Hypoxia induces apoptosis via two independent pathways in Jurkat cells: differential regulation by glucose

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Malhotra, Ricky, Zhiwu Lin, Claudius Vincenzi, and Frank C. Brosius III. Hypoxia induces apoptosis via two independent pathways in Jurkat cells: differential regulation by glucose. Am J Physiol Cell Physiol 281: C1596–C1603, 2001.—Glucose uptake and metabolism inhibit hypoxia-induced apoptosis in a variety of cell types, but the underlying molecular mechanisms remain poorly understood. In the present study, we explore hypoxia-mediated cell death pathways in Jurkat cells in the presence and absence of extracellular glucose. In the absence of extracellular glucose, hypoxia caused cytochrome c release, caspase 3 and poly(ADP-ribose)polymerase cleavage, and DNA fragmentation; this apoptotic response was blocked by the caspase 9 inhibitor z-LEHD-FMK. The presence of extracellular glucose during hypoxia prevented cytochrome c release and activation of caspase 9 but did not prevent apoptosis in Jurkat cells. In these conditions, overexpression of the caspase 8 inhibitor v-FLIP prevented hypoxia-mediated cell death. Thus hypoxia can stimulate two apoptotic pathways in Jurkat cells, one dependent on cytochrome c release from mitochondria that is prevented by glucose uptake and metabolism, and the other independent of cytochrome c release and resulting from activation of the death receptor pathway, which is accelerated by glucose uptake and metabolism.

cytochrome c; viral FLICE inhibitory protein; death receptor; caspases

HYPOXIA AND REOXYGENATION are well-known causes of cellular injury and death in a variety of cell types. Over the past several years, it has become clear that hypoxia and reoxygenation can trigger apoptotic cell death as well as necrosis in a number of cell types (3, 7, 9, 16, 20, 21). Apoptosis is a genetically controlled program that is associated with specific morphological changes, including cell rounding and shrinkage, plasma membrane blebbing, nuclear condensation, and DNA fragmentation (14). Apoptosis is implemented by molecular pathways that ultimately result in activation of a family of cysteine proteases termed caspases (1). These caspases act in a proteolytic cascade that includes initiator and effector caspases. In mammals, two main caspase cascades have been delineated. The first pathway links death receptors expressed at the cell surface to an upstream initiator caspase, caspase 8 (23). After ligation of the death receptors by the appropriate ligands [e.g., Fas ligand or tumor necrosis factor (TNF)], adapter molecules such as Fas-associated death domain (FADD) and TNF receptor-associated death domain are recruited, which in turn bind to procaspase 8 molecules. In close proximity, the procaspase 8 molecules cleave one another to become activated. When caspase 8 is activated by this receptor-mediated process, it proceeds to cleave and activate downstream effector caspases, such as caspases 3, 6, and 7. In the second apoptotic pathway, also known as the mitochondrial cell death pathway, diverse extracellular stresses, such as hypoxia, ultraviolet (UV) irradiation, and growth factor withdrawal, induce the release of cytochrome c from the mitochondria into the cytoplasm (12). Cytochrome c, apoptosis promoting factor-1, and dATP (or ATP) form a complex with and activate procaspase 9, which in turn cleaves and activates downstream effector caspases (19).

We recently reported that glucose uptake and glycolytic metabolism specifically interrupt hypoxia-induced apoptosis in rat neonatal cardiac myocytes via inhibition of the mitochondrial apoptotic pathway (21). We also showed that overexpression of the facilitative glucose transporter, GLUT-1, reduces hypoxia-mediated apoptosis in vascular smooth muscle cells (20). It is unknown whether such glucose-induced inhibition occurs in all cell types or whether glucose metabolism can also inhibit death receptor-mediated apoptotic pathways. To further investigate the pathways involved in hypoxia-induced apoptosis, we therefore studied apoptotic signaling in Jurkat cells, a lymphoblastic cell line that has been extensively used for studying receptor-mediated cell death pathways but has not been used to investigate hypoxia-mediated apoptotic events. We found that hypoxia triggers two distinct apoptotic pathways in Jurkat cells. A mitochondrial apoptotic pathway involving cytochrome c release and caspase 9 activation occurred during hypoxia in the absence of glucose in Jurkat cells, as in other cells (11, 20, 21), and could be prevented by inhibiting caspase 9 activation. Interestingly, despite prevention of cytochrome c release and caspase 9-me-
diated cell death, glucose failed to inhibit hypoxia-induced apoptosis in Jurkat cells and, in fact, augmented biochemical and morphological features of apoptosis in these cells. This cytochrome c-independent apoptosis was largely prevented by overexpression of E8, a viral FLICE inhibitory protein (v-FLIP) that inhibits receptor-mediated apoptosis (28). These data suggest that in Jurkat cells, hypoxia triggers apoptosis through both a cell death receptor pathway and a mitochondrial pathway that are differentially regulated by glucose uptake and metabolism.

MATERIALS AND METHODS

Cell culture, hypoxia, and reoxygenation conditions. Jurkat cells (from the laboratory of Dr. Vishva Dixit) were grown in high glucose containing RPMI medium (GIBCO BRL) with 10% fetal calf serum, 0.1 mM nonessential amino acids, and 2 mM glutamine. For hypoxia experiments, cells were washed three times with serum- and glucose-free medium and suspended in glucose-free medium or medium containing variable concentrations of glucose. Cells were then placed in a Plexiglas chamber, and a constant stream of water-saturated 95% N2-5% CO2 was maintained over the culture as previously reported (11, 20, 21). To lower the PO2 to <5 mmHg, Oxyrase, a mixture of bacterial membrane monoxygenases and dioxygenases (Oxyrase, Ashland, OH), was added to the culture medium at a final concentration of 2% as previously reported (11, 20, 21). After variable periods of hypoxia, cells were harvested or reoxygenated for 2 h by replacing the medium with fresh serum and glucose-containing RPMI medium and placing it in a conventional tissue culture incubator at 37°C in an atmosphere of 95% air-5% CO2. In some experiments, cells were preincubated in 100 μM of the non-specific caspase inhibitor benzylxycarbonyl-valinylalanlyaspartylfluoromethyl ketone (z-VAD-FMK), the more specific caspase 3 inhibitor benzylxycarbonyl-aspartylglutamylvalinylanlyaspartylfluoromethyl ketone (z-DEVD-FMK), or the caspase 9 inhibitor z-Leu-Glu (OMe)-His-Asp(OMe)-FMK-TFA (z-LEHD-FMK) (Enzyme Systems Products, Livermore, CA), as previously reported (11, 20, 21, 30).

DNA fragmentation. Cells were washed twice with PBS and resuspended in 500 μl of TNE buffer (10 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 10 mM EDTA). The cells were lysed with 2% SDS, and the lysates were incubated with 0.25 μg/μl RNase A (Roche Molecular Biologicals) for 1 h at 37°C, followed by incubation in 0.2 μg/μl of proteinase K (Roche) at 50°C for 2 h. The samples were extracted twice with phenol/chloroform. Genomic DNA was precipitated with ethanol and then dissolved in 50 μl of TE (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA). After spectrophotometric determination of the nucleic acid content, DNA samples (4 μg) were electrophoresed on 1.5% agarose gels to visualize laddering.

Morphological analysis. Cells were washed with PBS and fixed with 20% methanol. The cell pellets were resuspended in PBS with 1 μM propidium iodide (Sigma, St. Louis, MO) as previously reported (20). The cells were mounted on slides and counted under a fluorescence microscope. Cells with nuclear fragmentation or condensation were characterized as apoptotic cells. In other experiments, cells were washed with 1× PBS and resuspended in trypan blue solution (Sigma). The cells that failed to exclude trypan blue dye were characterized as dead cells (either necrotic or late apoptotic). For some experiments, Jurkat cells were preincubated with z-LEHD-FMK and stained with the karyophilic dye Hoe-33258. In these experiments, the cells were rinsed in PBS and fixed for 30 min in 4% paraformaldehyde at room temperature. After a rinse in PBS, the cells were permeabilized in 0.1% Triton X-100 in 0.1% sodium citrate. The cells were rinsed twice in PBS and then stained with HOE-33258 (5 μg/ml) for 10 min at room temperature. After a final rinse in PBS, the cells were stained with moiwol, an antifade agent, and visualized under UV light with a Leitz Orthoplan microscope. The percentage of apoptotic cells displaying chromatin condensation and nuclear fragmentation was determined. For all experiments, 100 cells were counted in five different fields.

Cytochrome c release. Mitochondrial and cytosolic fractions were prepared using a modification of our previously published protocol (21). Cells (3×10⁷) were washed with PBS, suspended in 10 mM HEPES, pH 7.4, 1 mM EDTA, 250 mM sucrose, and homogenized with a type B Dounce (glass/glass) tissue grinder. The homogenates were centrifuged at 10,000 g for 30 min to obtain a pellet highly enriched in mitochondria. This pellet was resuspended in the same buffer and recentrifuged while the supernatant was further centrifuged at 160,000 g for 1 h. The supernatant from this final ultracentrifugation represented the cytosolic fraction. The resulting protein samples were electrophoresed on 15% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Immunoblotting was performed as previously reported (21) using the cytochrome c antibody (Pharmingen, San Diego, CA) at a final concentration of 1 μg/ml in 5% nonfat dry milk. To ensure that cytochrome c release was not due to physical disruption of mitochondria during isolation, both the mitochondrial and cytosolic fractions were probed with monoclonal antibodies (MAB) to cytochrome oxidase (subunit IV; MAb 20E8-C12 at a dilution of 0.1 μg/ml; Molecular Probes, Eugene, OR), an enzyme complex bound to the outer leaflet of the inner mitochondrial membrane. Signals were detected by enhanced chemiluminescence (ECL, Amersham).

Cleavage of poly(ADP-ribose)/polymerase. For immunoblot analysis of poly(ADP-ribose)/polymerase (PARP), the cells were rinsed in cold PBS, pH 7.4, and then collected into a defined volume of lysis buffer (62.5 mM Tris, pH 6.8, 2% SDS, 8 M deionized urea, 10% glycerol, and protease inhibitors) and sonicated for 15 s. After loading buffer was added, the samples were incubated at 65°C for 15 min and resolved on a 7.5% SDS-PAGE gel. Immunoblotting for PARP was performed as previously described (21) using a MAb that specifically detects rat, mouse, or human PARP (MAB SA-250; clone C-2–10; BIOMOL Research Labs, Plymouth Meeting, PA) at a 1:5,000 dilution. Visualization of the signal was by ECL (Amersham).

Caspase cleavage. Whole cell lysates were prepared in 50 mM Tris·HCl, pH 8.0, 150 mM NaCl, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, and 1% Triton X-100. Cytosolic fractions were prepared as noted earlier. Protein samples were separated on 12% SDS-polyacrylamide gels, and immunoblotting was performed as indicated earlier with a 1:1,000 dilution of caspase 3, caspase 6, or caspase 7 antibodies (gifts from Dr. Vishva Dixit). These antibodies were raised against synthetic peptides from the p20 subunits that are cleaved from the respective caspases during apoptosis. The antibodies specifically recognize both the proform and the cleaved p20 subunits of human caspases 3, 6, and 7. Equal protein loading for all caspase Westerns was determined by staining the blots with Ponceau S solution. Samples from Jurkat cells that had been exposed to 2 μM staurosporine for 2 h were prepared in parallel with the experimental samples and used as positive controls.
Creation and characterization of E8-expressing cell lines. To inhibit cell death receptor-mediated caspase 8 activation in Jurkat cells, we utilized a mammalian expression vector encoding the equine herpes virus 2 protein E8, a v-FLIP. The expression vectors were generated by cloning E8-myc (13) (after PCR amplification from herpes virus 2 genomic DNA) into the XhoI/HindIII sites of the vector pcDNA3 (Invitrogen). Subconfluent flasks of Jurkat cells were transfected with 2 μg of recombinant E8 plasmid cDNA (pcDNA3.1MycHisA) by the use of Fugene (Boehringer Mannheim) according to the manufacturer’s specifications. Both recombinant E8-expressing pools of cells and cells transfected with the empty vector pcDNA3 alone were isolated in 400 μg/ml of G418 (GIBCO BRL). These pools of cells were then maintained in RPMI 1640 plus 10% FCS and 200 μg/ml of G418 for 7–10 days and subsequently used for hypoxia experiments. E8 overexpression was confirmed by immunoblotting with an anti-myc antibody directed against the COOH-terminal myc tag.

RESULTS

Hypoxia with reoxygenation induces caspase-dependent apoptosis. Jurkat cells exposed to hypoxia for 3–5 h in glucose- and serum-deficient medium followed by reoxygenation in complete medium for 2 h demonstrated apoptotic changes of plasma membrane blebbing, nuclear condensation, and some nuclear fragmentation. All of these typical morphological apoptotic changes could be blocked by preincubation with the broad spectrum caspase inhibitor z-VAD-FMK (Fig. 1) or with the caspase 9 peptide inhibitor z-LEHD-FMK. Jurkat cells exposed to 3 and 5 h of hypoxia in glucose- and serum-deficient medium followed by reoxygenation also displayed substantial DNA fragmentation (Fig. 2A). However, cells exposed to hypoxia alone (without reoxygenation) in glucose- and serum-deficient medium did not demonstrate such changes (Fig. 2B). Exposure of cells to glucose- and serum-deficient medium without hypoxia also failed to induce apoptotic changes whether or not the incubations were followed by incubation in complete medium. Similarly, DNA fragmentation was not detected during hypoxia but only after reoxygenation. DNA fragmentation could be blocked by preincubation with the caspase inhibitors z-VAD-FMK, z-DEVD-FMK (Fig. 2C), or z-LEHD-FMK (not shown). Cleavage of the death substrate PARP occurred only after reoxygenation and was prevented by preincubation with the caspase inhibitors z-VAD-FMK and z-DEVD-FMK (Fig. 2D). Similarly, the effector caspases, caspase 3, caspase 6 (not shown), and caspase 7, were cleaved only with reoxygenation (Fig. 2E). Thus hypoxia in the absence of glucose caused apoptosis in Jurkat cells only after a period of reoxygenation. This requirement for reoxygenation was different than shown previously for cardiac myocytes and vascular smooth muscle cells that underwent hypoxia-induced apoptosis without reoxygenation (20, 21) but was similar to H9c2 cells, a myogenic cell line, which required reoxygenation after hypoxia to show apoptotic changes (11).

Hypoxia without reoxygenation causes cytochrome c release. Cytochrome c translocation from the mitochondria to the cytosol was detected after 3 h of hypoxia and did not require reoxygenation (Fig. 3A) in contrast to other hypoxia-stimulated apoptotic events. The release of cytochrome c was a specific event and not due to physical disruption of mitochondria since no signal for cytochrome oxidase could be detected in the cytosolic fractions of Jurkat cells exposed to hypoxia (data not shown). Neither the relatively nonspecific caspase inhibitor z-VAD-FMK (Fig. 3B) nor the more specific caspase 3 inhibitor z-DEVD-FMK (not shown) blocked...
cytochrome c release, either before or after reoxygenation, suggesting that the process was caspase independent in these cells, similar to previous findings in cardiac myocytes (21). These findings imply that cytochrome c release is upstream of caspase activation in hypoxia-induced apoptosis.

Glucose prevents cytochrome c release but does not prevent apoptosis in Jurkat cells. Jurkat cells exposed to 3–5 h of hypoxia in the presence of 3 mM glucose underwent typical apoptotic morphological and biochemical changes in the absence of reoxygenation (Fig. 4A). The death substrate PARP was also cleaved during hypoxia in the presence of glucose (Fig. 4B) and caspase 3, and caspase 6 was also cleaved (Fig. 4C). In contrast, cytochrome c release was completely prevented during hypoxia in the presence of glucose (Fig. 4D). Thus in the presence of glucose, a mitochondrion-independent apoptotic process occurred during hypoxia in Jurkat cells.

Overexpression of v-FLIPs attenuates hypoxia-induced cell death in Jurkat cells in the presence of glucose. To determine whether this cytochrome c-independent apoptosis was due to some factor in the medium that induced receptor-mediated apoptosis, we incubated normoxic Jurkat cells in conditioned medium from cells exposed to hypoxia. This incubation failed to induce either PARP cleavage (Fig. 5) or any morphological features of apoptosis (not shown).

To further examine whether death receptor activation was involved in the cytochrome c-independent apoptotic pathway triggered by hypoxia in the presence of glucose, we expressed the v-FLIP E8 in Jurkat cells. E8 expression should inhibit all death receptor-mediated apoptotic pathways by preventing caspase 8 (FLICE) activation. Western blot analysis demonstrated significant expression of E8-myc in FLIP-transfected cells (Fig. 6A). Exposure of the E8-expressing Jurkat cells to 6 h of hypoxia in the presence of glucose failed to induce apoptosis, as demonstrated by morphological changes (Figs. 6B and 7). In addition, control cells showed cleavage of the death substrate PARP to its characteristic 85-kDa fragment, whereas E8-expressing cells showed little PARP cleavage (Fig. 6C). Interestingly, exposure of E8-expressing cells to hypoxia in glucose-deficient medium did not have any appreciable effect on the degree of apoptosis, as demonstrated by the number of apoptotic cells by Hoechst staining (Fig. 7), again suggesting that the apoptosis seen in glucose-deficient medium occurred via a
caspase 8-independent and death receptor-independent pathway.

Inhibitor of caspase 9 prevents apoptosis in Jurkat cells in the absence of glucose. To finally determine whether a mitochondrial pathway was required for hypoxia-induced apoptosis either in the absence or presence of glucose, Jurkat cells were exposed to 6 h of hypoxia after preincubation in 100 μM z-LEHD-FMK. This caspase 9 inhibitor significantly suppressed apoptosis when the cells were exposed to hypoxia/reoxygenation in the absence of glucose. Staurosporine (2 μM) was used as a positive control. For caspase inhibitor studies, Jurkat cells were preincubated with 100 μM of z-DEVD-FMK (B). Immunoblot analysis demonstrated caspase 3 and caspase 6 cleavage in Jurkat cells exposed to 3 and 5 h of hypoxia in the presence (gIc) and absence (no gIc) of glucose. The cleaved p20 fragments of the activated caspases 3 and 6, respectively, were detected only after reoxygenation in glucose-deficient conditions and without reoxygenation in glucose-containing medium. More pronounced caspase cleavage was seen in the presence of glucose. The caspase inhibitors z-VAD-FMK and z-DEVD-FMK (100 μM each) completely prevented cleavage of the proform of caspase 6 (C). Immunoblot analysis demonstrated prevention of release of cytochrome c in cytosolic fractions of Jurkat cells exposed to 3, 4, 5, and 7 h of hypoxia in the presence of glucose but not in its absence (D). C, control.

DISCUSSION

It is well known that hypoxia can lead to cellular injury and death. Only relatively recently, however, has it been appreciated that hypoxia can induce apoptosis as well as necrotic cell death. We and others (8, 9, 11, 16, 21) have previously shown that hypoxia induces apoptosis via a mitochondrial pathway involving the release of cytochrome c and subsequent caspase activation. Likewise, in Jurkat cells, hypoxia followed by a short period of reoxygenation was associated with cytochrome c translocation, caspase and PARP cleavage, and morphological evidence of apoptosis. Because cytochrome c release occurred before reoxygenation, cytochrome c release was apparently upstream of caspase 3, 6, and 7 cleavage. This finding was supported by the inability of nonspecific caspase inhibitors to block cytochrome c translocation, despite their ability to inhibit downstream events such as PARP cleavage, nuclear fragmentation, and DNA laddering.
tigators to inhibit apoptosis induced by hypoxia and serum deprivation in neonatal rat cardiac myocytes (30).

Interestingly, compared with other cells, significant differences in Jurkat cell responses to hypoxia were noted in the presence of glucose. In our previous studies, provision of glucose or overexpression of glucose transporters inhibited cytochrome c release and prevented apoptosis in cardiac myocytes and A7r5 cells, a vascular smooth muscle cell line (20, 21). However, in Jurkat cells, there was no protection garnered by exposure to glucose under similar conditions. Indeed, there was even more extensive caspase and PARP cleavage in the presence than in the absence of glucose, despite prevention of cytochrome c release. It appears that the cytochrome c-independent apoptosis required activation of death receptors, since the apoptotic process was inhibited by constitutive expression of an inhibitor of death receptor pathways that utilize caspase 8 as the initiator caspase. Whether hypoxia induces release of a ligand for such death receptors (e.g., Fas ligand, TNF) is unknown, although the lack of apoptosis in response to conditioned medium (Fig. 5) makes that possibility somewhat less likely. Alternatively, hypoxia may induce oligomerization of death receptors leading to activation of the receptor-mediated cell death pathway. Exposure of mammalian cells to extracellular stresses, such as UV light and hyperosmolarity, has been demonstrated to cause clustering of cell surface TNF receptors as well as other cytokine and growth factor receptors leading to their activation (5, 25). It is conceivable that extracellular stresses like hypoxia may induce clustering and activation of these receptors, leading to programmed cell death. Alternatively, hypoxia may induce clustering of procaspase 8 molecules through other mechanisms that have yet to be identified. In any case, this signaling complex could then recruit, via the receptor-bound adapter protein FADD, several procaspase 8 molecules, resulting in a high local concentration of procaspase 8, which is sufficient to drive intermolecular proteolytic activation of the receptor-associated procaspase 8 molecules (23, 24). Because Jurkat cells possess a large complement of death receptors, it would not be surprising for hypoxia to induce such a pathway in these cells. The critical upstream activators of the mitochondrial pathway in hypoxia are even less well understood. A number of recent studies have demonstrated that hypoxia-induced reduction in cellular ATP may be an important determinant of cell death (10, 18, 26) and may also serve as a switch in the cell’s decision between apop-

**Fig. 6.** Overexpression of E8 proteins prevent hypoxia-induced cell death in Jurkat cells. A: Western blot analysis of both vector- and E8-transfected pools of Jurkat cells after immunoblotting with an anti-myc antibody as described in MATERIALS AND METHODS. Quantitation of apoptotic cells based on morphological features after staining with the HOE-33258 dye is shown. Values are means ± SE; n = 5; P < 0.05. C, untransfected control cells; V, empty vector-transfected cells; E8, cells transfected with the viral FLICE inhibitory protein (v-FLIP); Apo-1, cells treated with the anti-Fas antibody as a positive control. B: effect of overexpressing the v-FLIP E8 in Jurkat cells, followed by hypoxia for 6 h in the presence of glucose. C: immunoblot analysis for hypoxia-induced PARP cleavage in vector- and E8-overexpressing pools of Jurkat cells.

**Fig. 7.** Caspase 9 inhibitor prevents apoptosis by inhibiting the mitochondrial cell death pathway. Jurkat cells were exposed to hypoxia for 6 h under various experimental conditions as indicated. The number of apoptotic cells was counted based on morphological features revealed after staining with the HOE-33258 dye. The data shown are from 4 separate experiments (n = 4), and values represent means ± SE. The caspase 9 inhibitor z-LEHD-FMK was added at a final concentration of 100 mM. E8, v-FLIP. P < 0.0001.
tosis, and necrosis. For example, in cultured kidney cells, cell death during hypoxia/reoxygenation was shown to follow a pattern wherein depletion of cellular ATP stores, either by hypoxia or mitochondrial uncouplers, was linked to protein translocation of the proapoptotic molecules Bax and cytochrome c, but final caspase activation occurred only after restoration of cellular energy stores by reoxygenation (26). These results suggest that low but measurable ATP levels may trigger apoptosis but that apoptotic caspase activation may actually be interrupted by exceedingly low ATP levels, causing the cells to undergo necrosis instead. Although these ATP-dependent mechanisms were not formally tested by our studies, our results on the timing of cytochrome c release and caspase activation are consistent with very low ATP levels triggering cytochrome c release, but at the same time inhibiting downstream caspase activation. Another important regulator of the mitochondrial apoptotic pathway in hypoxia is the proapoptotic Bcl-2 family member Bax. Translocation of Bax to the mitochondria triggers cytochrome c release in many cells during and after hypoxia (26). In addition, levels of Bcl-2, which prevent cytochrome c release, are reduced by hypoxia in several systems (17). These processes occur both during hypoxia as well as during reoxygenation. More proximal regulators of Bax and cytochrome c release during hypoxia may include hypoxia-inducible factor-1α (HIF-1α) and p53. HIF-1α levels are rapidly induced by hypoxia (29), and HIF-1α induces apoptosis, in part, via augmentation of p53 levels (2). In addition, HIF-1α may also be directly responsible for activating Nip3, a proapoptotic member of the Bcl-2 family (4) that provides a further link between hypoxia and apoptosis. It has been demonstrated that p53 also stimulates cytochrome c release through its action on Bax (15). Hypoxia may also induce cytochrome c release through other mitochondrial mechanisms, such as direct effects on Bax phosphorylation, the activity of the adenine nucleotide translocator, or mitochondrial membrane permeability, all of which have been shown to affect cytochrome c release. Hypoxia may also directly suppress the protein levels of Bcl-2, thereby promoting cytochrome c release. This activation may be mediated through activation of the nuclear transcription factor (NF-κB) by hypoxia (22).

We have shown that glucose metabolism prevents cytochrome c release during hypoxia, but the mechanisms by which glucose inhibits this event are still unclear. Chi et al. (6) induced apoptosis in murine blastocysts by inhibiting glucose transport using an antisense approach. Apoptosis was blocked in Bax (−/−) cells, suggesting that Bax was necessary for apoptosis and that glucose and glucose transport somehow inhibited Bax translocation. The protective effect of glucose on hypoxia-mediated apoptosis may also be associated with the ability of glucose to decrease intracellular Ca2+ concentration during hypoxia, promoting the upregulation of Bcl-2 and promoting the inactivation of another proapoptotic Bcl-2 family member, Bad (27). Finally, we have demonstrated that glucose prevents HIF-1α and p53 accumulation in hypoxic cells (unpublished data), which may thereby prevent the translocation and activation of Bax and reduce cytochrome c release. However, the mechanism of this effect is yet to be established.

The present observations of hypoxia-induced apoptosis in Jurkat cells provide a platform to further identify the pro- and antiapoptotic pathways that are generated in various cells by hypoxia. The precise role of glucose uptake and metabolism on the activities, levels, and translocation of Bax, p53, HIF-1α, NF-κB, and other factors should help provide further insight into the molecular mechanisms that control both mitochondrial- and death receptor-associated apoptosis during hypoxia and reoxygenation.

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REFERENCES

16. Kang PM, Haunstetter A, Aoki H, Usheva A, and Izumo S. Morphological and molecular characterization of adult cardiomy-


