implicating E2F in the physiological regulation of PUMA expression. These data provide a novel direct link between E2F and the apoptotic machinery and may explain the increased sensitivity of cells with a defective RB/E2F pathway to chemotherapy.

The retinoblastoma (RB)³ tumor suppressor pRB is a pivotal negative regulator of cell cycle progression whose inhibitory activity is largely attributed to its association with members of the E2F family of transcription factors (1). E2Fs are best known for their involvement in the timely activation of genes required for cell cycle progression (1). However, it is currently clear that E2Fs have important roles in regulating both cell proliferation and apoptosis. Indeed, ectopic expression of E2F1 and, in some settings, E2F2 and E2F3 also results in apoptosis (2, 3). Moreover, loss of either E2F1 or E2F3 suppresses apoptosis in RB-deficient mouse embryos (4, 5). E2F1-induced apoptosis occurs via both p53-dependent and p53-independent pathways, and it has been demonstrated that a number of E2F-regulated genes, including p14ARF, p19ARF, p73, Apaf-1, and caspases, contribute to this E2F1-induced apoptosis (6–12). Nevertheless, the molecular mechanisms underlying E2F1-induced apoptosis are not fully understood.

Bcl-2 homology 3 (BH3)-only proteins are members of the Bcl-2 protein family that trigger apoptosis. They share with each other and with the rest of the Bcl-2 family only a nine-amino acid BH3 domain. BH3-only proteins serve to integrate diverse apoptotic stimuli into a common cell death pathway governed by other multidomain Bcl-2 family members. Two such multidomain pro-apoptotic proteins, BAK and BAX, serve as essential effectors of cell death induced by BH3-only proteins (13).

We now show that E2F1 regulates the expression of the pro-apoptotic BH3-only proteins PUMA, Noxa, Bim, and Hrk/DP5. Furthermore, increased Noxa and PUMA levels mediate E2F1-induced apoptosis.

EXPERIMENTAL PROCEDURES

Cell Culture—NIH3T3 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% bovine calf serum. SAOS2 expressing the murine ecotropic receptor and mouse primary fibroblasts derived from mice deficient in p53 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Cells were maintained at 37 °C in a humidified 8% CO2-containing atmosphere. To induce activation of ER-E2F1 and ER-E7, cells were treated with 400 nm 4-hydroxytamoxifen (OHT) for the times indicated (Figs. 1, 3, 5, 7, and 8). Cycloheximide (10 μg/ml) was used for 4 h.

Plasmids—The following plasmids have been described previously: pRcCMV-HA-E2F1, pCMV-HA-Gal, pBabe-E2F1, and pBabe-HA-ER-E2F1 (14); pBabe-HA-ER-E7 (15); PUMA-luciferase (16); Noxa-luciferase and Noxa-mut-luciferase (17); and Bim-luciferase (18). pBabe-HA-ΔP1 (127–410) was generated by transferring the ΔP1 insert from pCMV-ΔP1 (127–410) (19) to pBabe-puro.

To create Hrk/DP5-luciferase, a 390-bp fragment of the Hrk/DP5 putative promoter region from −356 to +34 was generated by PCR using genomic DNA of NIH3T3 cells as template and the primers 5′-GGGATCTTTTCGCATTACGG-3′ and 5′-GGGACACGGGCACATGGC-3′. This fragment was cloned into pGL3-basic-Luc. To generate pRETRO-SUPER constructs, the pRETRO-SUPER vector (20) was digested with BglII/HindIII and ligated with the double strand oligonucleotides. Sequences of oligos are available upon request.

Transfection and Infection Assays—NIH3T3 cells were transfected by LipofectAMINE reagent (Invitrogen). Infection, cell lysis, β-galactosidase, and luciferase assays were performed essentially as described (14). 24 h after infection, 2 μg/ml puromycin or 300 μg/ml hygromycin B was added to the cultures for 24 or 72 h, respectively.

Reverse Transcription (RT)-PCR and Western Blotting—Total RNA was extracted from the cells using the TRI Reagent method (TR-118; Molecular Research Center, Inc.). RT-PCR was performed as described previously (14). Sequences of primers for distinct genes are available upon request. Western blot analysis was performed essentially as described (14).

Chromatin Immunoprecipitation—Approximately 10⁶ cells were cross-linked by the addition of formaldehyde directly to the growth medium (final concentration, 1%). Cross-linking was stopped after 10
E2F1 Regulates Expression of BH3-only Proteins

Fig. 1. Over-expressed or deregulated E2F elevates levels of BH3-only genes. A, NIH3T3 cells were infected with a pBabe-puro retroviral vector (vector) or with a retrovirus expressing E2F1 (E2F1). Total RNA was extracted from the cells, and RT-PCR was performed using specific primers for the PUMA, Noxa, Bim, Hrk/DP5, and GAPDH genes. B, NIH3T3 cells were infected with a retrovirus expressing ER-E7. Cells were then treated with OHT for 20 h (+) or not treated (−). Total RNA was extracted from the cells, and RT-PCR was performed using specific primers for the PUMA, Noxa, Bim, Hrk/DP5, and GAPDH genes. C, NIH3T3 cells containing either ER-E2F1 (upper panel) or ER-E7 (lower panel) were treated with OHT for the times indicated at the top of each lane. Proteins were extracted from the cells, and equal amounts of protein (determined by Bradford assay) were used for Western blot analysis with an anti-PUMA antibody (IMG-459), an anti-Noxa antibody (sc-11719), and an anti-GAPDH antibody (MAB374; Chemicon).

Fig. 2. Expression of BH3-only genes is growth related. NIH3T3 cells were starved for 48 h with 0.5% serum, and then serum was added to the medium to a final concentration of 20%. At the indicated times after serum addition, cells were harvested for FACS analysis and RNA extraction. RT-PCR was performed on the total RNA using specific primers for the PUMA, Noxa, Bim, Hrk/DP5, and elongation factor 1 (EF1) genes. Cell cycle distribution as determined by FACS analysis is presented (bottom panel).

Fig. 3. PUMA, Noxa, Bim, and Hrk/DP5 are direct E2F targets. NIH3T3 cells containing ER-E2F1 were incubated with OHT for the times indicated at the top of each lane. Where indicated, cycloheximide (CHX) was added for 4 h prior to harvesting. Total RNA was extracted from the cells, and RT-PCR was performed using specific primers for the PUMA, Bim, and GAPDH (A) or the Noxa, Hrk/DP5, and GAPDH genes (B).

The antibody used is anti-E2F1 (sc-193; Santa Cruz Biotechnology). Sequences of primers used for PCR are available upon request.

FACS Analysis—Cells were trypsinized and fixed with 70% ethanol (4 °C overnight). After fixation, cells were centrifuged for 5 min at 1200 g at 4 °C, and pellets were washed with PBS. Finally, cells 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 was diluted 1:10 with dilution buffer (10 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.
rpm and incubated for 30 min at 4 °C in 1 ml of PBS, centrifuged, and resuspended in PBS containing 5 mg/ml propidium iodide and 50 μg/ml RNase A for 20 min at room temperature. Fluorescence intensity was analyzed using a Becton Dickinson flow cytometer.

**Colony Formation Assay**—Equal numbers of cells were plated on 60-mm plates and grown for 2 weeks in the absence or presence of OHT (300 nM). Then cells were fixed using formaldehyde and stained with crystal violet.

**RESULTS**

To determine whether E2F regulates the expression of BH3-only proteins, we examined the effect of E2F1 on the expression of four members of the BH3-only family, namely PUMA, Noxa, Bim, and Hrk/DP5. Introduction of E2F1 into NIH3T3 cells by retrovirus-mediated gene transfer resulted in a significant increase in the endogenous mRNA levels of all four BH3-only genes (Fig. 1A). Murine Bim has three isoforms, and all three are induced by E2F1 (Fig. 1A). To study the regulation of expression of BH3-only genes by endogenous E2F, we infected NIH3T3 cells with a retrovirus containing an inducible human papilloma virus HPV16 E7 (ER-E7) protein that disrupts RB/E2F complexes, thereby leading to the deregulation of E2F activity. The ligand OHT, which activates the ER-E7 fusion protein, led to a significant increase in endogenous mRNA levels of these four BH3-only genes (Fig. 1B). These data strongly suggest that deregulated endogenous E2F elevates the expression of endogenous PUMA, Noxa, Bim, and Hrk/DP5. The positive effect of E2F on the expression of representative BH3-only genes could also be confirmed at the level of the corresponding proteins (Fig. 1C).

The fact that deregulation of endogenous E2F induces expression of PUMA, Noxa, Bim, and Hrk/DP5 (Fig. 1, B and C) suggests that these genes are physiological targets of E2F. In support of this notion, mRNA levels of all four genes increase as quiescent cells reenter S phase (Fig. 2). This growth-regulated expression of PUMA, Noxa, Bim, and Hrk/DP5 is similar to the previously demonstrated pattern of expression of many bona fide E2F target genes.

To determine whether BH3-only proteins are direct targets of E2F, we infected NIH3T3 cells with a retrovirus expressing ER-E2F1 (21). Induction of E2F1 by the addition of OHT to these cells led to an increase in mRNA levels of PUMA, Noxa, Bim, and Hrk/DP5 (Fig. 3). Importantly, the E2F1-induced increase in mRNA levels of these four BH3-only genes was detected also in the presence of the protein synthesis inhibitor, cycloheximide. These data indicate that de novo protein synthesis is not required for E2F1-induced up-regulation of these four genes, suggesting that they are direct targets of E2F.

Interestingly, expression of these genes was induced with different kinetics; whereas the expression of PUMA and Bim was already maximal 4 h after the addition of OHT (Fig. 3A), the induction of Noxa and Hrk/DP5 was detectable only 8 h after induction (data not shown) and was maximal 16 h post induction (Fig. 3B).

Sequence analysis of the promoters of the four BH3-only genes demonstrated that the murine Noxa, Bim, and PUMA promoters contain putative E2F binding sites at positions -109, -3, and -8, respectively (Fig. 5B, and data not shown).
In some cases the promoters of these BH3-only genes have not been formally characterized, but the genomic sequences upstream to the transcription start sites of human Noxa and both human and murine Hrk/DP5 contain putative E2F binding sites. The human PUMA promoter does not contain any consensus E2F-binding sites; however, various promoters were shown to recruit E2F via a mechanism independent of the defined consensus site (22).

Responsiveness of the PUMA, Hrk/DP5, Noxa, and Bim promoters to E2F was studied using luciferase reporter plasmids that contain their promoters. The studied promoter fragments were the −428/+67 region of the human PUMA promoter (16), the −356/+34 region of the murine Hrk/DP5 genomic sequence upstream to the transcription start site (as determined by Ref. 23), the −183/+146 region of the murine Noxa promoter (17), and the −699/+96 region of the murine Bim promoter (18).

Co-transfection of either the PUMA-Luc reporter or the Hrk/DP5-Luc reporter together with an E2F1 expression vector into NIH3T3 cells resulted in E2F1-induced activation of 26- and 13.6-fold, respectively (Fig. 4, A and B). The PUMA and Hrk/DP5 promoters were not activated by an E2F1 mutant, E2F1E132, which does not bind DNA, although E2F1E132 was expressed at levels similar to or higher than wild type E2F1 (Fig. 4, A and B). The Noxa and the Bim promoters exhibited a qualitatively similar pattern of regulation; co-transfection of either the Noxa-Luc reporter or the Bim-Luc reporter together with an E2F1 expression vector into NIH3T3 cells resulted in a 4-fold E2F1-induced activation (Fig. 4, C and D). Furthermore, the Noxa and the Bim promoters were not activated by E2F1E132 (Fig. 4, C and D).

Expression of both PUMA and Noxa is regulated by p53 (16, 17, 24, 25). E2F1 can up-regulate p53 levels and activity via
E2F1 Regulates Expression of BH3-only Proteins

To demonstrate the involvement of endogenous E2F1 in regulating the expression of these four BH3-only genes, we performed a chromatin immunoprecipitation analysis in growing NIH3T3 cells using an antibody directed against E2F1. The promoter fragments that were amplified are −250/+47 of PUMA, −311/+25 of Noxa, −219/+57 of Bim, and −356/+34 of Hrk/DP5. We observed a significant enrichment of all four promoters when using the anti-E2F1 antibody (Fig. 6). Such enrichment was not detected after amplification of an unrelated genomic DNA fragment (Fig. 6). These data demonstrate interaction of endogenous E2F1 with the promoters of these four BH3-only genes. Taken together with the ability of both ectopically expressed E2F1 and E7 to up-regulate these genes, these findings strongly suggest that endogenous E2F1 has a role in regulating the expression of PUMA, Noxa, Bim, and Hrk/DP5.

To test whether induction of BH3-only proteins plays an important role in E2F1-induced apoptosis, we first studied the effect of reducing Noxa expression on E2F1-induced apoptosis. To that end, NIH3T3 cells expressing ER-E2F1 were infected with retroviruses expressing either Noxa-specific siRNA, irrelevant siRNA, or an empty retroviral vector. Expression of siRNA that inhibits Noxa expression, but not irrelevant siRNA, abolished E2F1-induced elevation of Noxa mRNA (Fig. 7A). Activation of E2F1 resulted in apoptotic cell death, as determined by appearance of cells with Sub-G1 DNA content. Importantly, this E2F1-induced apoptosis was significantly inhibited by the Noxa-specific siRNA but not by irrelevant siRNA (Fig. 7, B and C). Next, we studied the effect of reducing PUMA expression on the survival and growth of cells overexpressing E2F1 by using a colony formation assay. To that end, SAOS2 cells expressing ER-E2F1 were infected with retroviruses expressing either PUMA-specific siRNA or irrelevant siRNA. Expression of siRNA that inhibits PUMA expression, but not irrelevant siRNA, abolished E2F1-induced elevation of PUMA mRNA (Fig. 8A). Activation of E2F1 resulted in a significant reduction in the number of colonies, most probably due to apoptosis. Inhibition of PUMA expression by the PUMA-specific siRNA led to a considerable increase in the number of colonies (Fig. 8B). These data indicate that E2F1-mediated up-regulation of Noxa contributes to E2F1-induced apoptosis. Of note, SAOS2 cells used for the PUMA siRNA experiments, do not contain a functional p53, and p53 levels were unaffected by the activation of E2F1 in the NIH3T3 cells used for the Noxa siRNA experiments (data not shown).

To test whether E2F mediates the expression of any of the BH3-only genes in response to stimuli that ordinarily induce them, we analyzed the induction of PUMA after genotoxic stress. As can be seen in Fig. 9, levels of the PUMA protein are increased when mouse embryo fibroblasts derived from p53−/− mice are treated with either cisplatin or doxorubicin. Importantly, infection of these p53−/− cells with a dominant negative mutant of the heterodimeric partner of E2F, DP1 (19), inhibited this damage-induced elevation in the levels of PUMA (Fig. 9), implicating endogenous E2F in the regulation of PUMA expression in response to stress.

**DISCUSSION**

Our data indicate that ectopic expression of E2F1 or deregulation of the RB/E2F pathway results in coordinated up-regulation of four members of the BH3-only family, namely PUMA, Noxa, Bim, and Hrk/DP5. Furthermore, we show that endogenous E2F1 binds to the promoters of these four BH3-only genes. Importantly, inhibition of expression of either Noxa or PUMA significantly diminished E2F1-induced apoptosis, supporting the conclusion that regulation of BH3-only protein expression by E2F1 contributes to its apoptotic function. We
also show that in cells lacking E2F activity, because of the expression of a dominant negative mutant of DP1, the elevation of PUMA levels in response to DNA damage is abrogated, indicating that E2F mediates, at least in part, the regulation of PUMA expression in response to stress.

Ectopic expression of each one of the four BH3-only genes studied here, PUMA, Noxa, Bim, and Hrk/DP5, can result in apoptosis (16, 17, 23–25, 27). However, it is believed that the different proteins mediate death in response to distinct stimuli; levels of PUMA and Noxa increase in response to DNA damage, including that caused by γ-irradiation and chemotherapeutic drugs, and both genes are transcriptionally regulated by p53 (16, 17, 24, 25), yet PUMA mRNA is also induced by p53-independent apoptotic stimuli (24). Levels of Bim increase upon the withdrawal of cytokines or nerve growth factor in hematopoietic cells or neurons, respectively (28, 29). In addition, Bim plays a role in apoptosis in response to Taxol or Ca²⁺ flux and is believed to be a critical physiological regulator of homeostasis in hematopoietic cells (30). Levels of Hrk/DP5 are high in embryonic neuronal tissues that undergo apoptosis (23), and in cultured neurons its expression is increased after nerve growth factor withdrawal (23) or exposure to β-amyloid (31). Interestingly, β-amyloid-induced apoptosis of cultured neurons was shown to be mediated by E2F1 (32).

A recent screen using a DNA microarray demonstrates that ectopic expression of E2F1 elevates levels of Bad and Bid, two additional members of the BH3-only family that have not been shown previously to be transcriptionally regulated (33). Interestingly, up-regulation of pro-apoptotic genes is not the only effect of E2F on the Bcl-2 family, and it was shown to repress the expression of the anti-apoptotic member of the family, Mcl-1 (34, 35). In addition, E2F1 can also affect the expression of Bcl-2, but the exact nature of this effect is currently under debate and, although some studies indicate that E2F1 down-regulates the expression of Bcl-2, others demonstrate that it up-regulates Bcl-2 levels (36–38). Identification of other E2F-regulated Bcl-2 family members and, in particular BH3-only genes, awaits further studies.

Thus, the list of BH3-only genes that are regulated by E2F may still grow. Regardless of the final number of such genes, the coordinated E2F-induced elevation of several BH3-only proteins described here suggests a mechanism whereby in-

**Fig. 7. Noxa mediates E2F1-induced apoptosis.** NIH3T3 cells containing ER-E2F1 were infected with either the pRetroSuper siRNA vector (vector), pRetroSuper siRNA containing a nonspecific siRNA (nonspecific), or a pRetroSuper containing siRNA that inhibits Noxa expression (Noxa). After incubation with OHT for the indicated times, cells were harvested for either FACS analysis or extraction of total RNA. A, RT-PCR was performed on the total RNA using specific primers for the NOXA and GAPDH genes. B, flow cytometric analysis following induction of E2F1 for the times indicated at the left of each panel. The percentage of cells with sub-G₀ DNA content is indicated. C, the bar graph depicts the apoptotic rate of the samples presented in B. The percentage of apoptosis (% Apoptosis) is presented relative to the apoptosis in cells infected with pRetroSuper siRNA vector, which is depicted as 100%.
with an anti-PUMA antibody (IMG-459) and an anti-tubulin antibody. Proteins were extracted from the cells, and equal amounts of protein deficient in p53 were infected with a pBabe retroviral vector (up-regulation of PUMA).

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increased E2F activity may sensitize cells to a variety of apopto-

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REFERENCES

1. DeGregori, J. (2002) Biochim. Biophys. Acta 1602, 131–150
2. Phillips, A. C., and Vousden, K. H. (2001) Apoptosis 6, 173–182
3. Ginsberg, D. (2002) FEBS Lett. 529, 122–125
4. Tsai, K. Y., Hu, Y., Macleod, K. F., Crowley, D., Yamashita, L., and Jacks, T. (1998) Mol. Cell 2, 293–304
5. Ziebold, U., Renz, T., Caron, A., and Lees, A. J. (2001) Genes Dev. 15, 386–391
6. Bates, S., Phillips, A. C., Clark, P. A., Stott, F., Peters, G., Ludwig, R. L., and Vousden, K. H. (1998) Nature 395, 124–125
7. Robertson, K. D., and Jones, P. A. (1998) Mol. Cell. Biol. 18, 6457–6473
8. Irwin, M., Marin, M. C., Phillips, A. C., Seelan, R. S., Smith, D. L., Liu, W., Flores, E. R., Tsai, K. Y., Jacks, T., Vousden, K. H., and Kaelin, W. (2000) Nature 407, 645–648
9. Stiewe, T., and Pützer, B. M. (2000) Nat. Genet. 26, 464–469
10. Moroni, M. C., Hickman, E. S., Denchi, E. L., Caprara, G., Colli, E., Cecconi, F., Muller, H., and Hein, K. (2001) Nat. Cell Biol. 3, 552–558
11. Furukawa, Y., Nishikura, N., Furukawa, Y., Satoh, M., Eode, H., Iwase, S., Yamada, H., Matsuda, M., Kano, Y., and Nakamura, M. (2002) J. Biol. Chem. 277, 39760–39768
12. Nabholtz, J., Papel, O., Davuluri, A. V., McCurrach, M. E., Jacobson, M. D., Narita, M., Zhang, M. Q., Lazebnik, Y., Bar-Sagi, D., and Lowe, S. W. (2002) Nat. Cell Biol. 4, 859–864
13. Letti, A., Bassik, M. C., Walsnes, L. B., Soricelli, M. D., Weiler, S., and Korsmeyer, S. J. (2002) Cancer Cell 2, 183–192
14. Berkovich, E., and Ginsberg, D. (2002) Oncogene 21, 161–167
15. Berkovich, E., Lamed, Y., and Ginsberg, D. (2003) Cell Cycle 2, 127–133
16. Yu, A., Zhang, L., Hwang, P. M., Kimiz, K. W., and Vogelstein, B. (2001) Mol. Cell 7, 673–682
17. Oda, E., Okhi, R., Murasawa, H., Nemoto, J., Shibue, T., Yamashita, T., Tokino, T., Taniguchi, T., and Tanaka, N. (2000) Science 288, 1053–1058
18. Bouillet, P., Zhang, L. C., Huang, D. C., Webb, G. C., Bottema, C. D., Shore, P., Eyre, H. J., Sutherland, G. R., and Adams, J. M. (2001) Mamm. Genome 12, 161–168
19. Wu, C. L., Classon, M., Dyson, N., and Harlow, E. (1996) Mol. Cell. Biol. 16, 3698–3706
20. Brummelkamp, T., Bernards, R., and Agami, R. (2002) Science 296, 550–553
21. Vigo, E., Muller, H., Prosperini, E., Hateboer, G., Cartwright, P., Moroni, M. C., and Helin, K. (1999) Mol. Cell. Biol. 19, 6379–6385
22. Weimann, A. S., Yan, P. S., Obeyese, M. J., Huang, T. H., and Parnham, P. J. (2002) Genes Dev. 16, 235–244
23. Imaiizumi, K., Tsuchiya, M., Imai, Y., Wanaka, A., Takeda, M., and Tohyama, M. (1999) J. Biol. Chem. 274, 18442–18448
24. Han, J., Flemington, C., Houghton, A. B., Hare, M., Zambetti, G. P., Lutz, F., R. J., Zhu, L., and Chittenden, T. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 11318–11323
25. Nakano, K., and Vousden, K. H. (2001) Mol. Cell 7, 683–694
26. Imaiizumi, K., Morihara, T., Mori, Y., Katayama, T., Tsuda, M., Furuyama, T., Wanaka, A., Takeda, M., and Tohyama, M. (1999) J. Biol. Chem. 274, 7973–7981
27. Giovannelli, A., Keramaris, E., Morris, R. J., Hsu, T. O., O’Hare, M., Dyson, N., Robertson, G. S., Slack, R. S., and Park, D. S. (2000) J. Biol. Chem. 275,
33. Stanelle, J., Stiewe, T., Theseling, C. C., Peter, M., and Putzer, B. M. (2002) Nucleic Acids Res. 30, 1859–1867
34. Dong, Y. B., Yang, H. L., Elliott, M. J., Liu, T. J., Stilwell, A., Atienza, C., Jr., and McMasters, K. M. (1999) Cancer 86, 2021–2033
35. Croxton, R., Ma, Y., Song, L., Haura, E. B., and Cress, W. D. (2002) Oncogene 21, 1359–1369
36. Yang, H. L., Dong, Y. B., Elliott, M. J., Liu, T. J., and McMasters, K. M. (2000) Clin. Cancer Res. 6, 1579–1589
37. Gomez-Manzano, C., Mitlianga, P., Fueyo, J., Lee, H. Y., Hu, M., Spurgers, K. B., Glass, T. L., Koul, D., Liu, T. J., McDonnell, T. J., and Yung, W. K. (2001) Cancer Res. 61, 6693–6697
38. Eischen, C. M., Packham, G., Nip, J., Fee, B. E., Hiebert, S. W., Zambetti, G. P., and Cleveland, J. L. (2001) Oncogene 20, 6983–6993