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Hypoxic preconditioning protects human brain endothelium from ischemic apoptosis by Akt-dependent survivin activation

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Zhang Y, Park TS, Gidday JM. Hypoxic preconditioning protects human brain endothelium from ischemic apoptosis by Akt-dependent survivin activation. Am J Physiol Heart Circ Physiol 292: H2573–H2581, 2007. First published March 2, 2007; doi:10.1152/ajpheart.01098.2006.—Preconditioning-induced ischemic tolerance is well documented in the brain, but cell-specific responses and mechanisms require further elucidation. The aim of this study was to develop the in vitro model of ischemic-tolerance in human brain microvascular endothelial cells (HBMECs) and to examine the roles of phosphatidylinositol 3-kinase (PI3-kinase)/Akt and the inhibitor-of-apoptosis protein, survivin, in the ability of hypoxic preconditioning (HP) to protect endothelium from apoptotic cell death. Cultured HBMECs were subjected to HP, followed 16 h later by complete oxygen and glucose deprivation (OGD) for 8 h; cell viability was quantified at 20 h of reoxygenation (RO) by the 3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium) bromide assay. HBMECs were examined at various times after HP or OGD/RO using immunoblotting and confocal laser scanning immunofluorescence microscopy for appearance of apoptotic markers and expression of phosphorylated (p)-Akt and p-survivin. Causal evidence for the participation of the PI3-kinase/Akt pathway in HP-induced protection and p-survivin upregulation was assessed by the PI3-kinase inhibitor LY-294002. HP significantly reduced OGD/RO-induced injury by 50% and also significantly reduced the OGD-induced translocation of apoptosis-inducing factor (AIF) from mitochondria to nucleus and the concomitant cleavage of poly(ADP-ribose) polymerase-1 (PARP-1). PI3-kinase inhibition blocked HP-induced increases in Akt phosphorylation, reversed the effects of HP on OGD-induced AIF translocation and PARP-1 cleavage, blocked HP-induced survivin phosphorylation, and ultimately attenuated HP-induced protection of HBMECs from OGD. Thus HP promotes an antiapoptotic phenotype in HBMECs, in part by activating survivin via the PI3-kinase/Akt pathway. Survivin and other phosphorylation products of p-Akt may be therapeutic targets to protect cerebrovascular endothelium from apoptotic injury following cerebral ischemia.

cell-specific receptors and signaling pathways. Recently, ischemic tolerance to oxygen and glucose deprivation (OGD)-induced necrotic death of cultured cerebrovascular endothelial cells was documented (4). Whether preconditioning also protects cerebral endothelium from ischemia-induced apoptotic cell death remains an important unanswered question. Moreover, the mechanistic basis of cerebral endothelial cell tolerance is not known.

Akt is a cytosol-localized serine/threonine kinase that, when activated by phosphorylation at serine-473, threonine-308, and perhaps other sites in response to growth factors, cytokines, and other stress stimuli, is recruited to the plasma membrane where it activates multiple downstream targets to enhance cell survival (48, 49). Phosphatidylinositol 3-kinase (PI3-kinase) is largely responsible for Akt phosphorylation (49, 52). Although the expression of phosphorylated (p)-Akt increases in hippocampal homogenates of rats (38) and gerbils (58) following ischemic preconditioning, whether Akt activation occurs within cerebral endothelium in response to hypoxic preconditioning (HP) and whether such activation might provide significant protection to these specific cells are unknown. Moreover, the downstream phosphorylation targets of p-Akt under these conditions have yet to be identified, although many are recognized as antiapoptotic (12, 15, 49).

Survivin is a member of the “inhibitor of apoptosis” (IAP) gene family of proteins that is widely expressed in cancer cells, where it can suppress both caspase-dependent and -independent apoptotic cell death (34, 35, 55). While barely detectable in normal tissues, survivin expression can be “reactivated” by specific growth factors and other prosurvival stimuli (42, 44, 50). Whether preconditioning with mild hypoxia is one such stimulus is unknown. Also requiring elucidation is how survivin is regulated at both the transcriptional and translational levels. Akt is not thought to be the prototypical kinase (41, 55) for posttranslational modification of survivin activity; however, recent studies documenting the blockade of the protective effect of survivin against apoptosis by PI3-kinase inhibition (21, 31, 43, 44, 62) argue in favor of such a possibility. In short, the potential role for survivin in establishing the antiapoptotic phenotype characteristic of ischemic tolerance in endothelial cells or other resident brain cells has yet to be defined.

Thus we undertook the present study to document the ability of HP to reduce apoptotic cell death in human cerebral endo-

THE CELL-SPECIFIC AND MECHANISTIC basis of cerebral ischemic tolerance, whereby an increased resistance to injury is induced by “preconditioning” the brain with a sublethal stress (18, 22), still requires considerable elucidation. Cultured cells provide an appropriate model to help identify the molecular underpinnings of preconditioning-induced ischemic tolerance in a given cell type. Preconditioning studies conducted in cultured neurons (8) and mixed neuronal-glial cultures (47) have identified
thelium and to begin to elucidate the mechanistic basis for such apoptotic resistance. We tested the hypothesis that the PI3-kinase/Akt pathway participates in establishing the ischemia-tolerant phenotype following HP and that p-Akt-mediated phosphorylation of the IAP protein survivin is one antiapoptotic effector mediating protection of cerebral endothelium against ischemia-reperfusion injury.

METHODS

Cerebral endothelial cell culture. Human brain microvascular endothelial cells (HBMECs) (13) were a generous gift from Dr. Sean P. Colgan (Harvard Medical School) and cultured on 0.1% collagen I-coated dishes or plates, as described previously (61). Cell purity was assessed by periodic immunostaining for prototypical endothelial- and glial-specific markers and routinely examined for the prototypical cobblestone appearance under phase; similar validation studies were performed in a previous study, as well as uptake of fluorescent-acetylated LDL (13).

HP protocol. Because the preconditioning OGD recovery protocol we adopted spanned 5 days in total, we initiated 3 consecutive days of HP when HBMECs were at 70% confluence, as follows: after being washed three times, cultures were transferred to a 37°C incubator within a hypoxic chamber (93% nitrogen-5% CO2-2% oxygen), and HBSS media were replaced with medium 199 (M199; without serum) that was prebubbled for 5 min with the same gas mix to provide a media PO2 (14.7 mmHg) equivalent to that in the ambient air of the chamber. This first exposure to hypoxia lasted 3 h, followed by a 1-h period of normoxia (21% oxygen-5% CO2-74% nitrogen), after which they were returned to the chamber and given fresh hypoxic media for a second 3-h hypoxia exposure. With each return to normoxia, oxygenated complete M199 containing 20% serum was added. Three consecutive days of these same paired 3-h hypoxia exposures constituted the HP stimulus. Measures of p-Akt and p-survivin were obtained 16 h following the last day of the preconditioning stimulus, at a time coinciding with the initiation of OGD.

In some experiments, LY-294002 (Sigma, St. Louis, MO), a well-established inhibitor of PI3-kinase (53), was added to the cells 30 min before each HP stimulus. The concentration used, 20 μmol/l, was chosen based on similar tens of micromolar dose ranges reported in the literature as effective and on results of pilot studies wherein we did measure p-Akt levels after LY-294002 administration by immunoblotting to document its effect.

OGD and reoxygenation, and cell viability determinations. Cells with or without previous HP treatment were subjected to an 8-h period of lethal OGDreoxygenation (RO) starting 16 h after the last HP stimulus as described in detail in an earlier publication (61). In brief, OGD was induced by placing the cells in a 37°C incubator housed within an anoxic chamber (95% nitrogen-5% CO2) and replacing the media with HBSS (without serum) that was bubbled for at least 5 min with the same gas mix; measurements of media PO2 (3.7 mmHg) revealed a level of near complete anoxia (<0.5% oxygen) that was identical to that measured in the ambient air of the chamber. RO was undertaken at 37°C by providing the cells media (M199) with 1% serum and normoxic conditions (21% oxygen-5% CO2-74% nitrogen). At 4 h of RO, poly(ADP-ribose) polymerase (PARP) cleavage and apoptosis-inducing factor (AIF) translocation were determined by immunoblotting; at 20 h of RO, HBMEC viability was quantified by a reduction of 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide, as described previously (61).

Laser scanning confocal immunofluorescence microscopy. For laser confocal immunofluorescence microscopy of p-Akt, von Willebrand Factor, glucose transporter-1 (Glut-1), glial fibrillary acidic protein (GFAP), cleaved PARP-1, AIF, and the inhibitor of apoptosis family protein survivin, HBMECs were seeded on round glass coverslips (BD Biosciences, San Diego, CA) coated with 1% collagen-I in six-well plates. At appropriate time points after different treatments, medium was removed, cells were washed and fixed with 4% paraformaldehyde for 10 min and then permeabilized and blocked for 1 h at room temperature in PBS containing 5% normal serum and 0.3% Triton X-100. Cells were incubated overnight at 4°C in blocking buffer containing 1:200 rabbit anti-human p-Akt (Cell Signaling Technology, Beverly, MA), 1:200 mouse anti-human von Willebrand factor antibody (Lab Vision, Fremont, CA), 1:200 mouse anti-human Glut-1 antibody (a gift from Dr. William R. Frazier, Dept. of Biochemistry, Washington Univ., St. Louis, MO), 1:200 mouse anti-human GFAP antibody (a gift of Belinda McMahan, Dept. of Ophthalmology, Washington Univ.), mouse anti-human cleaved PARP-1 antibody (1:200; Cell Signaling Technology), rabbit anti-human AIF antibody (1:200; Chemicon International, Temecula, CA), rabbit anti-cleaved p-survivin antibody (Thr34) (1:50; Santa Cruz, Santa Cruz, CA), followed by fluorescein Alexa-560-conjugated anti-mouse or anti-rabbit IgG (1:500) (Molecular Probes, Eugene, OR) for 1 h at room temperature. Nuclear DNA was stained with 4′,6-diamidino-2-phenylindole. The specimens were observed at ×40 under a laser-scanning confocal microscope (Carl Zeiss, LSM 5 PASCAL) at the excitation wavelength of 405 and 560 nm. To distinguish the colors, especially the merge color easily, the original colors of blue for nucleus and red for proteins were adjusted to red and green, respectively.

Subfractions and immunoblotting. For PARP-1 cleavage and AIF translocation, HBMECs were collected at 4 h of normoxic RO after OGD (with and without prior HP), and nuclear extracts were obtained (61). In brief, cells were washed with ice-cold PBS, scraped from dishes, and centrifuged (600 g for 5 min); the pellet was then resuspended in lysis buffer containing (in mM) 20 HEPES-KOH (pH 7.4), 10 NaCl, 1.5 MgCl2, 1 EDTA, 1 EGTA, and 250 sucrose and 1× protease inhibitor cocktail. Following centrifugation (600 g for 10 min), the supernatant was discarded and the pellet was washed and resuspended in nuclear extraction buffer [containing (in mM) 20 Tris-HCl (pH 7.5), 1.5 MgCl2, 420 NaCl, and 0.2 EDTA and 25% glycerol, 0.5% Triton X-100, 0.1% Nonidet P-40, and 1× protease inhibitor cocktail], vortexed, and shaken for 30 min. The suspension was then centrifuged (14,000 g for 10 min), and the supernatant, which contained the nuclear fraction, was transferred to a prechilled microcentrifuge tube. The primary antibodies used to probe the blots overnight at 4°C were a mouse anti-human-cleaved PARP-1 antibody (1:1,000; Cell Signaling Technology) and a rabbit anti-human AIF antibody (1:2,000; Chemicon International). p-Akt and p-survivin immunoblotting was performed on whole cell lysates, obtained 16 h after the last preconditioning stimulus at the time when sister cultures were exposed to OGD. Blots were probed overnight at 4°C with a rabbit anti-human p-Akt (for phosphorylation at Ser473) [1:1,000; Cell Signaling Technology] and a rabbit anti-human p-survivin antibody (1:500; Santa Cruz). For all blots, incubations with horseradish peroxidase-conjugated secondary antibodies were followed by detection of immunoreactive proteins using enhanced chemiluminescence (Cell Signaling Technology). After being scanned, protein bands were quantified with ImagePro Plus software and standardized for statistical analysis.

Statistics. All results are presented as means ± SE. For single and multiple comparisons among the experimental groups, Mann-Whitney nonparametric rank sum tests or ANOVA on rank tests, respectively, were used to calculate significance. In each case, significance was defined as P < 0.05.

RESULTS

Phenotypic confirmation. HBMECs exhibited the characteristic “cobblestone” appearance of confluent endothelial cells...
HP improves HBMEC viability following simulated ischemia. We assessed the effects of different durations of HP on cellular viability to an 8-h OGD insult that caused a moderate level of injury (39% cell death) in untreated HBMECs; improvements in cell viability at 20 h of RO were dependent on the duration of HP and whether hypoxia was continuous or intermittent (Fig. 2). Significant, but modest, cytoprotection (29 ± 1% cell death; 26% protection) was achieved by 3 consecutive days of 4 h of continuous HP. Increasing the duration of HP to 6 h reduced cell death to 26 ± 3% (33% protection). However, exposing HBMECs to the same 6 h of hypoxia in two 3-h episodes (separated by 1 h of normoxia) reduced OGD/RO-induced cell death to 19 ± 3% (51% protection). We adopted this HP protocol (3 h + 3 h) for the rest of the experiments reported herein. The preconditioning itself did not cause any noticeable cell injury in terms of cell viability loss and apoptotic marker appearance (data not shown).

HP reduces OGD-induced apoptosis of HBMECs. By subcellular fractionation analysis, we reconfirmed our earlier finding (61) that apoptosis, reflected by a significant increase in mitochondrial translocation from mitochondria to nuclei and a significant increase in nuclear PARP-1 cleavage following OGD (Fig. 3, A and B). With respect to PARP, confocal microscopy imaging with an antibody specific for cleaved PARP reveals an obvious increase in nuclear PARP cleavage following OGD and the ability of prior HP to attenuate this cleavage event (Fig. 3D, middle and bottom). HP prior to OGD attenuated this translocation, as reflected by reduced nuclear AIF expression (Fig. 3D, bottom). Together, these results indicate that HP-induced improvements in cell viability following OGD/RO (Fig. 2) are the result of a reduction in apoptotic cell death.

HP increases PI3-kinase/Akt pathway signaling. Since the PI3-kinase/Akt survival pathway has been implicated in the antipapoptotic effect of a variety of interventions in endothelial (36, 48, 62) and other cells (26, 49, 51), we examined whether HP affects levels of p-Akt in our HBMEC model. Sixteen hours after the last HP treatment, at a time just before when the cells were subjected to OGD, we found a threefold increase in p-Akt levels (Fig. 4A), which was revealed by confocal fluorescence microscopy to be primarily cytosolic (Fig. 4B). The addition of the PI3-kinase inhibitor LY-294002 during HP effectively blocked the HP-induced increase in Akt phosphorylation (Fig. 4, A and B). At the concentration we used in the current study, no noticeable toxicity of LY-294002 was observed. Moreover, this pharmacological intervention reversed the effects of HP on OGD-induced nuclear AIF translocation and cleavage of nuclear PARP-1, as evidenced by immunoblotting (Fig. 5A) and confocal microscopy (data not shown) and, in turn, blocked HP-induced HBMEC cytoprotection (Fig. 5B). Thus increased p-Akt levels appear critical to the HBMEC protection afforded by HP, secondary to reductions in AIF translocation and PARP-1 cleavage.

Survivin is a phosphorylation target of p-Akt. To begin to address the downstream targets of p-Akt involved in mediating HP-induced antiapoptotic protection, we measured changes in p-survivin protein levels following HP. As shown in the immunoblotting in Fig. 6A, we found that HP led to significant increases in cellular levels of p-survivin at the time just before when the cells were subjected to OGD. We also documented that this increase in p-survivin levels was significantly attenuated if PI3-kinase was inhibited during HP. These changes were confirmed by confocal microscopy. Under control conditions, p-survivin was expressed at low levels in the nucleus.
but the intensity of p-survivin expression was notably enhanced in response to HP (Fig. 6B, middle). The resultant attenuation of HP-induced increases in p-survivin protein levels by LY-294002 was evidenced by lower p-survivin staining intensities, similar to those observed under control conditions (Fig. 6B, bottom). Together, these results implicate that a p-Akt-dependent phosphorylation of survivin contributes to the antiapoptotic effect of HP on HBMECs.

**DISCUSSION**

Results of the present study indicate that HP increases the resistance of cerebral endothelial cells to ischemia-reperfusion-induced apoptotic cell death. HP-mediated protection was characterized by the attenuation of nuclear AIF translocation and nuclear PARP-1 cleavage. Our studies showed that this reduction in apoptosis resulted, in part, from the phosphorylation-based activation of the IAP protein survivin, secondary to an HP-induced activation of PI3-kinase and the formation of p-Akt.

The cerebrovascular endothelium is an extremely active tissue responsible for regulating the trafficking of cells, substrates, and other molecules across the blood-brain barrier; for controlling vasomotor tone and reactivity; and for maintaining homeostasis at the blood-vascular wall interface (2). Postischemic microvascular dysfunction involving each of these aforementioned regulatory properties is now a well-established sequela in the stroke brain (17), and, as such, protection of the “neurovascular unit” is considered an essential component of stroke therapy (27). For obvious reasons, cell culture models of cerebrovascular endothelium are useful for elucidating mechanisms of ischemic injury and protection that are considerably more difficult to identify in vivo. To date, although cultured cerebral endothelial cells from rat (23) and bovine (40) brain have been employed to study mechanisms of cellular tolerance to TNF-α (23) and heat stress (40), only one model on ischemic tolerance has been published (4) using mouse cerebral endothelial cells. The present study is the first to demonstrate preconditioning-induced protection against OGD-induced apoptotic injury in human cerebral endothelial cells, underscoring the potential clinical applications of cerebrovascular preconditioning. We also show for the first time that a noninjurious preconditioning regimen involving hypoxia, not brief ischemia, can serve as an effective preconditioning stimulus. As alluded to above, the protection afforded by our HP stimulus against OGD-induced HBMEC death was antiapoptotic in nature, as evidenced in our model as reductions in the...
caspase-independent nuclear translocation of AIF and the caspase-dependent cleavage of nuclear PARP-1, both of which correlate with transferase-mediated dUTP nick-end labeling positivity and other markers of apoptosis (61). The ability of HP to abrogate apoptotic cell death in cerebrovascular endothelium is in line with observations that preconditioning can prevent AIF translocation and/or PARP-1 cleavage in other cells (30, 46). Taken together with the recent finding in mouse cerebrovascular endothelial cell culture that preconditioning reduces the OGD-induced release of intracellular LDH (4), a prototypical marker of necrotic cell death (32), one would conclude that preconditioning acts on multiple cell death pathways in cerebral endothelium to reduce ischemia-induced death and dysfunction.

Our finding that HP promoted a PI3-kinase-dependent increase in the phosphorylation of Akt in HBMECs is consistent with similar observations of hypoxia-induced Akt phosphorylation in other cell types (3, 6, 56, 60). The detailed mechanisms by which PI3-kinase is activated by hypoxia require further elucidation (11, 60). Whether p-Akt is causal to HP-induced cytoprotection appears to be stimulus, cell type, and model dependent. In cultured neurons, Akt does not appear to be essential to OGD preconditioning-induced protection against more severe OGD-mediated injury (24, 26). However, when hypoxia is used as the preconditioning stimulus, protection of cultured neurons from ischemia and other death-inducing stimuli is p-Akt dependent (56). Moreover, the resistance of neuronal PC12 cells to serum withdrawal-induced apoptosis afforded by concomitant hypoxia is also dependent on Akt (3), as was HP-induced protection of cultured hepatocytes (9) and cardiomyocytes (51). Demonstrating a role for Akt in cerebral ischemic tolerance in vivo has been controversial (39, 58) and

Fig. 4. HP activates the phosphatidylinositol 3-kinase (PI3-kinase)/Akt pathway in HBMECs. A: immunoblots show HP-induced elevations in phosphorylated (p)-Akt 16 h after the last HP treatment, which was significantly blocked by the PI3-kinase inhibitor LY-294002 (LY, 20 μmol/l). Total Akt (t-Akt) levels were unaffected by HP. Values are means ± SE; n = 3 experiments. *P < 0.05 vs. CTL; #P < 0.05 vs. HP. B: representative immunoconfocal fluorescence microscopy images (from 3 independent experiments) with green for p-Akt, nuclear staining by DAPI in red, and the merged image showing the block of HP-induced increases in cytosolic and nuclear p-Akt by LY-294002. Scale bar = 20 μm.

Fig. 5. Inhibition of Akt phosphorylation blocks markers of apoptosis and HP-induced protection of HBMECs. A: immunoblots show that HP-induced reductions in nuclear translocation of AIF and c-PARP-1 in HBMECs are significantly attenuated by PI3-kinase inhibition with LY-294002. Values are means ± SE; n = 3 experiments. *P < 0.05 vs. OGD; #P < 0.05 vs. HP without LY-294002. B: inhibition of p-Akt formation blocks HP-induced protection of OGD-induced HBMEC injury. The significant protection afforded by HP against the OGD-induced 40% loss in cell viability was completely abrogated by PI3-kinase inhibition with LY-294002. Values are means ± SE; n = 3 experiments. *P < 0.05 vs. control; #P < 0.05 vs. OGD; †P < 0.05 vs. HP + OGD.
may reflect the use of brief global hypoxia-ischemia as a preconditioning stimulus in these models and/or the differential extent to which neurons, glia, and endothelial cells contributed to elevations in tissue homogenate levels of p-Akt and the inability to identify which cells account for the effects of intracerebroventricularly administered PI3-kinase inhibitors (39, 58). Our present results indicate that cerebral endothelial cell preconditioning by hypoxia requires p-Akt. Other endothelial cell models of HP also show a dependence on Akt for the induced tolerance exhibited by these cells to a variety of injurious insults (1, 62). Collectively, the data suggest that Akt may be critical to HP-induced protection in this particular vascular cell (48), independent of the tissue of origin, and that, with cerebral ischemic tolerance, non-Akt-dependent survival pathways may be operative in neurons and glia.

The mechanisms by which Akt exerts cytoprotective, pro-survival effects require further clarification. Considerable evidence indicates that p-Akt exerts antiapoptotic effects through the phosphorylation and deactivation of multiple targets, including Bad, caspase 9, glycogen synthase kinase-3, and the FOXO family of forkhead transcription factors. Activation of the PI3-kinase/Akt pathway inhibits the opening of the mitochondrial permeability transition pore (29), thereby preventing AIF release and translocation. Our demonstration by PI3-kinase inhibition that OGD-triggered AIF translocation from the mitochondria to the nucleus was negatively regulated by HP-induced increases in p-Akt is consistent with this effect. p-Akt-dependent reductions in the extent of ischemia-induced nuclear PARP-1 cleavage may also contribute to HP-induced increases in apoptotic resistance, as we observed in the present study, consistent with findings in other cells (20).

Survivin, a member of the IAP gene family, can be phosphorylated by cyclin-dependent kinase-1, cyclic AMP, and protein kinase C (41, 55), but a similar regulatory modification by p-Akt is not widely recognized. The findings that PI3-kinase inhibition blocked the upregulation of survivin protein expression by growth factors in CD34+ human mononuclear cells (21) and blocked survivin-mediated protection against the proapoptotic effects of serum deprivation (43), TNF-related apoptosis-inducing ligand (31), matrix detachment (44), Fas (54), and oxidative stress (62) are consistent with survivin being a downstream phosphorylation target of Akt. Our finding that p-survivin levels are increased in response to HP and that such an effect is attenuated in the presence of the PI3-kinase inhibitor indicates that p-survivin upregulation in cerebral endothelium by HP is p-Akt dependent. The fact that stimulus-induced increases in levels of survivin messenger RNA were also blocked by PI3-kinase inhibition (31, 43, 54) indicate that Akt may regulate survivin both transcriptionally and posttranslationally and that the mechanisms of constitutive expression of survivin may differ from stimulus-induced survivin gene transcription.

Although survivin is well established as a survival factor in the embryogenesis and oncology literature, our study is the first to document a role for p-survivin in establishing the antiapoptotic phenotype characteristic of ischemic tolerance in endothelial cells or other resident brain cells. Normally, survivin expression in resting endothelial cells is low (28) but can be upregulated in a cell cycle-dependent manner by VEGF (42, 50) and angiopoietin-1 (44) as part of an angiogenic phenotype. In addition, survivin upregulation may be an endogenous response on the part of endothelial cells to combat injury-inducing stimuli like ischemia (14) and other lesions (7). The present results indicate that mild hypoxia may also serve to upregulate survivin expression, consistent with findings in cultured endothelial cells from coronary artery (62) and in cancer cell lines (57). In vivo, hypoxia caused a doubling of survivin mRNA levels in brain homogenates (14), and we observed increases in p-survivin protein levels in whole cell lysates of adult mouse brain for 24 h after systemic hypoxia.

Fig. 6. Survivin is a downstream phosphorylation target of Akt in HP-induced protection against apoptotic cell death in HBMECs. A: immunoblots show that HP-induced elevations in p-survivin levels in HBMECs are significantly attenuated by the PI3-kinase inhibitor LY-294002. Values are means ± SE; n = 3 experiments. *P < 0.05 vs. CTL; #P < 0.05 vs. HP without LY-294002. B: HP-induced increases in p-survivin are shown in representative immunofluorescence microscopy images, wherein green is p-survivin, red shows DAPI-stained nuclei, and yellow is merged image showing reductions in HP-induced elevations in p-survivin by LY-294002 from 3 independent experiments. Scale bar = 20 μm.
OGD-induced apoptosis afforded by HP. That this protection was documented in human endothelium underscores the potential for translational application of endothelial preconditioning regimens to patients with stroke. Further studies are needed to elucidate other downstream targets of p-Akt that may mediate cerebral endothelial protection from ischemic injury as well as to define the mechanistic basis of the antiapoptotic effect of survivin in ischemic endothelium.

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