Molecular basis for the regulation of hypoxia-inducible factor-1α levels by 2-deoxy-D-ribose

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Abstract. The angiogenic factor, platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/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, inhibits the upregulation of hypoxia-inducible factor (HIF) 1α, BNIP3 and caspase-3 induced by hypoxia. In the present study, we investigated the molecular basis for the suppressive effect of 2-deoxy-D-ribose on the upregulation of HIF-1α. 2-Deoxy-D-ribose enhanced the interaction of HIF-1α and the von Hippel-Lindau (VHL) protein under hypoxic conditions. It did not affect the expression of *HIF-1α*, prolyl hydroxylase *(PHD)1/2/3* and *VHL* mRNA under normoxic or hypoxic conditions, but enhanced the interaction of HIF-1α and PHD2 under hypoxic conditions. 2-Deoxy-D-ribose also increased the amount of hydroxy-HIF-1α in the presence of the proteasome inhibitor MG-132. The expression levels of TP are elevated in many types of malignant solid tumors and, thus, 2-deoxy-D-ribose generated by TP in these tumors may play an important role in tumor progression by preventing hypoxia-induced apoptosis.

Introduction

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 the angiogenic factor, platelet-derived endothelial cell growth factor (PD-ECGF) (2,3). TP stimulates chemotaxis and [^{3}H]thymidine incorporation by endothelial cells *in vitro* and has an angiogenic activity *in vivo* (4-7). We previously demonstrated that TP enzymatic activity is indispensable for its 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 when compared to that in the adjacent non-neoplastic tissues (8-10). Under hypoxic conditions, TP can also enhance the growth of tumor cells and confer resistance to apoptosis induced by hypoxia (11). 2-Deoxy-D-ribose was able to partially prevent hypoxia-induced apoptosis in human leukemia HL-60 cells (12,13). TP also inhibited upregulation of HIF-1α, BNIP3 and caspase-3 under hypoxic conditions (14). These findings indicated that TP has another function apart from angiogenesis.

To elucidate the mechanism by which 2-deoxy-D-ribose suppresses HIF-1α levels under hypoxic conditions, we examined the effect of 2-deoxy-D-ribose on key regulators of HIF-1α: von Hippel-Lindau (VHL) and prolyl hydroxylase (PHD)2.

Materials and methods

Reagents and antibodies. The monoclonal anti-HIF-1α antibody was purchased from Transduction Laboratories (Lexington, KY, USA). The rabbit anti-VHL polyclonal antibody (FL-181) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A rabbit anti-PHD2 polyclonal antibody was from Bethyl Laboratories (Montgomery, TX, USA). A rabbit anti-hydroxy-HIF-1α (Pro564) was from Cell Signaling Technology (Boston, MA, USA).

Cell lines and induction of hypoxia. Human leukemia HL-60 cells were maintained in RPMI-1640 containing 10% fetal calf

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serum. 2-Deoxy-D-ribose was added to the culture medium and then hypoxia (1% O_2) was induced in a Personal Multigas Incubator (Astec).

Immunoblotting analysis. Samples were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli. Proteins in the gel were electrophoretically transferred onto polyvinylidene difluoride membranes (Immobilon-P transfer membrane; Millipore, Bedford, MA, USA) using Bio-Rad Trans-Blot SD apparatus. The membrane was treated with buffer A [350 mM NaCl, 10 mM Tris-HCl (pH 8.0), 0.05% Tween 20] containing 3% skimmed milk for 1 h and incubated with the indicated antibody (1:1,000) in buffer A containing 3% skimmed milk for 1 h. Following four washes with buffer A (10 min each), the membrane was incubated with peroxidase-conjugated horse anti-mouse IgG diluted 1:1,000 in buffer A containing 3% skimmed milk for 1 h. Following washing with buffer A, the membrane was developed using the enhanced chemiluminescence western blotting detection system (Amersham Pharmacia, Buckinghamshire, UK).

RT-PCR method. Total cellular RNA was extracted using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). 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 mM of each primer, 2 units of enzyme mixture including SuperScript II RT, Platinum Taq DNA polymerase, and 1X buffer with 1.2 mM $MgSO_4$ were maintained at 50˚C for 20 min, and then at 94˚C for 2 min, and PCR was performed as follows. The PCR profile consisted of 30 cycles at 94˚C for 15 sec, 55˚C for 30 sec, and 70˚C for 30 sec. The primers for RT-PCRs were designed based on human sequences in GenBank. These sequences used the following primers: PHD1, 5'-gccagtgctgggctgatggagg-3' and 5'-cgcagtggcggatgacggcgtc-3'; PHD2, 5'-tgcatgaacaa gcacggcatct-3' and 5'-atatacatgtcacacatcttcc-3'; PHD3, 5'-ga ctgcgtcctggagcgcgtca-3' and 5'-atatccgcaggatcccaccatg-3'; VHL, 5'-atgccccggagggcggagaactggg-3' and 5'-tcaatctcccatcc gttgatgtgca-3'; HIF-1α, 5'-tcgaggcctctgtgatgagg-3' and 5'-ggc ctctgtgatgaggcttt-3'; GAPDH, 5'-agaacatcatccctgcctctactgg-3' and 5'-aaaggtggaggagtgggtgtcgctg-3'.

Immunoprecipitation. For immunoprecipitation experiments, FLAG tagged VHL or PHD2 and HIF-1α cDNA (1 μg each) were transiently transfected into COS or HL-60 cells plated in 6-cm diameter dishes. Twenty four hours following transfection, the cells were treated for 5 h under hypoxic conditions and cells were harvested. The cells were suspended in 200 μg of whole cell extract buffer [10 mM HEPES (pH 7.9), 400 mM NaCl, 0.1 mM EDTA, 5% (vol/vol) glycerol, 1 mM DTT, 1 mM APMSF], and centrifuged at 12,500 rpm for 30 min at 4˚C. Subsequent to protein extraction, 300 μg of total proteins was incubated with anti-FLAG or anti-PHD2 antibodies at 4˚C for 1 h. Thirty microliters of a 50% slurry of protein G-Sepharose 4B in TEG buffer [20 mM Tris-HCl (pH 7.9), 1 mM EDTA, 10% glycerol, 1 mM DTT], containing 150 mM NaCl and 0.1% Triton X-100, were then added to reaction mixtures and incubated for 12 h at 4˚C with rotation. Following a rapid centrifugation, the resulting pellets were washed three times with TEG buffer, and the immunoprecipitated proteins were analyzed by immunoblotting using an anti-HIF-1α antibody.

Results

Effect of 2-deoxy-D-ribose on the expression of HIF-1α under hypoxic conditions. We determined the effect of 2-deoxy-D-ribose on the HIF-1a protein level under hypoxic conditions. As shown in Fig. 1A, we could not detect HIF-1α in HL-60 cells under a normoxic condition. When HL-60 cells were incubated under hypoxic conditions, the HIF-1α protein level was markedly increased (Fig. 1A). Treatment of the cells with 2-deoxy-D-ribose substantially suppressed the level of HIF-1α in the cells under hypoxic conditions. We next determined the effect of 2-deoxy-D-ribose on the expression of *HIF-1α* mRNA levels by RT-PCR. *HIF-1α* mRNA levels were not affected by hypoxia, and treatment of the cells under hypoxic conditions by 2-deoxy-D-ribose did not alter the levels of *HIF-1α* mRNA (Fig. 1B).

Effect of 2-deoxy-D-ribose on the interaction of HIF-1α and VHL. The HIF-1α protein is continuously synthesized and degraded under a normoxic condition, while it accumulates rapidly following exposure to hypoxic conditions. HIF-1α interacts with VHL and is degraded via the ubiquitin-proteasome pathway under a normoxic condition. As the oxygen concentration decreases, PHDs become inactive and the HIF-1α protein is consequently stabilized. One possible mechanism for the attenuation of HIF-1α caused by 2-deoxy-D-ribose under hypoxic conditions might be the enhanced interactions of HIF-1α with VHL and consequent degradation of HIF-1α. To assess the interactions between HIF-1α and VHL in the presence or absence of 2-deoxy-D-ribose under hypoxic conditions, COS cells were cotransfected with HIF-1α and VHL-expressing plasmids. After 24 h following transfection, the cells were exposed for 5 h to hypoxic conditions in the presence or absence of 2-deoxy-D-ribose. As shown in Fig. 2A, incubation of cells under hypoxic conditions resulted in a marked increase in HIF-1α protein levels, and treatment with 2-deoxy-D-ribose substantially suppressed HIF-1α in the cells under hypoxic conditions. To examine the effect of 2-deoxy-D-ribose on the interaction between VHL and HIF-1α, the cell lysates were immunoprecipitated with an anti-FLAG antibody and the coprecipitated HIF-1α was detected with an anti-HIF-1α antibody. Treatment of COS cells with 2-deoxy-D-ribose enhanced interaction of HIF-1α with VHL under hypoxic conditions (Fig. 2B).

Effect of 2-deoxy-D-ribose on the expression of PHD1/2/3 and VHL. To determine the effect of 2-deoxy-D-ribose on the expression of *PHD1/2/3* and *VHL* in HL-60 cells, the cells were cultured under normoxic or hypoxic conditions in the presence or absence of 2-deoxy-D-ribose. As shown in Fig. 3A, the expression of PHD2 was detected but those of *PHD1* and *PHD3* mRNA were not detected by RT-PCR. 2-Deoxy-D-ribose did not affect the expression of PDH2 mRNA and protein levels under normoxic and hypoxic conditions in HL-60 cells (Fig. 3).

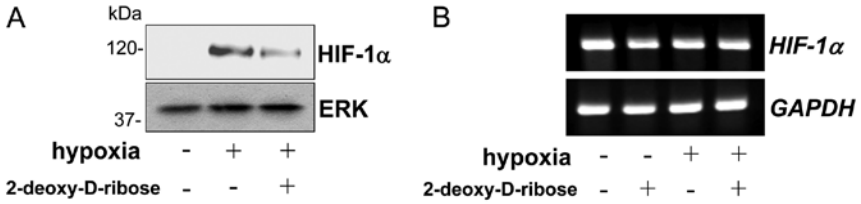

Figure 1. Effect of 2-deoxy-D-ribose on the expression of HIF-1α under hypoxic conditions. (A) The cells were incubated under hypoxic conditions for 18 h, and cell lysates were prepared from the cells. HIF-1α was detected by immunoblotting with an anti-HIF-1α antibody. (B) HL-60 cells were cultured under normoxic or hypoxic conditions for 24 h. Total RNA was extracted and the expression of HIF-1α was evaluated by RT-PCR.

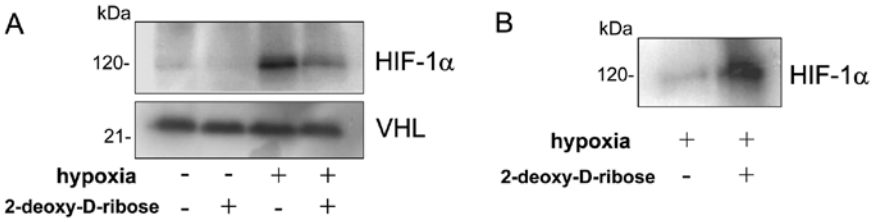

Figure 2. Effect of 2-deoxy-D-ribose on the interaction between VHL and HIF-1α. (A) COS cells were transiently cotransfected with VHL and HIF-1α in the presence of MG-132, and the cells were cultured under normoxic or hypoxic conditions for 5 h. HIF-1α and VHL were detected by immunoblotting with an anti-HIF-1α and anti-VHL antibody, respectively. (B) COS cells were transiently cotransfected with VHL and HIF-1α plasmids in the presence of MG-132, and the cells were cultured under normoxic or hypoxic conditions for 5 h, and cell lysates were prepared from the cells. Following immunoprecipitation of proteins with an anti-FLAG antibody, HIF-1α was detected by immunoblotting using an anti-HIF-1α antibody.

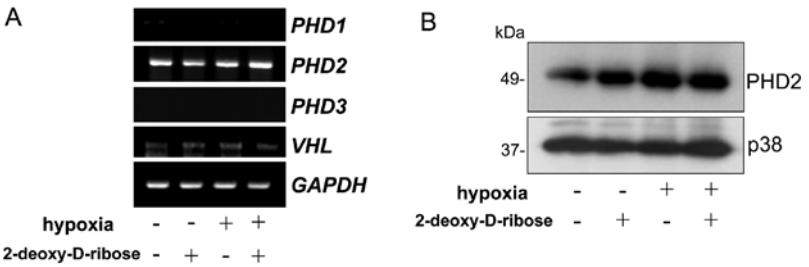

Figure 3. Effect of 2-deoxy-D-ribose on the expression of PHD1, PHD2, PHD3 and VHL. (A) HL-60 cells were cultured under normoxic or hypoxic conditions for 24 h. Total RNA was extracted and the expression of PHD1, PHD2, PHD3, VHL and GAPDH was evaluated by RT-PCR. (B) The cells were incubated under hypoxic conditions for 24 h and cell lysates were prepared from the cells.

Effect of 2-deoxy-D-ribose on the interaction between HIF-1a and PHD2. 2-Deoxy-D-ribose may decrease HIF-1α protein levels by affecting the interaction between HIF-1α and PHD2 and consequently modulating its degradation. To assess the effect of 2-deoxy-D-ribose on the interaction between HIF-1α and PHD2, HL-60 cells were cultured under normoxic or hypoxic conditions in the presence or absence of 2-deoxy-D-ribose. Whole cell extracts were prepared and immunoprecipitated with an anti-HIF-1α antibody and coprecipitated PHD2 was detected with an anti-PHD2 antibody. Hypoxia attenuated the interaction between HIF-1α and PHD2. Treatment of the cells with 2-deoxy-D-ribose augmented the interaction between HIF-1α and PHD2 under hypoxic conditions (Fig. 4A).

Effect of 2-deoxy-D-ribose on the levels of hydroxy-HIF-1a. HIF-1α can be hydroxylated by proline hydroxylase under normoxic conditions. Hydroxylation of HIF-1α leads to its binding to VHL and ubiquitin-mediated degradation (30,31). To further characterize the mechanisms leading to decreased

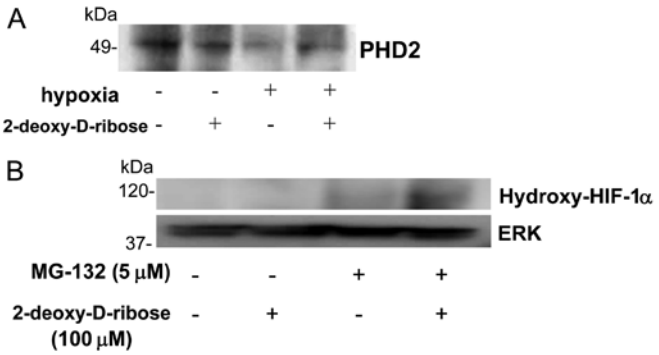

Figure 4. Effect of 2-deoxy-D-ribose on the interaction between PHD2 and HIF-1α, and the levels of hydroxy-HIF-1α. (A) Effect of 2-deoxy-D-ribose on the interaction between PHD2 and HIF-1α. HL-60 cells were cultured under normoxic or hypoxic conditions for 24 h and cell lysates were prepared from the cells. Following immunoprecipitation of proteins with an anti-PHD2 antibody, HIF-1α was detected by immunoblotting using an anti-HIF-1α antibody. (B) Effect of 2-deoxy-D-ribose on the levels of hydroxy-HIF-1α. HL-60 cells were cultured in the presence or absence of 2-deoxy-D-ribose or MG-132 for 5 h, and cell lysates were prepared from the cells. Hydroxy-HIF-1α was detected by immunoblotting using an anti-hydroxy-HIF-1α antibody.

HIF-1α levels by 2-deoxy-D-ribose, we analyzed the levels of hydroxy-HIF-1α. Direct analysis of the hydroxylation state of HIF-1α was carried out using an antibody specifically directed against hydroxy-HIF-1α. To visualize hydroxy-HIF-1α, the cells were treated with the proteasome inhibitor MG-132 to prevent the proteasomal degradation of HIF-1α. As shown in Fig. 4B, 2-deoxy-D-ribose increased the amounts of hydroxy-HIF-1α in the presence of MG-132. This finding suggests that 2-deoxy-D-ribose enhances proline hydroxylase activity.

Discussion

Previous studies have 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 KB epidermoid carcinoma cells (11,13) suggesting that it may be a downstream mediator of the TP function. 2-Deoxy-D-ribose prevented the hypoxia-induced activation of caspase-3 and -9 in HL-60 cells (12). TP-overexpressing Jurkat cells were also resistant to hypoxia-induced apoptosis. The induction of caspase-3 activity and the expression of HIF-1α and BNIP3 were found to be suppressed under hypoxic conditions in TP-expressing Jurkat cells (14).

HIF-1α protein is rapidly accumulated under hypoxia and degraded under a normoxic condition (15-17). The accumulated HIF-1α under hypoxia dimerizes with HIF-1β and translocates into the nucleus. HIF-1 binds to the hypoxia response element (HRE) on nuclear DNA, recruits coactivators p300/CBP, and promotes gene transcription of various genes (18,19). Many tumors contain a hypoxic microenvironment, a condition associated with resistance to anticancer agents and poor prognosis. HIF-1α is upregulated in a broad range of tumors and is involved in angiogenesis, invasion and altered energy metabolism (20). There appears to be a delicate balance between the pro- and anti-tumorigenic effects of HIF-1α. Several gene products such as BNIP3, RTP801 and Noxa were identified as HIF-1α-responsive pro-apoptotic proteins. Hypoxia-mediated BNIP3 expression is regulated by HIF-1α that directly binds to a consensus HRE in the *BNIP3* promoter (21). Overexpression of BNIP3 has been shown to be cytotoxic in a number of tumor cell lines (22-25). Mitochondria may be a direct target of death signals mediated via BNIP3 under hypoxic conditions. BNIP3 induced by HIF-1α binds to mitochondria and opens the mitochondrial permeability transition pore (26). BNIP3 is a key regulator of mitochondrial function and cell death of ventricular myocytes during hypoxia (26). We previously demonstrated that TP suppressed the level of BNIP3 under hypoxic conditions, and 2-deoxy-D-ribose, a downstream mediator of the TP function, accelerated the proteasome-mediated degradation of HIF-1α by enhancing the ubiquitination under hypoxic conditions (12,14).

O_2-dependent regulation of the HIF-1α protein is mediated by a functional domain of 200 amino acids located on the carboxy terminal to the PAS domain, which was named the oxygen-dependent degradation (ODD) domain (27). HIF-1α prolyl hydroxylases utilize O_2 and α-ketoglutarate as a substrate to generate 4-hydroxyproline at residues 402 and/or 546 of human HIF-1α (28,29). Three such prolyl hydroxylases 1-3 (PHD1-3) were identified in mammalian cells. Under normoxic conditions, the hydroxylation of specific proline residues of HIF-1α by PHD2 promotes the interaction of HIF-1α with the VHL protein and consequently ubiquitination and proteasomal degradation of HIF-1α (30,31). Although PHD1 and PHD3 can hydroxylate HIF-1α *in vitro*, HIF does not appear to be their physiological target in the cell (32). During hypoxia, the reduced levels of proline hydroxylation lead to HIF-1α degradation, causing an increase in its levels.

2-Deoxy-D-ribose had no effect on *HIF-1α* mRNA expression levels indicating that 2-deoxy-D-ribose affected the level of HIF-1α at the protein level. In a previous study, we also demonstrated that 2-deoxy-D-ribose accelerated proteasome-mediated degradation of HIF-1α by enhancing the ubiquitination of HIF-1α (12). In this study, we demonstrated that 2-deoxy-D-ribose increased the interaction of HIF-1α with VHL and PHD2 under hypoxic conditions, and levels of proline hydroxy-HIF-1α (Figs. 2 and 4). A potential mechanism of the enhanced levels of proline hydroxy-HIF-1α by 2-deoxy-D-ribose might be the increased level of α-ketoglutarate. The cosubstrates oxygen and α-ketoglutarate as well as the cofactors Fe^{2+} and ascorbate are required for PHD activity. 2-Deoxy-D-ribose is phosphorylated to 2-deoxy-D-ribose 5-phosphate which is then cleaved by deoxy-D-ribose phosphate aldolase to acetoaldehyde and glyceraldehyde-3-phosphate, which then produces pyruvate. α-ketoglutarate and alanine are produced by enzymatic transamination reaction between glutamate and pyruvate (33,34). α-Ketoglutarate produced by 2-deoxy-D-ribose may have increased PHD activity.

2-Deoxy-D-ribose is produced by the catalytic action of TP and is the downstream mediator of TP function. TP is expressed at higher levels in a wide variety of solid tumors when compared to adjacent non-neoplastic tissues. TP may thus play an important role in the progression of tumors by producing 2-deoxy-D-ribose from thymidine and decreasing the level of HIF-1α.

References

1. Iltzsch MH, Kouni MH and Cha S: Kinetic studies of thymidine phosphorylase from mouse liver. Biochemistry 24: 6799-6807, 1985.
2. Furukawa T, Yoshimura A, Sumizawa T, Haraguchi M, Akiyama S, Fukui K, Ishizawa M and Yamada Y: Angiogenic factor. Nature 356: 668, 1992.
3. Sumizawa T, Furukawa T, Haraguchi M, Yoshimura A, Takeyasu A, Ishizawa M, Yamada Y and Akiyama S: Thymidine phosphorylase activity associated with platelet-derived endothelial cell growth factor. J Biochem 114: 9-14, 1993.
4. Haraguchi M, Miyadera K, Uemura K, Sumizawa T, Furukawa T, Yamada K, Akiyama S and Yamada Y: Angiogenic activity of enzymes. Nature 368: 198, 1994.
5. Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, Usuki K, Takaku F, Risau W and Heldin CH: Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 338: 557-562, 1989.
6. Miyazono K, Okabe T, Urabe A, Takaku F and Heldin CH: Purification and properties of an endothelial cell growth factor from human platelets. J Biol Chem 262: 4098-4103, 1987.
7. Miyadera K, Sumizawa T, Haraguchi M, Yoshida H, Konstanty W, Yamada Y and Akiyama S: Role of thymidine phosphorylase activity in the angiogenic effect of platelet-derived endothelial cell growth factor/thymidine phosphorylase. Cancer Res 55: 1687-1690, 1995.

8. Takebayashi Y, Akiyama S, Akiba S, Yamada K, Miyadera K, Sumizawa T, Yamada Y, Murata F and Aikou T: Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma. J Natl Cancer Inst 88: 1110-1117, 1996.
9. Takebayashi Y, Yamada K, Miyadera K, Sumizawa T, Furukawa T, Kinoshita F, Aoki D, Okumura H, Yamada Y, Akiyama S and Aikou T: The activity and expression of thymidine phosphorylase in human solid tumours. Eur J Cancer 32A: 1227-1232, 1996.
10. Takebayashi Y, Miyadera K, Akiyama S, Hokita S, Yamada K, Akiba S, Yamada Y, Sumizawa T and Aikou T: Expression of thymidine phosphorylase in human gastric carcinoma. Jpn J Cancer Res 87: 288-295, 1996.
11. Kitazono M, Takebayashi Y, Ishitsuka K, Takao S, Tani A, Furukawa T, Miyadera K, Yamada Y, Aikou T and Akiyama S: Prevention of hypoxia-induced apoptosis by the angiogenic factor thymidine phosphorylase. Biochem Biophys Res Commun 253: 797-803, 1998.
12. Ikeda R, Furukawa T, Kitazono M, Ishitsuka K, Okumura H, Tani A, Sumizawa T, Haraguchi M, Komatsu M, Uchimiya H, Ren XQ, Motoya T, Yamada K and Akiyama S: Molecular basis for the inhibition of hypoxia-induced apoptosis by 2-deoxy-D-ribose. Biochem Biophys Res Commun 291: 806-812, 2002.
13. Ikeda R, Che XF, Ushiyama M, Yamaguchi T, Okumura H, Nakajima Y, Takeda Y, Shibayama Y, Furukawa T, Yamamoto M, Haraguchi M, Sumizawa T, Yamada K and Akiyama S: 2-Deoxy-D-ribose inhibits hypoxia-induced apoptosis by suppressing the phosphorylation of p38 MAPK. Biochem Biophys Res Commun 342: 280-285, 2006.
14. Ikeda R, Tajitsu Y, Iwashita K, Che XF, Yoshida K, Ushiyama M, Furukawa T, Komatsu M, Yamaguchi T, Shibayama Y, Yamamoto M, Zhao HY, Arima J, Takeda Y, Akiyama S and Yamada K: Thymidine phosphorylase inhibits the expression of proapoptotic protein BNIP3. Biochem Biophys Res Commun 370: 220-224, 2008.
15. Wang GL, Jiang BH, Rue EA and Semenza GL: Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci USA 92: 5510-5514, 1995.
16. Jiang BH, Semenza GL, Bauer C and Marti HH: Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O_2 tension. Am J Physiol 271: C1172-C1180, 1996.
17. Salceda S and Caro J: Hypoxia-inducible factor 1α (HIF1α) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J Biol Chem 272: 22642-22647, 1997.
18. Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P and Giallongo A: Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. J Biol Chem 271: 32529-32537, 1996.
19. Ebert BL and Bunn HF: Regulation of transcription by hypoxia requires a multiprotein complex that includes hypoxia-inducible factor 1, an adjacent transcription factor, and p300/CREB binding protein. Mol Cell Biol 18: 4089-4096, 1998.
20. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D and Keshert E: Role of HIF-1α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 394: 485-490, 1998.
21. Kothari S, Cizeau J, McMillan WE, Israels SJ, Bailes M, Ens K, Kirshenbaum LA and Gibson SB: BNIP3 plays a role in hypoxic cell death in human epithelial cells that is inhibited by growth factors EGF and IGF. Oncogene 22: 4734-4744, 2003.
22. Graham RM, Frazier DP, Thompson JW, Haliko S, Li H, Wasserlauf BJ, Spiga MG, Bishopric NH and Webster KA: A unique pathway of cardiac myocyte death caused by hypoxia-acidosis. J Exp Biol 207: 3189-3200, 2004.
23. Chen G, Ray R, Dubik D, Shi L, Cizeau J, Bleackley RC, Saxena S, Gietz RD and Greenberg AH: The E1B 19K/Bcl-2-binding protein Nip3 is a dimeric mitochondrial protein that activates apoptosis. J Exp Med 186: 1975-1983, 1997.
24. Chen G, Cizeau J, Velde CV, Park JH, Bozek G, Bolton J, Shi L, Dubik D and Greenberg A: Nix and Nip3 form a subfamily of pro-apoptotic mitochondrial proteins. J Biol Chem 274: 7-10, 1999.
25. Velde CV, Cizeau J, Dubik D, Alimonti J, Brown T, Israels S, Hakem R and Greenberg AH: BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore. Mol Cell Biol 20: 5454-5468, 2000.
26. Regula KM, Ens K and Kirshenbaum LA: Inducible expression of BNIP3 provokes mitochondrial defects and hypoxia-mediated cell death of ventricular myocytes. Circ Res 91: 226-231, 2002.
27. Huang LE, Gu J, Schau M and Bunn HF: Regulation of hypoxia-inducible factor 1alpha is mediated by an O_2 dependent degradation domain via the ubiquitin proteasome pathway. Proc Natl Acad Sci USA 95: 7987-7992, 1998.
28. Bruick RK and McKnight SL: A conserved family of prolyl-4-hydroxylases that modify HIF. Science 294: 1337-1340, 2001.
29. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ and Ratcliffe PJ: *C. elegans* EGL9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 107: 43-54, 2001.
30. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS and Kaelin WG Jr: HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science 292: 464-468, 2001.
31. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW and Ratcliffe PJ: Targeting of HIF-α to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 292: 468-472, 2001.
32. Berra E, Benizri E, Ginouves A, Volmat V, Roux D and Pouyssegur J: HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1α in normoxia. EMBO J 22: 4082-4090, 2003.
33. Feron O: Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. Radiother Oncol 92: 329-333, 2009.
34. DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S and Thompson CB: Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci USA 104: 19345-19350, 2007.