Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 342 (2006) 280–285
BBRC
www.elsevier.com/locate/ybbrc

2-Deoxy-D-ribose inhibits hypoxia-induced apoptosis by suppressing the phosphorylation of p38 MAPK

Ryuji Ikeda [a], Xiao-Fang Che [b], Mina Ushiyama [a], Tatsuya Yamaguchi [a], Hiroshi Okumura [c], Yuichi Nakajima [b], Yasuo Takeda [a], Yoshihiko Shibayama [a], Tatsuhiko Furukawa [b], Masatatsu Yamamoto [b], Misako Haraguchi [b], Tomoyuki Sumizawa [d], Katsushi Yamada [a], Shin-ichi Akiyama [b,*]

[a] *Department of Clinical Pharmacy and Pharmacology, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan*
[b] *Department of Molecular Oncology, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan*
[c] *First Department of Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan*
[d] *Department of Environmental Toxicology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi, Kitakyushu 807-8555, Japan*

Received 24 January 2006
Available online 6 February 2006

Abstract

An angiogenic factor, platelet-derived endothelial cell growth factor/thymidine phosphorylase (TP), stimulates the chemotaxis of endothelial cells and confers resistance to apoptosis induced by hypoxia. 2-Deoxy-D-ribose, a degradation product of thymidine generated by TP enzymatic activity, partially prevented hypoxia-induced apoptosis. 2-Deoxy-D-ribose inhibited hypoxia-induced phosphorylation of p38 mitogen-activated protein kinase (MAPK) but not c-jun NH_2-terminal kinase/stress-activated protein kinase in human leukemia HL-60 cells. 2-Deoxy-D-ribose also suppressed the levels of Bax attached to mitochondria under hypoxic conditions. SB203580, a specific inhibitor of the p38 MAPK, suppressed the hypoxia-induced apoptosis of HL-60 cells. These findings suggest that one of the molecular bases for resistance to hypoxia-induced apoptosis conferred by 2-deoxy-D-ribose is the inhibition of the p38 signaling pathway. The expression levels of TP are elevated in many malignant solid tumors and thus the 2-deoxy-D-ribose generated by TP in these tumors may play an important role in tumor progression by preventing hypoxia-induced apoptosis.

Keywords: 2-Deoxy-D-ribose; Apoptosis; p38 MAPK; Hypoxia; Bax

Thymidine phosphorylase (TP; EC 2.4.2.4) catalyzes the reversible conversion of thymidine, deoxyuridine, and their analogs to their respective bases and 2-deoxy-D-ribose-1-phosphate [1]. TP is identical to an angiogenic factor, platelet-derived endothelial cell growth factor (PD-ECGF) [2,3]. TP stimulates chemotaxis of endothelial cells in vitro and has an angiogenic activity in vivo [4–7]. Recently, we have demonstrated that TP enzymatic activity is indispensable for angiogenic activity [4,7]. Among the degradation products generated by TP enzymatic activity, 2-deoxy-D-ribose, a dephosphorylated product derived from 2-deoxy-D-ribose-1-phosphate, also displays chemotactic activity in vitro and angiogenic activity in vivo. TP is expressed at higher levels in a wide variety of tumors than in the adjacent non-neoplastic tissues [8–10]. 2-Deoxy-D-ribose was able to partially prevent hypoxia-induced apoptosis indicating that another function of TP besides angiogenesis is to inhibit the apoptosis of tumor cells under hypoxic conditions [11,12].

Mitogen-activated protein kinases (MAPK) comprise a family of serine/threonine kinases that function as critical

* Corresponding author. Fax: +81 99 265 9687.
E-mail address: akiyamas@m3.kufm.kagoshima-u.ac.jp (S. Akiyama).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.01.142

mediators of signal transduction pathways [13]. Members of the MAP kinase superfamily include extracellular signal-regulated kinase (ERKs), Jun NH_2-terminal kinase (JNK), and p38 MAPK. ERKs (ERK1 and ERK2) are activated in response to mitogen or growth factor stimulation [14]. In contrast, JNK and p38 MAPK are activated by a variety of cellular stresses including hypoxia, ultraviolet light, hyperosmolarity, heat shock, and proinflammatory cytokines [15]. The p38 MAPK pathway mediates apoptosis following UV irradiation by inducing the release of mitochondrial cytochrome *c* into the cytosol, followed by pro-caspase 3 activation [16]. The inhibition of p38 counteracted both apoptosis and cytochrome *c* release as well as caspase 3 activity without affecting the processing of pro-caspase 8 [16].

To elucidate the mechanism by which 2-deoxy-D-ribose can prevent hypoxia-induced apoptosis, we examined the effect of 2-deoxy-D-ribose on key regulators of hypoxia-induced apoptosis including MAPK.

Materials and methods

Reagents and antibodies. 2-Deoxy-D-ribose was purchased from Sigma Chemicals Co. (St. Louis, MO). Monoclonal anti-Bax (B-9), anti-ERK1, anti-JNK, anti-p38, phospho-ERK1, anti-phospho-JNK, and anti-phospho-p38 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). SB203580 was from Calbiochem (San Diego, CA).

Cell lines and induction of hypoxia. Human leukemia HL-60 and Jurkat cells were maintained in RPMI1640 containing 10% fetal calf serum. 2-Deoxy-D-ribose or SB203580 was added to the culture medium and then hypoxia was induced with a Gas Pak Pouch Anaerobic System (Becton Dickinson and Company, Cockeysville, MD).

Immunoblot analysis. Samples were subjected to 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) according to the method of Laemmli [17]. Proteins were electrophoretically transferred onto polyvinylidene difluoride membranes (Immobilon-P transfer membrane; Millipore, Bedford, MA) using Bio-Rad Transblot SD apparatus [18]. The membrane was treated with buffer A (350 mM NaCl, 10 mM Tris–HCl [pH 8.0], and 0.05% Tween 20) containing 3% skimmed milk for 1 h and incubated with the indicated antibody (1:1000) in buffer A containing 3% skimmed milk for 1 h. Following four washes with buffer A (10 min each), the membrane was incubated with a peroxidase-conjugated horse anti-mouse IgG diluted 1:1000 in buffer A containing 3% skimmed milk for 1 h. The membrane was washed with buffer A and developed using the enhanced chemiluminescence Western blotting detection system (Amersham Biosciences, Buckinghamshire, UK).

Measurement of apoptotic cells. Apoptotic cells were quantitatively evaluated by flow cytometry. Cells were cultured under normoxic or hypoxic conditions, then harvested and washed once with PBS. For flow cytometry, cells (1×10^6) were suspended in 100 μl of PBS, thoroughly mixed with 100 μl of COULTER DNA-Prep LPR (COULTER, Miami, FA), and then 2 ml of COULTER DNA-Prep Stain was added and again mixed thoroughly. The mixtures were incubated for 15 min at room temperature. The DNA content and the sub-G_1 fraction, representing apoptotic cells, were determined as previously described [11].

Measurement of mitochondrial membrane potential. To quantify the mitochondrial membrane potential, cells were incubated in buffer containing 1 μl MitoSensor for 30 min at 37 °C. The cells were washed with PBS and analyzed by flow cytometry. Fluorescence was observed with λ_{ex} at 488 nm and λ_{em} at 530 nm.

Subcellular fractionation from HL-60 cells. Human leukemia HL-60 cells (5×10^5/ml) were washed twice with ice-cold PBS and then resuspended in 2 volumes of buffer B [20 mM Hepes–KOH (pH 7.5), 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA-2Na, 1 mg/ml aprotinin ,and 1 mM *p*-aminophenylmethanesulfonylfluoride (APMSF)]. Following incubation on ice for 15 min, the cells were disrupted by passage through a 29-gauge needle 40 times. The nuclei were centrifuged at 750*g* for 10 min at 4 °C. The supernatant was centrifuged at 10,000*g* for 25 min at 4 °C. This pellet was resuspended in buffer B and used as the mitochondrial fraction. The supernatant was further centrifuged at 100,000*g* for 60 min at 4 °C and the resulting supernatant from this final centrifugation was used as the S100 fraction.

Acridine Orange staining. Apoptosis was evaluated by acridine orange staining [19]. Cells were incubated for 24 h at 37 °C in RPMI 1640 containing 10% fetal calf serum in the presence or absence of 10 μM 2-deoxy-D-ribose under hypoxic conditions. The cells were stained with 3 μM acridine orange for 15 min, then washed with PBS and examined by confocal microscopy with λ_{ex} at 488 nm [19].

RT-PCR method. Total cellular RNA was extracted using Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). RT-PCR was performed with the SuperScript One-Step RT-PCR system and gene-specific primers according to the manufacturer's instructions (Invitrogen). Reaction mixtures containing total RNA (500 ng of each), 0.2 mM dNTPs, 0.2 μM of each primer, enzyme mixture including SuperScript II RT, Platinum *Taq* DNA polymerase, and 1× buffer with 1.2 mM $MgSO_4$ were maintained at 50 °C for 20 min, then at 94 °C for 2 min, and PCR was performed as follows: the PCR profile was 30 cycles at 94 °C for 15 s, 55 °C for 30 s, and 70 °C for 30 s. The primers for RT-PCRs were designed based on human sequences in GenBank. These sequences used the following primers: Bax, 5′-GCCGCC GTGGACACAGACTCCC-3′, and 5′-AGATGGTGAGCGAGGCGG TGAG-3′; GAPDH, 5′-AGAACATCATCCCTGCCTCTACTGG-3′ and 5′-AAAGGTGGAGGAGTGGGTGTCGCTG-3′.

Statistical analysis. Difference between groups was tested by 1-way ANOVA. Data are presented as means ± SD. Differences were considered significant at $p < 0.05$.

Results

The effect of 2-deoxy-D-ribose on cell morphology under normoxic and hypoxic conditions

The effect of 2-deoxy-D-ribose on cell morphology under normoxic or hypoxic conditions was determined in Jurkat cells by staining with acridine orange. Under normoxic conditions, no apoptotic cells were observed in non-treated Jurkat cells and treatment with 2-deoxy-D-ribose had no effect on cell morphology (Figs. 1A and B). In hypoxic conditions, chromatin condensation in apoptotic bodies was observed in Jurkat cells (Fig. 1C). 2-Deoxy-D-ribose prevented this hypoxia-induced change in morphology (Fig. 1D).

The effect of 2-deoxy-D-ribose on hypoxia-induced apoptosis in HL-60 cells

To investigate the effect of 2-deoxy-D-ribose on hypoxia-induced apoptosis, we measured the sub-G_1 proportion of HL-60 cells by flow cytometric analysis. Hypoxia increased the proportion of the sub-G_1 fraction in HL-60 cells in a time-dependent manner. The proportion of the sub-G_1 fraction of HL-60 cells treated with 2-deoxy-D-ribose was also increased under hypoxic condition, but it was lower than that of HL-60 cells without 2-deoxy-D-ribose (Fig. 2).

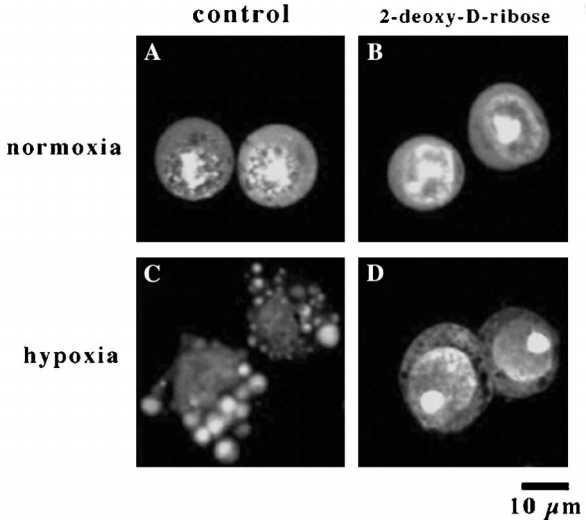

Fig. 1. The effect of 2-deoxy-D-ribose on hypoxia-induced cell morphology. Representative morphology of Jurkat cells grown under normoxic (A,B) or hypoxic (C,D) conditions for 24 h in the absence (A,C) or presence (B,D) of 2-deoxy-D-ribose. Fluorescence micrographs of cells stained with acridine orange are shown. No apoptotic nuclei were observed in untreated Jurkat cells under normoxic conditions. Hypoxia-induced nuclear DNA condensation is observed in untreated Jurkat cells, but not in cells treated with 2-deoxy-D-ribose. Bar = 10 μm.

The effect of 2-deoxy-D-ribose on mitochondria depolarization induced by hypoxia in HL-60 cells

Mitochondrial membrane alterations are a characteristic of apoptotic cell death. We therefore examined the effect of 2-deoxy-D-ribose on the mitochondrial membrane potential of HL-60 cells under normoxic or hypoxic condition.

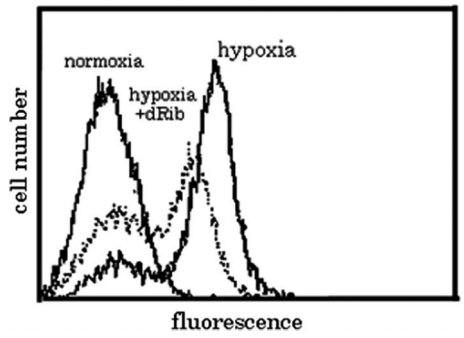

Fig. 3. The effect of 2-deoxy-D-ribose on mitochondria depolarization induced by hypoxia in HL-60 cells. HL-60 cells were incubated in the presence or absence of 10 μM 2-deoxy-D-ribose under normoxic or hypoxic conditions for 36 h. Mitochondrial membrane potential was quantified by flow cytometry following staining with the potentiometric dye Mitosensor. The cells were analyzed by flow cytometry. Fluorescence was observed with λ_{ex} at 488 nm and λ_{em} at 530 nm.

Green fluorescence of the mitochondrial-specific dye Mitosensor in the cells under hypoxic condition was more intensive than in the cells under normoxic condition (Fig. 3). These findings suggest that hypoxia caused mitochondrial depolarization in HL-60 cells. Cells treated with 2-deoxy-D-ribose inhibited the hypoxia-induced loss of mitochondrial membrane potential in HL-60 cells.

The effect of 2-deoxy-D-ribose on the phosphorylation of p38 MAPK, JNK, and ERK under hypoxic conditions

One of the mechanisms of hypoxia-induced apoptosis is the activation of JNK and p38 MAPK [20,21]. The

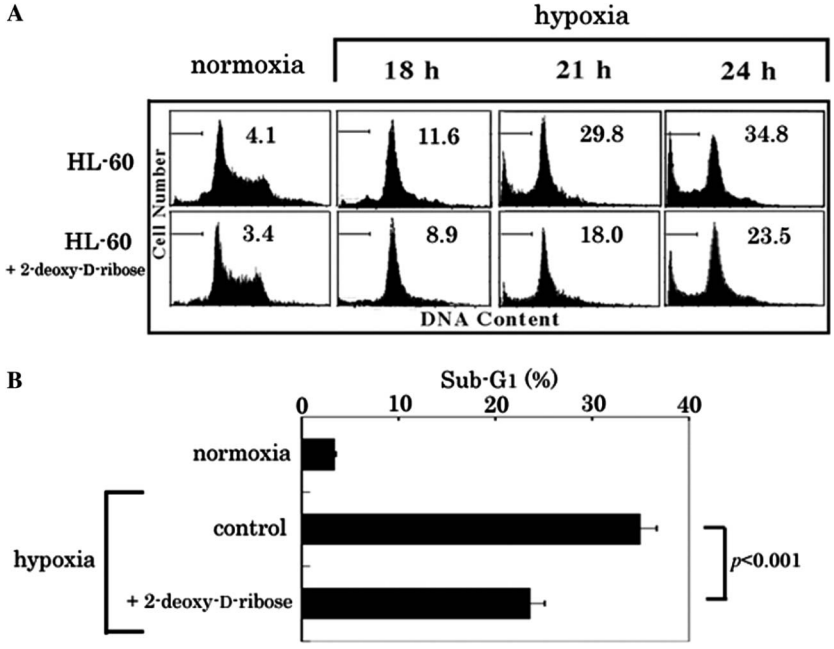

Fig. 2. The effect of 2-deoxy-D-ribose on the proportion of sub-G_1 fraction. HL-60 cells were cultured under normoxic conditions or hypoxic conditions for indicated times in the presence or absence of 2-deoxy-D-ribose and analyzed by flow cytometry (A). The numbers in the figure indicate the proportion of the sub-G_1 cell fraction. The proportion of sub-G_1 fraction in HL-60 cells treated with 2-deoxy-D-ribose for 24 h was less than in non-treated cells (B).

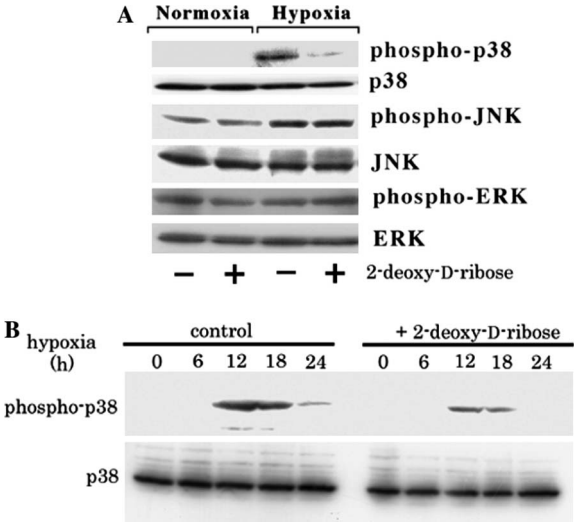

Fig. 4. The effect of 2-deoxy-D-ribose on the activation of MAP kinase in HL-60 cells under hypoxic conditions. HL-60 cells treated with 2-deoxy-D-ribose (10 μM) were incubated under normoxic or hypoxic conditions for 12 h. Cell lysates were prepared and the activation of p38, JNK, and ERK was assessed by Western blotting using phospho-specific antibodies. The total amount of each MAP kinase was assessed using specific antibodies against non-phosphorylated protein.

dominant negative mutant of JNK inhibited hypoxia-induced apoptosis [21]. To determine the potential role of p38 MAPK, JNK, and ERK in hypoxia-induced apoptosis, we first examined the effects of hypoxia on the phosphorylation of p38 MAPK, JNK, and ERK. As shown in Fig. 4A, the phosphorylation of p38 MAPK and JNK, but not ERK, was increased under hypoxic condition. The phosphorylation of p38 was observed when cells were incubated in a hypoxic environment for 12 h. After 24 h of hypoxia, the level of phosphorylated p38 was considerably decreased. 2-Deoxy-D-ribose suppressed the phosphorylation of p38 MAPK in cells under hypoxic condition. Phosphorylated p38 MAPK was not detected after incubation for 24 h in the presence of 2-deoxy-D-ribose under hypoxic condition (Fig. 4B).

The effect of SB203580 on hypoxia-induced apoptosis in HL-60 cells

To investigate the role of p38 MAPK in apoptosis, we compared the proportion of apoptotic cells in the presence or absence of p38 MAPK inhibitor SB203580 under normoxic or hypoxic conditions. The cells treated with SB203580 strongly reduced the proportion of sub-G_1 fraction under hypoxic conditions (Fig. 5). These results suggested that the phosphorylation of p38 MAPK caused apoptosis in HL-60 cells.

The effect of 2-deoxy-D-ribose on Bax translocation under hypoxic conditions

The activation of p38 MAPK promoted translocation of Bax from cytosol to mitochondria and cell death [22,23].

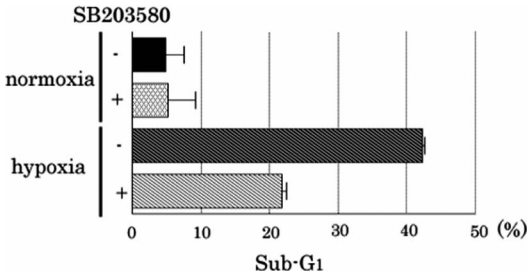

Fig. 5. The effect of p38 MAP kinase inhibitor SB203580 on hypoxia-induced apoptosis. HL-60 cells were cultured under normoxic or hypoxic conditions as indicated in the presence or absence of 10 μM SB203580 for 30 h. The sub-G_1 fractions were measured by flow cytometric analysis.

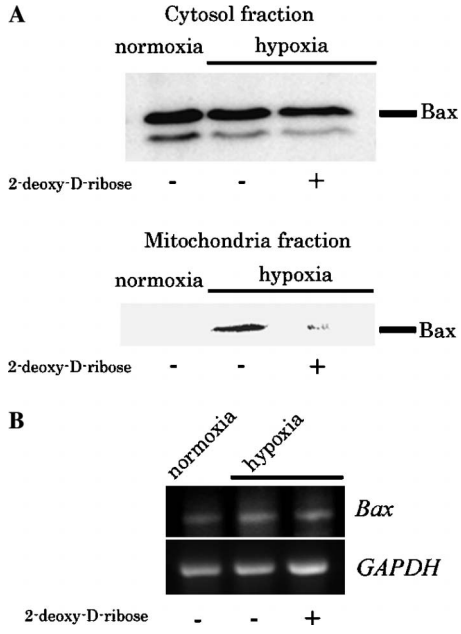

Fig. 6. The effect of 2-deoxy-D-ribose on the hypoxia-induced Bax protein level associated with mitochondria and cytosol. HL-60 cells were incubated under normoxic or hypoxic conditions in the presence or absence of 2-deoxy-D-ribose. To determine the protein levels, mitochondrial fractions and cytosol fraction were prepared and subjected to immunoblot analysis using an antibody against Bax as indicated (A). The expression of Bax in HL-60 cells under normoxic or hypoxic condition in the presence or absence of 2-deoxy-D-ribose. Total RNA was extracted, and RT-PCR was performed with Bax and GAPDH (B).

We therefore examined the effect of 2-deoxy-D-ribose on Bax translocation from cytosol to the mitochondria under hypoxic conditions. The cells were first incubated for 18 h under hypoxic conditions. Mitochondrial fractions and cytosol fraction were then prepared and subjected to immunoblot analysis using an anti-Bax antibody. The immunoblot analysis showed that significant amounts of Bax levels were observed in the cytosol under normoxic or hypoxic condition in the HL-60 cells. The levels of Bax attached to mitochondria were increased under hypoxic conditions (Fig. 6A). 2-Deoxy-D-ribose considerably decreased the level of Bax attached to mitochondria under hypoxic conditions. As shown in Fig. 6B, total RNA was

extracted under normoxic or hypoxic condition for 24 h in the presence or absence of 2-deoxy-D-ribose in HL-60 cells, and then RT-PCR was performed. 2-Deoxy-D-ribose failed to reduce the expression of *Bax* mRNA in HL-60 cells under hypoxic condition (Fig. 6B).

Discussion

Previous studies demonstrated that TP confers resistance to apoptosis induced by hypoxia and that the enzymatic activity of TP is required for this effect. 2-Deoxy-D-ribose, a degradation product of thymidine generated by TP activity, can also prevent hypoxia-induced apoptosis in human epidermoid carcinoma KB cells [11]. Both TP and 2-deoxy-D-ribose inhibited the up-regulation of hypoxia-inducible factor (HIF)-1α, the down-regulation of Bcl-2 and Bcl-X_L, mitochondrial cytochrome *c* release, and caspase 3 activation. 2-Deoxy-D-ribose also prevented the hypoxia-induced apoptosis of human leukemia HL-60 cells [12]. Our findings suggest that 2-deoxy-D-ribose is a downstream mediator of TP function [11,12].

The activation of p38 MAPK by cellular stresses was coupled to apoptosis signaling [24] but also to cell survival [25,26]. These findings suggest that distinct p38 MAPK isoforms are selectively involved in the cell death and survival signal pathways. Exposure of the cells to moderate hypoxia stimulated the phosphorylation of p38α and γ [27]. p38α and p38β MAPK isoforms were specifically blocked by SB203580, which suppressed hypoxia-induced apoptosis. Cells lacking p38α MAPK are more resistant to apoptosis induced by serum deprivation and an anti-Fas monoclonal antibody, indicating that p38α has an important role in apoptosis [24].

The activation of p38 MAPK was required for translocation of Bax to mitochondria, cytochrome *c* release, and apoptosis induced by UVB irradiation in human keratinocytes [23]. The p38 MAPK activity was indispensable for NO-mediated apoptosis and the regulation of Bax translocation to the mitochondria in neurons [22]. Hypoxia was shown to cause translocation of Bax to mitochondria and the release of cytochrome *c* into cytosol in HeLa cells [28]. p38 MAPK phosphorylation was also enhanced by hypoxia in this study. Exposure of HL-60 cells to hypoxia increased the level of the proapoptotic Bcl-2 family member Bax attached to the mitochondria. These findings indicated that the p38 MAPK–Bax pathway plays a critical role in apoptosis induced by different stimuli in different cells.

Bax induced the release of cytochrome *c* from mitochondria into cytosol and caused mitochondria dysfunction [28–31]. Proapoptotic Bax is a critical mediator of the mitochondrial pathway of caspase activation. We therefore examined the effect of 2-deoxy-D-ribose on the levels of Bax attached to mitochondria in cells incubated under hypoxic conditions. 2-Deoxy-D-ribose inhibited the hypoxia-induced translocation of Bax from the cytosol to the mitochondria under hypoxic conditions.

These findings suggest that the inhibition of Bax translocation from the cytosol to mitochondria by 2-deoxy-D-ribose may be mediated by the inhibition of p38 activation. The effect of 2-deoxy-D-ribose on apoptosis may therefore be mediated by decreasing the phosphorylation of p38 MAPK. Further study is needed to elucidate the mechanism of the suppression of the p38 MAPK phosphorylation by 2-deoxy-D-ribose.

2-Deoxy-D-ribose is produced by the catalytic action of TP, which is expressed at high levels in a wide variety of solid tumors compared to the adjacent non-neoplastic tissues. We have previously demonstrated that 2-deoxy-D-ribose as well as TP promoted the angiogenesis and chemotaxis of endothelial cells [7]. TP may thus play an important role in the progression of tumors by producing 2-deoxy-D-ribose from thymidine.

References

[1] M.H. Iltzsch, M.H. Kouni, S. Cha, Kinetic studies of thymidine phosphorylase from mouse liver, Biochemistry 24 (1985) 6799–6807.
[2] T. Furukawa, A. Yoshimura, T. Sumizawa, M. Haraguchi, S. Akiyama, K. Fukui, M. Ishizawa, Y. Yamada, Angiogenic factor, Nature 356 (1992) 668.
[3] T. Sumizawa, T. Furukawa, M. Haraguchi, A. Yoshimura, A. Takeyasu, M. Ishizawa, Y. Yamada, S. Akiyama, Thymidine phosphorylase activity associated with platelet-derived endothelial cell growth factor, J. Biochem. (Tokyo) 114 (1993) 9–14.
[4] M. Haraguchi, K. Miyadera, K. Uemura, T. Sumizawa, T. Furukawa, K. Yamada, S. Akiyama, Y. Yamada, Angiogenic activity of enzymes, Nature 368 (1994) 198.
[5] F. Ishikawa, K. Miyazono, U. Hellman, H. Drexler, C. Wernstedt, K. Hagiwara, K. Usuki, F. Takaku, W. Risau, C.H. Heldin, Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor, Nature 338 (1989) 557–562.
[6] K. Miyazono, T. Okabe, A. Urabe, F. Takaku, C.H. Heldin, Purification and properties of an endothelial cell growth factor from human platelets, J. Biol. Chem. 262 (1987) 4098–4103.
[7] K. Miyadera, T. Sumizawa, M. Haraguchi, H. Yoshida, W. Konstanty, Y. Yamada, S. Akiyama, Role of thymidine phosphorylase activity in the angiogenic effect of platelet-derived endothelial cell growth factor/thymidine phosphorylase, Cancer Res. 55 (1995) 1687–1690.
[8] Y. Takebayashi, S. Akiyama, S. Akiba, K. Yamada, K. Miyadera, T. Sumizawa, Y. Yamada, F. Murata, T. Aikou, Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma, J. Natl. Cancer Inst. 88 (1996) 1110–1117.
[9] Y. Takebayashi, K. Yamada, K. Miyadera, T. Sumizawa, T. Furukawa, F. Kinoshita, D. Aoki, H. Okumura, Y. Yamada, S. Akiyama, T. Aikou, The activity and expression of thymidine phosphorylase in human solid tumours, Eur. J. Cancer A 32 (1996) 1227–1232.
[10] Y. Takebayashi, K. Miyadera, S. Akiyama, S. Hokita, K. Yamada, S. Akiba, Y. Yamada, T. Sumizawa, T. Aikou, Expression of thymidine phosphorylase in human gastric carcinoma, Jpn. J. Cancer Res. 87 (1996) 288–295.
[11] M. Kitazono, Y. Takebayashi, K. Ishitsuka, S. Takao, A. Tani, T. Furukawa, K. Miyadera, Y. Yamada, T. Aikou, S. Akiyama, Prevention of hypoxia-induced apoptosis by the angiogenic factor thymidine phosphorylase, Biochem. Biophys. Res. Commun. 253 (1998) 797–803.
[12] R. Ikeda, T. Furukawa, M. Kitazono, K. Ishitsuka, H. Okumura, A. Tani, T. Sumizawa, M. Haraguchi, M. Komatsu, H. Uchimiya, X.Q.

Ren, T. Motoya, K. Yamada, S. Akiyama, Molecular basis for the inhibition of hypoxia-induced apoptosis by 2-deoxy-D-ribose, Biochem. Biophys. Res. Commun. 291 (2002) 806–812.
[13] R.J. Davis, Signal transduction by the JNK group of MAP kinases, Cell 103 (2000) 239–252.
[14] J.Y. Fang, B.C. Richardson, The MAPK signaling pathways and colorectal cancer, Lancet Oncol. 6 (2005) 322–327.
[15] A.R. Nebreda, A. Porras, p38 MAP kinases: beyond the stress response, Trends Biochem. Sci. 25 (2000) 257–260.
[16] Z. Assefa, A. Vantieghem, M. Garmyn, W. Declercq, P. Vandenabeele, J.R. Vandenheede, R. Bouillon, W. Merlevede, P. Agostinis, p38 mitogen-activated protein kinase regulates a novel, caspase-independent pathway for the mitochondrial cytochrome *c* release in ultraviolet B radiation-induced apoptosis, J. Biol. Chem. 275 (2000) 21416–21421.
[17] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature (Lond) 227 (1970) 680–685.
[18] J.K. Andersen, Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose, J. Biochem. Biophys. Methods 10 (1984) 203–209.
[19] X.X. Wu, Y. Mizutani, Y. Kakehi, O. Yoshida, O. Ogawa, Enhancement of Fas-mediated apoptosis in renal cell carcinoma cells by adriamycin, Cancer Res. 60 (2000) 2912–2918.
[20] W. Zou, J. Zeng, M. Zhuo, W. Xu, L. Sun, J. Wang, X. Liu, Involvement of caspase-3 and p38 mitogen-activated protein kinase in cobalt chloride-induced apoptosis in PC12 cells, J. Neurosci. Res. 67 (2002) 837–843.
[21] M. Kunz, S. Ibrahim, D. Koczan, H.J. Thiesen, H.J. Kohler, T. Acker, K.H. Plate, S. Ludwig, U.R. Rapp, E.B. Brocker, G.N. van Muijen, E. Flory, G. Gross, Activation of c-Jun NH_2-terminal kinase/stress-activated protein kinase (JNK/SAPK) is critical for hypoxia-induced apoptosis of human malignant melanoma, Cell Growth Differ. 12 (2001) 137–145.
[22] S. Ghatan, S. Larner, Y. Kinoshita, M. Hetman, L. Patel, Z. Xia, R.J. Youle, R.S. Morrison, p38 MAP kinase mediates bax translocation in nitric oxide-induced apoptosis in neurons, J. Cell Biol. 150 (2000) 335–347.
[23] A. Van Laethem, S. Van Kelst, S. Lippens, W. Declercq, P. Vandenabeele, S. Janssens, J.R. Vandenheede, M. Garmyn, P. Agostinis, Activation of p38 MAPK is required for Bax translocation to mitochondria, cytochrome *c* release and apoptosis induced by UVB irradiation in human keratinocytes, FASEB J. 18 (2004) 1946–1948.
[24] A. Porras, S. Zuluaga, E. Black, A. Valladares, A.M. Alvarez, C. Ambrosino, M. Benito, A.R. Nebreda, P38 alpha mitogen-activated protein kinase sensitizes cells to apoptosis induced by different stimuli, Mol. Biol. Cell 15 (2004) 922–933.
[25] N. Hendrickx, C. Volanti, U. Moens, O.M. Seternes, P. de Witte, J.R. Vandenheede, J. Piette, P. Agostinis, Up-regulation of cyclooxygenase-2 and apoptosis resistance by p38 MAPK in hypericin-mediated photodynamic therapy of human cancer cells, J. Biol. Chem. 278 (2003) 52231–52239.
[26] S. Okamoto, D. Krainc, K. Sherman, S.A. Lipton, Antiapoptotic role of the p38 mitogen-activated protein kinase-myocyte enhancer factor 2 transcription factor pathway during neuronal differentiation, Proc. Natl. Acad. Sci. USA 97 (2000) 7561–7566.
[27] P.W. Conrad, R.T. Rust, J. Han, D.E. Millhorn, J.D. Beitner, Selective activation of p38alpha and p38gamma by hypoxia. Role in regulation of cyclin D1 by hypoxia in PC12 cells, J. Biol. Chem. 274 (1999) 23570–23576.
[28] V. Mikhailov, M. Mikhailova, K. Degenhardt, M.A. Venkatachalam, E. White, P. Saikumar, Association of Bax and Bak homo-oligomers in mitochondria. Bax requirement for Bak reorganization and cytochrome *c* release, J. Biol. Chem. 278 (2003) 5367–5376.
[29] V. Mikhailov, M. Mikhailova, D.J. Pulkrabek, Z. Dong, M.A. Venkatachalam, P. Saikumar, Bcl-2 prevents Bax oligomerization in the mitochondrial outer membrane, J. Biol. Chem. 276 (2001) 18361–18374.
[30] P. Saikumar, Z. Dong, Y. Patel, K. Hall, U. Hopfer, J.M. Weinberg, M.A. Venkatachalam, Role of hypoxia-induced Bax translocation and cytochrome *c* release in reoxygenation injury, Oncogene 17 (1998) 3401–3415.
[31] M.C. Wei, W.X. Zong, E.H. Cheng, T. Lindsten, V. Panoutsakopoulou, A.J. Ross, K.A. Roth, G.R. MacGregor, C.B. Thompson, S.J. Korsmeyer, Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death, Science 292 (2001) 727–730.