Apoptosis Signal-regulating Kinase 1 Is a Direct Target of E2F1 and Contributes to Histone Deacetylase Inhibitor-induced Apoptosis through Positive Feedback Regulation of E2F1 Apoptotic Activity*

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The oncogenic retinoblastoma protein (Rb)/E2F pathway links cellular proliferation control to apoptosis as a fail-safe mechanism to protect aberrant oncogenic transformation. We have previously shown that histone deacetylase inhibitors (HDACIs) activate the E2F1-Bim apoptotic pathway, leading to efficient cell killing in cancer cells with deregulated E2F1 activity. To identify additional gene cassettes that might contribute HDACI-induced apoptosis upon E2F1 activation, we investigated the apoptotic transcriptional network affected by HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) in cancer cells with inducible E2F1. Data analysis focusing on 220 apoptosis-related genes identified apoptosis signal-regulating kinase 1 (ASK1) as one of a few genes in addition to Bim that are substantially up-regulated by SAHA upon E2F1 activation. We show that ASK1 is directly regulated by E2F1 and that prevention of ASK1 induction by RNA interference decreases SAHA-induced apoptosis. We further show that the role of ASK1 in the SAHA apoptotic response is not associated with its downstream effectors p38 or JNK. Instead, ASK1 knockdown results in reduced E2F1 transcriptional activity, leading to decreased Bim induction by SAHA. Moreover, ASK1 expression reverses the negative effect of Rb on E2F1 activity. These results indicate that ASK1 induction by E2F1 provides positive feedback regulation of E2F1 activity via Rb inhibition, which allows an efficient E2F1-Bim activation. Thus, the concomitant induction of E2F1 targets ASK1 and Bim by HDACIs warrants an effective activation of E2F1-dependent apoptosis in response to SAHA.

The E2F family of transcription factors plays a critical role in overall cell cycle control. Members of the E2F family of transcription factors control cell proliferation by regulating the expression of genes required for S-phase entry and progression (1–3). As a fail-safe homeostasis mechanism, E2F’s are also equipped with apoptotic function to protect aberrant oncogenic transformation. E2F1 appears to be crucial for E2F-dependent apoptosis (4). Ectopic expression of E2F1 induces p53-dependent apoptosis both in vitro and in vivo (5–9) through the transactivation of p19ARF (10, 11) and thus alleviation of MDM2-mediated degradation of p53 (11–13). In addition, E2F1 can induce the expression of p73 (14, 15), Apaf-1 (16), caspases (17), and pro-apoptotic BH3-only proteins of the Bcl-2 family (18) and thus induces apoptosis through a p53-independent mechanism. Thus, pharmacologic activation of E2F1-mediated apoptosis in p53-deficient tumors can be explored to overcome the chemoresistance in these tumors.

E2Fs activity is negatively modulated by pRb tumor suppressor. Hypophosphorylated Rb binds to and sequesters the activating E2Fs (E2F1–3) (19), resulting in the repression of proliferation-associated genes. Inactivation of pRb through phosphorylation by cyclin-dependent kinases and other kinases results in increased E2F1 activity and subsequent transactivation of genes required for cell cycle progression, leading to aberrant cell proliferation (20). Although Rb disruption primarily occurs in retinoblastoma, Rb inactivation can be caused in many tumor types by alterations of other components in this regulatory machinery, such as loss of p16(INK4a) or overexpression of Cyclin D1 or cyclin-dependent kinase 4 (21).

Histone deacetylase inhibitors (HDACIs)2 are a new class of anticancer agents that inhibit tumor cell growth and survival. A number of mechanisms have been proposed to explain their anti-tumor activity (22–25). We previously showed that HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) and trichostatin A can promote oncogene E2F1-mediated apoptosis through the induction of pro-apoptotic Bcl2 family member Bim and that this apoptosis does not require p53 or p73 (26). As a result, cancer cells with deregulated E2F1 activity are sensitive to HDACIs. In this study, we interrogated the transcriptional response of the apoptotic network to HDAC inhibitor SAHA that is affected by E2F1 activity and identified ASK1 as an additional target of E2F1 that participates in HDACI-induced cell death. Contrary to an established role of ASK1 in regulating its downstream apoptotic signaling pathways, we have shown that ASK1 induction contributes to SAHA-induced apoptosis through positive feedback regulation of E2F1 apoptotic activity.

EXPERIMENTAL PROCEDURES

Cell Culture and Chemicals—p53 null HCT116 cells expressing inducible E2F1 were described previously (26). IMR90 and U2OS cells were from ATCC. IMR90-E1A cells were kindly provided by Dr. Claudio Brancolini (University of Di Udine, Italy). All cell culture reagents and media were from Invitrogen. SAHA was from Alexis Biochemicals (San Diego, CA).

Microarray Hybridization and Data Analysis—Total RNA was extracted with the use of TRIzol reagent (Invitrogen) and the Qiagen RNeasy kit according to the manufacturers’ instructions. For all experiments, the costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

* This work was supported by Agency for Science, Technology, and Research (A* Star) of Singapore. The authors thank Dr. Claudio Brancolini for providing IMR90-E1A cells. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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2 The abbreviations used are: HDACI, histone deacetylase inhibitor; 4-OHT, 4-hydroxytamoxifen; JNK, c-Jun N-terminal kinase; RB, retinoblastoma; CDK, cyclin-dependent kinase; SAHA, suberoylanilide hydroxamic acid; ChIP, chromatin immunoprecipitation; ER, estrogen receptor; ASK1, apoptosis signal-regulating kinase 1.
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In the previous study, we reported that HDACIs activate E2F1-dependent apoptosis and that the E2F1 target Bim plays a crucial role in this process. To fully characterize the genomic program and to identify additional molecular events that might participate in E2F1-dependent apoptosis in response to HDAC inhibition, we employed DNA microarray analysis and an E2F1-inducible HCT116 p53 null cell line (26). In this cell system, E2F1 is fused to the estrogen receptor (ER)-binding domain (30) and ER-E2F1 is activated by ER ligand 4-OHT. As described previously (26) and as shown here in Fig. 1A, HDAC inhibitor SAHA induced markedly increased apoptosis upon E2F1 activation by 4-OHT.

To determine the apoptotic program that contributes to SAHA-induced apoptosis as a result of E2F1 activation, we focused on 220 well annotated apoptosis-related genes represented in the 19k gene array. We began by defining apoptotic genes activated by 4-OHT over a 4-, 8-, and 24-h time course in ER-E2F1-expressing cells or cells expressing the empty vector that contain ER-binding domain only (ER). Gene expression data from each time point were cluster analyzed and displayed in TreeView software (28). As shown in Fig. 1B, 42 of 220 apoptosis-related genes showed increased expression after 4-OHT treatment in ER-E2F1 cells, but not in ER cells, indicating these apoptotic genes are potentially regulated by E2F1. Among E2F1-regulated apoptotic genes, BCL2L11, TP73, and CASP3 are previously known E2F1 targets and were strongly up-regulated by E2F1. In addition, we found for the first time that RUNX3, ATM, TPS3BPL, and RPS6KA1 were also substantially activated by E2F1.

To identify SAHA-responsive genes that reflect the E2F1 apoptotic activity, we next compared the expression patterns of apoptosis genes commonly and selectively activated by SAHA treatment in the presence and absence of 4-OHT. 49 genes were found to be up-regulated by SAHA for at least one time point regardless of the presence of 4-OHT (Fig. 1C). As previously described, we found that SAHA strongly activated multiple genes implicated in the apoptosis program. Among them are those involved in the intrinsic apoptotic pathway (31), such as Bcl2-family members (BCL2L11, BCL10, and CASP3). Genes belonging to the receptor-mediated death pathway (TNFRSF1B, TNFRSF10D, and TNFRSF13) were also induced by SAHA, albeit to lesser extents. Among them, 15 were E2F1 targets as marked in Fig. 1C. We reasoned that, if SAHA activates the E2F1 apoptotic program, then we should find a set of genes whose expression can be further induced by SAHA upon E2F1 activation. Indeed, cluster analysis revealed a subset of genes whose expression was markedly enhanced by SAHA upon E2F1 activation by 4-OHT (Fig. 1C, cluster A). Notably, in cluster A MAP3K5,
FIGURE 1. Cell death response and expression analysis of apoptosis genes associated with SAHA and E2F1. A. ER-E2F1-expressing or control ER-expressing cells were treated with 1 μM SAHA in the presence or absence of 4-OHT. After 48 h, cells were harvested and stained for active anti-caspase-3. Percentages of cells positive for active caspase-3 are indicated. B. E2F1-regulated apoptosis genes. Microarray analysis as illustrated in Cluster and TreeViewer shows E2F1-dependent genes in ER-E2F1 and ER cells treated with 4-OHT. Red represents up-regulation relative to the untreated control (black). C. SAHA-responsive genes in ER-E2F1 cells in the presence or absence of 4-OHT. Genes in boxes are putative E2F1 targets identified in panel B.
which encodes apoptosis signal-regulating kinase 1 (ASK1) and appeared to be weakly induced by E2F1 alone, was strongly upregulated by SAHA upon E2F1 activation. In the same cluster were BCL2L11 (Bim) and CASP3, which had been described in our previous study (26). These observations support two conclusions: 1) SAHA activates E2F1 apoptotic activity in a target gene-specific manner, and 2) ASK1 is an E2F1-regulated target and its expression can be activated by SAHA.

Both Exogenous and Endogenous E2F1 Regulate ASK1 Expression—To confirm the results obtained from the gene expression analysis, reverse transcription PCR experiments were performed to examine the ASK1 mRNA levels in HCT116 cells expressing ER-E2F1, ER, or a DNA binding-defective mutant of E2F1 (E132) fused to ER. In agreement with the microarray data, activation of E2F1 by 4-OHT resulted in an increase in ASK1 mRNA level, but not in cells expressing ER or ER-E132 (Fig. 2A, left panel). Consistently, ASK1 protein accumulated following 4-OHT treatment in ER-E2F1-expressing cells (Fig. 2A, right panel). To investigate whether ASK1 expression is associated with the endogenous E2F1, we arrested the human osteosarcoma cells U2OS in G0/G1 phase by serum starvation (Fig. 2B, left panel). Expression of ASK1 was analyzed by Western blotting (right panel). Corresponding to the enhanced E2F1 activity, we detected an increase in ASK1 expression as cells

**FIGURE 2.** E2F1 induces ASK1 mRNA and protein accumulation. A, p53 null HCT116 cells were infected with an empty retrovirus (ER), a retrovirus expressing ER-E2F1 (E2F1) or ER-E2F1-E132 (E132). Cells were left untreated (−) or treated with 4-OHT (+) for the indicated duration. ASK1 mRNA and protein expression levels were analyzed by reverse transcription PCR (left panel) and Western blot (right panel), respectively. B, U2OS cells had been synchronized in G0 (0 h) by serum starvation for 48 h and then reentered the cell cycle after serum stimulation. The corresponding cell cycle distribution is shown (left panel). Proteins were extracted from cells at different times after release into the cell cycle. E2F1, ASK1, and Cyclin E were analyzed by Western blotting (right panel). C, Western blot analysis of ASK1, p73, and α-tubulin protein in U2OS and IMR90 cells infected with the control adenovirus (−) or an adenovirus expressing E1A (+).
entered S-phase, together with the bona-fide E2F1 targets p73 and Cyclin E. Thus, ASK1 expression correlated with endogenous E2F1 expression. To further substantiate this conclusion, we also used the adenovirus-expressing oncoprotein E1A (Ad-E1A) to infect U2OS and IMR90 cells. E1A binds to and inactivates Rb family members (1, 32), resulting in the activation of endogenous E2F1 (9, 33). Thus, cells overexpressing E1A will have enhanced E2F1 activity and therefore increased expression of E2F1 target genes. Indeed, we found that Ad-E1A infection resulted in the up-regulation of ASK1 and p73 in both U2OS and IMR90 cells (Fig. 2C). These observations suggest that activation of endogenous E2F1 is able to induce the expression of ASK1.

**ASK1 Is a Direct Target of E2F1**—To determine whether the E2F1 induction of ASK1 is regulated at the level of transcription, the ASK1 promoter was isolated and subcloned into a luciferase reporter plasmid. Fig. 3 illustrates the promoter region of the ASK1 gene, including the putative E2F-binding sites as well as the deletion mutant for reporter constructs. As shown in Fig. 3B, the promoter activity of the 1.0-kb 5′-proximal region of the ASK1 gene can be markedly activated by increasing amounts of E2F1 plasmid. To determine the potential E2F binding region that mediates the induction, we next measured ASK1 promoter activity using various deletion mutants. The deletion constructs containing the region between 1000 and 256 were not responsive to E2F1 (Fig. 3C). In contrast, E2F1 can activate the -273/+125 promoter up to 12-fold, suggesting that the putative E2F1-binding site within -273/+125 is a functional E2F1-responsive element in the ASK1 promoter.

To examine the in vivo recruitment of E2F1 to the ASK1 gene promoter, we used ER-E2F1- or a DNA binding-deficient mutant (ER-E132)-expressing cells to perform the ChIP assay. In the absence of 4-OHT, anti-E2F1 immunoprecipitated the proximal region of the ASK1 gene promoter containing the E2F1 motif in both ER-E2F1- and ER-E132-expressing cells, whereas control IgG did not (Fig. 3D). This indicates that endogenous E2F1 binds to the ASK1 promoter. In the presence of 4-OHT, which activates ER-E2F1, recruitment of E2F1 to the ASK1 promoter was substantially increased in ER-E2F1 cells, whereas the activation of binding mutant ER-E132 failed to do so. In light of these results, we conclude that E2F1 activates ASK1 transcription and ASK1 is a direct target of E2F1.

**ASK1 Regulates E2F1 Activity through a Positive Feedback Mechanism**—E2F1 activity is negatively regulated by pRB. pRB hyperphosphorylation inactivates Rb, resulting in increased E2F1 activity. ASK1 has been recently shown to interact with and inactivate pRB (34). This observation raises the possibility that ASK1 induction by E2F1 might lead to pRB inhibition and thus provide a positive feedback loop on E2F1 activity. Given this possibility, we determined whether ASK1 knockdown by siRNA could impair the activation of E2F1 targets. In ER-E2F1-expressing cells, activation of E2F1 by 4-OHT resulted in the induction of ASK1 as well as other E2F1 targets p73, Bim, and Cyclin E. We found that depletion of ASK1 by siRNA not only prevented its own induction by 4-OHT but also substantially impaired the induction of p73, Bim, and Cyclin E (Fig. 4A). This result suggests that E2F1-medi-
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**FIGURE 4.** ASK1 regulates E2F1 target gene expression through Rb inactivation. A, ER-E2F1-expressing cells were transfected with nonspecific control siRNA (NC siRNA) or ASK1-specific siRNA for 48 h and then either left untreated (−) or treated with 4-OHT. The expression of ASK1, Bim, Cyclin E, and p73 was analyzed by Western blotting. B, HCT116 cells were co-transfected with a luciferase reporter plasmid containing the Bim promoter, together with E2F1, Rb, or ASK1 expression vector. In addition, ASK1 expression plasmid was cotransfected with E2F1 to determine the effect of ASK1 on E2F1-mediated activation of the Bim promoter in the presence or absence of Rb overexpression. Data are expressed as means ± S.E. from three experiments. *, p < 0.01, E2F1 + ASK1 versus E2F1; **, p < 0.01, E2F1 + Rb + ASK1 versus E2F1 + Rb, based on Student’s t-test.

E2F1 induction permits a positive feedback effect on E2F1 activity, leading to the sufficient induction of E2F1 target genes.

To investigate whether ASK1 activates E2F1 activity through inhibition of Rb, we used the E2F1-responsive Bim promoter to test the effect of ASK1 on E2F1/Rb-mediated regulation of Bim promoter activity. In the luciferase reporter assay, ectopic expression of E2F1 activated the Bim promoter; as expected, this E2F1 function was inhibited by co-expression of pRB (Fig. 4B). Further introduction of the ASK1 expression plasmid reversed the negative effect of Rb on E2F1-mediated activation of the Bim promoter (Fig. 4B). Thus, these results support the conclusion that ASK1 induction by E2F1 results in Rb inactivation and thereby increases E2F1 activity through a positive feedback loop.

**SAHA Promotes E2F1-mediated ASK1 Induction**—Microarray analysis as shown in Fig. 1 indicates that ASK1, like Bim, is weakly regulated by E2F1; however, this regulation can be significantly augmented following HDAC inhibition by SAHA. This observation is further validated through Western blot analysis in ER-E2F1-expressing cells (Fig. 5A). To examine whether the increased ASK1 induction by SAHA upon E2F1...
activation is associated with the increased E2F1 recruitment to the ASK1 promoter, we performed the ChIP assay. Indeed, under SAHA treatment in the presence of 4-OHT, E2F1 binding to the ASK1 promoter was markedly increased (Fig. 5B).

To investigate whether endogenous E2F1 is required for ASK1 induction by SAHA, we compared IMR90 and IMR90 cells stably expressing E1A oncoprotein. Consistent with the higher E2F1 activity in IMR90-E1A cells, these cells express a higher level of ASK1 as compared with the IMR90 cells and after SAHA treatment ASK1 expression was further induced (Fig. 5C). To determine a definite role of E2F1 in SAHA-induced ASK1 expression, we used the E2F1 siRNA to inhibit E2F1 expression in U2OS cells and examined its effect on ASK1 expression after SAHA treatment. As shown in Fig. 5D, SAHA induced ASK1 expression over time in U2OS cells and that cells treated with E2F1 siRNA, but not control siRNA, efficiently abolished ASK1 induction. Taken together, these results indicate that both exogenous and endogenous E2F1 are required for the induction of ASK1 in response to SAHA.

**E2F1-ASK1 Activation Contributes to Apoptosis Induction by SAHA**—

We next examined the role of ASK1 in SAHA-induced apoptosis resulting from enhanced E2F1 activity. To this end, we used ASK1 siRNA to reduce ASK1 expression in ER-E2F1-expressing cells and observed a marked reduction in the level of apoptosis following SAHA treatment upon E2F1 activation (Fig. 6A). Consistent with the role of ASK1 on the positive feedback regulation on E2F1 activity, we found that ASK1 depletion resulted in marked reduction of Bim in response to SAHA (Fig. 6B).

**DISCUSSION**

The oncogenic pRB-E2F pathway is frequently deregulated in human cancers. Our previous study has shown that HDACIs target this pathway for apoptosis induction and that an important mediator in this process is the proapoptotic Bcl-2 family Bim (26). To identify additional gene elements that might be involved in this process, we interrogated the genomic response of the HDACI inhibitor SAHA and focused on the gene expression changes of 220 apoptosis-related genes. Our study is in line with previous studies showing that HDACIs affect the expression of multiple genes involved in both the intrinsic apoptotic and receptor-mediated apoptotic pathways (31). Consistent with a possible role of E2F1 in the SAHA response, a number of E2F1 targets such as BCL2L11 (encodes Bim), CASP3, APAF1, TNFRSF10D, and MAP3K5.

**FIGURE 6.** Suppression of ASK1 expression inhibits SAHA-induced apoptosis upon E2F1 activation. A, ER-E2F1-expressing cells were transfected with NC siRNA and ASK1 siRNA and treated with SAHA in the presence or absence of 4-OHT. Cell death was determined by fluorescence-activated cell sorting analysis. Mean results of three independent experiments are shown with S.D. B, cells were treated as in panel A. The levels of ASK1, Bim, phospho-p38, p38, phospho-JNK, and JNK were analyzed by Western blotting. C, model of feedback regulation of ASK1 on the E2F1-Bim apoptotic pathway in response to SAHA treatment.
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ACKNOWLEDGMENTS—We thank Dr. Kristian Helin for the ER-E2F1 plasmids, inductions that is central to inducing apoptosis.

Although previous microarray studies have indicated that ASK1 might be regulated by E2F1 (37, 38), it is not clear whether ASK1 is directly regulated by E2F1. In addition, its function in the E2F1 pathway has not been studied. We have shown here, using ChIP assay, RNA interference, as well as a dominant-negative mutant of E2F, that ASK1 is a direct target of E2F1 and E2F1 activity is required for ASK1 induction by SAHA. ASK1 is involved in multiple signaling pathways leading to apoptosis (35, 36). It has been reported that ASK1-mediated apoptosis is achieved through the phosphorylation and activation of proapoptotic p38 and JNK signaling pathways in certain cells. In fact, we did not observe increased p38 or JNK phosphorylation in response to SAHA upon E2F1 activation, suggesting that the conventional role of ASK1 in apoptotic function does not contribute to this process. Importantly, we showed that ASK1 knockdown by RNA interference impaired the induction of other E2F1 targets including Bim, p73, and Cyclin E. We thus propose that an important function of ASK1 induction lies in the feedback regulation of E2F1 activity.

E2F1 activity is regulated via various upstream components, including Rb, p16, and cyclin-dependent kinase activity. In addition, E2F1 activity might also be regulated through a feedback mechanism mediated through its target genes. For instance, it has been shown that E2F1 induces the expression of cyclin-dependent kinase inhibitor p27, resulting in negative feedback regulation of E2F1 transcriptional activity through inhibition of cyclin-dependent kinase activity and Rb hyperphosphorylation (39). Consistent with a previous report that ASK1 inhibits Rb function (34), we have shown that Rb-mediated repression of Bim promoter activation by E2F1 can be reversed by ASK1 overexpression. We propose that ASK1 induction by E2F1 provides positive feedback regulation of E2F1 through Rb inhibition. Thus, E2F1 can be regulated by both positive and negative feedback mechanisms through its target genes. The suppression of Bim induction and inhibition of apoptosis induction by SAHA following ASK1 knockdown suggests that ASK1 induction contributes to SAHA-induced cell death by potentiating the E2F1-Bim apoptotic network. Although the role of ASK1 in E2F1-mediated apoptosis needs to be further evaluated, our data suggest that the concomitant inductions of ASK1 and Bim reflect the efficiency of the mechanism through which E2F2 supports sustained Bim induction that is central to inducing apoptosis.

REFERENCES

1. Dyson, N. (1998) Genes Dev. 12, 2245–2262
2. Ishitani, S., Huang, E., Zuanz, H., Spang, R., Leone, G., West, M., and Nevins, J. R. (2001) Mol. Cell. Biol. 21, 4681–4699
3. Ben, R., Cam, H., Takahashi, Y., Volkert, T., Terragni, J., Young, R. A., and Dynlacht, B. D. (2002) Genes Dev. 16, 245–256
4. Lazzarini Denchi, E., and Helin, K. (2005) EMBO Rep. 6, 661–668
5. 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
6. Wu, X., and Levine, A. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3602–3606
7. Pierce, A. M., Gimenez-Conti, B., Schneider-Brousard, R., Martinez, L. A., Conti, G. L., and Johnson, D. G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8858–8863
8. Pan, H., Yin, C., Dyson, N. J., Harlow, E., Yamazaki, L., and Van Dyke, T. (1998) Mol. Cell. 2, 283–292
9. Macleod, K. F., Hu, Y., and Jacks, T. (1996) EMBO J. 15, 6178–6188
10. Zhu, J. W., DeRyckere, D., Li, F. X., Wan, Y. Y., and DeGregori, J. (1999) Cell Growth Differ. 10, 829–838
11. Bates, S., Phillips, A. C., Clark, P. A., Scott, P., Peters, G., Ludwig, R. L., and Vousden, K. H. (1998) Nature 395, 124–125
12. Emonee, A., Roussel, M. F., and Sherr, C. J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3993–3998
13. Kamijo, T., Weber, J. D., Zambetti, G., Zindy, F., Roussel, M. F., and Sherr, C. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8292–8297
14. Steewe, T., and Putzer, B. M. (2000) Nat. Genet. 26, 464–469
15. Sherry, C. J. (1996) Science 274, 1672–1677
16. Sherry, C. J., and McCormick, F. (2002) Cancer Cell 2, 103–112
17. Nebriosa, A., Clarke, N., Voltz, E., Germainin, E., Ambrosion, C., Bontempo, P., Alvarez, R., Schiavone, E. M., Ferrara, F., Bresciani, F., Weiss, A., de Lera, A. R., Gronemeier, H., and Altucci, L. (2005) Nat. Med. 11, 77–84
18. Understed, J. S., Sowa, Y., Xu, W. S., Shao, Y., Dokmanovic, M., Perez, G., Ngo, L., Hsu, G., Jiang, X., and Marks, P. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 102, 673–678
19. Inzinger, A., Moneistri, S., Ronsoni, S., Gelmetti, V., Marchesi, F., Viale, A., Altucci, L., Nerli, C., Minucci, S., and Pelicci, P. G. (2005) Nat. Med. 11, 71–76
20. Zhao, Y., Tan, J., Zhuang, L., Jiang, X., Liu, E. T., and Yu, Q. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 16090–16095
21. Yu, Q., He, M., Lee, N. H., and Liu, E. T. (2002) J. Biol. Chem. 277, 13059–13066
22. Eisen, M. B., Spellman, P. T., Brown, P. O., and Botstein, D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14863–14868
23. Urist, M., Tanaka, T., Poyurovykh, M. V., and Prives, C. (2004) Genes Dev. 18, 3041–3054
24. Vigo, E., Muller, H., Prosperini, E., Hateboer, G., Cartwright, P., Moroni, C., and Helin, K. (1999) Mol. Cell. Biol. 19, 6379–6395
25. Peart, M. J., Smyth, G. K., van Laar, R. K., Bowtell, D. D., Richon, V. M., Marks, P. A., Holloway, A. J., and Johnstone, R. W. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 3697–3702
26. Sherr, C. J. (2001) Nat. Rev. Mol. Cell. Biol. 2, 731–737
27. Huford, R. K., Jr., Cohbrink, D., Lee, M. H., and Dyson, N. (1997) Genes Dev. 11, 1447–1463
28. Dasgupta, P., Betts, V., Rastogi, S., Joshi, B., Morris, M., Brennan, B., Ordonez-Ercan, D., and Chellappan, S. P. (2004) J. Biol. Chem. 279, 38762–38767
29. Tobiume, K., Matsuzawa, A., Takahashi, T., Nishitoh, H., Morita, K., Takeda, K., Minowa, O., Miyazono, K., Noda, T., and Ichijo, H. (2001) EMBO Rep. 2, 222–228
30. Mizumura, K., Takeda, K., Hashimoto, S., Horie, T., and Ichijo, H. (2005) J. Cell Physiol.
31. Muller, H., Bracken, A. P., Vernell, R., Moroni, M. C., Christians, F., Grassilli, E., Prosperini, E., Vigo, E., Oliner, J. D., and Helin, K. (2001) Genes Dev. 15, 267–285
32. Shanell, J., Stiewe, T., Theseling, C. C., Peter, M., and Putzer, B. M. (2002) Nucleic Acids Res. 30, 1859–1867
33. Wang, C., Hou, X., Mehtapatra, S., Shao, Y., Cress, W. D., Pledger, W. J., and Chen, J. (2003) J. Biol. Chem. 280, 12339–12343