Nuclear phosphoprotein HMGA1a, high mobility group A1a, (previously HMGI) has been investigated during apoptosis. A change in the degree of phosphorylation of HMGA1a has been observed during apoptosis induced in four leukemic cell lines (HL60, K562, NB4, and U937) by drugs (etoposide, camptothecin) or herpes simplex virus type-1. Both hyper-phosphorylation and de-phosphorylation of HMGA1a have been ascertained by liquid chromatography-mass spectrometry. Hyper-phosphorylation (at least five phosphate groups/HMG A1a molecule) occurs at the early apoptotic stages and is probably related to HMGA1a displacement from DNA and chromatin release from the nuclear scaffold. De-phosphorylation (one phosphate or no phosphate groups/HMGA1a molecule) accompanies the latter formation of highly condensed chromatin in the apoptotic bodies. We report for the first time a direct link between the degree of phosphorylation of HMGA1a protein and apoptosis according to a process that involves the entire amount of HMGA1a present in the cells and, consequently, whole chromatin. At the same time we report that variously phosphorylated forms of HMGA1a protein are also mono-methylated.

Among nonhistone nuclear proteins of mammalian cells, a family of three proteins called HMGA1a, HMGA1b, and HMGA2 (previously termed HMGI, HMGY, and HMGI-C, respectively) have aroused great interest in many laboratories over the last few years, due to the variety of biological processes in which they are involved (Refs. 1–4 and references therein). HMGA1a and HMGA1b are very similar, differing by only 11 amino acid residues, because they are the splicing products of the same gene (5), whereas HMGA2 is the product of another gene (6). These proteins are composed of about a hundred residues, and all contain three characteristic short basic regions, called AT-hooks, that interact with AT-rich stretches of DNA in the minor groove (7, 8).

A property that is characteristic not only of the three proteins under discussion, but also of other HMG proteins, is a C-terminal domain having a very high content of acidic residues (1, 9). In HMGA1a, HMGA1b, and HMGA2 the acidic C-terminal domain is constitutively phosphorylated in vivo by CK2 (10–12), but additional sites for cell-cycle-dependent phosphorylation by other kinases have also been reported (13–15). Levels of high MVA1a, HMGA1b, and HMGA2 proteins have been found in embryonic cell lines or tissues as well as in neoplastic cell lines and tumors, but they are absent or expressed at very low levels in normal cells (1, 12, 16–23).

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EXPERIMENTAL PROCEDURES

Cell Cultures and Treatments—The four cell lines HL60, K562, NB4, and U937 were cultured in RPMI 1640 medium containing 10% fetal bovine serum at 37 °C. Induction of apoptosis was carried out by three base-unpairing regions; kbp, kilobase pair(s); HSV-1, herpes simplex virus type-1; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; HPLC, high pressure liquid chromatography.
Apoptosis and HMGA1a Phosphorylation

An Electrophoretic Retarded Band of HMGA1a Protein Appears in Leukemic Cells during Apoptosis—Protein extracts from four leukemic cell lines (K562, HL60, NB4, and U937) have been analyzed by acetic acid/urea electrophoresis, which showed, in the extract from apoptotic cells, a double band at the migration position of the HMGA1a protein, in comparison with the single band obtained from control cells (Fig. 1A). To verify if the retarded band could be due to the new apoptotic form(s) of HMGA1a, the regions comprising HMGN2 and HMGA1a of the acid/urea first dimension (Fig. 1A) were analyzed by SDS two-dimensional analysis. The pattern obtained was blotted for Western identification using a polyclonal antibody raised against an N-terminal peptide common to both HMGA1a and HMGA1b proteins (5, 23) (Fig. 1B). From the result reported in Fig. 1B, it is evident that the antibody identified both normal HMGA1a and HMGA1b proteins as well as the retarded band, which can consequently be considered as one or more new forms of HMGA1a appearing during apoptosis.

Both Hyper-phosphorylation and De-phosphorylation of HMGA1a Takes Place in Leukemic Cells during Apoptosis—To understand the nature of the HMGA1a form(s) generated during apoptosis, the same extracts of Fig. 1 have been separated by HPLC, and the eluted protein was detected by both UV absorption (Fig. 2, A and B) and LC-MS total ion count (Fig. 2, C and D). LC-MS molecular masses of HMGA1 components have been determined (Fig. 2, E and F): control K562 cells contain two forms of HMGA1a protein having masses of 11,745.9 and 11,826.0 Da, respectively. The predicted mass of human HMGA1a protein without modifications (i.e. 106 amino acid residues, no initial methionine) is 11,544.8 Da, which becomes 11,586.8 Da if N-terminal acetylation at the first serine is assumed, as found in preceding studies. It is possible to infer this also from both the mass spectrometric data recently reported by Reeves and coworkers (11, 19, 29) and by tryptic digestion experiments as reported below. Because one phosphate group increases the mass of a protein by 80 Da, the two forms of HMGA1a protein found in control K562 can be considered as the di-phosphorylated (found, 11,745.9 Da; calculated, 11,746.8 Da) and the tri-phosphorylated (found, 11,826.0 Da; calculated, 11,826.8 Da) modifications. This result is in agreement with preceding data that reported in vitro and in vivo phosphorylation of the three last serines at the C-terminal of both HMGA1a and HMGA1b proteins (i.e. serines 98, 101, and 102) as consensus sites for CK2 (10–12). The extract from K562 apoptotic cells contains, in addition to the di-phosphorylated and tri-phosphorylated forms present in the control cells,
other forms whose molecular masses are consistent with hyper-phosphorylated forms (four phosphate groups, 11,905.4 Da and five phosphate groups, 11,986.0 Da) and with de-phosphorylated forms (one phosphate group, 11,665.4 Da and zero phosphate group, 11,585.5 Da). Assuming that LC-MS peak intensity is approximately proportional to the amount of protein, it is possible to estimate that the sample from K562 control cells of Fig. 2E contains about 50% of di-phosphorylated HMGA1a protein and about 50% of tri-phosphorylated, whereas in the apoptotic sample (Fig. 2F) the main form is tri-phosphorylated, all other forms being present at lower percentage.

LC-MS data shown in Fig. 3A indicate that in all four analyzed cell lines the main forms of HMGA1a are those that are di-phosphorylated and tri-phosphorylated. The same data from apoptotic cells (Fig. 3B) show both hyper-phosphorylated and de-phosphorylated forms. However, inspection of the data indicates that the degree of phosphorylation is not only cell type-dependent but also related to the percentage of apoptotic cells present in the analyzed cell sample. In fact, di-phosphorylated and tri-phosphorylated forms of HMGA1a are the main molecules in both HL60 and NB4 cells, which contain 98% of apoptotic cells, whereas in K562, having 77% of apoptotic cells, the tri-phosphorylated molecule is the main form accompanied by 50% of di-phosphorylated, whereas in the control sample (Fig. 2F) the main form is tri-phosphorylated, all other forms being present at lower percentage.

To obtain further confirmation that the identified forms of HMGA1a protein are due to phosphorylation, HPLC-purified protein samples from both control and apoptotic K562 cells were alkaline phosphatase-treated. This removed phosphate groups from all phosphorylated forms of HMGA1a and gave only the unphosphorylated, N-terminally acetylated molecule, having molecular masses of 11,586.2 Da (control) and 11,585.9 Da (apoptotic) (data not shown).

Tryptic Digestion of HMGA1a from both Control and Apoptotic K562 Cells: Identification of Phosphorylated Peptides—Tryptic fragments of HPLC-purified HMGA1a from both control and apoptotic K562 cells were analyzed by LC-MS spectrometry. 26 different peptides, spanning the entire HMGA1a sequence, have been identified and are reported in Fig. 4A. For each of the identified peptides, a search for the phosphorylated forms was carried out in both control and apoptotic samples, and the following conclusions were reached: (i) in both control and apoptotic cells N-terminal fragments were acetylated; (ii) in control K562 cells the main phosphorylated fragment was the 88- to 106-amino acid peptide present as the...
di- or tri-phosphorylated forms; (iii) in apoptotic K562 cells the 88- to 106-amino acid peptide was mainly unphosphorylated; one phosphate group has been detected in fragments 1–23, 73–83, and 74–83; mono- and di-phosphorylated forms have been identified for the fragments 26–54, 30–54, and 30–57. This means that the additional phosphates in the hyper-phosphorylated forms are located inside the protein molecule and that the de-phosphorylation process starts by removing the phosphate groups from the C-terminal side.

On the basis of literature data (10–14, 29, 30), we have drawn the scheme shown in Fig. 4A, where all the putative sites for hyper-phosphorylation of the HMGA1a protein are shown together with the constitutive C-terminal modified serines. Data summarized in Fig. 4B indicate that it could be
possible to find HMGA1a molecules bearing up to seven phosphate groups at a time. LC-MS data shown in Fig. 3B clearly indicate up to five phosphate groups; however, very low amounts of HMGA1a having six or seven phosphate groups have been detected (data not shown). In any case, it must be pointed out that phosphorylation and de-phosphorylation processes could partially overlap.

Hyper-phosphorylation of HMGA1a Precedes its De-phosphorylation during Apoptosis—From preceding data, results show that the hyper-phosphorylation of HMGA1a and its de-phosphorylation should be related to different events that take place at different times during apoptosis and concern different regions of the molecule. To clarify this point, we have carried out a time course at the beginning of apoptotic induction. To this end, HL60 cells were treated with 136 μM etoposide for 30 min and after washing left to proceed toward apoptosis quantified by FACSscan (%). The degree of phosphorylation of HMGA1a protein was evaluated at the beginning of the experiment (Control, 0 h), after 30 min of etoposide treatment, and after 1, 2, and 3 h from washing. Phosphorylated forms are reported as follows: 0P, 0+1P (A); 2P + 3P (B); 4P + 5P (C). All reported values are the results of three independent experiments. B, DNA fragmentation in the time course of apoptosis of HL60 cells was analyzed on 2% agarose gel and visualized with ethidium bromide.

The time course shows that hyper-phosphorylation of HMGA1a starts as soon as etoposide treatment begins, reaching a maximum 1 h after drug removal when the forms having one or no phosphate groups begin to appear. Phosphate groups introduced at an early stage of the apoptotic process are added to the two or three phosphate groups already present at the C-terminal of the molecule and produce the 4P and 5P (or more phosphorylated) modifications. De-phosphorylation starts with the removal of the constitutive phosphate groups, i.e. C-terminal phosphates (serines 98, 101, and 102) probably when phosphorylation inside the HMGA1a molecule is still active. This is the reason why di- and tri-phosphorylated forms can have a constitutive origin (i.e. control cells or cells at very early stages of the process) or an apoptotic origin, deriving from the removal of constitutive phosphate groups from molecules bearing four or five (or more) phosphates (i.e. hyper-phosphorylated forms). Further support for the hypothesis that hyper-phosphorylation of HMGA1a protein should be related to the early events of the apoptotic process derives from the electrophoretic analysis of the DNA reported in Fig. 5B where the maximum level of HMGA1a phosphorylation observable after 1 h does correspond to high molecular weight digested DNA, whereas the subsequent de-phosphorylation process correlates with the formation of low molecular weight DNA fragments.

Caspase Inhibition Evidences HMGA1a Hyper-phosphorylation as an Early Event of Apoptosis—It is well known that topoisomerase I and II poisons such as etoposide and camptothecin cause apoptosis through cell cycle block and activation of a group of cysteine proteases called caspases (31–35). Inhibition of caspase activity slows down or stops the advance of the apoptotic process and could allow one to obtain information on its very early events. Therefore, we used the caspase inhibitor Z-VAD-fmk (35–38) on HL60 etoposide-treated cells and both DNA fragmentation and HMGA1a phosphorylation were analyzed. To understand to which nuclear morphologic change phosphorylation of HMGA1a protein could be related during apoptosis, we carried out microscopic observation of aliquots of the same cells stained with 4,6-diamidino-2-phenylindole. Fig. 6A shows that using 25 μM Z-VAD-fmk (lane 2) DNA fragmentation is almost blocked, and small differences in the chromatin status are observable comparing representative cells from the control to the 25 μM Z-VAD-fmk-treated cells (Fig. 6B, lane 1 and 2). Moreover, mass data (Fig. 6C, 2) show that hyper-phosphorylated forms (4P and 5P) have been produced. If a lower Z-VAD-fmk concentration is used (10 μM, Fig. 6A, lane 3), an increased digestion of DNA is observable, but it is lower, however, than the substantial nucleosomal cleavage reached after 2 h of etoposide treatment in the absence of Z-VAD-fmk (Fig. 6A, lane 4). Consistently, an increased level of hyper-phosphorylation has been detected in this protein sample: note that the 4P peak in mass spectrum 3 (Fig. 6C) has the same intensity as the 2P peak. At this stage, in which a clear beginning of DNA fragmentation is seen (Fig. 6A, lane 3), chromatin starts to condense showing a typical alteration of nuclear organization (Fig. 6B, 3) but not yet forming well defined apoptotic bodies as those observed after reported 2 h treatment of cells without Z-VAD-fmk (Fig. 6B, 4). In this last sample both hyper-phosphorylation and de-phosphorylation are observable (Fig. 6C, 4), because only about 30% of cells are definitively apoptotic (see Fig. 5A), while the remaining cells are still running through the preceding steps of the apoptotic process. It is then obvious that protein mixtures extracted from such a composite system would contain both hyper-phosphorylated forms (i.e. early stages of apoptosis) and de-phosphorylated forms (i.e. late stage of apoptosis). In conclusion, we think that inhibition experiments using Z-VAD-fmk clearly show that hyper-phos-
phorylation of HMGA1a is an early apoptotic event and, at the same time, demonstrate that the level of phosphorylation of this protein is truly linked to the apoptosis of leukemic cells.

U937 Leukemic Cells Induced to Apoptosis by Herpes Simples Virus 1 (HSV-1) Show Degrees of HMGA1a Phosphorylation Similar to That Found in Cells Induced by Nonviral Agents—To verify that the degree of phosphorylation of HMGA1a during apoptosis is independent of the agent capable of triggering the signal pathway that leads to apoptosis, we analyzed protein extracts from apoptotic U937 cells induced by herpes simplex virus type-1 (HSV-1) (25). In Fig. 7, LC-MS data of the HMGA1a protein from U937 apoptotic cells are compared with those of control mock cells, and it is possible to see that a massive de-phosphorylation of HMGA1a takes place during apoptosis. Moreover, it is noteworthy that methylation of HMGA1a as revealed in drug-induced leukemic cells (Fig. 3B) is also observable in virus-induced U937 cells. We conclude that both alteration of phosphorylation and methylation are related to the apoptotic process per se rather than to the agent used to induce apoptosis.

DISCUSSION

This report concerns the study of post-translational modifications of HMGA1a protein during apoptosis induced in four leukemic cell lines (HL60, K562, NB4, and U937). These cell lines show constitutive expression of the two proteins HMGA1a and HMGA1b, whereas the HMGA2 has not been detected; HMGA1a is the predominant species as compared with HMGA1b. In fact, from Coomassie Blue-stained electrophoretic patterns we evaluated that the ratio HMGA1a/HMGA1b in both HL60 and K562 cells is about 10:1 (data not shown). The present paper deals only with the most abundant protein (i.e. HMGA1a), but a similar behavior was ascertained also for HMGA1b during apoptosis, from both a careful inspection of the Western analysis shown in Fig. 1 and mass data not shown. Electrophoretic patterns have been also used to evaluate the molecular ratio between histone H1 and HMGA1a that indicated a ratio of about 20:1 in control HL60 cells (data not shown). This means that in these cells there is on average one HMGA1a molecule for every 20 nucleosomes, assuming about one histone molecule is bound to the linker DNA of each nucleosome (39).

What could the function for this high amount of HMGA1a protein be? It is not conceivable that it is entirely involved in the formation of specific protein entities that regulate transcription of specific genes.

Involvement of HMGA1a protein has been reported not only at promoter regions of specific genes, where a limited amount of
protein appears to be necessary, but also at more global nuclear structures related to higher order chromatin bound to the nuclear matrix and forming distinct nucleoprotein loops. Such structures have been called MARs (matrix attachment regions) or SARs (scaffold attached regions), and it has been shown that they contain specialized AT-rich DNA regions with high unwinding aptitude termed BURs (base-unpairing regions) (40–42). BURs specifically bind HMGA1a and HMGA1b proteins (43), although this should involve DNA regions different from those bound to histone H1. In fact, it has been reported that HMGA1a displaces histone H1 from chromatin and nucleosome-sensitive chromatin releases HMGA1a, HMGA1b, and HMGA2 proteins but not histone H1 (41, 44, 45). At the same time, in an immunocytochemical study we have demonstrated that topoisomerase IIα and HMGA1a-HMGA1b proteins colocalize in the interphase nucleus of HeLa cells (46). The mutually exclusive localization of histone H1 and HMGA1a protein could account for a different involvement of these two factors in the processes of chromatin condensation/de-condensation, which are in turn related to the phosphorylation of these proteins, both well known substrates for cyclin-dependent kinases p34-Cdc2 and Cdk2 (13, 14, 47). H1 phosphorylation due to these kinases appears to be related to mitosis (48–52) rather than to apoptosis, whereas data from this report indicate a relationship of HMGA1a phosphorylation with apoptosis and preceding data associated HMGA1a phosphorylation by p34-Cdc2 with mitosis (13, 14).

As reported under “Results,” in addition to the C-terminal constitutive phosphates due to CK2, HMGA1a protein bears other phosphate groups that could derive from the action of different kinases. The constitutive C-terminal phosphorylation due to CK2 is not directly involved in DNA binding alteration, although an indirect effect could be elicited by affecting protein tertiary structure (53). On the other hand, hyper-phosphorylation of HMGA1a protein could be related to its displacement from DNA; this results in a more open chromatin structure that is a more accessible substrate for nucleases, which produce large DNA fragments at the very early stages of apoptosis, together with lamin degradation. DNA fragmentation may have the release of MARs from nuclear scaffold as a first step followed by chromatin unfolding that allows progressive DNA digestion. Large DNA fragments (20–50 kbp) should thus be related to HMGA1a hyper-phosphorylation and initial chromatin condensation as shown in Fig. 6. Further DNA fragmentation generates DNA ladders that are characteristic of the highly condensed chromatin of apoptotic bodies. This last event is related to de-phosphorylated HMGA1a protein as shown in Fig. 3B for U937 cells. It is worthwhile to mention that at neither the initial stage of apoptosis nor the later stage of apoptotic bodies formation is there loss of HMGA1a protein (data not shown). We wish to point out that the phosphorylation/de-phosphorylation process evidenced for HMGA1a involves the total amount of protein present in the cell and, consequently, chromatin as a whole. Very recently, phosphorylation of both histones H2B and H2AX has been reported during apoptosis of HL60 cells induced by etoposide (54, 55). Phosphorylation of these histones, detected by 32P autoradiography, concerns only a fraction of the total protein (about 5–10% in the case of histone H2B) and has been related to the early phase of DNA fragmentation during apoptosis. These results are consistent with our data on hyper-phosphorylation of HMGA1a as an early event of apoptosis in which some histones, HMGA1a and HMGA1b proteins, and other proteins of the nuclear scaffold are substrates of a programmed process of phosphorylation that initiates cell death. However, apoptotic hyper-phosphorylation of HMGA1a is a quantitatively more important event both for the number of new phosphorylation sites (at least four) and the involvement of the entire amount of protein. Moreover, the following massive de-phosphorylation, leading to a completely de-phosphorylated form (here observed for the first time), could be one of the events required for an irreversible chromatin condensation, just as the final committed apoptosis is. The de-phosphorylation of HMGA1a protein parallels another de-phosphorylation process recently described for H1 histone during apoptosis of HL60 cells. In fact, Kratzmeier et al. (56) reported that histone H1 sub-types become rapidly de-phosphorylated upon apoptosis induction and interpret this phenomenon as an important event for the process of chromatin condensation and/or chromatin fragmentation. Our data are consistent with the apoptotic de-phosphorylation of histone H1 and demonstrate, for the first time, that phosphorylation/de-phosphorylation of HMGA1a is involved in the apoptotic process and that HMGA1a could be considered as a structural element in the chromatin of leukemic cells. Moreover, a characteristic mono-methylation of HMGA1a has been evidenced that could reserve further information on stress-exposed cells.

We have demonstrated that, at least in the cells studied, alteration in the degree of phosphorylation of HMGA1a is independent of the agent that induces apoptosis, i.e. drug or virus. However, it is necessary to recall that HMGA1a protein is not present or present at very low levels in normal cells, whereas its expression is increased in transformed cells. Therefore, the link between alteration of constitutive HMGA1a phosphorylation, chromatin, and apoptosis should refer only to cells characterized by high levels of this protein. If, on one hand, this limits the extension of the observed phenomenon to all cells, on the other hand, it constitutes an interesting difference between normal and neoplastic cells, which could provide a possible way to induce or at least to influence apoptosis only in the latter. This aspect of the question, concerning the differences of nuclear organization and matrix protein composition in cancer and normal cells, is a promising area for application in both cancer diagnosis and prognosis (57).

**REFERENCES**

1. Bustin, M., and Reeves, R. (1996) Prog. Nucleic Acids Res. Mol. Biol. 84, 35–1068, O.
2. Bustin, M. (1999) Mol. Cell. Biol. 19, 5237–5246.
3. Jansen, E., Petit, M. R., Shoenmakers, E. F. P. M., Torik, A. Y., and Van de Ven, W. J. M. (1999) *Gene Ther. Mol. Biol.* 3, 387–395.
4. Brunetti, A., Manfioletti, G., Chieffi, E., Goldfine, I. D., and Foti, D. (2001) *FASEB J.* 15, 492–500.
5. Johnson, K. R., Lehn, D. A., Elton, T. S., Barr, P. J., and Reeves, R. (1988) *J. Biol. Chem.* 263, 18338–18342.
6. Manfioletti, G., Giancotti, V., Bandiera, A., Buratti, E., Sauvière, P., Cary, P., Crane-Robinson, C., and Goodwin, G. H. (1991) *Nucleic Acids Res.* 19, 6783–6797.
7. Huth, J. R., Beley, C. A., Nissen, M. S., Evans, J. N., Reeves, R., Gronenborn, A. M., and Clore, G. M. (1997) *Nat. Struct. Biol.* 4, 657–665.
8. Mantovani, F., Covacevutsch, S., Rustighi, A., Sgarra, R., Heath, C., Goodwin, G. H., and Manfioletti, G. (1998) *Nucleic Acids Res.* 26, 14334–14339.
9. Johns, E. W. (1982) *The HMG Chromosomal Proteins*, Academic Press, New York.
10. Palvimo, J., and Linnala-Kankunnen, A. (1989) *FEBS Lett.* 257, 101–104.
11. Palvimo, J., Linnala-Kankunnen, A., Marino, G., Pucic, P., Goodwin, G. H., Manfioletti, G., and Giancotti, V. (1992) *J. Biol. Chem.* 267, 22486–22489.
12. Giancotti, V., Bandiera, A., Buratti, E., Fusco, A., Marzari, R., Coles, B., and Goodwin, G. H. (1991) *Eur. J. Biochem.* 199, 211–216.
13. Reeves, R., Langan, T. A., and Nissen, M. S. (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88, 1671–1675.
14. Nissen, M. S., Langan, T. A., and Reeves, R. (1991) *J. Biol. Chem.* 266, 19945–19952.
15. Schwanbeck, R., and Wisniewski, R. J. (1997) *J. Biol. Chem.* 272, 27476–27483.
16. Giancotti, V., Berlingieri, M. T., Di Fiore, P. P., Fusco, A., Vecchio, G., and Crane-Robinson, C. (1985) *Cancer Res.* 45, 6051–6057.
17. Giancotti, V., Pani, B., D’Andrea, P., Berlingieri, M. T., Di Fiore, P. P., Fusco, A., Vecchio, G., Philip, B., Crane-Robinson, C., Nicolas, B. H., Wright, C. A., and Goodwin, G. H. (1987) *EMBO J.* 6, 1981–1987.
18. Giancotti, V., Buratti, E., Perissin, L., Zorzert, S., Balmain, A., Portella, G., Fusco, A., and Goodwin, G. H. (1989) *Exp. Cell Res.* 184, 538–545.
19. Giancotti, V., Bandiera, A., Ciani, L., Santoro, D., Crane-Robinson, C.,...
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20. Chiappetta, G., Avantaggiato, V., Visconti, R., Fedele, M., Battista, B., Trapasso, F., Merciai, B. M., Fidanza, V., Giancotti, V., Santoro, M., Simone, A., and Fusco, A. (1996) Oncogene 13, 2439–2446
21. Zhou, X., and Chada, K. (1998) Keio J. Med. 47, 73–77
22. Chiappetta, G., Tallini, G., De Biasio, M. C., Manfioletti, G., Martinez-Tello, F. J., Pentimalli, F., de Nigris, F., Mastro, A., Botti, G., Fedele, M., Berger, N., Santoro, M., Giancotti, V., and Fusco, A. (1998) Cancer Res. 58, 4193–4198
23. Bandiera, A., Bonifacio, D., Manfioletti, G., Mantovani, F., Rustighi, A., Zanconati, F., Fusco, A., Di Bonito, L., and Giancotti, V. (1998) Cancer Res. 58, 426–431
24. Del Bino, G., Lassota, P., and Darzynkiewicz, Z. (1991) Exp. Cell Res. 193, 27–35
25. Mastino, A., Sciortino, M. T., Medici, M. A., Perri, D., Ammendolia, M. G., Grelli, S., Amici, C., Pernice, A., and Guglielmino, S. (1997) Cell Death Differ. 4, 629–638
26. Morana, S. J., Wolf, C. M., Li, J., Reynolds, J. E., Brown, M. K., and Eastman, A. (1996) J. Biol. Chem. 271, 18263–18271
27. Zhu, N., and Wang, Z. (1997) Anal. Biochem. 246, 155–158
28. Wilm, M., and Mann, M. (1996) Anal. Chem. 68, 1–8
29. Banks, G. C., Li, Y., and Reeves, R. (2000) Biochemistry 39, 8333–8346
30. Xiao, D.-M., Pak, J. H., Wang, X., Sato, T., Huang, F. L., Chen, H.-C., and Huang, K.-P. (2000) J. Neurochem. 74, 392–399
31. Froelich-Ammon, S. J., and Oberson, O. (1995) J. Biol. Chem. 270, 21429–21432
32. Martins, L. M., Mesner, P. W., Kottke, T. J., Basi, G. S., Sinha, S., Tung, J. S., Vingen, P. A., Madden, B. J., Takahashi, A., McCormick, D. J., Earnshaw, W. C., and Kaufman, S. H. (1999) Blood 93, 4283–4296
33. Liu, W.-M., Guerra-Vladusic, F. K., Kurakata, S., Lupu, R., and Kaufman, S. H. (1999) Cancer Res. 59, 5695–5703
34. Hirokawa, F., and Fackelmayer, F. O. (1999) Biochemistry 36, 8276–8283
35. Liu, W.-M., Guerra-Vladusic, F. K., Kurakata, S., Lupu, R., and Kaufman, S. H. (1999) J. Biol. Chem. 274, 31775–31783
36. Choi, K. S., Eom, Y. W., Kang, Y., Ha, M. J., Rhee, H., Yoon, J.-W., and Kim, S.-J. (1999) J. Biol. Chem. 274, 31775–31783
37. Shibata, K., Inagaki, M., and Ajiko, K. (1996) Eur. J. Biochem. 192, 87–93
38. Shibata, K., and Ajiko, K. (1998) J. Biol. Chem. 273, 18431–18434
39. Marushige, Y., and Marushige, K. (1995) Anticancer Res. 15, 267–272
40. Yoshida, M., Usui, T., Tsujimura, K., Inagaki, M., Beppu, T., and Horinouki, S. (1997) Exp. Cell Res. 232, 225–233
41. Hendzel, M. J., Nishioka, W. K., Raymond, Y., Allis, C. D., Bezett-Jones, D. P., and Ting, J. P. (1998) J. Biol. Chem. 273, 24470–24478
42. Wang, D.-Z., Ray, P., and Boothby, M. (1995) J. Biol. Chem. 270, 22924–22932
43. Ajiro, K. (2000) J. Biol. Chem. 275, 439–443
44. Rogakou, E. P., Nieves-Neira, W., Boon, C., Pommier, Y., and Bonner, W. M. (2000) J. Biol. Chem. 275, 9396–9399
45. Krautmemeier, M., Ahlg, W., Hanceke, R., and Doenecke, D. (2000) J. Biol. Chem. 275, 30478–30486
46. Bosman, F. T. (1999) Virchows Arch. 435, 391–399
A Link between Apoptosis and Degree of Phosphorylation of High Mobility Group A1a Protein in Leukemic Cells
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