Pivotal Role of Reactive Oxygen Species as Intracellular Mediators of Hyperthermia-induced Apoptosis*

Received for publication, February 29, 2000, and in revised form, April 18, 2000
Published, JBC Papers in Press, April 25, 2000, DOI 10.1074/jbc.M001629200

Dörthe M. Katschinski‡‡, Kristina Boos‡, Susann G. Schindler†, and Joachim Fandrey¶

From the ‡Institute of Physiology, Medical University of Lübeck, D-23538 Lübeck, Germany and the ¶Institute of Physiology, University of Essen, D-45122 Essen, Germany

The effects of cellular antioxidant capacity on hyperthermia (HT)-induced apoptosis and production of antiapoptotic heat shock proteins (HSPs) were investigated in HL-60 cells and in HL-60AR cells that are characterized by an elevated endogenous catalase activity. Exposure of both cell lines to 43 °C for 1 h initiated apoptosis. Apoptosis peaked at 3–6 h after heat exposure in the HL-60 cells. Whereas HL-60AR cells were partially protected against HT-induced apoptosis at these early time points, maximal levels of apoptosis were detected later, i.e. 12–18 h after heat exposure. This differential induction of apoptosis was directly correlated to the induction of the antiapoptotic HSP27 and HSP70. In particular, in the HL-60 cells HSP27 was significantly induced at 12–18 h after exposure to 43 °C when apoptosis dropped. In contrast, coinciding with the late onset of apoptosis in HL-60AR cells at that time HL-60AR cells lacked a similar HSP response. In line with the higher antioxidant capacity HL-60AR cells accumulated reactive oxygen species to a lesser degree than HL-60 cells after heat treatment. Protection from HT-induced apoptosis as well as diminished heat-induced HSP27 expression was also observed after cotreatment of HL-60 cells with 43 °C and catalase but not with superoxide dismutase. These data emphasize the pivotal role of reactive oxygen species for HT induced pro- and antiapoptotic pathways.

Hyperthermia (HT) has a potential as an antineoplastic treatment modality when combined with radiation or chemotherapy (1). Thus, the biological effects of elevated temperatures have been studied extensively. Cell death is apparent after application of a critical temperature load (2, 3). Previously, we and others were able to demonstrate that HT-induced cell death can at least partly be attributed to the induction of apoptosis (2–4). Despite this effect, exposure of malignant cells to elevated temperatures also elicits a well regulated cellular defense including the production of HSPs (5, 6). In particular, induction of HSP27 and HSP70 has been shown to exert the protective role of HSP by inhibition of apoptosis (7–11). The exact mechanism(s) of heat-induced pro- and antiapoptotic pathways, however, are currently not entirely clear.

Several studies indicate the involvement of heat-mediated oxidative stress in HT-induced cytotoxicity. After exposure to heat-increased levels of superoxide anions, hydrogen peroxide and nitric oxide as well as increased lipid peroxidation products have been found in various cell lines and in tumor tissue (12–16). The origin of the ROS, however, remains to be determined. Noteworthy, ROS are also known to induce HSPs that are critical for cellular thermo resistance and the development of thermotolerance (2, 17, 18). Thus, heat-induced oxidative stress seems to play a pivotal role, i.e. the induction of apoptotic cell death and induction of antiapoptotic HSPs. Via manipulation of the activity of antioxidant enzymes one should be enabled to determine the importance of ROS for heat-mediated apoptosis and the induction of cellular defense. SOD, CAT, and glutathione peroxidase are the main cellular ROS degrading enzyme systems. SOD converts the superoxide radical (O2-) into hydrogen peroxide, which is metabolized by CAT and glutathione peroxidase. Therefore, the aim of this study was to investigate whether the antioxidative capacity is affecting HT-mediated apoptosis and HSP production. To this end we investigated HT-induced apoptosis in the parental leukemia cell line HL-60 as well as in HL-60AR cells that are characterized by elevated endogenous CAT activity (19, 20) and the modulation of HT-induced apoptosis and HSP induction by exogenous treatment with CAT or SOD.

MATERIALS AND METHODS

Chemicals and Antibodies—The mouse anti-Bcl-2, mouse anti-Bcl-XL, and rabbit anti-Bax antibodies as well as the appropriate horse-radish peroxidase-labeled secondary antibodies were obtained from Santa Cruz (Santa Cruz, CA) and used according to the manufacturer’s recommendations. The mouse monoclonal anti-HSP27 and anti-HSP70 antibodies were purchased from Stressgen (Victoria, Canada). CAT, SOD, and all other chemicals were obtained from Sigma.

Cell Lines and Culture Conditions—The human leukemia cell lines HL-60 and HL-60AR were a kind gift from Dr. K. Bhalla (Medical University of South Carolina, Charleston, SC). Endogenous CAT enzyme activity in HL-60 and HL-60AR cells was determined to be 53.3 ± 17.8 and 176.3 ± 55.8 μmol min⁻¹ mg protein⁻¹. The cells were grown in RPMI 1640 with 10% fetal bovine serum in a humidified 5% CO₂ in air atmosphere at 37 °C. For heat exposure cells were seeded in 25-cm² tissue culture flasks, allowed to grow for 24 h, and then exposed to 43 °C for 1 h. After heat treatment, cells were returned to 37 °C. Cell viability was determined using the trypan blue exclusion test. Before each experiment cells were determined to have a viability of >90%. Time zero was considered the point at which the cells were removed from the water bath after heat treatment. All experiments were repeated at least three times. Data shown in the graphs for various parameters represent the means ± S.D., and data sets were compared using the Student’s t test.

Temperature Treatment—The 25-cm² tissue culture flasks were placed into plastic freezer bags and filled with 5% CO₂/air before sealing. The flasks were placed into a gently shaking water bath maintained at 43 °C ± 0.1 °C for 1 h. The medium in the flasks equilibrated...
to the water bath temperature in about 5 min. The temperature in the water bath was controlled using a thermostator temperature probe, which was regularly calibrated to a tenth degree in the range of 20–50 °C. After the heat treatment the flasks were removed from the freezer bags and returned to the 37 °C incubator.

Quantitation of DNA Fragmentation—Extent of DNA fragmentation was determined by a modified method described in Ref. 21. At different time points after heat exposure cells were collected and resuspended in 100 μl of lysis buffer (5 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.5% Triton-X). After incubation for 20 min at 4 °C, the suspension was centrifuged at 14,000 rpm for 20 min, and subsequently the fragmented DNA was recovered from the supernatant. The pellets were sonicated for 15 s in 100 μl of lytic buffer. The amount of DNA in both fractions was determined by a fluorometric method using 10 μg/ml DAPI as described in Ref. 22. The fluorescence intensity was measured using 360-nm excitation wavelength and 460-nm emission wavelength (Cytofluor™ 2350 Millipore, Eschborn, Germany). The percentage of DNA fragmentation was defined as the ratio of the amount of fragmented DNA to the total amount of DNA.

Preparation of Cell Lysates and Determination of DEVD-AMC Cleavage—Cells were exposed to 43 °C for 1 h. At frequent intervals thereafter, cells were collected by centrifugation and resuspended in 100 μl of lysis buffer (100 mM Hepes, pH 7.5, 10% sucrose, 0.1% CHAPS, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride). Cell lysate (30 μg of protein/ sample) with 12 μM of the fluorogenic peptide substrate DEVD-AMC in a 96-well microtiter plate at room temperature. The cleavage of DEVD-AMC was monitored by AMC liberation in a fluorescence plate reader (Cytofluor™ 2350, Millipore) using 360-nm excitation and 460-nm emission wavelength. Fluorescence was measured every 60 s during a 60-min period, and fluorescence units were converted to pmol amounts of AMC using a standard curve generated with free AMC (Sigma). Control experiments confirmed that the release of substrate was linear with DEVD-AMC and protein concentration. Addition of the competitive inhibitor DEVD-CHO (50 μM) to the samples blocked DEVD-AMC cleavage.

Quantitation of Apoptotic Cells—At various time points after heat treatment, cells were treated with 3.7% paraformaldehyde for 10 min followed by fixation onto glass slides with 70% ethanol. After rehybridization glass slides were stained with Hoechst dye (1.5 μg/ml) for 5 min, washed with phosphate-buffered saline, and mounted in 2.5% Dabco (1,4-diazabicyclo[2.2.2]octane). Nuclei were visualized using a Zeiss fluorescence microscope. Apoptotic nuclei were expressed as a percentage of total nuclei.

Western Blot Analysis—Cells were spun down, washed with phosphate-buffered saline, and lysed with 50 mM Tris buffer, pH 8.2, 120 mM NaCl, 100 mM NaF, 0.5% Nonidet P-40, 200 mM sodium orthovanadate, 10 μg/ml aprotinin, 10 μg/ml phenylmethylsulfonylfluoride, and stored at −80 °C. Protein (50 μg) was run on a polyacrylamide gel and blotted onto nitrocellulose membranes by semi-dry electroblotting (Bio-Rad); membranes were stained with Ponceau S to verify equal protein loading on nitrocellulose membranes. Blots were probed with anti-Bcl-2 (200:1), Bax, Bcl-X, HSP27, HSP70, or actin (diluted: 1:200, 1:200, 1:200, 1:1000, 1:1000, and 1:1000, respectively) and detected with horseradish peroxidase-conjugated antibodies (dilution: 1:2000) and the ECL system (Amersham Pharmacia Biotech).

Determination of Intracellular Generation of ROS—The production of intracellular ROS was estimated fluorometrically using the oxazine sensitive fluorescent probe 2′,7′-dichlorofluorescin diacetate (DCF; Sigma). Before heat exposure, cells were washed with phosphate-buffered saline and incubated with 5 μM DCF for 30 min at 37 °C. Subsequently cells were washed and resuspended in DCF free RPMI 1640. Cells were then exposed to 37 or 43 °C for 1 h. At frequent time intervals thereafter, samples were analyzed using a Cytofluor™ 2350, Millipore fluorometer. Fluorescence of DCF was detected at an excitation and emission wavelengths of 485 and 530 nm, respectively.

RNA Extraction and Northern Blot Analysis—Total RNA was isolated according to the method described in Ref. 23. 15 μg of total RNA were subjected to electrophoresis in denaturing 1% agarose gels containing 0.7 mol/liter formaldehyde. RNA was transferred onto nylon membranes (Nytran Plus, Schleicher & Schuell) with a vacuum blotting apparatus (Amersham Pharmacia Biotech). Filters were cross-linked with UV light dried at 80 °C for 2 h and prehybridized for 4 h at 42 °C in 45% formamide, 5× SSC, 5× Denhardt’s solution, 0.1% SDS, and 100 μg/ml sonicated denatured salmon testis DNA. Hybridizations were performed in fresh solution supplemented with the radioactive probe (0.5 to 2 × 10⁶ cpm/ml) for 2 days at 42 °C. Hybridization probes were polymerease chain reaction generated fragments. Polymerease chain reaction fragments were [α-32P]dCTP-labeled with a commercially available kit (MBI Fermentas, St. Leon-Rot, Germany). After hybridization, the filters were washed twice for 15 min in 2× SSC/0.1% SDS at 50 °C and then twice in 0.1× SSC/0.1% SDS at 60 °C. Filters were sealed in plastic bags and analyzed by exposure to imaging plates for a Bio Imaging Analyzer (BAS 1000; Fuji, Düsseldorf, Germany).

RESULTS

Hyperthermia-induced Apoptosis in HL-60 and HL-60AR Cells—Exposure of HL-60 to 43 °C for 1 h resulted in significant apoptotic cell death as demonstrated by DNA fragmentation, caspase 3 activation, and quantitation of apoptotic nuclei after Hoechst staining (Fig. 1). To test for the involvement of ROS in HT-induced apoptosis, HL-60 cells were incubated with CAT (500 units/ml) or SOD (50 units/ml) during heat exposure. As shown in Fig. 2 treatment of HL-60 cells with heat in the presence of CAT but not SOD partially protected from HT-induced apoptosis 3 h after heat exposure. Moreover, modulation of apoptosis by antioxidant enzymes was demonstrated by heat exposure of the parental HL-60 cells and the HL-60AR cells, for which an increased endogenous CAT enzyme activity has been described (also see “Materials and Methods”). In the HL-60 cells, apoptosis peaked at 3–6 h and disappeared by 18–24 h after treatment, whereas HL-60AR cells showed a late onset of apoptosis. Peak apoptosis was not observed until 12–18 h after exposure to 43 °C for 1 h (Fig. 1).

Hyperthermia-mediated Generation of Intracellular ROS—Determination of intracellular ROS in HL-60 and HL-60AR cells after exposure to 43 °C for 1 h confirmed the accumulation of ROS in response to heat as described earlier by others (Ref. 14 and Fig. 3). However, hyperthermia induced a significantly higher accumulation of ROS in HL-60 cells compared with HL-60AR cells. This is in line with the higher antioxidative capacity of the HL-60AR cells via their increased endogenous CAT enzyme activity (20).

Expression of Members of the Bcl-2 Family as Well as HSP27 and HSP70 after Heat Treatment—Members of the Bcl-2 protein family play an important role for pro- but also antiapoptotic pathways. Interestingly, basal Bcl-2 protein expression was significantly higher in the HL-60AR cells than in the parental HL-60 cells. In contrast, basal expression of Bcl-XL and Bax was not altered in the HL-60AR cells. Treatment of HL-60 and HL-60AR cells with 43 °C did not affect the protein levels of the antiapoptotic Bcl-2 and Bcl-XL as well as the proapoptotic Bax at any time (Fig. 4A). In contrast, exposure of both cell lines to 43 °C induced HSP27 and HSP70 mRNA and protein levels (Fig. 4), but the response markedly differed between HL-60 and HL-60AR cells. Following exposure to 43 °C for 1 h HSP70 mRNA levels in HL-60 cells peaked at 3 h after treatment (Fig. 4B). In contrast, in HL-60AR cells the induction of HSP70 mRNA was delayed for 3 h and reached its maximum 6 h after treatment. Similar results were obtained for HSP27. HL-60 cells presented with detectable basal HSP27 mRNA and protein levels and a rapid induction after exposure to heat. Both the induction of HSP27 and HSP70 in the HL-60 cells coincided with the drop of hyperthermia-induced apoptosis. In contrast, in the HL-60AR cells an increased endogenous HSP27 mRNA and protein levels were only moderately induced after exposure to heat. To further determine the effects of the cellular redox status on HSP27 expression, HL-60 cells were treated with CAT or SOD in addition to HT. As demonstrated in Fig. 5, heat-induced HSP27 induction was diminished by co-treatment of HL-60 cells with heat and CAT but not with SOD.

DISCUSSION

In the present study we found that HT exerted its cytotoxic effect via induction of apoptosis in the human leukemia cells HL-60. These data are consistent with previous reports using...
HL-60 cells and other human leukemia cell lines (2–4). We further demonstrate that the induction of apoptotic cell death by heat was modulated by the antioxidant capacity of the cells. CAT, glutathione peroxidase, and SOD are the main cellular ROS degrading enzyme systems. SOD converts the superoxide radical into hydrogen peroxide, which is metabolized by CAT and glutathione peroxidase. Treatment of HL-60 cells with CAT conferred partial protection against the induction of heat-induced apoptosis, whereas SOD did not affect the heat-induced apoptotic cell death. Thus, with respect to HT-induced apoptosis, hydrogen peroxide but not superoxide appears to be of greater importance. In line with this assumption, different effects of hydrogen peroxide and superoxide anions on apoptosis are reported from studies with vascular smooth muscle cells and neutrophils (24).

ROS degrading enzymes like CAT and SOD are poorly cell permeable, and nonphysiological concentrations are needed to affect intracellular ROS concentrations. Although previous reports demonstrate that exogenous delivery of ROS degrading enzymes is sufficient to affect intracellular ROS concentrations (25–27), data from cells with endogenously elevated CAT activity would be much more convincing. Therefore, to further test the hypothesis of whether hydrogen peroxide is involved in HT-induced apoptosis, we made use of the closely related parental leukemia cell line HL-60 and its subline HL-60AR. HL-60 AR cells have increased endogenous CAT enzyme levels (19). In line with the experiments in which exogenous ROS degrading enzymes were used to modulate intracellular ROS levels, the induction of apoptosis was significantly different in HL-60 and the HL-60AR cells. Although apoptosis

---

**FIG. 1.** Induction of apoptosis after heat exposure of HL-60 and HL-60AR cells. HL-60 and HL-60AR cells were treated with 43 °C for 1 h. At frequent time intervals thereafter, cells were assayed for apoptotic morphology using the DNA-specific fluorochrome Hoechst 33258 (a), DNA fragmentation (b), or caspase 3 activity (c) as described under “Materials and Methods.” Data points represent three independent experiments. Bars, S.D.

**FIG. 2.** Modulation of hyperthermia-induced apoptosis and caspase 3 activity by ROS scavengers. HL-60 cells were exposed to 43 °C for 1 h in the presence of CAT (500 units/ml) or SOD (50 units/ml). After heat treatment, cells were washed and resuspended in fresh medium in the absence of ROS modulating molecules. 3 h later cells were stained with the DNA-specific fluorochrome Hoechst 33258 and assayed for the extent of apoptosis.

**FIG. 3.** Intracellular generation of ROS as a function of heat exposure in HL-60 and HL-60AR cells. HL-60 and HL-60AR cells were exposed to 37 or 43 °C for 1 h. At frequent time intervals thereafter, intracellular generation of ROS was determined using the fluorescent probe DCF as described under “Materials and Methods.”
HL-60 cells were exposed to 43 °C for 1 h. HL-60 and HL-60AR cells were treated with 43 °C for 1 h. b, expression of HSP27 and HSP70 mRNA expression as a function of temperature treatment in HL-60 and HL-60AR cells. HSP27 and HSP70 mRNA were detected as described under “Materials and Methods.”

Fig. 4. Changes in protein (a) and mRNA (b) expression after exposure of HL-60 and HL-60AR cells to 43 °C for 1 h. HL-60 and HL-60AR cells were treated with 43 °C for 1 h. a, expression of Bel-2, Bcl-XL, Bax, HSP27, and HSP70 protein expression as a function of temperature treatment in HL-60 and HL-60AR cells. Cellular protein was extracted from HL-60 and HL-60AR cells up to 24 h after exposure to 43 °C for 1 h. b, expression of HSP27 and HSP70 mRNA expression as a function of temperature treatment in HL-60 and HL-60AR cells. HSP27 and HSP70 mRNA were detected as described under “Materials and Methods.”

was seen as early as 3–6 h after exposure to 43 °C for 1 h in the HL-60 cells. HL-60AR cells seemed to be partially protected against HT-induced apoptotic cell death at this time point. The partial resistance of HL-60AR cells at this time point was correlated to significantly diminished HT-induced ROS levels in HL-60AR cells, possibly because of the higher antioxidative capacity of the HL-60AR cells. The origin of the ROS has not yet been identified and needs further investigation. ROS are produced when oxygen is consumed in the electron transport chain reaction, and mitochondria can be a major source of ROS.

ROS production by mitochondria during apoptosis has already been considered by several investigators (28–31). However, assuming that HL-60 and HL-60AR cells differ in their levels of hydrogen peroxide through higher CAT activity in HL-60AR cells, this difference does not explain the observation that 12–18 h after heat exposure when apoptosis dropped in the HL-60 cells maximal levels of apoptotic cell death were detected in HL-60AR cells. To gain insight into the mechanism for this late onset of apoptosis, different proteins with regulatory function in apoptotic pathways were investigated. Important general regulators of apoptotic cell death are the Bel-2 proteins and the HSPs, i.e. HSP27 and HSP70 (32–35). Whereas Bel-2 proteins affect the apoptotic pathway via interaction with the mitochondrial membrane potential, cytochrome c, and finally the caspases, HSPs seem to exert their apoptotic effect downstream of caspase 3-like proteases (11, 36, 37).

Basal Bel-2 expression was significantly higher in the HL-60AR cells compared with the HL-60 cells. After exposure to heat, however, protein expression of Bel-2, Bel-XL, and Bax was not significantly altered. Thus, it seems that these members of the Bel-2 family are not involved in the HT-mediated induction of apoptosis. These data are in agreement with earlier reports of our group and others (3, 4). In contrast, HT-dependent induction of HSP27 and HSP70 directly correlated with the apoptotic effect in HL-60 and HL-60AR cells. Whereas HSP27 and HSP70 mRNA and protein expression was significantly increased after exposure to heat in the HL-60 cells, the induction of HSP27 in particular was markedly diminished in the HL-60AR cells. This differential HSP expression in the HL-60 and HL-60AR cells implies that the HT-induced increase of HSPs is dependent on the accumulation of ROS. Strong support for this assumption was the observation that HSP27 expression was reduced by CAT. Taken together and in agreement with earlier reports (2, 17, 38), our data support the notion that the induction of HSP27 is dependent on the cellular redox status. However, because the influence of the increased CAT activity was more pronounced for HSP27 compared with HSP70, one has to assume a higher sensitivity of HSP27 by the cellular redox status. A differential expression of HSP27 and HSP70 is in line with reports of others and may be due to specific transcriptional regulatory elements (2).

Transcriptional regulation of heat shock genes requires the activation of the HSF-1, which binds to the consensus heat shock element located in the promoter region of the heat shock genes. Activation of HSF-1 requires phosphorylation, trimerization, and nuclear translocation. Recent studies suggest that hydrogen peroxide increases nuclear translocation and DNA binding activity of HSF-1 (39, 40), which is in line with our observation reported here and makes a redox mechanism in heat-induced signal transduction pathways during apoptosis very likely.

Understanding of the regulation of HSP induction may be important for anticancer strategies, because it has been demonstrated that HSP27 can limit the efficacy of antitumor agents and enhance tumorigenicity (41, 42). Induction of HSPs in the HL-60 cells coincided with the drop of HT-induced apoptosis. HSPs may be induced as a cellular defense, which would support the concept of an inverse relationship of HSP induction and HT-induced cytotoxicity (43). As a consequence of the above hypothesis, the lack of protection via diminished HSP27 and, to lesser degree, HSP70 induction in the HL-60AR cells is sensitizing these cells to undergo apoptosis at later time points.

Our data underscore the role of ROS in HT-induced apoptosis. However, the role of HT-induced accumulation of ROS appears to be ambiguous. Although a decrease in HT-mediated
ROS generation via a higher endogenous antioxidative capacity confers partial resistance against HT-induced apoptosis at first, it might also affect the cellular defense, i.e. HSP production. Thus, ROS represent a common denominator of both pro- and antiapoptotic HT-induced pathways.

Acknowledgment—We thank Dr. Bhalla (Medical University of South Carolina, Charleston, SC) for providing HL60AR cells.

REFERENCES

1. Robins, H. I., Cohen, J. D., and Neville, A. J. (1992) Clinical Thermology (Gauthier, M., ed) Springer-Verlag, Berlin
2. Gorman, A. M., Heavey, B., Creagh, E., Cotter, T. G., and Samali, A. (1999) FEMS Lett. 445, 98–102
3. Katschinski, D., Robins, H. I., Schad, M., Frede, S., and Fandrey, J. (1999) Cancer Res. 59, 3404–3410
4. Takasu, T., Lyons, J. C., Park, H. J., and Song, C. W. (1998) Cancer Res. 58, 2504–2508
5. Li, G. C., and Werb, Z. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3218–3222
6. Wu, C. (1995) Annu. Rev. Cell Dev. Biol. 11, 441–469
7. Jaattela, M., Wissing, D., Bauer, P. A., and Li, G. C. (1992) EMBO J. 11, 3507–3512
8. Samali, A., and Cotter, T. G. (1996) Exp. Cell Res. 223, 163–170
9. Mehlen, P., Schulze-Osthoff, O. K., and Arrigo, A. P. (1996) J. Biol. Chem. 271, 16510–16514
10. Mosser, D. D., Caron, A. W., Bourget, L., Denis, L. C., and Massie, B. (1997) Mol. Biol. Cell. 17, 5317–5327
11. Jaattela, M., Wissing, D., Kokholm, K., Kallunki, T., and Egeblad, M. (1998) EMBO J. 17, 6124–6134
12. Yoshikawa, T., Kokura, S., Tainaka, K., Itani, K., Oyamada, H., Kaneko, T., Naito, Y., and Kondo, M. (1993) Cancer Res. 53, 2326–2329
13. Davidson, J. F., Whyte, B., Bissinger, P. H., and Schiestl, R. H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5116–5121
14. Flanagan, S. W., Moseley, P. L., and Buettner, G. R. (1998) FEMS Lett. 431, 285–290
15. Frank, J., Kelleher, D. K., Pompeila, A., Thews, O., Biesalski, H. K., and Vaupel, P. (1996) Cancer Res. 56, 2693–2698
16. Matsumoto, H., Hayashi, S., Hashitabshi, M., Ohnishi, K., Ohtsubo, T., Kitai, R., Shiyoa, H., Ohnishi, T., and Kano, E. (1999) Cancer Res. 59, 3239–3244
17. Schoneniger, L. O., Andreoni, K. A., Ott, G. R., Rizzi, T. H., Bulkey, G. B., Udeelman, R., Burchard, J. F., and Buchman, T. G. (1994) Gastroenterology 106, 177–184
18. Malyshev, I. Y., Malugin, A. V., Gelubeva, L. Y., Zenina, T. A., Manukhina, E. B., Mikoyan, V. D., and Vanin, A. F. (1996) FEMS Lett. 391, 21–23
19. Bhalla, K., Hindenburg, A., Taub, R. N., and Grant, S. (1985) Cancer Res. 45, 3657–3662
20. Lenehan, P. F., Gutierrez, P. L., Wagner, J. L., Milak, N., Fisher, G. R., and Ross, D. D. (1995) Cancer Chemother. Pharmacol. 35, 377–386
21. Wylie, A. H., Kerr, J. F., and Currie, A. R. (1980) Int. Rev. Cytol. 68, 251–306
22. Brunk, C. F., Jones, K. C., and James, T. W. (1979) Anal. Biochem. 92, 497–500
23. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
24. Rollet, L. E., Grange, M. J., Elbim, C., Marquetty, C., Guogerot, P. M., and Pasquier, C. (1998) Free Radic. Biol. Med. 24, 563–572
25. Sandstrom, P. A., and Buttke, T. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 90, 4708–4712
26. Takeuchi, A., Miyamoto, T., Yamati, K., Masulo, Y., Hayashi, M., Hayashi, H., and Onozaki, K. (1994) Cancer Res. 55, 1586–1589
27. Sundaresan, M., Yu, Z., Ferrans, V. J., Irani, K., and Finkel, T. (1995) Science 270, 296–299
28. Kinningham, K. K., Oberley, T. D., Lin, S., Mattingly, C. A., and St. Clair, D. K. (1999) FASEB J. 13, 1601–1610
29. Lemasters, J. J., Nieminen, A. L., Qian, T., Trusc, L. C., Elmore, S. P., Nishimura, Y., Crowe, R. A., Cascio, W. E., Bradham, C. A., Brenner, D. A., and Herman, B. (1998) Biochim. Biophys. Acta 1366, 177–196
30. Li, J. J., and Oberley, L. W. (1997) Cancer Res. 57, 1991–1998
31. Wong, G. H., McHugh, T., Weber, R., and Goodell, D. V. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4372–4376
32. Garrido, C., Ottavi, P., Fromentin, A., Hbmann, A., Arrigo, A. P., Chaffert, B., and Mehlen, P. (1997) Cancer Res. 57, 2661–2667
33. Adams, J. M., and Cory, S. (1998) Science 281, 1322–1326
34. Jaattela, M. (1999) Exp. Cell Res. 248, 30–43
35. Brar, B. K., Stephanou, A., Wagstaff, M. J., Coffin, R. S., Marber, M. S., Engelmann, G., and Latchman, D. S. (1999) J. Mol. Cell Cardiol. 31, 135–146
36. Kluck, R. M., Bossy, W. E., Green, D. R., and Newmeyer, D. D. (1997) Science 275, 1322–1326
37. Guenal, I., Sidrini-de, F. C., Gauzer, S., and Mignotte, B. (1997) Oncogene 15, 347–360
38. Bornman, L., Baladi, S., Richard, M. J., Tyrrell, R. M., and Polla, B. S. (1999) J. Cell. Physiol. 178, 1–8
39. Jacquier-Sarin, M. R., and Polla, B. S. (1996) Biochem. J. 318, 187–193
40. Jornot, L., Petersen, H., and Junod, A. F. (1997) FEBS Lett. 416, 381–386
41. Ciocca, D. R., Fuqua, S. A., Lock, L. S., Tuth, D. O., Welch, W. J., and McGuire, W. L. (1992) Cancer Res. 52, 3648–3654
42. Garrido, C., Fromentin, A., Bonnette, B., Favre, N., Moutet, M., Arrigo, A. P., Mehlen, P., and Solary, E. (1998) Cancer Res. 58, 5495–5499
43. Riabowol, K. T., Mizzen, L. A., and Welch, W. J. (1988) Science 242, 433–436
