Arsenic trioxide (As$_2$O$_3$) is highly effective for the treatment of acute promyelocytic leukemia, even in patients who are unresponsive to all-trans-retinoic acid therapy. As$_2$O$_3$ is believed to function primarily by promoting apoptosis, but the underlying molecular mechanisms remain largely unknown. In this report, using cDNA arrays, we have examined the changes in gene expression profiles triggered by clinically achievable doses of As$_2$O$_3$ in acute promyelocytic leukemia NB4 cells. CASPASE-10 expression was found to be potently induced by As$_2$O$_3$. Accordingly, caspase-10 activity also substantially increased in response to As$_2$O$_3$ treatment. A selective inhibitor of caspase-10, Z-AEVD-FMK, effectively blocked caspase-3 activation and significantly reduced As$_2$O$_3$-induced apoptosis. Interestingly, treatment of NB4 cells with As$_2$O$_3$ markedly increased histone H3 phosphorylation at serine 10, an event that is associated with acetylation of the lysine 14 residue. Chromatin immunoprecipitation assays revealed that As$_2$O$_3$ potently enhances histone H3 phosphoacetylation at the CASPASE-10 locus. These results suggest that the effect of As$_2$O$_3$ on histone H3 phosphoacetylation at the CASPASE-10 gene may play an important role in the induction of apoptosis and thus contribute to its therapeutic effects on acute promyelocytic leukemia.

Acute promyelocytic leukemia (APL) accounts for 10–15% of adult myeloid leukemias with 3,500–5,500 new cases diagnosed annually (1, 2). The vast majority of APL patients harbor the chromosomal translocation t(15,17)(q22,q21) involving the retinoic acid receptor a (RARA) gene on chromosome 15 and the promyelocytic leukemia (PML) gene on chromosome 17, generally giving rise to two fusion genes, PML-RARα and RARα-PML (1, 3). Studies using transgenic mice have demonstrated that the protein product of the PML-RARα fusion gene is primarily responsible for the leukemogenic property of this characteristic translocation (4, 5). All-trans-retinoic acid (ATRA), a physiologically active derivative of vitamin A, can induce complete remission in most APL patients associated with an enhancement of differentiation pathways (6). Recent studies (7–9) have provided strong evidence that the induction of leukemia by the PML-RARα protein relies on its ability to repress gene transcription by recruiting transcription repressor complexes. Pharmacological doses of ATRA stimulate the release of the transcription repressor complexes from PML-RARα, thereby activating the transcription of genes critical for normal granulocytic differentiation. However, ATRA is not curative, and resistance rapidly develops usually within 10 months of therapy (2, 6). Therefore, alternative therapies are necessary.

Recently, As$_2$O$_3$ was identified as a potent anti-leukemic agent for treating not only newly diagnosed but also relapsed APL patients (2, 10–13), and it is remarkably effective in ATRA-refractory patients (2, 12). However, the mechanisms underlying its therapeutic effects are not well understood (2, 11, 13). As$_2$O$_3$ induces a drastic reorganization of the nucleus characterized by restoration of intact PML nuclear bodies followed by a progressive degradation of the PML-RARα fusion protein (14, 15). Although As$_2$O$_3$ induces partial differentiation of leukemic cells (13), the apoptosis-promoting effect of As$_2$O$_3$ is believed to play a prominent role in the remission of APL as large numbers of apoptotic myelocytes have been documented in bone marrow and peripheral blood of As$_2$O$_3$-treated APL patients (2, 11, 13). As$_2$O$_3$ has been shown to trigger the early collapse of mitochondrial transmembrane potential (16, 17), the release of cytochrome c (10), and the activation of caspases-1, -2, -3, and -8 (2, 16, 18).

To elucidate the key molecular events involved in mediating As$_2$O$_3$-induced apoptosis, we have employed the APL-derived NB4 cell line exposed to clinically relevant concentrations of As$_2$O$_3$ and analyzed the changes in gene expression profiles using cDNA array. CASPASE-10 was among the genes upregulated with this treatment. Interestingly, treatment with As$_2$O$_3$ also resulted in a significant increase in histone H3 phosphoacetylation, an event that is associated with altered gene transcription (19–21). Importantly, selective inhibition of caspase-10 substantially abolished As$_2$O$_3$-triggered caspase-3 activation and significantly reduced As$_2$O$_3$-induced apoptosis. Chromatin immunoprecipitation (ChIP) assays revealed that As$_2$O$_3$ potently enhances histone H3 phosphoacetylation at the CASPASE-10 locus. These results suggest that As$_2$O$_3$-induced histone H3 phosphoacetylation may be implicated in altering gene transcription profile and apoptosis and play an important role in the therapeutic effect of As$_2$O$_3$ on APL.

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Treatment, and Assessment of Apoptosis—** NB4 cells were cultured in RPMI 1640 medium with 10% fetal bovine serum, 10 mM HEPES, and 2 mM glutamine. As$_2$O$_3$ was from Sigma, and Z-AEVD-FMK was from Calbiochem.

**Pharmacological Doses of ATRA**

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FMK was from Enzyme Systems (Livermore, CA). Flow cytometry and estimation of sub-G1, apoptotic populations were performed as described previously (22). Where indicated, percentages of dead cells were assessed by direct cell counting after trypan blue staining using a hemacytometer. For caspase activity determinations, 100-μg aliquots of cell lysate were prepared and used following the manufacturers’ recommendations to assay caspase-2 (BioVision, Mountain View, CA), caspase-3 (Molecular Probes, Eugene, OR), or caspase-10 activity (R&D, Minneapolis, MN) by fluorometry.

Northern and Western Blotting—Total RNA was isolated using a Nucleospin RNA II isolation kit (Clontech, Palo Alto, CA), and Northern blot analysis was performed as described previously (23). Caspase-10 mRNA was detected through the simultaneous use of two radiolabeled oligonucleotides (5′-CAGGAATACCTGCTCTGCAGGGAA-GTGGGTTGCTGTCAGGGTTTCAGA-3′ and 5′-TGGGCTATGATATAA-GAGAGTTCTCAGAGGGAAAGGTTTCCTC-3′) as probes. Signals were quantitated with a PhosphorImager (Amersham Biosciences).

Caspases-2, -3, and DNA fragmentation factor-45 were detected by Western blot analysis using monoclonal antibodies (Transduction Laboratories, Lexington, KY). Caspase-8 was detected by Western blotting using a rabbit polyclonal antibody (BD Biosciences). Active caspase-3 was detected by Western blotting using a rabbit polyclonal antibody (BD Biosciences). Active caspase-3 was detected by Western blotting using a rabbit polyclonal antibody (BD Biosciences). Active caspase-3 was detected by Western blotting using a rabbit polyclonal antibody (BD Biosciences). Active caspase-3 was detected by Western blotting using a rabbit polyclonal antibody (BD Biosciences).

**RESULTS**

**As$_2$O$_3$ Stimulates Apoptosis and Induces CASPASE-10 Expression in NB4 Cells**—Treatment of NB4 cells with As$_2$O$_3$ led to a time-dependent cleavage of caspase-2 and caspase-3 as indicated by the progressive decrease in the unprocessed caspases. However, it did not affect the expression of another apoptosis-related protein, DNA fragmentation factor-45 (Fig. 1A). Confirming the previous report that As$_2$O$_3$ activates caspase-3 in APL cells in vivo (12), we observed a substantial increase in caspase-3 activity after 24 h in As$_2$O$_3$-treated NB4 cells. The activity of caspase-3 reached its maximal levels at 48–72 h (Fig. 1B). In addition, treatment with As$_2$O$_3$ caused...
the appearance of an apoptotic sub-G1 population (Fig. 1C) and a marked increase in cell death (Fig. 1D). It has been demonstrated that that As2O3 at concentrations above 0.5 μM induces apoptosis in APL cells (10). Consistent with this report, we also observed a substantial increase in caspase-3 activity in cells treated with 0.8 and 1.6 μM As2O3 but not in cells treated with lower doses of As2O3 (0.2 and 0.4 μM). The increase was detected by both Western blotting using an antibody specific for the active/cleaved caspase-3 (Fig. 2A) and fluorometric assays (Fig. 2B). Similarly, a significant increase in caspase-2 activity was detected by fluorometric assays in cells treated with 0.8 and 1.6 μM As2O3 but not in cells treated with lower doses (0.2 and 0.4 μM) (Fig. 2C). The substantial increases in caspase-2 and caspase-3 activity in cells treated with 0.8 and 1.6 μM As2O3 were reflected in a significant increase in trypan blue-positive cells (Fig. 2D). It has been reported that in patients receiving daily intravenous As2O3 infusion, the plasma As2O3 concentration stays within a range between 0.5 and 3 μM for the most time with a peak level between 4.2 and 6.7 μM shortly after infusion (10). Based on this report, we chose a highly effective and clinically relevant dose of 1.6 μM for most of the subsequent analyses.

To understand the molecular basis for the As2O3-induced apoptosis, we performed a large-scale gene expression analysis to identify genes regulated by As2O3. Total RNA was isolated from either untreated or As2O3-treated cells, and radiolabeled reverse transcripts were used to hybridize cDNA arrays containing 1,176 human gene transcripts closely related to a variety of cancers (Atlas Human Cancer Arrays, Clontech). As2O3 consistently caused a ≥2-fold change in the expression of 25 genes on the DNA array, representing ~2% of genes analyzed. Among the most interesting genes up-regulated by As2O3 was CASPASE-10 (Fig. 3A), which has been shown to play an important role in apoptosis (26, 27).

To further characterize the effect of As2O3 on CASPASE-10 expression, Northern blot analysis was performed. Consistent with the cDNA array results, CASPASE-10 mRNA levels increased markedly with the As2O3 treatment, reaching its highest expression (~7-fold) after 12 h of treatment and gradually declining thereafter (Fig. 3B).

The effect of As2O3 on caspase-10 activity was examined using fluorometric assays. As2O3 treatment resulted in a time-dependent increase in caspase-10 activity, which peaked between 48 and 72 h after the addition of As2O3 to the medium, and decreased abruptly thereafter, possibly because of protein degradation during the late stages of apoptosis (Fig. 4A). The activation of caspase-10 by As2O3 was dose-dependent. Significant activation of caspase-10 only occurred in cells treated with 0.8 and 1.6 μM As2O3 (Fig. 4B). Importantly, treatment with the caspase-10 inhibitor Z-AEVD-FMK led to a significant reduction of the apoptosis triggered by As2O3 as indicated by the considerable decrease in cell death (Fig. 4C). These observations demonstrate the important role of caspase-10 in As2O3-mediated cytotoxicity.

To further address the role of caspase-10 in the apoptosis cascade, the effect of the selective caspase-10 inhibitor Z-AEVD-FMK on caspase-3 activation was examined (Fig. 5).
In cells treated with \( \text{As}_2\text{O}_3 \), an ~20-fold increase in caspase-3 activity was detected after 48 h using fluorometric assay. Importantly, the caspase-10 inhibitor Z-AEVD-FMK at a very low concentration of 2 \( \mu \text{M} \) substantially attenuated the \( \text{As}_2\text{O}_3 \)-induced caspase-3 activity, decreasing caspase-3 activity by 75% at the 48-h time point (Fig. 5A). The partial inhibition of caspase-3 by Z-AEVD-FMK was confirmed by Western blot analysis using an antibody that specifically recognizes the active/cleaved caspase-3 (Fig. 5B). It is worth noting that although higher concentrations of Z-AEVD-FMK further decreased caspase-3 activity, even at a high concentration of 50 \( \mu \text{M} \), the caspase-10 inhibitor was unable to completely abolish the \( \text{As}_2\text{O}_3 \)-induced caspase-3 activation (Fig. 5C). Likewise, the high concentrations of Z-AEVD-FMK (50 \( \mu \text{M} \)) only partially blocked the apoptotic process triggered by \( \text{As}_2\text{O}_3 \) (Fig. 5D). These observations suggest that in addition to caspase-10, other early caspase(s) may also contribute to the activation of caspase-3 by \( \text{As}_2\text{O}_3 \). Indeed, a cleaved form of caspase-8 was detected by Western blotting in cells treated with \( \text{As}_2\text{O}_3 \) (Fig. 5B), confirming the earlier report by Kitamura et al. (18) that caspase-8 is rapidly activated in response to \( \text{As}_2\text{O}_3 \) treatment and plays a significant role in \( \text{As}_2\text{O}_3 \)-induced apoptosis.

\( \text{As}_2\text{O}_3 \) Induced Phosphorylation of Serine 10 and Acetylation of Lysine 14 on Histone H3—We have previously shown that arsenite-stimulated MAPK phosphatase-1 induction is associated with an increase of phosphoacetyl-histone H3 on its chromatin (23). To assess whether similar chromatin changes were implicated in the \( \text{As}_2\text{O}_3 \)-mediated induction of apoptosis, we examined histone H3 modification in NB4 cells treated with different doses of \( \text{As}_2\text{O}_3 \) using antibodies specific for various modified histone H3 molecules (Fig. 6A). As shown in Fig. 5A, lower doses of \( \text{As}_2\text{O}_3 \) (0.2 and 0.4 \( \mu \text{M} \)) did not significantly alter the level of either phospho-histone H3 (p-Ser-10) or phosphoacetyl-histone H3 (p-Ser-10/Ac-Lys-14). However, at higher doses (0.8 and 1.6 \( \mu \text{M} \)), \( \text{As}_2\text{O}_3 \) induced a significant increase in both the phospho-histone H3 (p-Ser10) and phosphoacetyl-histone H3 (p-Ser10/Ac-Lys14) levels. The effect of \( \text{As}_2\text{O}_3 \) on histone H3 phosphorylation did not appear to be caused by a nonspecific inhibition of the histone deacetylase activity, because \( \text{As}_2\text{O}_3 \) treatment did not result in a significant increase in either Lys-14-acetylated histone H3 or hyperacetylated histone H3 (Ac-Lys-9/Ac-Lys-14) (Fig. 6A). Histone H3 phosphorylation at Ser-10 and dual modification (phosphorylation at Ser-10 and acetylation at Lys-14) occurred with similar kinetics in \( \text{As}_2\text{O}_3 \)-treated cells (Fig. 6B), supporting the notion that acetylation at the Lys-14 residue and phosphorylation at the Ser-10 residue are coupled events (19). Comparable loading of the crude histone proteins among the various samples was verified by Western blotting using a monoclonal antibody recognizing total histone H3.

\( \text{As}_2\text{O}_3 \) Stimulates the Phosphoacetylation of Histone H3 at the Chromatin of the CASPASE-10 Locus—To examine whether histone H3 modification occurs in vivo at the chromatin of \( \text{CASPA}SE-10 \), ChIP assays were performed using an antibody specifically against dually modified histone H3 (23). To ascertain that equal amounts of starting chromatin material were used, DNA amplifications were carried out by real-time PCR using input chromatin DNA (not subject to immunoprecipitation) from both untreated and \( \text{As}_2\text{O}_3 \)-treated cells and a pair of primers specific for \( \text{CASPA}SE-10 \) (Fig. 7A, left panel). The very similar \( \text{CASPA}SE-10 \) amplification curves and \( C_T \) values, which represent the number of PCR cycles required to reach a threshold set arbitrarily at 0.5, from both the untreated and \( \text{As}_2\text{O}_3 \)-treated input DNA indicated that the starting chromatin materials used in the subsequent ChIP assays were comparable. To detect histone H3 phosphoacetylation, chromatin solutions were incubated with a rabbit polyclonal antibody specifically recognizing phosphoacetyl-histone H3 (phospho-
Ser-10/Ac-Lys-14) and protein A. Genomic DNA present in the immunoprecipitates was extracted and analyzed by real-time PCR using primers specific for \textit{CASPASE-10} (Fig. 7, right panel). A substantial enhancement in histone H3 phosphoacetylation at the \textit{CASPASE-10} gene was detected after As$_2$O$_3$ treatment. The $C_T$ value was reduced from 32.6 (untreated) to 28.7 (As$_2$O$_3$-treated), corresponding to a 15-fold increase in chromatin-bound phosphoacetyl-histone H3. The specificity of the PCR reaction was indicated by the presence of a single band of the anticipated size on agarose gel after separating the final PCR products (Fig. 7B, upper panel). To test the specificity of the immunoprecipitation assay procedures, mock ChIP assays were carried out using rabbit pre-immune serum. The \textit{CASPASE-10} sequence was virtually undetectable in the mock immunoprecipitates (Fig. 7B, upper panel). Importantly, As$_2$O$_3$ appeared to stimulate histone H3 phosphoacetylation only at the chromatin of a small subset of genes\(^2\) including \textit{CASPASE-10} (Fig. 7). Phosphoacetyl-histone H3 was absent at the transcriptionally inactive \textit{\textit{β}}\textit{-GLOBIN} chromatin in both untreated and As$_2$O$_3$-treated cells (Fig. 7B, lower panel), further illustrating the specificity of such modification in As$_2$O$_3$-stimulated cells.

\section*{DISCUSSION}

As$_2$O$_3$ has been shown to constitute effective therapy for APL patients relapsing from or refractory to ATRA treatment (10, 13). As$_2$O$_3$ and ATRA appear to exert their anti-leukemic effects through partially overlapping mechanisms including those leading to the restoration of granulocyte differentiation (6, 10, 12, 13, 28). However, As$_2$O$_3$ and ATRA therapies exhibit important differences. For example, As$_2$O$_3$ can induce complete remission in t(15,17)-positive APL patients, regardless of their sensitivity to ATRA. In addition, As$_2$O$_3$ can induce a high rate of molecular conversion from positive to negative for t(15,17) translocation. This is indicative of the elimination of the neoplastic progenitors that is unusual after treatment with ATRA alone (10, 12, 13). Recent studies (2, 12, 13, 29) have provided considerable support for the notion that the anti-leukemic effect of As$_2$O$_3$ lies primarily in its ability to induce apoptosis. Both \textit{in vivo} and \textit{in vitro}, As$_2$O$_3$ has been found to induce apoptosis of APL cells at clinically achievable concentrations (10–13). Here, we have confirmed that As$_2$O$_3$-induced apoptosis is associated with activation of the late caspases includ-

\(^2\) J. Li and Y. Liu, unpublished observations.
even at a high concentration of 50/\text{H9262} modification at 24 h. Tone H3 modification was examined via Western blotting using anti-
were extracted and separated on NuPAGE gel using MES buffer. His-
ment and plays a role in As2O3-induced apoptosis (18). It is
(Figs. 4
\text{caspase-3} activation (Fig. 5), whereas caspase-8 was still active, resulting in attenuated
ence of Z-AEVD-FMK, caspase-10 activity was inhibited,
caspase-8 contribute to the activation of caspase-3. In the pres-
possible that both caspase-10 and the structurally related
caspase-8 is activated in APL cells in response to As2O3 treat-
abolish caspase-3 activation, although it significantly delayed
consistent with our results that Z-AEVD-FMK did not totally
induced caspase-3 activation. Nevertheless, it is important to
note that caspase-10 is probably only partially responsible for
executing As2O3-induced apoptosis, because Z-AEVD-FMK even at a high concentration of 50 µM only partially inhibited
the apoptotic process (Figs. 4B and 5D). This observation is
consistent with our results that Z-AEVD-FMK did not totally abolish caspase-3 activation, although it significantly delayed caspase-3 activation (Fig. 5). Indeed, it has been reported that caspase-8 is activated in APL cells in response to As2O3 treat-
ment and plays a role in As2O3-induced apoptosis (18). It is
possible that both caspase-10 and the structurally related caspase-8 contribute to the activation of caspase-3. In the presence of Z-AEVD-FMK, caspase-10 activity was inhibited, whereas caspase-8 was still active, resulting in attenuated caspase-3 activation (Fig. 5, A–C) and decreased apoptosis (Figs. 4C and 5D).
A critical role for histone deacetylase-dependent transcriptional repression by PML-RARα has recently been established in the pathogenesis of APL (7–9, 30). Indeed, the stimulation of histone modification is emerging as a common property shared by many effective APL therapeutic regimens including ATRA and various histone deacetylase inhibitors (11, 31–33). Here, we have examined the effect of As2O3 on histone H3 modification. Clinically relevant doses of As2O3 were found to potentially enhance histone H3 phosphorylation and phosphoacetylation (Fig. 5), modifications that are similar to those reported to occur following treatment with mitogens and stress agents (20, 23, 34). The role that histone H3 phosphorylation and acetylation plays in activating gene transcription has recently been documented (20, 34, 35). Here, we show that As2O3 strongly increases histone H3 phosphoacetylation at the CASPASE-10 locus (Fig. 6). Thus, enhanced histone phosphoacetylation at the CASPASE-10 chromatin is likely to contribute to the observed increase in CASPASE-10 expression, caspase-10 activation, and subsequent apoptosis.
As2O3 may promote apoptosis through multiple mechanisms. Caspase-10 activation is probably only part of the apoptotic execution machinery, because As2O3 causes 2-fold change in the expression of only ~2% of genes on the DNA array (25 genes of 1,176). In this regard, As2O3 enhances gene expression in a relatively selective manner. This finding is consistent with our observation that neither As2O3 significantly altered the levels of hyperacetylated (Ac-Lys-9/Ac-Lys-14) at the global levels (Fig. 5), nor did it induce histone H3 acetylation at the β-GLOBIN locus (Fig. 6B). Although it is unclear how the relative specificity of As2O3 is achieved, we can speculate that it may involve the degradation of the PML-RARα and a protein kinase that can phosphorylate histone H3 at Ser-10. Interestingly, it has been shown that As2O3 induces the phosphorylation of the SMRT protein, the dissociation of SMRT from its nuclear receptor partner including PML-RARα, and the trans-
port of SMRT from the nucleus into the cytoplasm through a MAPK-mediated mechanism (36). Moreover, it has been reported that As₂O₃-induced apoptosis of NB4 cells is partially blocked by a pharmacological inhibitor of MAPK kinase; thus, further supporting a significant role of MAPK in the process (36). MAPK can activate two downstream kinases, MSK1 and P90RSK2 (37, 38), which are shown to directly phosphorylate histone H3 at Ser-10. It has been shown that both epidermal growth factor and stress stimulate histone H3 phosphoacetylation at the promoters of ATRA-regulated genes (39, 40). MSK1 is by promoting histone H3 phosphoacetylation and degradation of PML-RARα at the promoters of ATRA-regulated genes through a process regulated at least in part by MAPKs. Further characterization of the mechanisms involved in this process may aid in the development of more effective less toxic therapeutic regimens for APL.

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REFERENCES

1. de The, H., and Chelbi-Alix, M. K. (2001) Oncogene 20, 7136–7139
2. Soignet, S. L. (2001) Oncologist 6, Suppl. 2, 11–16
3. Goddard, A. D., Borrow, J., Freemont, P. S., and Solomon, E. (1991) Science 254, 1371–1374
4. Brown, D., Kogan, S., Lagasse, E., Weissman, I., Alcalay, M., Pelcic, P. G., Atwater, S., and Bishop, J. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2551–2556
5. He, L. Z., Trizic, C., Rivi, K., Peruzzi, D., Pelcic, P. G., Soares, V., Cattoretti, G., and Pandolfi, P. P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5302–5307
6. Degos, L., and Wang, Z. Y. (2001) Oncogene 20, 7140–7145
7. Powell, B. L. (2001) Curr. Opin. Oncol. 13, 8–13
8. Kogan, S. C., Hong, S. H., Shultz, D. B., Privalsky, M. L., and Bishop, J. M. (2000) Blood 95, 1541–1550
9. Piazza, F., Gurrieri, C., and Pandolfi, P. P. (2001) Oncogene 20, 7216–7222
10. Chen, G. Q., Zhu, J., Shi, X. G., Ni, J. H., Zhong, H. J., Jin, X. L., Teng, W., Li, X. S., Xiong, S. M., Shen, Z. X., Sun, G. L., Ma, J., Zhang, P., Zang, T. D., Gatin, C., Naoe, T., Chen, S. J., Wang, Z. Y., and Chen, Z. (1996) Blood 88, 1052–1061
11. Chen, Z., Chen, G. Q., Shen, Z. X., Chen, S. J., and Wang, Z. Y. (2001) Semin. Hematol. 38, 26–36
12. Soignet, S. L., Maslak, P., Wang, Z. G., Jhanwar, S., Calleja, E., Dardashiti, L. J., Corne, D., DelBasso, A., Gahrniere, J., Scheinberg, D. A., Pandolfi, P. P., and Warren, R. P., Jr. (1998) N. Engl. J. Med. 339, 1341–1348
13. Zhang, T. D., Chen, G. Q., Wang, Z. G., Wang, Z. Y., Chen, S. J., and Chen, Z. (2001) Oncogene 20, 7146–7153
14. Shao, W., Funelli, M., Ferrara, F. F., Riccioni, R., Rosenauer, A., Davison, K., Lamp, W. W., Waxman, S., Pelcic, P. G., Lo, C. F., Avizziati, G., Testa, U., Peschle, C., Gambacorti-Passerini, C., Neri, C., and Miller, W. H., Jr. (1998) J. Natl. Cancer Inst. 90, 124–133
15. Zhu, J., Lallemant-Breitenbach, V., and de The, H. (2001) Oncogene 20, 7257–7265
16. Jing, Y., Bai, D., Chalmers-Redman, R. M., Tattton, W. G., and Waxman, S. (1999) Blood 94, 2102–2111
17. Cai, X., Shen, Y. L., Zhu, Q., Jia, P. M., Yu, Y., Zhou, L., Huang, Y., Zhang, J. W., Xiong, S. M., Chen, S. J., Wang, Z. Y., Chen, Z., and Chen, G. Q. (2000) Leukemia 14, 282–270
18. Kitamura, K., Minami, Y., Yamamoto, K., Akao, Y., Kiyoi, H., Saito, H., and Naoe, T. (2000) Leukemia 14, 1743–1750
19. Cheung, P., Allis, C. D., and Sassone-Corsi, P. (2000) Cell 103, 263–271
20. Cheung, P., Tanner, K. G., Cheung, W. L., Sassone-Corsi, P., Denu, J. M., and Allis, C. D. (2000) Mol. Cell 5, 905–915
21. Mizzen, C. A., and Allis, C. D. (2000) Science 289, 2280–2289
22. Gong, J., Trapaneo, F., and Darzynkiewicz, Z. (1994) Anal. Biochem. 218, 314–319
23. Li, J., Geroso, M., Butter, D., Barnes, J., Keyse, S. M., and Liu, Y. (2001) Mol. Cell. Biol. 21, 8213–8224
24. Hadano, S., Yanagisawa, Y., Skag, J., Fichter, K., Nasir, J., Martindale, D., Koop, B. F., Scherer, S. W., Nicholson, D. W., Rouleau, G. A., Ikeda, J., and Hayden, M. R. (2001) Genomics 71, 200–213
25. Heuserprumte, M., Berclare, I., Gail, J. L., Van Geet, C., Ferrant, A., Malhaire, Y., Thonnard, J., Vaerman, J. L., and Philippe, P. (1996) Hum. Genet. 98, 77–79
26. Wang, J., Chun, H. J., Wong, W., Spencer, D. M., and Lenardo, M. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 13884–13888
27. Wang, J., Zheng, L., Lebot, A., Chan, F. K., Dale, J., Sneller, M., Yau, X., Puck, J. M., Straus, S. E., and Lenardo, M. J. (1999) Cell 98, 47–58
28. Tallman, M. S., Andersen, J. W., Schiffer, C. A., Appelbaum, F. R., Feusner, P. H., Ogden, A., Shepherd, L., Willman, C., Bloomfield, C. D., Rowe, J. M., and Wiernik, P. H. (1997) N. Engl. J. Med. 337, 1021–1028
29. Warrell, R. P., Jr. (1999) Haematologica 84, 75–77
30. Pandolfi, P. P. (2001) Oncogene 20, 3116–3127
31. Warrell, R. P., Jr., He, L. Z., Richon, Y., Calleja, E., and Pandolfi, P. P. (1998) J. Natl. Cancer Inst. 90, 1621–1625
32. He, L. Z., Tolentino, T., Grayson, P., Zhong, S., Warrell, R. P., Jr., Rikfink, R. A., Marks, P. A., Richon, V. M., and Pandolfi, P. P. (2001) J. Clin. Invest. 108, 1211–1330
33. Amin, M. H., Seedor, S., and Alkan, S. (2001) Br. J. Haematol. 115, 287–297
34. Clayton, A. L., Rose, S., Barratt, M. J., and Mahadevan, L. C. (2000) EMBO J. 19, 3714–3726
35. Nowak, S. J., and Corves, V. G. (2000) Genes Dev. 14, 3003–3013
36. Hong, S. H., Yang, Z., and Privalsky, M. L. (2001) Mol. Cell. Biol. 21, 7172–7182
37. Sassone-Corsi, P., Mizzen, C. A., Cheung, P., Crosio, C., Monaco, L., Jacquot, S., Hanauer, A., and Allis, C. D. (1999) Science 285, 886–891
38. Thomson, S., Clayton, A. L., Hazzalin, C. A., Rose, S., Barratt, M. J., and Mahadevan, L. C. (1999) EMBO J. 18, 4779–4793